Hopanoids and sporulenes of a few bacteria and insights into sporulenes of *Bacillus subtilis*

Thesis submitted for the degree of
Doctor of Philosophy

By

Smita Nandardhane
(Reg. No. 17LPPH11)

Research Supervisor
Prof. Ch. Venkata Ramana

Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad – 500046
INDIA

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(A Central University established in 1974 by an Act of the Parliament) HYDERABAD 500046

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This is to certify that **Ms. Smita Nandardhane** has carried out the research work embodied in the present thesis under the supervision and guidance of **Prof. Ch. Venkata Ramana** for the full period prescribed under the Ph.D. ordinances of this University. We recommend her thesis entitled **"Hopanoids and sporulenes of a few bacteria and insights into sporulenes of *Bacillus subtilis*"** for submission for the degree of Doctor of Philosophy to the University.

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HEAD
Department of Plant Sciences

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School of Life Sciences
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हैदराबाद / Hyderabad-500 046.

26/6/2023

SUPERVISOR
Prof. Ch. Venkata Ramana

Prof. Ch. Venkata Ramana Ph.D
Department of Plant Sciences School of Life Sciences
University of Hyderabad, P.O. Central University,
Hyderabad - 500 046, India

UNIVERSITY OF HYDERABAD

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DECLARATION

I, **Smita Nandardhane**, hereby declare that this thesis entitled **"Hopanoids and sporulenes of a few bacteria and insights into sporulenes of *Bacillus subtilis*"** submitted by me under the guidance and supervision of **Prof. Ch. Venkata Ramana** is an original and independent research work. I, hereby declare that this work is original and has not been submitted previously in part or in full to this University or any other University or Institution for the award of any degree or diploma.

Nandardhane

Smita Nandardhane
(Ph.D. student)

26/6/2023

Supervisor
Prof. Ch. Venkata Ramana

Prof. Ch. Venkata Ramana Ph.D.
Department of Plant Sciences School of Life Sciences
University of Hyderabad, P.O. Central University,
Hyderabad - 500 046, India

UNIVERSITY OF HYDERABAD
(A Central University established in 1974 by an Act of the Parliament) **HYDERABAD 500046**

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This is to certify that the thesis entitled **"Hopanoids and sporulenes of a few bacteria and insights into sporulenes of *Bacillus subtilis*"** submitted by **Ms. Smita Nandardhane**, bearing registration number **17LPPH11**, in partial fulfilment of the requirements for the award of Doctoral of Philosophy in Department of Plant Sciences, School of Life Sciences, University of Hyderabad, is a bonafide work carried out by her under my guidance and supervision. This thesis is free from plagiarism and has not been submitted in any part or in full to this or any other University or Institute for the award of any degree or diploma.

Parts of the thesis have been:

A. Authored in the following publications:

1. **Smita N**, Indu B, Anusha R, Sasikala Ch and Ramana Ch V (2023) In silico analysis of sporulene biosynthesis pathway genes in the members of the class *Bacilli*. *Archives of Microbiology*. 205: 233.

2. Lhingjakim KL*, **Smita N***, Kumar G*, Jagadeeshwari U, Ahamad S, Sasikala Ch and Ramana Ch V (2022) *Paludisphaera rhizosphaereae* sp. nov., a new member of the family *Isosphaeraceae*, isolated from the rhizosphere soil of *Erianthus ravennae*. *Antonie van Leeuwenhoek*. 115:1073-1084. (*, the authors contributed equally)

B. Presented at the following conferences:

1. Presented a poster at the "National Conference on Frontiers in Plant Biology" (Feb. 2020), University of Hyderabad, Hyderabad.
2. Presented poster at the "59th Annual Conference of Association of Microbiologists of India" (Dec. 2018), University of Hyderabad, Hyderabad.
3. Presented an oral presentation at 13th Plant Science colloquium "Sporulenes of a few bacilli and their role in *Bacillus subtilis* under stress conditions" (Oct. 2021), University of Hyderabad, Hyderabad.

Further, the student has passed the following courses towards the fulfilment of the coursework requirement for Ph.D. degree.

S. No.	Course	Name	Credits	Results
1	PL801	Research methodology/Analytical techniques	4	Pass
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हैदराबाद विश्वविद्यालय / University of Hyderaba
हैदराबाद / Hyderabad-500 046.

26/6/2023

SUPERVISOR
Prof. Ch. Venkata Ramana

Prof. Ch. Venkata Ramana Ph.D
Department of Plant Sciences School of Life Sciences
University of Hyderabad, P.O. Central University
Hyderabad - 500 046, India

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"यद् भावं तद् भवति"

"As you think, so you believe , as you believe, so you become"

It is said that, beliefs come from what you surround yourself with, cause energies are contagious and your environment will become you. Therefore, you should make a conscious effort to surround yourself with positive, nourishing, and uplifting people who believe in you and encourage you to go after your dreams and applaud your victories

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I thank God, the Almighty, for enabling me to overcome all of life's challenges and survive it all. I am eternally grateful for His/Her serene presence.

Smita…

विद्ये विना मती गेली।
मती विना निती गेली॥
निती विना गती गेली।
गती विना वित्त गेले।।
वित्त विना शुद्र खचले।
एवढे अनर्थ एका अविद्येने केले॥

"Without knowledge, wisdom was lost; without wisdom morals were lost; without morals development was lost; without development wealth was lost; without wealth shudras are ruined; all these disasters are due to lack of knowledge"

– Mahatma Phule

Dedicated to my beloved parents and my supervisor

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Abbreviations	Expansions
AA	Amino acid
ANOVA	Analysis of Variance
BCM	*Bacillus* Composite Medium
BGC	Biosynthetic Gene Clusters
BHPs	Bacteriohopanepolyols
BLAST	Basic Local Alignment Search Tool
BPHs	Biohopanoids
Bsa	Glutathione peroxidase
Ca (NO_3)	Calcium nitrate
$Ca2^+$	Calcium ions
CARD	Comprehensive Antibiotic Resistance Database
CAS	CRISPR-associated protein
CDOM	Chromophoric-Dissolved Organic Matter
CESRs	Cell Envelope Stress Responses
CFU	Colony Forming Units
CLE	Cortex-lytic enzyme
CotA	Copper dependent laccase enzyme
cotJC, katX1,yjqC	Gene encoding catalase enzyme
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DCM	Dichloromethane
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribose nucleic acid
DPA	Dipicolinic acid
EDTA	Ethylenediaminetetraacetic acid
EMBL	European Molecular Miology Laboratory
erg32/*hpnX*	Sterol desaturase family protein
FAME	Fatty acid methyl ester
$FeSO_4$	Ferrous sulphate
FPP	Farnesyl diphosphate
FtsZ	Cell division protein homologous to bacterial tubulin
GC-MS	Gas Chromatography-Mass Spectrometry
GCW	Germ cell wall
GerD	Germination receptor D
GerKB, YlaJ, YhnC	Germination factors
gL^{-1}	Gram per liter
gm	Gram
GO	Gene ontology

GPS	Global positioning system
GR	Germination receptor
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric chloride
HepS, HepT	Heptaprenyl diphosphate synthase
hopC	Gene encoding for squalene/phytoene desaturase
HPLC	High Performance Liquid Chromatography
hpnA	Gene encoding hopanoid associated sugar epimerase
hpnC	Gene encoding for squalene synthase
hpnG	Gene encoding for 5-methylthioadenosine nucleosidase
hpnO	Gene encoding for amino-bacteriohopanetriol synthase
hr	Hour
IPP	Isopentyl pyrophosphate
iTOL	Interactive Tree Of Life
K_2HPO_4	Di-potassium hydrogen phosphate
KCl	Potassium chloride
KEGG	Kyoto Encyclopedia of Gene and Genomes
KH_2PO_4	Potassium dihydrogen phosphate
KinA, KinC	Histidine sensor kinases
LC-MS	Liquid Chromatography- Mass Spectrometry
LGT	Lateral gene transfer
LPG	Lysylphosphatidylglycerol
LPSN	List of Prokaryotic names with Standing in Nomenclature
M	Molar
MEGA	Molecular evolutionary genetics analysis
MeOH	Methanol
MEP	Methylerythritol phosphate pathway
mg	Milligram
$MgSO_4$	Magnesium sulphate
min	Minute
ml	Milliliter
mM	Millimolar
$MnCl_2$	Manganese chloride
MUSCLE	Multiple Sequence Comparison by Log-Expectation
MVA	Mevalonate pathway
NA	Nutrient agar
Na_2HPO	Di-Sodium hydrogen phosphate
NaCl	Sodium chloride

NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NAM	N-acetylmuramic acid
NaOH	Sodium hydroxide
NB	Nutrient broth
NCBI	National center for biotechnological information
NH_4HCO_3	Ammonium bicarbonate
NJ	Neighbor-joining
nm	Nanometer
O_2	Oxygen
PAH	Polycyclic Aromatic Hydrocarbons
PBS	Phosphate buffer
PCR	Polymerase chain reaction
PDA	Photodiode array detector
PE	Phosphatidylethanolamine
PG	Peptidoglycan
PhG	Phosphatidylglycerol
PI	Propidium iodide
PVC	*Planctomycetes-verrucomicrobia-chlamydiae* superphylum
RAST	Rapid Annotation using Subsystem Technology
recA	DNA recombination and repair protein
RNA	Ribose nucleic acid
ROS	Reactive oxygen species
RT-PCR/qPCR	Real time-polymerase chain reaction
SafK, GroEL	Morphogenetic proteins
SAMe	Gene encoding for S-adenosyl-L-methionine
SASP	α/β-Type small acid-soluble proteins
SDS	Sodium dodecyl sulfate
SEM	Scanning Electron Microscopy
SHC	Squalene hopene cyclase catalyzes
SkfC	Sporulation killing factor
SMART	Simple modular architecture research tool
SodA /SodF	Superoxide dismutase
SqhC	Sporulenol synthase
TAE	Tris-base, acetic acid and EDTA
TCA	Trichloroacetic acid
TE	Tris-EDTA
TEM	Transmission Electron Microscopy
TLC	Thin Layer Chromatography
Tpx	Thiol peroxidase

TrxA	Thioredoxin
TS	Terpenoid synthase
UV	Ultra violet radiations
v/v	Volume per volume
VBNC	Viable but non-cultivable
w/v	Weight per volume
WT	Wild type
YkuU	Peroxiredoxin
YtpB	Tetraprenyl-β-curcumene synthase
µg	Microgram
μg^{-l}	Microgram per litre
µl	Microlitre
µm	Micrometer

Introduction

1. INTRODUCTION

The emergence of organic matter, that act as the building block for intricate biomolecule structures was the first of several transitions that were necessary for the rapid evolution of life forms. Though the exact origin of these molecules is unknown, experiments have shown that they might have arose under hypothetical prebiotic conditions. A living cell is made up of four such large sized biomolecules. Owning to their prominent size and major contribution to a cell, proteins, nucleic acids, carbohydrates and lipids are recognized as macromolecules which serve as the foundation of life (Cooper 2000).

1.1 Prelude to the cellular macromolecules

Macromolecules are polymers amalgamated from small organic monomers which are nuts and bolts of cellular backbone *viz*, a) twenty amino acids which gives rise to **proteins;** b) two sugars (ribose and deoxyribose) with five carbons each, five nitrogenous bases (adenine, cytosine, guanine, thymine, uracil) and phosphoric acid as the components of **nucleic acids;** c) a primary sugar D-glucose, which majority of **carbohydrates** are derives of; and d) a five component of membrane **lipid** (Chaffey et al. 2003).

1.1.1 Proteins

Proteins are long polymers of variety of combination of amino acids. Of all macromolecules, proteins have the widest range of functions and are one of the most prevalent (except water) organic molecules in living systems. While some proteins serve as structural components, others function as transporter, signal receptors and regulatory factors. Many of them have catalytic domain and act as enzyme, whereas some are toxins (Morris et al. 2022).

1.1.2 Nucleic acids

Nucleic acids are the linear nucleotide polymers. Deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) are the most vital macromolecules for survival and continuity

of life, as it holds the genetic blueprint of a cell and propagate it further. Certain RNA molecules function as structural and catalytic components of supramolecular complexes. Nucleic acid's unique sequence of bases gives it a distinctive identity and serves as a source of encoded genetic information (Minchin and Lodge 2019).

1.1.3 Carbohydrates

Carbohydrates are polymers of monosaccharides that are mostly aldehyde or ketone in nature, with additional hydroxyl groups (simple sugars such as glucose) (Cooper 2000). Carbohydrates primarily serve two purposes: they serve as fuel stores that produce energy and as extracellular structural components that contain particular protein-specific binding sites (Holesh et al. 2017).

1.1.4 Lipids

Lipids are broadly defined as hydrophobic group of organic molecules with nonpolar C-C or C-H bond thus soluble in organic solvent to an extent but insoluble in water (Finley and deMan 2018). Different classes of lipids play an important role in many biological processes, including the structural maintenance of cellular membranes, the storage of energy and the involvement in signaling pathways (Yang and Han 2016).

1.1.4.1 Classification of lipids

Lipids are divided into eight different categories based on their chemical structures: fatty acyls, sphingolipids, glycerophospholipids, glycerolipids, saccharolipids and polyketides that are formed from the condensation of ketoacyl groups. Whereas polyprenyl lipids (prenols, sterols, hopanoids and quinones) are produced as a result of isoprene unit condensation (Fahy et al. 2009, Fahy et al. 2011). In contrast to the extensive research on other group of lipids in prokaryotic systems, little is known about the role of polyprenyl lipids in bacterial membranes (López-Lara and Geiger 2017). However, in eukaryotic system sterol lipids like cholesterol and its derivatives in association with sphingolipids and phospholipids

forms an essential lipid component of the membrane (Simon and Ikonen 1997) and were studied in detail.

1.1.4.2 Lipidomics

Understanding cellular physiology and pathology requires a thorough examination of lipid molecules. The in detailed study of lipids thus branched into "lipidomics," in the similar context of genomics and proteomics; as a result, lipid biology has grown to be a significant area of study after genomic revolution and advances in systems biology (Han 2016). The term "lipidome" refers to the total profile of lipids found in a cell, tissue or organism. It is a subset of the term "metabolome," which also refers to other three major classes of macromolecules: the study of proteins (**proteomics**), sugars and nucleic acids (**genomics**) (Fahy et al. 2011).

1.2 Bacterial membrane lipids and importance

The lipid rich cell envelop is a multilayered structure which forms an exterior barrier and act as cell boundary. Membrane lipids are essential component in the physiology of bacterial cells. At first, it was believed that the lipids in membranes act as a static barrier but with more recent discoveries, lipids are characterized as a complex and dynamic membrane component that electively bridge the cell to extracellular environment (van Meer et al. 2008). Membranes are made up of more than thousand different lipid moieties, each of which has unique physicochemical characteristics that cause membrane microdomains to form. But it's still unclear why having such a wide variety of lipid species is necessary (Bramkamp and Lopez 2015). In general, phospholipids contribute to about 70 % and neutral glucolipids fills up the remaining 30 % of vegetative cell lipidome (van Tilburg et al. 2022). Phosphatidylglycerol (PhG) and phosphatidylethanolamine (PE) are the major phospholipids of the membrane and some other minor lipids includes lysylphosphatidylglycerol (LPG) and cardiolipin (Sohlenkamp et al. 2016). Cell membranes serve as the access point for cellular

import and export, a shield against toxic substances, provides protection against changing environmental milieu, and the site of numerous key metabolic activities (Silhavy et al. 2010). For the survival of cell, the envelope must be able to endure the stress factors and associated extreme external conditions. Envelope stress responses are required to maintain envelope homeostasis and to quickly repair any damage in order to assure integrity (Hews et al. 2019). Cell envelope stress responses (CESRs) are regulatory pathways that detect dangers and launch a defense mechanism, frequently modifying the inner membrane, peptidoglycan, lipopolysaccharides (in Gram-negative bacteria), teichoic acids (in Gram-positive bacteria), and other cellular components (Mitchell and Silhavy 2019).

1.3 Terpenoid lipids

Terpenoids are a subclass of terpenes that are produced through biochemical modifications and consist of oxygen molecules. The synthesis of terpenes and terpenoids occurs through the mevalonate pathway (MVA) and methylerythritol phosphate pathway (MEP) pathway (Oldfield and Lin 2012). Aldehydes, alcohols, ethers, esters, epoxides, phenols and ketones are the different types of terpenoids (Masyita et al. 2022). Whereas, citronellal, carvacrol, geraniol, linalyl, linalool, acetate, menthol, and thymol are some of the commonly known terpenoids examples (Hyldgaard et al. 2012). All these biologically active compounds harbor interesting properties like anti-bacterial, anti-cancers, anti-oxidants and anti-allergic (Guimarães et al. 2019, Wang et al. 2019). Triterpenoids are three isoprene unit oxygen containing terpenes with molecular formula of $C_{30}H_{48}$. Plants, animals, microorganisms all produce triterpenes, the most common of which is squalene that act as precursor to all the steroids and cyclic triterpenoids (Hillier and Lathe 2019).

1.4 Hopanoids

Hopanoids are sterol surrogate membrane lipids which are pentacyclic triterpenoids in nature and are widespread of all complex natural products (Fig. 1). With few exceptions,

most prokaryotic members lack sterols; however, several bacteria have been found to produce the poly isoprenoid lipids like hopanoid (Lodha et al. 2015). Despite being one of the most prevalent (in terms of mass) classes of natural products found on Earth's surface and mentioned in all of the fossil records, they are not well known by the majority of people, both inside and outside of the scientific community (Ourisson et al. 1979). Hopanoids were discovered for the first time from the resin component of a plant belonging to genus *Hopea* which was named after the botanist John Hope and that's how the term "hopane" was originated (Poralla 1999). In addition to a few higher plants, protists, ferns, mosses, fungi many eubacteria also produce hopanoids (Belin et al. 2018, Rohmer et al. 1984, Volkman 2005).

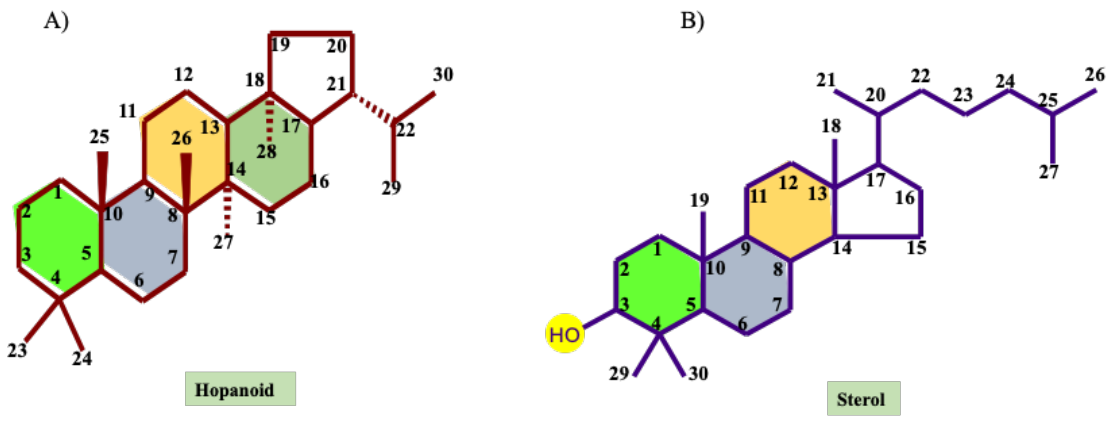


Fig. 1 A) Basic skeleton of hopanoids, containing four cyclohexane rings and one cyclopentane ring B) Basic skeleton of sterol, with tetracyclic ring structure

The structure of hopanoid contains four cyclohexane ring and one pentane ring whereas structure of sterol contains three cyclohexane ring and one pentane ring (source information adapted from Lodha et al. 2015).

Squalene, a C_{30} hydrocarbon molecule, serves as a biosynthetic precursor for both hopanoids and sterols. In contrast to sterols, which have three and one cyclohexane rings respectively, hopanoids have four and one cyclopentane rings. Additionally, sterols differ

from hopanoids in that they have a hydroxyl group at the C_3 position. Sterols exhibit chair-boat-chair-boat-open conformations, whereas hopanoids exhibit chair-chair-chair-chair-chair conformations of the cyclohexane ring (Kontnik et al. 2008). Due to their planar, rigid, hydrophobic and half-membrane-thick framework, sterol and hopanoid molecules can both readily intercalate into the phospholipid bilayer and interact with nearby fatty acids (Kannenberg et al. 1985, Sáenz et al. 2015). Hopanoids and sterols can therefore control the membrane's fluidity and permeability. There have been reports of several different hopanoid structures, such as the C_{30} hopanoids diploptene or hop-22(29)-ene (one of the simplest C_{30} hopanoid compounds) and diplopterol or hopan-22-ol. They have low occurrence in bacteria but can act as precursors for complex hopanoids like C_{35} bacteriohopanepolyols (BHPs), which have an extended C_5 side chain derived from D-ribose (Rohmer et al. 1984). They also exist as composite structures, such as hopanoids linked to a glycosyl moiety (Härtner et al. 2005, Talbot et al. 2008). Tetra-functionalized biohopanoids are mostly observed amongst complex hopanoids having variety of functional groups at C_{35} position. Bacteriohopane-32,33,34, 35-tetrol having hydroxyl group, aminobacteriohopane-32, 33, 34-triol having amino group are the examples of side chain containing hopanoids (Rohmer et al.1984).

1.4.1 Biohopanoids and geohopanoids

Biohopanoids (BPHs) are hopanoids commonly associated with various bacterial system. Whereas, the diagenesis of biohopanoids produces geohopanoids (Ourisson and Albrecht 1992). In the process of diagenesis many physical, chemical and biological transformations take place as sedimentary rocks are formed. Owning to their exceptionally stable behavior under extreme environments like highly acidic or alkali conditions and high temperatures, hopanoids form one of the very few classes of compounds that can endure the process of diagenesis (McGarvey and Croteau 1995). Once the biohopanoids are liberated into external environment the BPHs undergo sediment diagenesis, which transforms them

into simpler products such as hopanes, hopanoic acids and hopanols but they still retain the identity of their parent compound (Shunthirasingham and Simpson 2006).

1.4.2 Distribution of hopanoids

Hopanoids are synthesized by many aerobic bacteria although the presence of dioxygen is not obligate for hopanoid biosynthesis (Rohmer et al. 1984). Apart from oxygenic bacteria (Niu et al. 2021, Talbot and Farrimond 2007), hopanoids were also reported from few facultative anaerobic bacteria like, *Rhodospirillum* spp., *Rhodomicrobium* spp., *Rhodopseudomonas* spp. and *Zymomonas mobilis* (Hermans et al. 1991, Rohmer et al. 1984, Talbot and Farrimond 2007). However, there are also reports of hopanoids from anaerobic bacteria like, *Geobacter* spp., and *Desulfovibrio* spp., with some tolerance level to microquantities of oxygen (Blumenberg et al. 2006, Härtner et al. 2005). Additionally, it has been recorded that few anammox bacteria of phylum *Planctomycetota* which are strictly anaerobic in nature, occasionally produce hopanoids (van Niftrik and Jetten 2012). The majority of distribution studies on hopanoids have concentrated on the *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Acidobacteria*, *Planctomycetota*, and *Bacillota* (Damsté et al. 2004, Rohmer et al. 1984, Talbot et al. 2007, Talbot et al. 2008).

1.4.3 Hopanoid functions

Most of the studies have focused mainly on hopanoid biosynthesis while functional research has primarily been done on proteobacteria. Hopanoids are important for membrane order as its deficiency may lead to compromised cell membranes in turn affects the fluidity, permeability, ion conductivity, cell signaling and lateral segregation. Hopanoids are also considered to be involved in plant-microbe interaction, bacterial cell cycle and stress tolerance (Belin et al. 2018).

1.4.3.1 As biomarker

Geologists and paleobiologists referred hopanoids as molecular fossils in the evolution of Earth's surface environments. They could discover hopanoids even from very old samples which dated back more than 2500 billion years (Brocks et al. 2003). According to a distribution analysis of hopanoids, certain hopanoids may be related to particular bacterial taxa.

1.4.3.2 Membrane integrity and lipid ordering

The hydrophobic centers of bilayers are packed by hopanoid as it intercalates in phospholipid bilayers. The cell is protected by this architecture (with hopanoids in the membrane) under extreme pH conditions by preventing proton loss (Welander et al. 2009). Although studies have suggested that hopanoid may be important for lipid ordering, the mechanism and importance of lipid ordering in bacteria remain poorly understood.

1.4.3.3 Plant microbe interaction

Kulkarni et al. (2015) described that C_{35} hopanoids are necessary for growth in a symbiotic environment for the symbiosis between *B. diazoefficiens* and *A. afraspera*.

1.4.3.4 Cell cycle and stress tolerance

During the cell cycle events, hopanoids allocate the cell division apparatus in appropriate subcellular area and plays a crucial role in cell growth and division. Also, a study on *N. punctiforme*, a diazotrophic symbiotic cyanobacterium, suggested the role of hopanoids in stress tolerance and nutrient storage (Ricci et al. 2017).

1.5 Sporulenes

Sporulenes is a heptaprenyl lipid identified for the first time from the endospores of *B. subtilis*. It resembles to hopanoids in having a tetracyclic ring structure but differ from it having a pentacyclic ring (Fig. 2A). Structure of sporulene predicted based on HRMS analysis and MS fragmentation pattern revealed a typical sesterpene skeleton with

tetracyclic scalarane system as also observed in metabolites of marine sponge (Renoux and Rohmer 1986). Till date only three types of sporulenes have been identified based on the position of double bond in the basic skeleton of sporulene *viz*, sporulene A (saturation at 16-17 position), sporulene B (saturation at 17-35 position) and sporulene C (saturation at 15-16 position) (Kontnik et al. 2008).

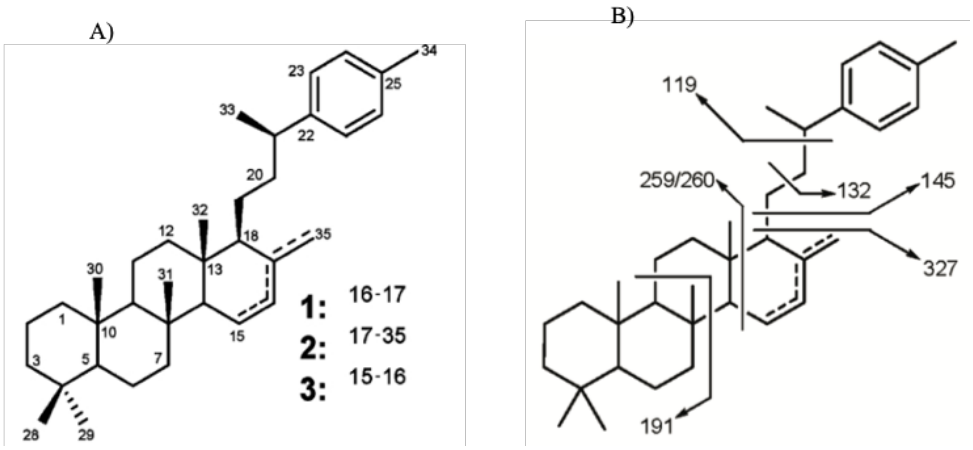


Fig. 2 A) Structure of sporulene a C_{35} terpenoid from *Bacillus subtilis* endospore
B) Major fragmentation scheme accounting for sporulene

Sporulene A, B and C (1-3) are represented based on the position of double bond in the skeleton of sporulene. Further the fragmentation scheme on the tetra-scalarane system represents major fragments recorded for sporulene on MS analysis. (source information is adapted from Kontnik et al. 2008).

All these sporulenes have equal molecular ion of *m/z* 474 and molecular formula of $C_{35}H_{54}$. The additional fragments characteristics of sporulene includes *m/z* 119, 145 and 132 along with the presence of ethylene- (C9H11+), butylene-methylphenyl (C11H13+) and propylene- (C10H12+) fragments respectively (Fig. 2B). These fragments signify the presence of an aromatic side-chain moiety in the structure (Kontnik et al. 2008). The roughly predicted abundance of sporulene in *B. subtilis* spore is approximately 0.1 mg/g dry weight of spores. Given that *B. subtilis* synthesize sporulenes only during

sporulation, their biological function(s) is/are related to endospores. According to previous reports, sporulenes showed a strong candidature for being one of the factors in alleviating oxidative stress in *Bacillus* endospores (Bosak et al. 2008, Checinska et al. 2012). The first hint about the proposed role of sporulene molecule came from superoxide dismutase (*sodF*), situated after *sqhC* gene in a two-operon system (Fig. 3) (Bosak et al. 2008).

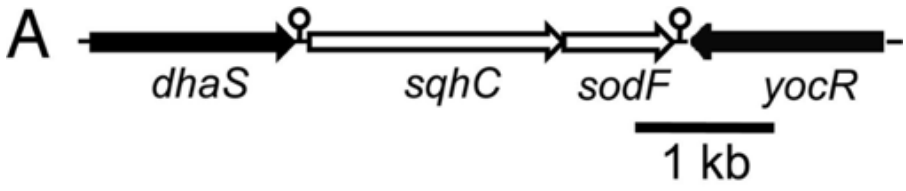


Fig. 3 *sqhC* operon in *Bacillus subtilis*

The arrangement of sqhC gene in an operon where, sqhC is located between dhaS and sodF gene (source information adapted from Bosak et al. 2008)

1.5.1 Sporulene biosynthesis pathway

The biosynthesis of sporulene is a five-step process, first three steps are enzyme catalyzed steps followed by two non-enzymatic steps (Fig. 4) (Takigawa et al. 2010). It starts with the synthesis of heptaprenyl diphosphate (compound I) from farnesyl diphosphate (FPP) and isopentyl pyrophosphate (IPP) with the help of a heterodimeric enzyme heptaprenyl diphosphate synthase where two components (HepS and HepT) are encoded by genes *hepS* and *hepT*. Then action of a novel terpene cyclase enzyme, tetraprenyl-β-curcumene synthase (TS/YtpB) encoded by gene *ytpB*, cyclizes the synthesis of extended prenyl-diphosphate strands to form tetraprenyl-β-curcumene (compound II). This enzyme is unique within itself as it does not share any sequence similarities with other existing terpene cyclase. Whereas, sporulenol synthase (SqhC) encoded by gene *sqhC*, shares striking similarity with squalene hopene cyclase catalyzes (SHC's) and brings about the cyclization of polycyclic skeleton of

sporulene intermediate (Bacitrol, compound III). The intermediate then undergoes two non-enzymatic reactions i.e autooxidation and thermal dehydration to finally form sporulenes (Christianson 2017, Sato 2013).

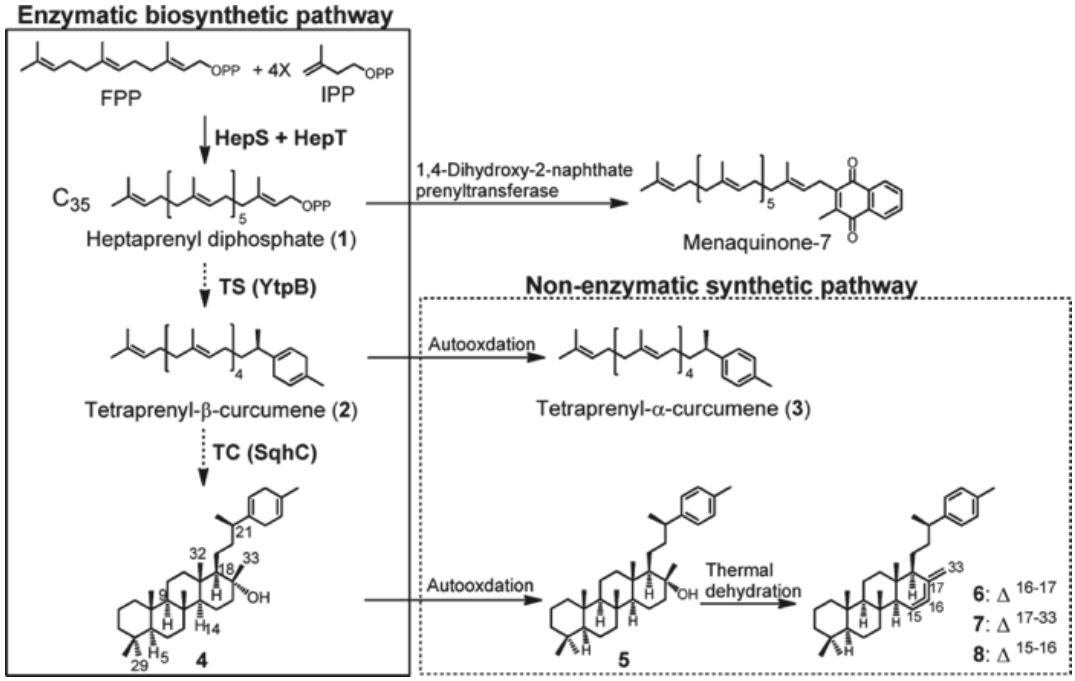


Fig. 4 Schematic representation of sporulene biosynthesis pathway in *Bacillus subtilis*

The enzymatic reactions are represented in solid box; where, (1): heptaprenyl diaphosphate, (2): tetraprenyl-β-curcumene, (3): tetraprenyl-α-curcumene, (4): an intermediate structure. Rest of the non-enzymatic reactions are represented in dotted box which leads to final structure of sporulenes. (source information adapted from Sato 2013).

1.6 Sporulenol synthase and squalene hopene cyclase

The enzyme sporulenol synthase (SqhC) and squalene hopene cyclase (SHC) are key enzymes in synthesis of sporulenes and hopanoids respectively and belong to class of terpenoid cyclase enzymes (Bosak et al. 2008, Christianson 2017). Earlier both the enzymes were considered as similar cyclase with varied substrate dependent activity. Further, it was confirmed that they are highly similar but not the same enzymes, both with

aspartic acid rich domain (DXXD), signature for class two terpenoids synthases (Hoshino and Sato 2002). For SHC, squalene is the substrate and hopanoids like deplopterol, deploptene, bacteriohopane and tetrahymanol are products. Whereas, sporulenol synthase uses tetraprenyl-β-curcumene as substrate to form sporulenes (Christianson 2017). The five-ringed skeleton of hopene is synthesized with major modifications of thirteen covalent bonds that lead to the formation of nine stereo centers, making hopanoid synthesis a complex single step reaction (Siedenburg and Jendrossek 2011). However, synthesis of sporulene involves protonation of the terminal C=C bond of substrate (enzymatic reaction) and non-enzymatic reactions (Christianson 2017, Sato 2013).

1.7 Survival strategies of bacteria

Survival is commonly described as maintaining viability in the face of adversity. Bacteria are highly adaptable organisms that have evolved a wide range of survival strategies to endure extreme environments, nutrient-limiting conditions, and exposure to toxic substances (Forchhammer 2021). One such strategy is the formation of endospores, which are metabolically inactive structures that can survive for years, and even decades, under harsh conditions such as extreme temperatures or desiccation (Tan and Ramamurthi 2014). Biofilm formation is another common survival strategy where bacterial cell form a complex matrix on a surface, providing them protection from antibiotics, immune surveillance, and other environmental stressors (Fazeli-Nasab et al. 2022). Bacteria can also induce DNA damage repair mechanisms, activate stress response pathways, or produce efflux pumps that export toxins out of the cell to survive against toxic substances. Some bacteria have evolved the ability to exchange genetic material through horizontal gene transfer, allowing them to acquire novel traits, such as antibiotic resistance or the ability to use alternative energy sources (Dawan and Ahn 2022, Huang et al. 2022). Some other commonly known mechanism of survival includes, development of stress responsive

cytoplasmic elements (CRCE), viable but non-cultivable state (VBNC), cyst formation (Haruta and Kanno 2015, Ramamurthy et al. 2014, Willdigg and Helmann 2021,). Over all, the ability of bacteria to adapt to various environmental stressors, via structural and physiological changes, is what enables their survival. This unique ability to adapt and survive under hostile conditions has allowed bacteria to thrive in nearly every corner of the world, including extreme habitats like deep-sea vents, hot springs, and glaciers.

1.7.1 Bacterial endospore

The goal of sporulation is to produce "endospore," a form of latent bacterial body which is metabolically inactive and able to withstand challenging environmental conditions till ideal growth conditions returns and therefore provides a kind of in-built resistance against unfavorable conditions. Some common bacteria that produce endospores include *Bacillus* and *Clostridium* species (Leggett et al. 2012, Tan and Ramamurthi 2014). *Bacillus subtilis* is widely distributed in nature and is capable of successfully adjusting to numerous environmental changes. The relatively simplistic process of sporulation and easy genetic tractability in *Bacillus subtilis* makes it highly appealing system to study cell differentiation and sporogenesis (Tan and Ramamurthi 2014).

1.7.1.1 Sporogenesis

Process of sporulation is also known as sporogenesis. The spore formation is triggered by activation of histidine sensor kinases (KinA to KinC), they transport phosphate over an extended phosphorelay, causing phosphorylation of transcription factor Spo0A, the key regulator of sporulation (McKenney et al. 2013). The onset of sporulation is marked with the asymmetric division of rod-shaped *Bacillus subtilis*, developing a polar septum that forms two genetically similar but physically different compartments: mother cell (larger compartment) and forespore, (smaller compartment), each of which undergoes distinct cell fates. For a short while, the exterior cell wall holding both compartments

together keep them side by side. After being flat at first, the polar septum starts to curve and forespore is swallowed by the mother cell (called as engulfment), resulting in a forespore that lives as a double membrane-bound, spherical structure inside the mother cell cytoplasm. The partially dehydrated mature forespore is eventually released into external environment through programmed cell lysis of mother cell (Fig. 5) (Tan and Ramamurthi 2014).

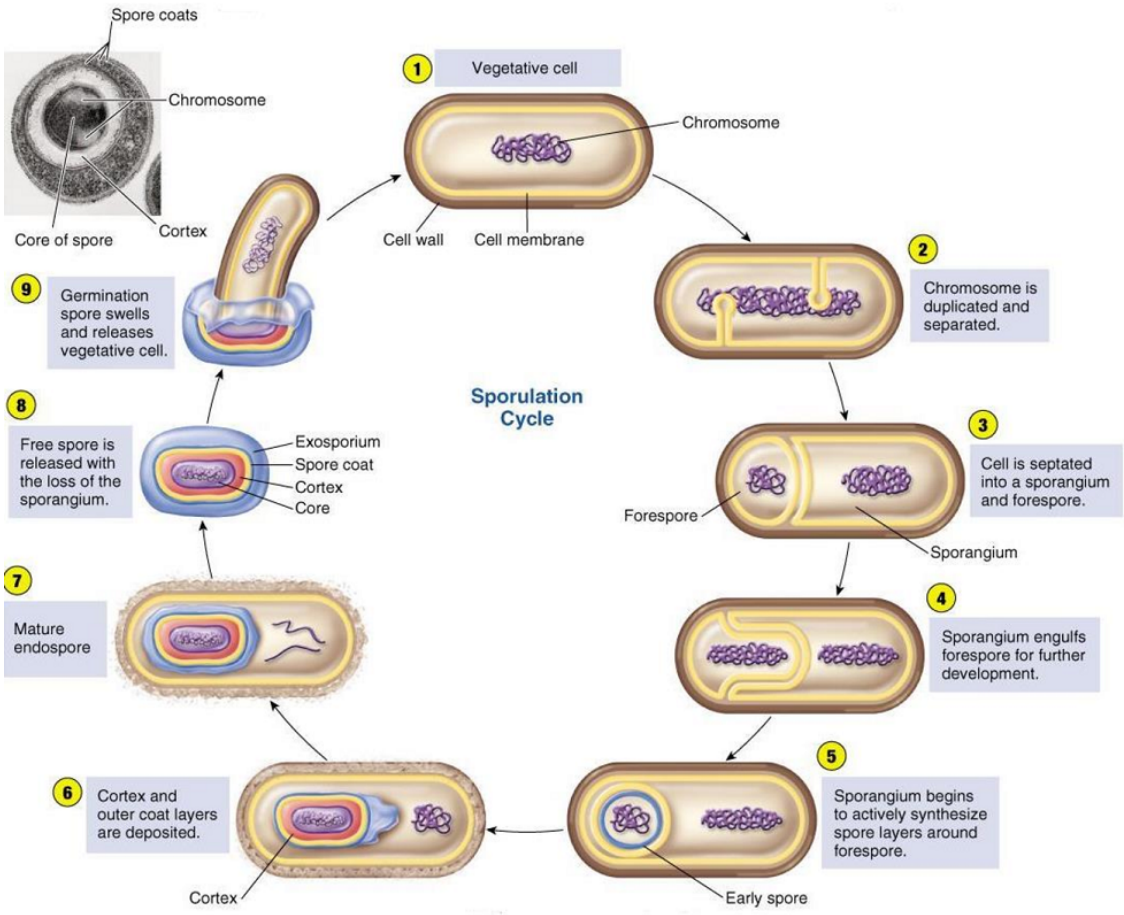


Fig. 5 The process of sporulation in endospore producing bacteria

(source information adapted from Mugadza 2017).

1.7.1.2 Structure and composition of endospore

The central core, which has much of its hydrogen peroxide (H_2O_2) replaced it with $Ca2^+$-dipicolinic acid, carries the genome. Core is enveloped by the inner spore membrane, a thin layer of peptidoglycan that resembles the vegetative cell (germ cell wall), spore cortex, outer membrane, protein coat and exosporium in some cases (Fig. 6) (McKenney et al. 2013).

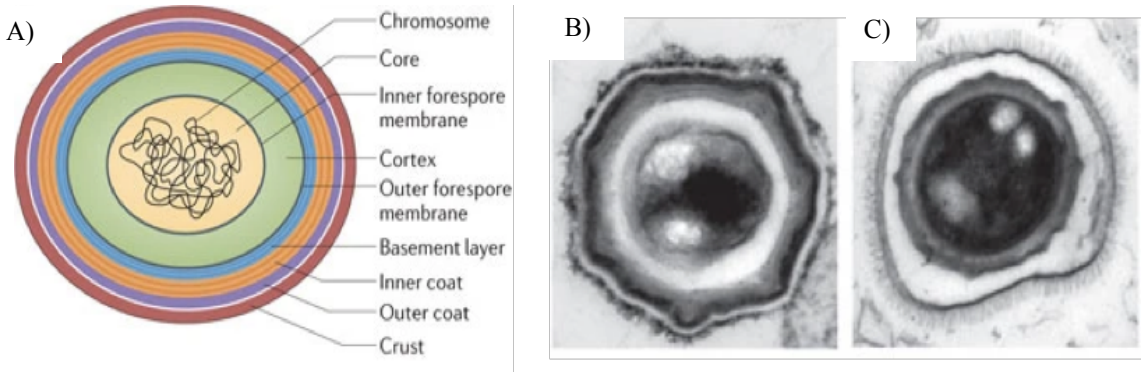


Fig. 6 Structure of bacterial endospore. A) Diagrammatic representation of ultrastructure of endospore B) and C) transmission electron micrographs of transverse section of endospore

(Source information adapted from McKenney et al. 2013, Nature Reviews Microbiology).

1.7.1.2.1 The spore core

Spore core is the heart of endospore, located at the center it harbors DNA, RNA, ribosomes, and majority of its enzymes (Setlow 2006). Water contributes only 28-57 % of the spores' wet weight in the core, making it mostly dehydrated. Specialty of the spore core is high levels of dipicolinic acid (DPA) which is present as 1:1 chelate with divalent cations, primarily $Ca2^+$ (Huang et al. 2007). DPA accounts for approximately 20 % of spore core dry weight. The factors inside the core have a significant impact on the spore's resistance traits, many of which play a role in preventing spore DNA damage (Leggett et al. 2012).

1.7.1.2.2 Inner spore membrane

The protective layer which surround spore core is inner spore membrane. The inner spore membrane composition seems quite similar to the plasma membrane of a vegetative cell. Both the membranes are composed of phosphatidylglycerol, cardiolipin, glucosaminyl phosphatidylglycerol and phosphatidyl ethanolamine (Leggett et al. 2012). In case of *Bacillus subtilis*, higher content of diglucosyl diacylglycerol is found as compared to spore (Griffiths and Setlow 2009).

1.7.1.2.3 Spore cortex

Peptidoglycan (PG) makes the major component of spore cortex that makes it resemble to vegetative cell PG in general but there are numerous spore-specific alterations that stand out, most notably the complete lack of teichoic acids from the N-acetylmuramic acid (NAM) residues in spore PG (Atrih et al. 1996). About 50 % of the NAM residues in spore cortex PG lack peptide side chains and are rather cyclized to produce muramic-δ-lactam, whereas almost 25 % of NAM residues possess L-alanine side chain only (Leggett et al. 2012).

1.7.1.2.4 Germ cell wall

The germ cell wall (GCW) is yet another PG structure found in bacterial spores. Owning to the difference in peptidoglycan composition, cortex peptidoglycan undergoes selective degradation during spore germination but not GCW however it develops into the cell wall during spore germination and outgrowth (Atrih et al. 1996).

1.7.1.2.5 Outer membrane

Once coat and cortex are synthesized which are major structures in spore, it is challenging to recognize the outer membrane in electron micrographs. Dormant spores from numerous species have reportedly known to have poorly defined or undetectable outer membranes (Holt et al. 1975, Leggett et al. 2012) The existence of an outer spore membrane

in the mature spore is supported by functional and biochemical data, despite the lack of strong morphological evidence.

1.7.1.2.6 Spore coat

The majority of the coat is made up of protein (50–80 %), but there also exists a little amount of carbohydrates (6 %) (Pandey and Aronson 1979). At least seventy different proteins, synthesized by the mother cell contribute to the protein fraction of the spore-coat, their localization to the spore surface starts immediately after engulfment (McKenney and Eichenberger 2012). The spore-coat protein includes, soluble fraction (70 %), that can be isolated through alkaline pH treatment using a cocktail of reducing and denaturing chemicals and insoluble fraction (30 %), that resists solubilization (Pandey and Aronson 1979). Functionally, the spore-coat acts as a first line of defense against big molecules that would otherwise be able to penetrate the spore cortex, including the PG-lytic enzyme lysozyme (Nicholson et al. 2000).

1.7.1.2.7 Exosporium

Exosporium is primarily composed of proteins which forms 43-52 % of its fraction, although it also contains 20-22 % carbohydrate and 15-18 % lipids molecules and negligible (~4 %) quantity of other components known as “ash” which includes calcium, magnesium along with some unidentified compounds (Leggett et al. 2012).

1.7.1.3 Bacterial spore resistance

Bacterial endospores in their dormant state are far more resistant as compared to corresponding vegetative cells to extreme conditions such as, high temperature, pressure, radiation, and toxic chemicals (Setlow 2000). In particular, oxidizing agents, such as H_2O_2, are the chemicals to which spores show increased resistance (Setlow and Setlow 1993, Setlow 2000). The impressive resistance of spore is unquestionably a result of the combination of structural, chemical, and biological characteristics. A protein rich spore coat guards the spore

from lytic enzymes like lysozymes (Riesenman and Nicholson 2000). Additionally, presence of a melanin like brown pigment produced by a copper dependent laccase enzyme (CotA) in spore outer coat helps to endure the oxidative damages (Checinska et al. 2012). Mineralization due to the presence of DPA and thick layer of spore cortex keeps the spore core highly dehydrated (low core water content) that provides protection against elevated temperature (Leggett et al. 2012). Inner spore membrane which is mostly impervious and thus act as barrier for large and toxic molecules (Setlow 2000). The last but most important factor in spore resistance is protection of the spore genetic material by α/β-type small acid-soluble proteins (SASP), a group of DNA associated proteins that prevent the possible DNA damage by reactive oxygen species and UV radiations (Setlow and Setlow 1993, Setlow 1995, 2000). In addition, catalase enzyme encoded by *cotJC* gene is commonly known for neutralizing the oxidative effects (Checinska et al. 2012) but it has not been found in case of *B. subtilis* spores (Seyler et al. 1997). Several other spore-associated proteins with antioxidant activity have been identified in *B. subtilis*, such as Sod A or Sod F (Wang et al. 2018).

1.7.1.4 Spore germination

The phenomenon of spore germination is associated with the characteristic loss of most of the spore structures. Under natural conditions spore germination commences with the encounter and interaction of nutrients like carbohydrates, amino acids purine nucleosides (in combination or individually), to the receptors on the spores' inner membrane. The process of germination is often marked with the release of DPA in the beginning along with the release of hydrogen ions and divalent cations which are eventually replaced with water resulting in change of pH (from 6.5 to 7.7) of spore core (Andryukov et al. 2019). The GR-mediated processes that result in CaDPA release during germination involves the GerD protein. The hydrolysis of the spore's massive peptidoglycan cortex by any of the two redundant cortex-lytic enzymes (CLEs), CwlJ or SleB, is one among the

subsequent processes in germination that are triggered by CaDPA release (Tan and Ramamurthi 2014). At first, germinating spore unloads its spore coat and cell wall hydrolases breaks down the cortex. Whereas, the germ cell wall is kept intact as to serve for the commencement of vegetative cell wall assembly. While it is less clear what will happen to the outer spore membrane (it may get lost during spore maturation process), the inner spore membrane develops into the plasma membrane (McKenney et al. 2013). These modifications play a crucial role in the subsequent surge in enzyme activity and, eventually, the metabolism of upcoming cells, which is accompanied by synthesis of macromolecules. The spore expands and increases in size as a result of both cell expansion brought on by reserve material and water absorption. A new vegetative cell arises from the ruptured spore membrane further along, when the membranes break due to pressure brought on by growth (McKenney et al. 2013).

1.8 Challenges in spore studies

1.8.1 Separation of spores from vegetative cells

The very first challenge in working with spore is their close association with vegetative cells. When working with spores it's very important to make sure that the spores are devoid of vegetative cells. Considering the spore origin and its composition similar vegetative cells (Leggett et al. 2012, McKenney et al. 2013) it is more likely that vegetative cell and its components may interfere with spore related study. Also, poor methods used for isolation of clean spore preparation may fail to separate cells and spore, that may lead to confusions in subsequent experiments as to the results obtained are from spore or vegetative cells. This makes it imperative to sperate vegetative cells from spores with the help of best available separation method to confirm complete absence of vegetative cells, while working with spores.

1.8.2 Lipids, fatty acids and protein extraction

The proteinaceous spore coat, often makes it difficult to analyze the constituents of internal spore structures. Also, spores are extremely challenging to lyse because of their architecture and composition. Thus, it becomes necessary to stripe off the spore-coat before extraction of polar lipids and fatty acid. The solubilization of the coat proteins from clean spore preparations has been documented in the literature using a variety of chemical, enzymatic, and physical approaches (Riesenman and Nicholson 2000).

In bacterial spores, about one-third of the total coat protein is resistant to the extraction techniques typically used to solubilize the majority of other proteins. The solubility of various coat fractions has also been observed to vary, which may make spore protein extraction even more difficult. The work in this area has resurged as a result of the accessibility of handful of whole genome sequences from various strains, expression analysis with the help of new genetic engineering techniques and high sensitivity and resolution of mass spectral analysis (Thorne et al. 2010).

1.9 Hydrogen peroxide and environmental concerns associated

Hydrogen peroxide was discovered in the year 1818 by Louis The´nard, later in 1891 it was identified as a biocide by B. W. Richardson (Linley et a. 2012). It is currently used as a disinfectant on a large scale whereas other H_2O_2 based products are being frequently used in the food and medical industries for dealing with microbial contaminations. As far as environmental production of H_2O_2 is concerned, rising environmental pollution causes UV radiation-based activation of harmful compounds as a result massive amount of H_2O_2 is being generated (Sánchez-Quiles and Tovar-Sánchez 2014). Although peroxide breaks down quickly into simple by-products such as H_2O (water) and O_2 (oxygen), certain microbial communities are gravely affected by the elevated concentrations of H_2O_2 produced as a result of photochemical reactions (Sen and

Imlay 2021). Although peroxide degrades rapidly, the cytotoxic and genotoxic nature of accelerated levels of H_2O_2 is of serious concern to microbial communities (Linley et a. 2012). There are reports stating about the toxic effects of H_2O_2 on marine phytoplankton community which affected their survival (Lin et al. 2018). The effects of H_2O_2 on microbial ecology is being studied while responses of microorganisms to it remain underexplored.

1.9.1 H_2O_2: Mode of action

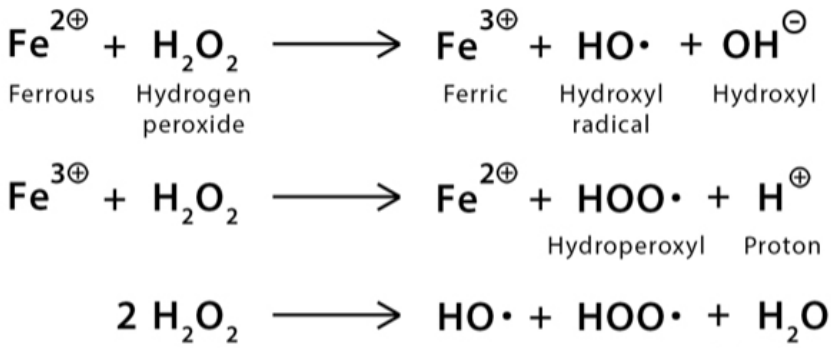


Fig. 7 Fenton's reaction *(Source information adapted from ChemistryLearner.com)*

Fenton's reaction is the reason why relatively harmless extracellular H_2O_2 turns into a lethal substance once inside the cell. H_2O_2 exhibits significant permeability across the membrane barrier due to its smaller size and lack of charge. In contrast to most other toxic compounds, H_2O_2 is unique in that it is stable in abiotic conditions at room temperature and neutral pH, but it quickly destroys any kind of cell by generating extremely reactive hydroxyl radicals (Sen and Imlay 2021). This life-specific reactivity follows the concentration of soluble iron, Fe (II), which is abundant inside cells but almost nonexistent outside of them. Fe (II) combines with H_2O_2 to generate the famous Fenton's reagent (Fig. 7). This reaction involves the formation of rather unstable superoxide (O_2^-) and highly reactive hydroxyl radical ($OH^·$) which attribute to the reactivity of reactive oxygen species (ROS) (Mahaseth and Kuzminov 2017). As a result, H_2O_2 is being continuously used as

effective biocidal agent even at its modest concentrations (Linley et al. 2012, Sen and Imlay 2021). Due to the cytotoxic and genotoxic risk posed by peroxide, all cells possess potent H_2O_2 scavengers which enable cells to survive even at higher concentrations of H_2O_2. It includes few intracellular and extracellular enzymes which plays role in oxidative stress response that may help in elevated resistance to reactive oxygen species (ROS). These enzymes include catalase KatX1, manganese catalase (BPUM_1305/ YjqC), superoxide dismutase (SodF), manganese superoxide dismutase (SodA), laccase (CotA), peroxiredoxin (YkuU), glutathione peroxidase (Bsa), thiol peroxidase (Tpx) or thioredoxin (TrxA). The most common of them are catalases which are induced under oxidative stress condition or produced near the stationary growth phase (Checinska et al. 2012). The catalases which are secreted in medium are more effective in neutralizing effects of ROS generating molecules like H_2O_2; mere cytoplasmic activity of catalase is not sufficient to manage the oxidative assault (Naclerio et al. 1995).

1.10 Bacterial members under study

The quest for hopanoids and sporulenes was carried out in phylum *Bacillota* and phylum *Planctomycetota* members.

1.10.1 Phylum *Bacillota*

Phylum *Bacillota* as renamed by Gibbons and Murray in 2021 was earlier known as phylum *Firmicutes*. Members of this phylum are mostly Gram-stain positive (Galperin 2016). The phylum contains seven classes, of which class *Bacilli* is currently divided into two orders as *Caryophanales* (nine families) and *Lactobacillales*, (six families) both the orders altogether have fifty families (Fig. 8) (https://www.bacterio.net/). Many members of the phylum *Bacillota* have the specific trait to form endospores. It has been found to be associated with four classes *Bacilli*, *Clostridia*, *Erysipelotrichia*, and *Negativicutes*, all of them contain identical sets of the essential sporulation proteins (Galperin 2016, Gopal et

al. 2015). Non-spore-forming organisms are also included in each of these classes; in some cases, they even share the same genus or even species as their spore-forming counterparts. A conserved group of sixty genes is required for the formation of spores; alterations in these genes can interfere the process of sporulation and drastically reduce the proportion of cells that can form spores, or even make the cells absolutely asporogenous (Onyenwoke et al. 2004). In the order *Lactobacillales* none of the members harbor spore formation properties, whereas both sporogenic and asporogenic members can be found in the order *Caryophanales*. The class *Clostridia* consists of four recognized orders, two of which, *Clostridiales* and *Thermoanaerobacterales* harbor spore-formers and other two classes *Halanaerobiales* and *Natranaerobiales* do not represent spore forming members (Galperin 2016).

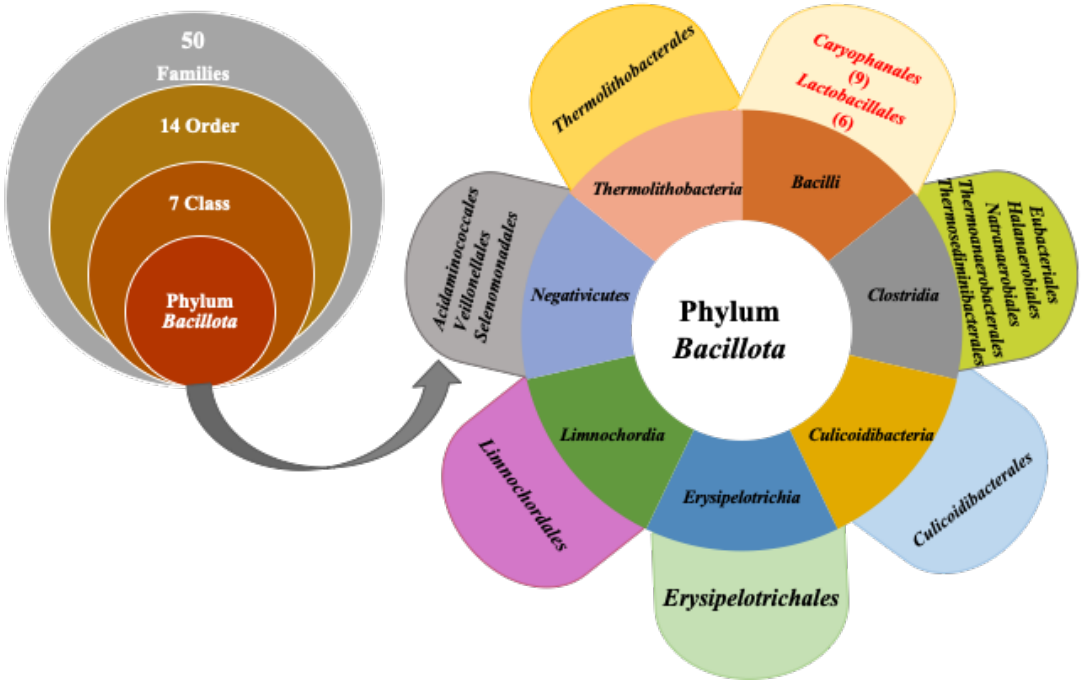


Fig. 8 Classification of phylum *Bacillota*

1.10.2 Phylum *Planctomycetota*

The phylum *Planctomycetota* forms a part of *Planctomycetes-Verrucomicrobia-Chlamydiae* (PVC) superphylum which also has *Lentisphaerae* and other related phyla having *Candidatus* status (Wagner and Horn 2006). The phylum's name was derived almost a century ago based on the fact that the very first member was categorized as a floating fungus by mistake (Kaboré et al. 2020). Currently, phylum *Planctomycetes* is subdivided into two classes as *Planctomycetia* and *Phycisphaerae* (Fukunaga et al. 2009, Krieg et al. 2010). Along with the members of above-mentioned classes, *Planctomycetota* members that can anaerobically oxidize ammonium make up the *Candidatus* anammox group, proposed under order *Brocadiales* (Jetten et al. 2010), as no described member of the order currently has any axenic culture. Of the two classes most members are described and characterized in class *Planctomycetia*. The phylum *Planctomycetota* harbors bacteria that possess characteristics which are unusual for prokaryotes *viz*, budding as mode of multiplication as they do not have the cell division protein FtsZ (bacterial tubulin-homolog) and complex cell structures with cytoplasmic membrane invasions (Kumar et al. 2021). Also, many members lack peptidoglycan in their cell wall assembly (Wiegand et al. 2018) Despite the fact that *Planctomycetota* are widespread, there are still fewer reported species and isolated strains that can be grown in axenic conditions, whereas a higher diversity of the same has been noted in metagenomes or environmental investigations (Wiegand et al. 2020). The recent description of a considerable number of novel species and their improved accessibility in terms of pure cultures show that *Planctomycetota* are receiving more and more attention.

1.11 Genome mining and identification of novel metabolites

Microorganisms routinely produce vast range of bioactive compounds, either as a part of conventional survival strategies or in response to external stress. Generally, standard

protocols are used for extraction and identification of such secondary metabolites from bacteria. Over the time, the rising discovery rates of bacteria made it difficult and laborious to identify and study the distribution of novel metabolites amongst them (Baltz 2019, Lebedeva et al. 2021). This inefficiency of the expensive conventional approaches resulted in their replacement with the cost-effective and rapid techniques to gather such information. As a result, identification of novel biosynthetic pathways now heavily relies on genome sequencing and the mining of microbial genomes for unexplored biosynthetic gene clusters (BGCs), many of which may encode for novel unidentified compounds (Tracanna et al. 2017). The recent advancements in the field of bioinformatics have opened the door for genome-based discovery methods. Although, the microbial metabolites are diverse in nature, the machinery involved in their biosynthesis is quite conserved also because the key biosynthesis enzymes share a high degree of amino acid sequence similarity thus genomic data can be screened for the existence of specific biosynthetic genes (Scherlach and Hertweck 2021) In future, the extensive availability of genomic data, sophisticated data mining techniques, continued evolution of novel culturing strategies, effective genome editing, and improved expression systems will subsequently overcome significant obstacles in gaining access to the hidden metabolic diversity from genomic and metagenomics data (Albarano et al. 2020).

1.12 Rationale behind the study

Few newly identified lipids are often overlooked molecules in the scientific society. Regardless of their critical function in structuring biological membranes, these lipids are frequently neglected, partly due to the difficulty in their quantification, synthesis and alterations when compared to nucleic acid. With all the extensive studies on hopanoids in different group of bacteria, very few studies are available on hopanoids of *Planctomycetota* members. This study was aimed in understanding the diversity of hopanoids amongst the

members of the phylum *Planctomycetota*. Further, the study was extended in understanding the diversity of sporulenes amongst the endospore producing members of class *Bacilli*, since its discovery was stopped with *Bacillus subtilis*. The following are the three major questions to which the thesis was focused in addressing on hopanoids and sporulenes

- Are there any unique hopanoids associated with *Planctomycetota* members?
- To what extent sporulenes are associated with endospore forming members?
- How does oxidative stress influence the *Bacillus* endospores?

1.13 OBJECTIVES

I. To investigate a few newly isolated bacteria for hopanoids and sporulenes among the members of :

(a) Phylum *Planctomycetota* (b) Class *Bacilli*

II. Insights into sporulenes of *Bacillus subtilis*

(a) Genomic insights (b) Functional insights

Materials and Methods

2. MATERIALS AND METHODS

2.1 Glassware and plasticware

The glassware and plasticware used under this study include beakers, conical flasks, side arm flasks, petri dishes, measuring cylinders, test tubes, boiling tubes, falcon tubes, PCR tubes, reagent bottles, centrifuge tubes, glass spreaders, 96 well microtiter-plate, pipettes and microcentrifuge tubes. The glassware used were of Borosil, Duran and Thermo Fisher Scientific make. The plasticware were of Tarsons make and pipettes were of Eppendorf brand.

2.2 Glassware cleaning

All the glassware were first soaked for 24 h in dilute solution of chromic acid [Add sodium/potassium dichromate (8 gm) in distilled water (80 ml), to this add concentrated sulphuric acid (8 ml)] washed with detergent (Teepol) and rinsed with tap water followed by airdrying.

2.3 Chemicals and solvents

The chemicals and reagents used in this study were of analytical grade procured from Sigma-Aldrich, Himedia, Thermo Fisher Scientific and Takara. Whereas, most of the solvent used for the experiments performed under this study were availed from Merck and 100% ethanol was obtained from Hayman. Single distilled and double distilled water used for media and buffer preparation was taken from the Millipore distillation plant at school facility. MilliQ water (deionized water) was also obtained from the deionizer plant at school facility (ELIX from Merck Millipore).

2.4 Buffers, solutions and reagents

Autoclaved deionized water and standard procedures were used to prepare buffers and solutions. The pH was maintained at room temperature as per buffer composition.

2.5 Major solutions

- Phosphate buffer saline (PBS, $g.l^{-1}$): sodium chloride (NaCl) - 8, potassium chloride (KCl) - 0.2, di-sodium hydrogen phosphate (Na_2HPO_4) -1.44 and potassium dihydrogen phosphate (KH_2PO_4) - 0.24; pH was adjusted to 7.4.
- Tris-EDTA buffer (TE): 10 ml of 1M Tris-buffer (pH, 10 to 11) was mixed with 2 ml of 0.5 M EDTA, the pH was adjusted to 8 and make up the volume to 1000 ml.
- Lysis buffer: 6 M Urea, 14.3 mM β-mercaptoethanol in 50 mM Tris-HCl.
- Germination solution: 10 mM L-alanine in10 mM Tris-HCl (pH 7.5).
- De-coating solution: 50 mM dTT in 8 M urea.
- p-Anisaldehyde stain: p-Anisaldehyde: perchloric acid: acetone: water (1:10:20:80)

2.6 Other solutions

Hydrogen peroxide (H_2O_2) [30 % (v/v)], sodium dodecyl sulfate (SDS) solution [10 % (v/v)], acetic acid [0.1 N (v/v)], sodium azide [1 % (w/v)], 100 $mg.ml^{-1}$ cycloheximide, tween 80 [0.01 % (v/v)], propidium iodide (PI) dye [1 $mg.ml^{-1}$ (w/v)], sodium hydroxide (NaOH) [0.1 N (w/v)]; 50 mM ammonium bicarbonate (NH_4HCO_3).

2.7 Culture media preparation

2.7.1 Nutrient broth/agar

Nutrient broth/ agar (NA/NB) was prepared as per the given composition ($g.l^{-1}$); peptone - 5, NaCl - 5, beef extract - 1.5, yeast extract 1.5; pH adjusted to 7.4.

2.7.2 *Bacillus* composite medium ($g.l^{-1}$)

L-Glutamic acid - 4, citric acid - 2, di-potassium hydrogen phosphate (K_2HPO_4) - 0.5, magnesium sulfate ($MgSO_4$) - 0.5, dehydrated nutrient broth powder - 1.3, sodium azide - 0.01 % and cycloheximide - 0.01 %.

2.7.3 Sporulation medium ($g.l^{-1}$)

Dehydrated nutrient broth powder - 8, MgSO4 - 1.2 % (10 $ml.l^{-1}$), 10 % KCl (10 $ml.l^{-1}$) after autoclaving filter sterilized calcium nitrate (Ca $(NO_3)_2$) [1 M, 1 $ml.l^{-1}$], manganese chloride ($MnCl_2$) [10 mM, 1 $ml.l^{-1}$] and 1 mM ferrous sulfate ($FeSO_4$) were added.

2.8 pH adjustment

pH of media and solutions was determined using calibrated (with standard buffer of pH range 4.2-7-9.2) M/s. Digisun electronics digital pH meter (model no. 707). The pH was adjusted with 5 N NaOH solution and 0.1 M hydrochloric acid (HCl).

2.9 Sterilization

The glassware, microtips, microcentrifuge tubes, centrifuge tubes, falcons, cotton plugs, deionized water, autoclavable buffers/solution and media were sterilized by autoclaving at 121^{o}C for about 15 min. However, the heat sensitive solutions like antibiotics and certain buffers were sterilized by passing through membrane filter (Millipore, 0.22 µm).

2.10 Enrichment, purification and growth conditions of isolates

Environmental samples like soil sediment, hot spring water, dried sponge collected from different geographical locations of India *viz.*, Western Ghats, Sikkim, Tamil Nadu, Orissa, Bihar and Jharkhand were enriched in bacillus composite medium (BCM) containing 0.01 % (w/v) of sodium azide and 0.01 % (w/v) cycloheximide. Enriched samples were given heat shock treatment in a hot water bath at 50 °C and 50 µl of the enrichment was plated on NA (HiMedia, M002). Isolated colonies were purified by repeated streaking on NA medium.

2.11 Bacterial strains and growth conditions

B. subtilis strains used in this study were *B. subtilis* PY79, a wild type (WT) strain and *B. subtilis* TB10, a *ΔsqhC* deletion mutant. NB medium (Himedia; M002) was used to grow *B. subtilis* strains at 37 °C in a table top shaker incubator at 180 rpm. In order induce sporulation, the cultures were exhausted in sporulation medium.

2.12 Growth analysis

The growth studies for both WT and *ΔsqhC* mutant were conducted first, in NB with (treated) and without (control) any treatment. Log phase cells of OD_{620} nm normalized to 0.5 were used as primary culture and inoculated (2% inoculum) into side arm flasks, containing growth media. Unlike control set, 5% ethanol (v/v); 5% butanol (v/v) and 0.005% H_2O_2 (v/v) (Himedia, PCT1511) was added to treated sets of both WT and *ΔsqhC* mutants at exponential growth phase. The absorbance was noted at OD_{620} nm and growth was recorded from zero h till stationary phase. Based on the results, another growth analysis study was conducted in sporulation medium with 0.005 % (v/v) hydrogen peroxide stress, in the similar fashion as carried out in NB. All the experiments were performed in biological triplicates.

2.13 Culture stock preparation and maintenance

All the bacterial strains isolated during this study and *Bacillus* strains received as gratis, were preserved in 50 % of sterilized glycerol. The glycerol stocks were maintained at -20 °C and -80 °C. However the bacterial strains which were continuously in use, were maintained on nutrient agar plates by repeated sub-culturing at room temperature (25 °C).

2.14 DNA extraction, sequencing and genome annotation

Log phase culture (OD_{620}=0.5 nm) was used for extracting DNA. Genomic DNA was extracted using Nucleo-pore gDNA Fungal Bacterial Mini Kit (Genetix Biotech Asia), as per the protocol provided along with the kit. 16S rRNA gene was PCR amplified (Table 1 and Table 2) with universal primers (F27, 5'GTTTGATCCTGGCTCAG3'; F8,5' AGAGTTTGATCCTGGCTCAG 3'; R1525, 5'AGAAAGGAGGTGATCCAGCC3'; R1492, 5'GTTTGATCCTGGCTCAG3'). Amplified PCR products were observed using 1 % agarose gel electrophoresis. Sequence results for 16SrRNA gene and whole genome of strain JC1005 were outsourced to Agrigenome, Pvt. Ltd. Hyderabad.

Content	Volume (µl)
Master Mix	50
H_2O_2	32
Forward Primer	4
Reverse Primer	4
DNA Template	10
Total	100

Table 1. Reaction mixture for amplification of 16Sr RNA gene sequence

Steps	Temperature (°C)	Time (min)
Initial denaturation	94	10
Denaturation	94	1
Annealing	52 to 54	1
Elongation	72	1
	Total 35 cycles	
Final extension	72	15

Table 2. PCR amplification program for 16S rRNA gene sequence

The genome sequence obtained was submitted into the Rapid Annotation using Subsystem Technology (RAST) (https://rast.nmpdr.org), a genome annotation server for detailed analysis. Genome was analyzed for gene encoding putative proteins involved in sporulene biosynthetic pathway. Genome of *B. subtilis* JC1005 was compared with the genome of reference strain *B. subtilis* PY79 using web server OrthoVenn (Wang et al. 2015). Further, the genome was also annotated and visualized using CGView server's PROKSEE software which included features such as GC Skew, Prokka-annoatation, CRISPER/CAS finder and CARD analysis (Grant et al. 2023).

2.15 Genome mining for hopanoids and sporulenes

2.15.1 Collection of genomes and gene mining

The genomes sequences of newly described *Planctomycetota* members (laboratory isolates) were submitted to RAST and genes encoding for hopanoid biosynthetic pathway proteins were searched from the annotated genome files. However, for the mining of sporulene biosynthesis genes amongst class *Bacilli* members, Two hundred and thirty-three validly published species belonging to order *Caryophanales* and seventy-one of the order *Lactobacillales,* were taken into account from the List of Prokaryotic names with Standing in Nomenclature (LPSN) (https://www.bacterio.net) and EZBioCloud database (www.ezbiocloud.net). The list of bacterial strains, putative proteins used for the identification of genes involved in sporulene biosynthesis pathway and corresponding accession numbers are mentioned in Table 10. Bacterial strains accessed for screening of sporulenes under this study were as per the state of the knowledge till December 2021. The protein FASTA sequences were retrieved from (.faa) files of the annotated genome assemblies available in NCBI database. The search was conducted for the respective gene encoding putative protein involved in sporulene biosynthesis (Table 3).

Name of gene/ protein	Gene code	Protein code	EC numbers
Heptaprenyl diphosphate synthase component I	*hepS*	HepS	EC 2.5.1.30
Heptaprenyl diphosphate synthase component II	*hepT*	HepT	EC 2.5.1.30
Tetraprenyl-β-curcumene synthase	*ytpB*	YtpB	EC 4.2.3.130
Sporulenol synthase	*sqhC*	SqhC	EC 4.2.1.137

Table 3. **List of genes and putative proteins involved in sporulene biosynthesis**

(Source information adapted from Sato 2013 and Takigawa et al. 2010).

2.15.2 Construction of phylogenetic tree and data visualization

16S rRNA gene sequences of the bacterial members were downloaded from EZBiocloud and NCBI database. The sequences were aligned using ClustalW algorithm (Madeira et al. 2019) and Neighbour-joining phylogenetic tree was constructed with 16SrRNA gene sequences of class *Bacilli* members using MEGA 7 (Kumar et al. 2016). iTOL interactive Tree Of Life (iTOL) (https://itol.embl.de) was used to annotate the tree. The advance iTOL editor tool (Letunic et al. 2021) was used to represent the distribution of proteins HepS, HepT, YtpB and SqhC.

2.15.3 Protein alignments, synteny and interaction network

The amino acid sequences in FASTA format were collected from the NCBI protein database or obtained from the .faa file of the annotated genome assembly. Alignments of amino acid sequences were conducted using LALIGN (https://www.ebi.ac.uk/Tools/psa/lalign/), for all putative proteins of sporulene biosynthesis pathway (*Bacillus* members marked with * in table. 10). The post alignment editing was done using an online tool ESPript (https://espript.ibcp.fr). Arrangements of

sporulene biosynthesis pathway gene amongst family *Bacillaceae* and genus *Bacillus* members was studied with the help of SynTax software (http://archaea.u-psud.fr/SyntTax) (Oberto et al. 2013). A string network of proteins involved in sporulene biosynthesis was constructed using network building feature of SMART (https: //smart.embl.de/) tool, other features of the same tool were was used for gene co-occurrence and conservation study (Letunic et al. 2021).

2.16 Sporulation and spore isolation

Bacterial cultures were subjected to sporulation by exhaustion in sporulation medium for 72 h. Isolated spores devoid of vegetative cells were obtained as describe by Bosak et al. (2008) with minor changes in the procedure. Spores were harvested from stationary phase culture by centrifugation at 6000 g for 10 min, followed by washing and incubation in 10 ml of Tris-EDTA buffer with 5 $mg.ml^{-1}$ lysozyme in it, at 37 °C for 1 h. Further, 2 ml of 10 % (w/v) SDS was added and incubated for 25-35 min at 37 °C. From this, the clean spore pellet was isolated by centrifugation at 6000 g for 15 min and supernatant was discarded. The final washing of pellet was done with 0.01 % (v/v) Tween-80 then with water and resuspended in dl-H_2O.

2.17 Total lipid extraction and partial purification

2.17.1 For hopanoid analysis

Lyophilized cells (0.1 to 0.2 gm) or centrifuged cell pellet (1 to 2 gm) were sonicated in 20 ml of dichloromethane: methanol: water (DCM: MeOH: H_2O; 5: 10: 4) for 30 min at 75 % power. It was then refluxed in the same solvent for 1 hr at 75 °C in soxhlet extraction mantle (Shital scientific industries, series no. 321012, cat no. 27; capacity: 360 Watt, 240 Volt). The refluxed mixture was centrifuged at 5000 g for 10 min and supernatant was collected. To the pooled supernatant, a combination of DCM: H_2O (10:10) was added,

thoroughly mixed and centrifuged for 10 min at 5000 g and biphasic separation was carried out. DCM layer was collected and concentrated to 1 ml (Lodha et al. 2015).

2.17.2 For sporulene analysis

The spore pellet was acid hydrolysed for about 36 h, in 12 % of HCl at 80 °C. The acidified spore suspension was the used for total lipid extraction as described above for hopanoids. The total lipid fraction collected from the spores was eluted into two fractions with hexane and a mixture of 75 % hexane with 25 % ethyl acetate on a SiO2-gel (LICpure, 60 to 120 mess) gravity column (32–64 µm) prepared in DCM. Followed by further purification by a SiO_2-gel gravity column prepared in hexane, finally collected the DCM fraction (Sato et al. 2013) and concentrated it to 1 ml.

2.17.3 Thin layer chromatography

The lipid fractions collected (for hopanoids and sporulenes) were subjected to two-dimensional thin layer chromatography on SiO_2 gel TLC plate (Merck, dimensions 9 × 9). DCM was used as mobile phase to separate the samples in two dimensions. For visualization of hopanoid and putative sporulene spots, the plates were developed with 0.1 % berberine chloride (Sigma, Cat No. B3251) in 100 % ethanol and observed in the UV-light range of 360 nm (Subhash et al. 2013). Additionally, *p*-anisaldehyde stain was also used to stain putative sporulenes for the total lipid fraction.

2.17.4 Gas chromatography mass spectrometry analysis

The total lipid fraction collected from cell and spores were derivatized with 50 µl of pyridine and 50 µl of acetic anhydride (1:1) at 80 °C in heat block for 40 min. Gas chromatography mass spectrometry (GC-MS) was carried out using Agilent 7890 GC instrument allied to Leco Pegasus TOF-MS with ESI ionization mode. Separation was conducted using DB-1HT column (30 m X 0.25 mm, 0.1 mm film thickness), where helium was the carrier gas with flow rate of 1.2 $ml.min^{-1}$. Total 1µl of sample was injected was

and GC was operated in full scan mode, the detector was operated at 70eV. (Bosak et al. 2008, Kontnik et al. 2008, Lodha et al. 2015). GC program along with other operating parameters, for the detection of hopanoids and sporulenes is mentioned below.

2.17.4.1 GC-MS program for hopanoids

The GC program for hopanoid analysis was initiated with 100 ^{o}C with a hold for 2 min, which was further ramped from 100 to 200 ^{o}C and 200 to 360 ^{o}C at 10 ^{o}C. min^{-1} and at 6 ^{o}C. min^{-1} respectively (the later temperature was held for 10 min). The source temperature and interface temperature was maintained at 200 ^{o}C and 350 ^{o}C respectively and acquisition delay was for 120 sec (Lodha et al. 2015).

2.17.4.2 GC-MS program for sporulenes

The ramping program for sporulene analysis started with 65 ^{o}C and attained the final temperature till 320 ^{o}C with a hold at particular intervals as described by Bosak et al. (2008). Source temperature of 250 °C, interference temperature of 350 ^{o}C and a total run time of 68 min with acquisition delay of 100 sec, was maintained.

2.18 Real time analysis

2.18.1 RNA extraction

Total RNA was extracted using the method described by Rio et al. (2010) with Trizol (Takara; RNAiso Plus; Cat No. 9109) reagent. The log phase culture, treated with 0.005 % (v/v) of H_2O_2 was collected at 0, 15, 30 and 60 min after treatment and centrifuged at 6000 rpm. Pellet was homogenised with liquid N_2, mixed with Trizol and incubated for 5 min at room temperature. To this 0.2 ml of chloroform was added and again incubated for 5 min. Above homogenate was centrifuged at 10,000 rpm for 15 min and aqueous layer was collected which was further mixed with 0.5 ml of isopropanol and RNA was allowed to precipitate by keeping it on stand-bye for about 5 min. RNA pellet was collected by

centrifuging at 12,000 rpm for 10 min and washed with 70 % of ice cold ethanol. The air dried pellet was then dissolved in DEPC treated water.

2.18.2 RNA quantification and cDNA synthesis and qPCR

Quantification of the isolated RNA was done using NanoDrop spectrophotometer (ND- 2000, Thermo fisher scientific, USA) at 260 nm and quality was accessed by checking the 260/230 and 260/280 ratios for contamination. The cDNA synthesis was carried out with equal concentration of RNA, according to the manufacturers protocol given by OneScript® cDNA synthase kit (abm® Canada). Finally, qPCR reaction was set as per (Table 4). Total sixty reactions were set for five genes, that included four genes of sporulene biosynthesis pathway and one housekeeping gene (*recA*) in biological triplicates (5X3=15) at four time points (15X4=60). Real time PCR was operated according to the programme given in (Table 5).

Content	Volume (µl)
SYBR® Premix Ex Taq™	5
ROX	0.5
Forward Primer	0.4
Reverse Primer	0.4
cDNA Template	volume=10ng
DEPC water	Make up the volume to 10
Total	10

Table 4. qPCR component and reaction for gene expression analysis

Steps	Temperature (°C)	Time (min.)
Initial denaturation	95	2:00
Denaturation	95	0:30
Annealing	52	0:25
Elongation	72	0:30
	Total 40 cycles	
Final extension	72	15

Table 5. qPCR program for real time analysis

2.19 Spore enumeration and viability assessment

Isolated spores (no vegetative cells) were serially diluted to 1 x 10^{-9} dilution in autoclaved milli-Q H_2O. Spore enumeration was done using hemocytometer, for 5 µl spore suspension from 1 x 10^{-9} dilution. Based on the spore count, 1 ml of spore suspensions were made for *B. subtilis* PY79 (WT) and *B. subtilis* TB47 (*ΔsqhC* mutant) having equal number of spores (~1 x 10^{-6} CFU.ml^{-1} spores). This final dilutions were treated with 0.5 % of H_2O_2, 0.5 % of glutaraldehyde, 70 % of ethanol, 0.05 % of sodium hypochlorite and 50 % of butanol for 1 h. Followed by wash and diluted with 1 ml of autoclaved milli-Q H_2O. 10 µl of this was plated on germinating medium and incubated at 37 °C. Parallelly, the spore viability assessment was performed with 0.03 %, 0.05 %, 0.3 %, 0.5 %, 1.0 %, 3.0 % and 5.0 % (v/v) of H_2O_2. The germinated spores were enumerated in terms of colony forming units (CFU).

2.20 Staining and microscopy

2.20.1 Scanning electron microscopy

Spores of WT and *ΔsqhC* mutant *B. subtilis* were treated with 0.05% H_2O_2 (v/v) for 1 h at room temperature. Whereas, the control set (untreated spores) was prepared by suspending spores in 50 mM phosphate buffer (PBS). After the incubation H_2O_2 was removed and spores were washed thrice to ensure removal of residual H_2O_2. Sample

preparation for SEM was perfomed as described by Kaláb et al. (2008). First, the spore pallets were suspended in 200 µl of 0.9 % saline and centrifuged for 10 min at 10,000 rpm. The spore pellet was then incubated in 2.4 % of glutaraldehyde for 30 min at room temperature followed by overnight fixation at 4 °C. The fixative was removed by centrifugation at 10,000 rpm for 10 min and washed thrice with 0.9 % saline followed by gradual washes with 20 %, 30 %, 50 %, 70 %, 90 % and 100 % of ethanol. Finally the spore pellets were resuspended in absolute ethanol and kept on stand-by for 20 min at room temperature. The sample slides were prepared by air drying samples on coverslip and mounted on SEM stubs using carbon tape. Imaging was done using FeSEM instrument (Olympus BH-2/Carl Zeiss LSM880/Philip XLO3).

2.20.2 Transmission electron microscopy

The cross sections of treated [0.05 % H_2O_2 (v/v)] and untreated spores of *B. subtilis* PY79 (WT) and *B. subtilis* TB47 (*ΔsqhC* Mutant) were observed using transmission electron microscopy (TEM; H-7500 Hitachi). The spores samples were cut into thin sections (120 nm) with microtome cutting and placed on copper grids (200 mesh). Prior to imaging gold sputtering was performed on the sample slide to finally ready the TEM sections to be observed (Indu et al. 2021).

2.20.3 Propidium iodide staining and confocal microscopy

Membrane permeability of spore was assessed with propidium iodide (PI) staining and confocal microscopy. Clean spore preparations (1 x 10^{-6} CFU.ml^{-1}) of both WT and *ΔsqhC* mutant *B. subtilis* were used for the experiment. Autoclaved spores suspension was considered as positive control (nonviable spores with membrane damage). Spores exposed to 0.05 % (v/v) of H_2O_2 (treated) and untreated spores of both the *Bacillus* strains were washed with sterilized milli-Q water and incubated with 5 µl of 1 mg.ml^{-1} of PI stain at

room temperature for 15 to 30 min. The stain was removed by 2-3 consecutive washes and spores were suspended in 100 µl of sterilized milli-Q water. The spore samples to be observed were mounted (5 µl) on glass slide. Images were captured with confocal microscope (Zeiss LSM880) with red filter (560-620 nm) and 60x lens.

2.21 Spore germination and dipicolinic acid extraction

Thirty mg dry weight each from spores of WT and sporulene mutant strain of *B. subtilis* was taken into three sets as A (Positive control), B (Treated) and C (Untreated). Set B spore were incubated with 0.05 % (v/v) of H_2O_2 for 1 h. However, the spores of set C were suspended in PBS (pH 7). Set A spores were autoclaved (121 lbs for 15 min), acidified with 0.1 N of acetic acid at room temperature for 1 h, followed by centrifugation at 1500 g for 10 min (Janssen et al. 1958). The supernatant was vacuum dried and re-dissolved in 1 ml methanol which was further concentrated to 200 µl and filtrated with 0.2 µm filter for HPLC analysis. Whereas, set B (treated) and C (untreated) spores were washed and germinated for 1 h in germination solution at 37 °C. Prior to germination heat shock was given at 70 °C followed by ice cooling for 15 min. For both set B and C, dipicolinic acid (DPA) released during germination was quantified from the supernatant and to quantify DPA retained in the spore, similar procedure was mimicked as for set A.

2.22 DPA quantification through HPLC analysis

The quantity of DPA released and retained was analysed through HPLC (Shimadzu, Japan with PDA M20A detector). A linear gradient programme with 1 % (v/v) acetic acid (Solvent A) and 100 % acetonitrile (Solvent B) was used for separation through Phenomenex C-18 column (Luna, 5 µm, 250 x 4.6 mm). The flow rate was set as 1.5 $ml.min^{-1}$. DPA solution (1 $mg.ml^{-1}$) was used as quantification standard. All the solvents used were of LC-MS grade.

2.23 Fatty acid and polar lipids and analysis

Extraction of polar lipids from spores, involved the removal of the proteinaceous coat by incubation in de-coating solution for 1 h at 0 °C, followed by two step washing with sterilized H_2O. Further, it was incubated with 0.1 M NaOH for 15 min at 0°C (Checinska et al. 2012). Finally, the spores were rewashed and digested with 20 $mg.ml^{-1}$ of lysozyme for 10 min at 0 °C and used for polar lipid extraction. The extraction of polar lipids was carried out as per the method suggested by Kates (1986) and Tindall (1990). The extracted lipids were run on two dimensional TLC using first dimension solvent as chloroform: methanol: H_2O (30: 10: 1) and second dimension solvent as chloroform: methanol: glacial acetic acid: H_2O (35: 7: 6: 1.5). The spots were developed with 20 % (w/v) of phosphomolybdic acid. For the fatty acid methyl ester (FAME) analysis of spores produced under control and treated [0.005 % (v/v), H_2O_2] condition, spores devoid of vegetative cells were used. The analysis was outsourced to M/s. Royal Life Sciences Pvt. Ltd, Secunderabad, India. Analysis was conducted as per MIDI (Microbial ID) Sherlock, USA by RSTBA6 method (www.midi-inc.com) as described by Sasser (1990).

2.24 Spore protein extraction and identification

Proteins of both the WT and *ΔsqhC* mutant *B. subtilis* spores were extracted according to trichloroacetic acid (TCA) method as described by Kaiser et al. (2015). Approximately, 10 mg of spores sample were incubated in 20 % (v/v) of trichloro acetic acid at -20 °C for 24 h, after which pellets were thawed extensively and centrifuged for 5 min at 16000 g. TCA treated spores were washed with ice cold ethanol and resuspended in lysis buffer followed by sonication (5-25 sec pulses with 15 sec of recovery period). The spore lysate obtained was further incubated for 1 h with vigorous shaking at 400 rpm at 60 °C. In the lysate 50 mM ammonium bicarbonate was added and overnight trypsin digestion was carried out at 37 °C. The spore protein extracts were outsourced to M/s. Sandor

Speciality Diagnostics Pvt. Ltd. Hyderabad and QTOF protein profiling was done. The protein samples were separated through BEH C-18 column (75 μm x 150 cm x 1.7 μm) attached to Nano Acquity Waters UPLC system with solvent A 0.1 % formic acid and solvent B: acetonitrile 0.1 % formic acid; separation was continued for 40 min. UniProta and PLGS-Waters search engine databases were used for protein identification.

Results

3. RESULTS

3.1 *In silico* and *In vitro* analysis of hopanoids and sporulenes amongst few *Planctomycetota* members

3.1.1 Laboratory isolates of *Planctomycetota* members

Planctomycetota strains used under this study were previously reported laboratory strains, isolated from sediment samples of Chilika lagoon. Whereas one of the members *Paludisphaera rhizosphaerae* JC665^T was isolated from rhizospheric soil of Loktak lake (Table 6).

3.1.2 Genome based inventory of hopanoid and sporulene pathway genes

Genomes of few novel isolates of phylum *Planctomycetota* (laboratory strains) were sequenced and the annotated genomes were screened for the presence of hopanoid and sporulene biosynthesis pathway genes (Table 6). Genome mining was started with the screening of genes encoding for squalene hopene cyclase (*shc*), the key enzyme in hopanoid biosynthesis and sporulenol synthase (*sqhC*), the key enzyme of sporulene biosynthesis. The *Planctomycetota* genomes harbouring *shc/sqhC* were subsequently mined for other genes involved in synthesis of hopanoids, sporulenes and their derivatives. Amongst the screened members *Paludisphaera rhizosphaerae* JC665^T and *Gimesia chilikensis* JC646^T showed the presence of all the hopanoid biosynthesis pathway genes in their genomes except for *hpnN* gene (Table 7). Other three *Planctomycetota* members *viz, Roseimaritima sediminicola* JC651^T, "*Roseiconus lacunae*" JC635^T and "*Roseiconus nitratireducens*" JC645^T showed the presence of only few of the hopanoid biosynthesis genes in their genomes (Table 7). However, we could not identify the genes involved in sporulene biosynthesis pathway in any of the screened *Planctomycetota* genomes (Table 6).

3.1.3 Phylogeny based on squalene hopene cyclase

A phylogenetic tree was constructed based on amino acid sequences of putative squalene hopene cyclase protein (SHC) of *Planctomycetota* members screened for hopanoid biosynthesis pathway genes along with putative SHC protein sequences of other bacterial members. The tree was generated with seven type strains of hopanoid producing bacteria, six type strains and two non-type strains of the genus *Bacillus* and five lab isolates of phylum *Planctomycetota* with *Verrucomicrobiales bacterium* VVV1 as outgroup.

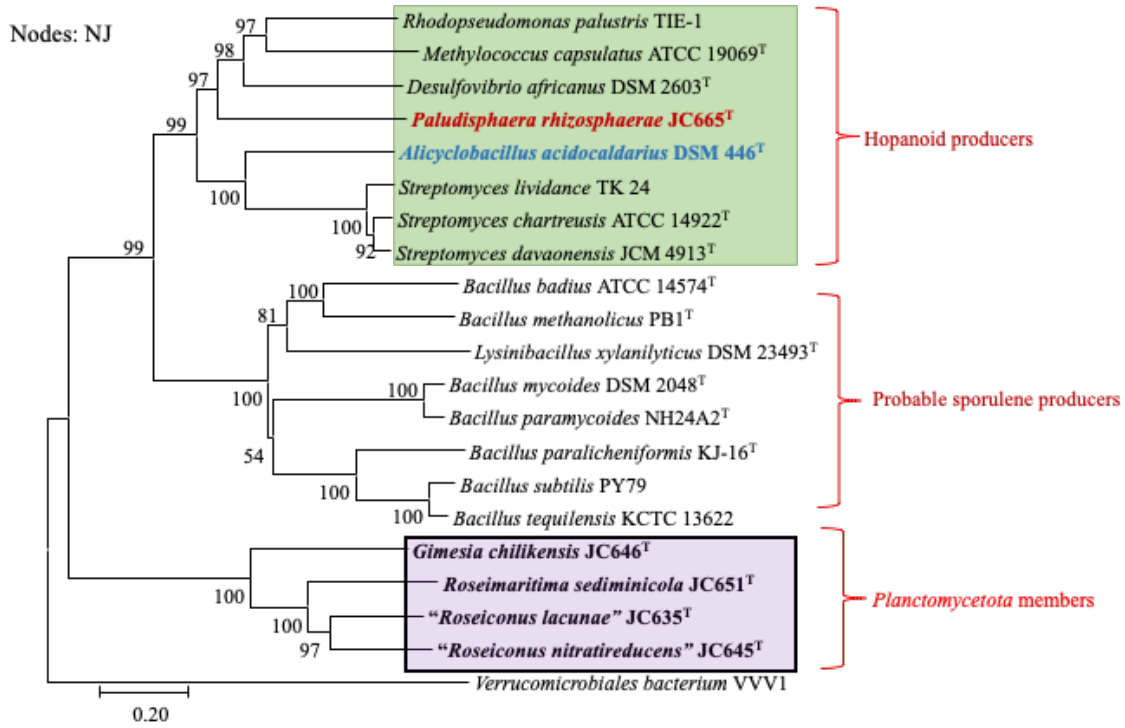


Fig. 9: Phylogenetic tree constructed based on amino acid sequences of SHC proteins of hopanoid producing bacteria, putative SqhC proteins of genus *Bacillus* members and putative SHCs of *Planctomycetota* members.

The amino acid sequences were aligned by MUSCLE sequence alignment and neighbor joining tree was constructed using MEGA 7 software. Bootstrap values based on 1000 replications are listed at branching points. Putative SHCs of hopanoid producers are highlighted by green box. Whereas, putative SHCs of Planctomycetota members are highlighted in bold letters and formed a distinct clade from rest of the bacterial members. Paludisphaera rhizosphaerae JC665^T cladded along with hopanoid producing members.

Source	Geographical locations and GPS coordinates	Strain no.	Highest 16S rRNA gene sequence identity	Identity (%)	Newly described taxa names	16S rRNA gene accession number	GeneBank accession no. for whole genome sequence	Culture deposition no.	Genes encoding for enzymes of hopanoid Biosynthesis pathway	Genes encoding for enzymes of sporulene biosynthesis pathway
Sediment	Chilika Lagoon, Odisha 19º 40' 12"N-85º 25' 48"E	JC646	*Gimesia maris* DSM 8797^T	97	*Gimesia chilikensis* JC646^T	LR132072	VTSR00000000	KCTC 72175 NBRC 113926	+	-
Sediments	Chilika Lagoon, Odisha 19º 40' 12"N-85º 25' 48"E	JC651	*Roseimaritima ulvae* UC8^T	98	*Roseimaritima sediminicola* JC651^T	LR133893	WIAD01000000	KCTC 72178 NBRC 113926	+	-
Sediments	Chilika Lagoon, Odisha 19º 40' 12"N-85º 25' 48"E	JC635	*Rhodopirellula baltica* SH1^T	94	*"Roseiconus lacunae"* JC635^T	LR132029	VSZO00000000	KCTC 72164 NBRC 113875	+	-
Sediments	Chilika Lagoon, Odisha 19º 40' 12"N-85º 25' 48"E	JC645	*Rhodopirellula rubra* LF2^T	94	*"Roseiconus nitratireducens"* JC645^T	LR132069	VWOX00000000	KCTC 72174 NBRC 113879	+	-
Rhizosphere soil	Loktak Lake, Manipur 24°30′21″ N 93°47′43″ E	JC665	*Paludisphaera borealis* PX4^T	95	*Paludisphaera rhizosphaerae* JC665^T	LR746340	JAALCR000000000	KCTC 72671 NBRC 114305	+	-

Table 6. Phylogenetic affiliation of *Planctomycetota* isolates along with its association to hopanoids and sporulene biosynthesis pathway

Samples collected from Chilika Lagoon, Odisha and Loktak Lake Manipur along with GPS co-ordinates. Novel strains isolated from respective samples with their closest phylogenetic member, based on the 16S rRNA gene sequence similarity. 16S rRNA gene accession numbers and GeneBank accession numbers for whole genome sequence along with strain deposition number of different culture collection centers are mentioned with respective strain. Genomes of all the listed strains were screened for the presence of genes encoding for enzymes of hopanoid biosynthesis pathway and genes encoding for enzymes of sporulene biosynthesis pathway. Where; +, present; -, absent.

Enzymes of hopanoid biosynthesis pathway	**Gene code**	**EC numbers**	***Paludisphaera rhizosphaerae* JC665^T**	***Gimesia chilikensis* JC646^T**	***Roseimaritima sediminicola* JC651^T**	***"Roseiconus lacunae"* JC635^T**	***"Roseiconus nitratireducens"* JC645^T**
Squalene hopene cyclase	*shc/hpnF*	5.4.99.17	+	+	+	+	+
Glycosyl transferase family 2, hpnB	*hpnI/hpnB*	2.4.1.-	+	+	-	-	-
5'-methylthioadenosine nucleosidase/ S-adenosylhomocysteine nucleosidase	*hpnG*	3.2.16/3.2.2.9	+	+	-	-	-
Radical SAM protein required for addition of adenosine to hopane skeleton	*hpnH*	3.20.20.70	+	+	-	-	-
Acetylornithine aminotransferase	*hpnO/argD*	2.6.1.11	+	+	+	+	+
Hopanoid-associated RND transporter	*hpnN*	-	-	-	-	-	-

Table 7. Genes involved in hopanoid biosynthesis pathway and their association to the screened *Planctomycetota* isolates

Hopanoid biosynthesis genes mined amongst the laboratory isolates of Planctomycetota members. The details of genes encoding for putative proteins involved in hopanoid biosynthesis pathway are as described by Tushar et al. 2014; Where; +, represents presence of gene; -, represents absence of gene.

In the resulting neighbour joining tree (NJ), hopanoid producing members, the genus *Bacillus* members and *Planctomycetota* members cladded separately, forming three distinct clades (Fig. 9). Interestingly, based on the putative SHC protein sequence identity *P. rhizosphaerae* JC665^T cladded with the hopanoid producing bacteria likely to *Alicyclobacillus acidocaldarius* DSM 446^T. It prompted us to look more into the details of hopanoid biosynthesis of *P. rhizosphaerae* JC665^T.

3.1.4 Non-canonical biosynthesis of few hopanoids in *P. rhizosphaerae* JC665^T

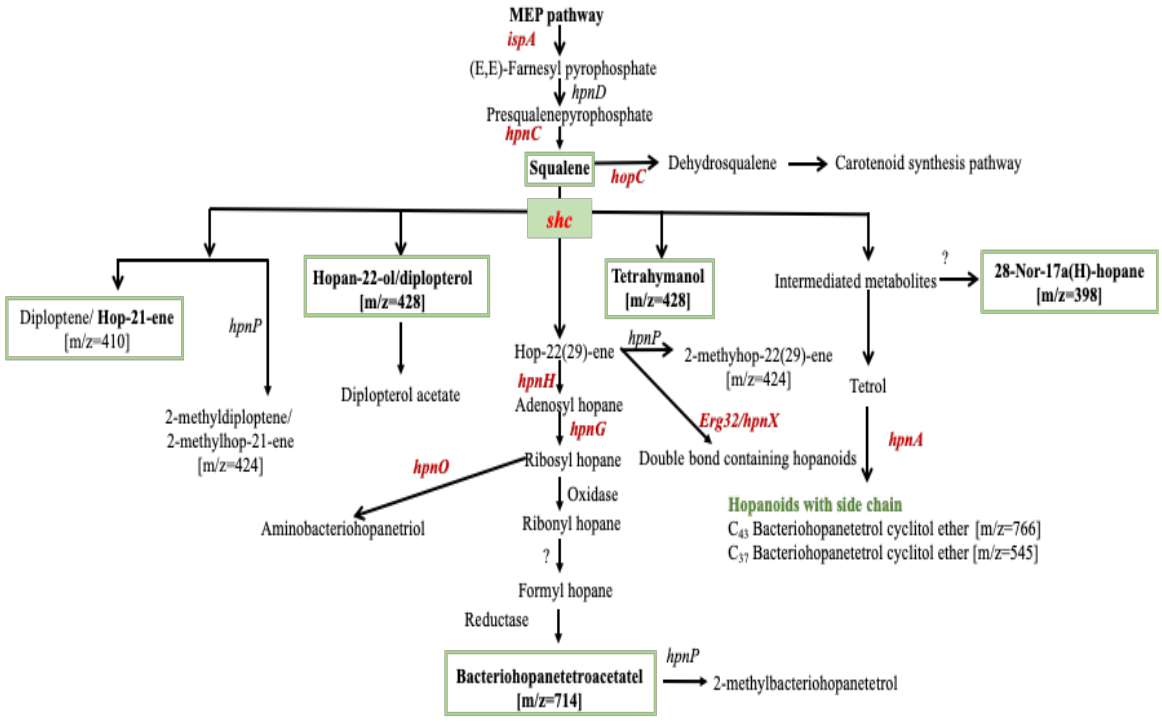


Fig. 10: Schematic representation of non-canonical hopanoid biosynthesis pathway in *P. rhizosphaerae* JC665^T

The representation is based on genes identified from the its genomes, GC-MS analysis and previous studies. All the genes identified showed nearly 50% to 60% gene identity with the hopanoid biosynthesis genes of Rhodopseudomonas palustris TIE-1(A reference organism in which hopanoid pathway is already studied). Genes in red: Hopanoid biosynthesis genes identified in P. rhizosphaerae JC665^T by genome mining; Metabolites in boxes: Precursor metabolites, hopanoids and its derivatives identified in endo-metabolome of P. rhizosphaerae JC665^T through gas chromatography.

Genome of *P. rhizosphaerae* JC665^T harboured majority of the genes encoding for putative hopanoid biosynthesis pathway proteins which includes, squalene synthase (*hpnC*), squalene/phytoene desaturase (*hopC*), squalene hopene cyclase (*shc*), radical S-adenosyl-L-methionine (SAMe) (*hpnH;* required for addition of adenosyl group to hopane skeleton), 5-methylthioadenosine nucleosidase (*hpnG*), acetylornithine aminotransferase/amino-bacteriohopanetriol synthase (*hpnO*), hopanoid associated sugar epimerase (*hpnA*) and sterol desaturase family protein (*erg32*/*hpnX*) (Fig. 10).

Hopanoid biosynthesis genes identified in *P. rhizosphaerae* JC665^T were compared with homologous genes of *Rhodopseudomonas palustris* TIE-1 using multiple sequence alignment. The squalene hopene cyclase (*shc;* gene encoding the key enzyme of hopanoid biosynthesis) of *P. rhizosphaerae* JC665^T showed 52 % amino acid identity with *shc* of *Rhodopseudomonas palustris* TIE-1 (Fig. S1), whereas all the other genes shared 50-60 % nucleotide identity. A few genes remained unidentified like, *hpnP* and *hpnB* which were previously observed to be involved in the synthesis of the intermediate hopanoids like ribonylhopane, methylated bacteriohopanepolyol and glycosyl group containing hopanoids in *Rhodomicrobium sp.* or *Rhodopseudomonas sp.* **(**Lodha et al. 2015).

3.1.5 Hopanoid inventory of *P. rhizosphaerae* JC665^T

In correspondence with the genomic information, when the endo-metabolome of *P. rhizosphaerae* JC665^T was analysed, few commonly known hopanoids such as bacteriohopanetetrol acetate, tetrahymanol, diplopterol, 2-methyldiplopterol were discovered. Whereas, few unidentified hopanoid like fragmentation spectra were also recorded (Table 8). The unidentified hopanoids represented the MS fragmentation spectra at *m/z*=442, *m/z*=383, *m/z*=384, *m/z*=431.

28-Nor-17a(H)-hopane, a hopanoid resembling geohopanoids (French et al. 2012) and hop-21-ene, a rare prokaryotic hopanoid (Pale-Grosdemange et al. 1998) were also identified in *P. rhizosphaerae* JC665^{T}. The reference fragmentation spectra for the hopanoids identified is given in (Fig. 11).

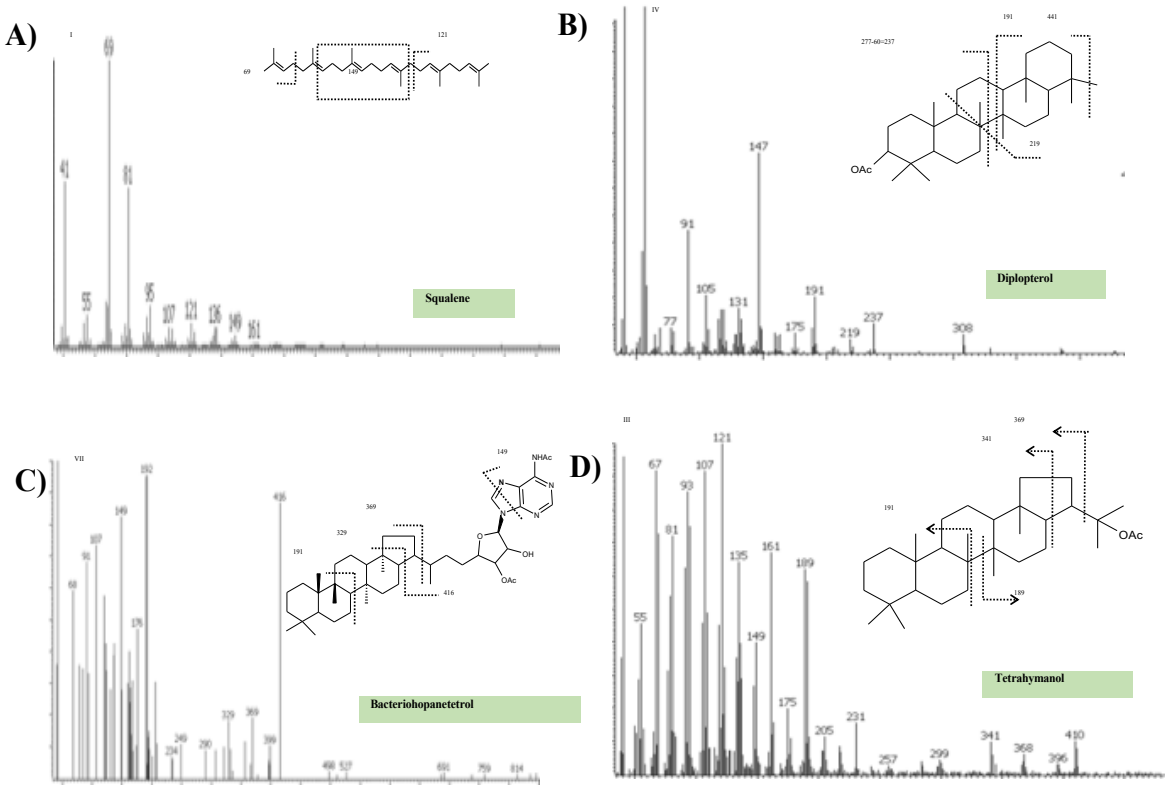


Fig. 11: Standard mass spectra recorded for few common hopanoids and its precursor

Identification of hopanoids of P. rhizosphaerae JC665^{T} was done with the help of comparative studies of mass spectra of hopanoids given above as described by Lodha et al. 2015. The libraries referred were NIST library of metabolites and hopanoid library. Where, A) MS fragmentation patter and predicted skeleton of squalene B) MS fragmentation patter and predicted skeleton of diplopterol C) MS fragmentation patter and predicted skeleton of bacteriohopanetetrol D) MS fragmentation patter and predicted skeleton tetrahymanol.

Acetylated and C30 hopanoids	Present/Absent
28-Nor-17a(H)-hopane [m/z=398]	+
Bacteriohopanetetrol acetate [m/z=714]	+
Hop-21-ene [m/z=410]	+
2-methylhop-22(29) ene [m/z=424]	-
Hop-22(29)-ene	-
Adenosyl hopane, Ribosyl hopane, Ribonyl hopane, Formyl hopane	-
2-methylhop-21-ene [m/z=424]	-
Tetrahymanol [m/z=428]	+
Hopane-22-ol/ Diplopterol [m/z=428]	+
2-methyldiplopterol	-
Hopanoids with side chain	-
Unidentified hopanoids	
UH3 [m/z=442]; UH7 [m/z= 383]; UH8 [m/z=384]; UH16 [m/z=431]	+

Table 8. List of hopanoids identified from the endometabolome of *P. rhizosphaerae* JC665^T

The hopanoids were identified through GCMS analysis. Where; +, Hopanoids identified; -, Hopanoid unidentified

3.2 Isolation of genus *Bacillus* members and genome sequence of *Bacillus subtilis* JC1005 with special reference to sporulene biosynthesis

3.2.1 Sample collection, source and geographical locations

Samples collected for isolation of *Bacilli* members were from different geographical locations of India like Tamil Nadu, Jharkhand, Orissa and Karnataka. Some of the samples were sourced from extreme environments such as water starved soil, dry sand, hot spring water and few were from other sources like marine sponge and soil sediments (Fig. 12).

Fig. 12: Sampling sites from different parts of India and samples collected for bacterial isolation

The samples collected and used for isolation of various bacterial members under this study includes sediment samples, dry soil, sponges, beach sand, pink deposits in water bodies salt crystals from different geographical locations of India like Tamil Nadu, Gujarat, Sikkim.

At the time of sample collection pH, salinity of the samples and GPS co-ordinates of the sampling site was recorded. All the samples were carefully brought to laboratory and enriched into suitable medium. The bacteria of interest were isolated, purified and the culture stocks were preserved in 50 % of glycerol at -20 °C.

3.2.2 Isolation and identification of *Bacillus* members

Total, seventy-five different samples were enriched in bacillus composition medium. From the enrichments, thirty-five bacterial isolates were obtained with the help of selective treatments such as pasteurization, use of cycloheximide and sodium azide. The isolates were purified by repetitive streaking on nutrient agar medium. 16S rRNA gene sequences of purified isolates were outsourced to Agrigenome Pvt. Ltd. and the quality sequences (1300-1450 nt.) were analysed using EzBiocloud or NCBI BLAST. Based on the 16S rRNA gene sequence analyses, fifteen isolates were identified as the members belonging to the genus *Bacillus* spp. (Table 9). All the isolates showed 99.9-100 % 16S rRNA gene sequence identity with previously reported *Bacillus* spp. except for one which had 98.7 % identity with *Mesobacillus selenatarsenatis* SF-1^T. For all the *Bacillus* strains isolated in the laboratory, 16S rRNA gene sequences were submitted in European Molecular Biology Laboratory (EMBL) and are available on National Centre for Biotechnology information (NCBI). Genomes of the previously reported *Bacillus* spp. which showed closest 16S rRNA gene sequence identity with our lab isolates listed in (Table 9) were screened for genes involved in hopanoid and sporulene biosynthesis. We could not identify hopanoid biosynthesis genes in any of the screened *Bacilli* genomes. However, all the genes of sporulene biosynthesis pathway were identified amongst the screened members (Table 9).

3.2.3 Phylogeny based on sporulenol synthase

A phylogenetic tree was created based on amino acid sequence of putative sporulenol synthase proteins (SqhC) of genus *Bacillus* members along with amino acid sequences of SHC protein of hopanoid producing members. The tree was constructed using twenty three type strains and two non-type strains of genus *Bacillus*, eight type strains of hopanoid producing bacteria with *Gemmata obscuriglobus* UQM 2246^T as out group.

Source	Geographical locations and GPS co-ordinates	Strain no.	Highest 16S rRNA gene sequence identity	Identity (%)	Accession number	Genes encoding for enzymes of hopanoid Biosynthesis pathway	Genes encoding for enzymes of sporulene biosynthesis pathway
From water body	Vedaranyam 10º 21' 11"N-79º 50' 41"E	JC1001	*Bacillus tequilensis* KCTC 13622 ^{T}	100	LS998695	-	+
Dry soil from aquaculture pond	Velankinni 10º 43' 36"N-79º 49' 47"E	JC1002	*Bacillus paralicheniformis* KJ-16^{T}	100	LS998696	-	+
Garden soil from pea plantation	Gangtok 27º 40' 11"N-88º 43' 37"E	JC1004	*Bacillus mycoides* DSM 2048^{T}	100	LS998020	-	+
Yak droppings	Yumthang valley Sikkim 27º 47' 35"N-88º 42' 27"E	JC1005	*Bacillus subtilis* subsp. *subtilis* NCIB 3610^{T} (NCBI genome accession no. JAAOLA000000000)	100	LS998021	-	+
Soil sediment from brown pond	Bihar 25º 13' 27"N-85º 15' 4"E	JC1006	*Lysinibacillus xylanilyticus* DSM23493 ^{T}	99	LS998024	-	+
Soil sediment	Jharkhand 25º 13' 27"N-85º 5' 11"E	JC1007	*Bacillus badius* MTCC1458 ^{T}	100	LS998023	-	+
Hot water from sulphur spring	Yumthang valley Sikkim 27º 47' 35"N-88º 42' 27"E	JC1008	*Bacillus paralicheniforms* KJ-16^{T}	99	LS998691	-	+
Water from stream of a river	Orissa 27º 40' 11"N-88º 43' 37"E	JC1009	*Bacillus subtilis* subsps. *spizizenii* W2	100	LS998693	-	+
Hot water from sulphur spring	Yumthang valley Sikkim 27º 47' 35"N-88º 42' 27"E	JC1010	*Bacillus paramycoides* NH24A2^{T}	100	LS998692	-	+
Soil sediment	Sikkim 27º 3' 31"N 88º 26' 20"E	JC1012	*Bacillus tequilensis* KCTC 13622^{T}	100	LS998694	-	+
Orange brown gelatinous layer on salt pan	Vedaranyam 10º 21' 11"N-79º 50' 41"E	JC1013	*Mesobacillus aurantius* JC1013^{T}	98	LS998022	-	+
Dark brown layer on salt crystal	Vedaranyam 10º 21' 11"N-79º 50' 41"E	JC1014	*Bacillus tequilensis* KCTC 13622 ^{T}	100	LS998697	-	+
Dry Sponge	Pattukkoittai 10º 14' 59"N-79º 16' 43"E	JC1015	*Bacillus tequilensis* KCTC 13622 ^{T}	100	LS998698	-	+
Black sponge in water	Mandapam 9º 16' 52"N-79º 10' 20"E	JC1016	*Bacillus tequilensis* KCTC 13622 ^{T}	100	LS998699	-	+

Source	Geographical locations and GPS co-ordinates	Strain no.	Highest 16S rRNA gene sequence identity	Identity (%)	Accession number	Genes encoding for enzymes of hopanoid Biosynthesis pathway	Genes encoding for enzymes of sporulene biosynthesis pathway
Soil from an organically grown field	Vettaikaran 10º 33' 35"N-79º 49' 48"E	JC1017	*Bacillus tequilensis* KCTC 13622 ^{T}	100	LS998700	-	+
Yeast (Isolated from soil sample)	Hyderabad 22º 98' N-71º 47'E	JC507^{T}	**Chryseobacterium candidae* JC507^{T}	100	LT838865	-	-
Epiphytic rhizosphere of an orchid, Vanda sp.	Western ghat,Karnataka 15°95′69″N and 73°99′47″E	JC501^{T}	**Paracoccus aeridis* JC501^{T}	100	LT799401	-	-

Table 9. The phylogenetic affiliation of the isolated strains, isolation source, location, accession numbers along with its association to hopanoids and sporulenes

*Samples collected from different geographical location of India along with GPS co-ordinates. Strains isolated from respective samples are listed with their closest phylogenetic member, based on the 16S rRNA gene sequence similarity. Accession numbers obtained after EMBL submission are mentioned with respective strain. Genomes of all the listed strains were screened for the presence of genes encoding for enzymes of hopanoid biosynthesis pathway and genes encoding for enzymes of sporulene biosynthesis pathway. Where +, present; -, absent; *, strains other than Bacillus members screened for hopanoid and sporulene pathway genes.*

Based on putative SqhC sequence identity, *Bacillus* members formed a separate clade from that the hopanoid producing members in the resulted neighbour joining (NJ) tree except for *Alicyclobacillus acidocaldarius* DSM 446^{T}. Considering the phylogenetic relatedness of the genus *Bacillus* members observed in the NJ tree (Fig. 13) *Bacillus subtilis* subsp. *subtilis* NCIB 3610^{T} was the closest phylogenetic neighbour to *Bacillus subtilis* PY79 (Fig. 13). However, *Bacillus subtilis* subsp. *subtilis* NCIB 3610^{T} showed 100 % 16S rRNA gene sequence identity with strain JC1005 (lab isolate) (Table 9) further used for sporulene analysis.

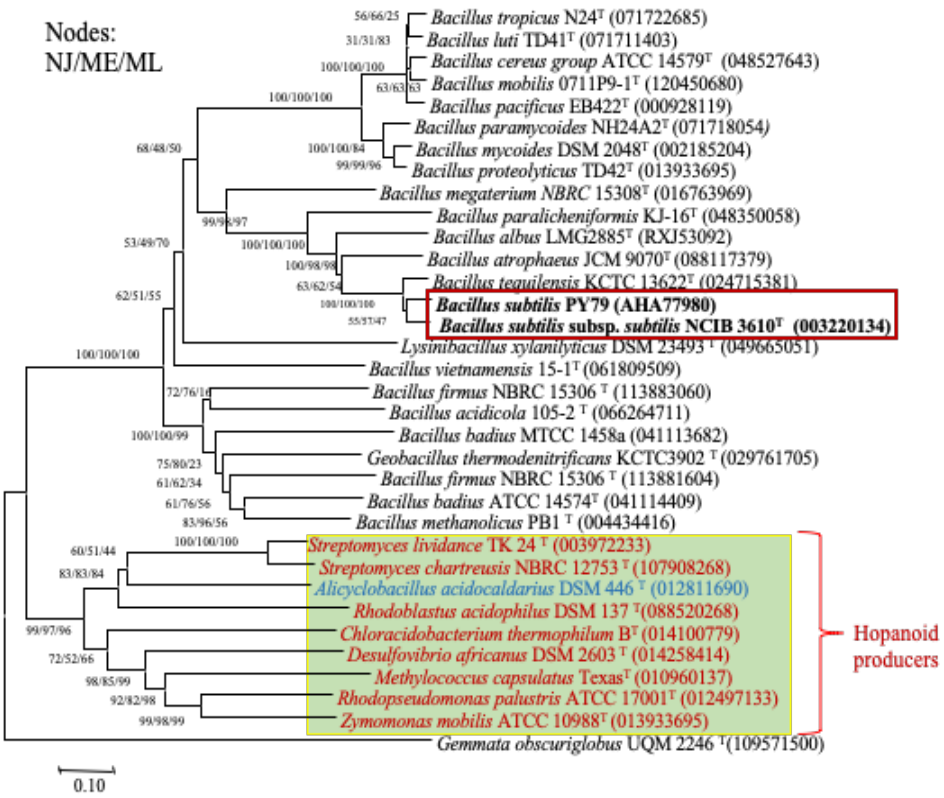


Fig.13: Phylogenetic tree constructed based on amino acid sequences of SHC of hopanoid producing bacteria and putative SqhC proteins of probable sporulene producer genus *Bacillus* members

The neighbor joining tree was constructed using MEGA 7 software and sequences were aligned by MUSCLE sequence alignment. Bootstrap values based on 1000 replications are listed at branching points. Putative SqhC of genus Bacillus members which are probable sporulene producers, formed a separated clade from that of SHC of hopanoid producing bacteria. SHCs of hopanoid producing bacteria is highlighted in red color and boxed in green.

3.2.4 16S rRNA gene sequence identity and genome analysis of strain JC1005

Strain JC1005 was isolated from Yak droppings collected in Yumthang Valley Sikkim (GPS co-ordinates of sampling site 27º 47' 35"N-88º 42' 27"E). 16S rRNA gene sequence analysis of strain JC1005 (1266 nt.) through NCBI BLAST and EZBioCloud revealed its highest (100 %) sequence identity with *Bacillus subtilis* subsp. *subtilis* NCIB 3610^{T}. Further, Illumina HiSeq 2500 platform was used for genome sequencing. The process of end pairing was used for the construction of libraries, paired end reads were generated through sequencing and the genome sequence was deposited in NCBI (JAAOLA000000000). The genome was annotated using RAST (Fig. 14) and CGView server's PROKSEE software, annotated genome represented all the genes encoding for putative proteins of sporulene biosynthesis pathway (Fig. 15).

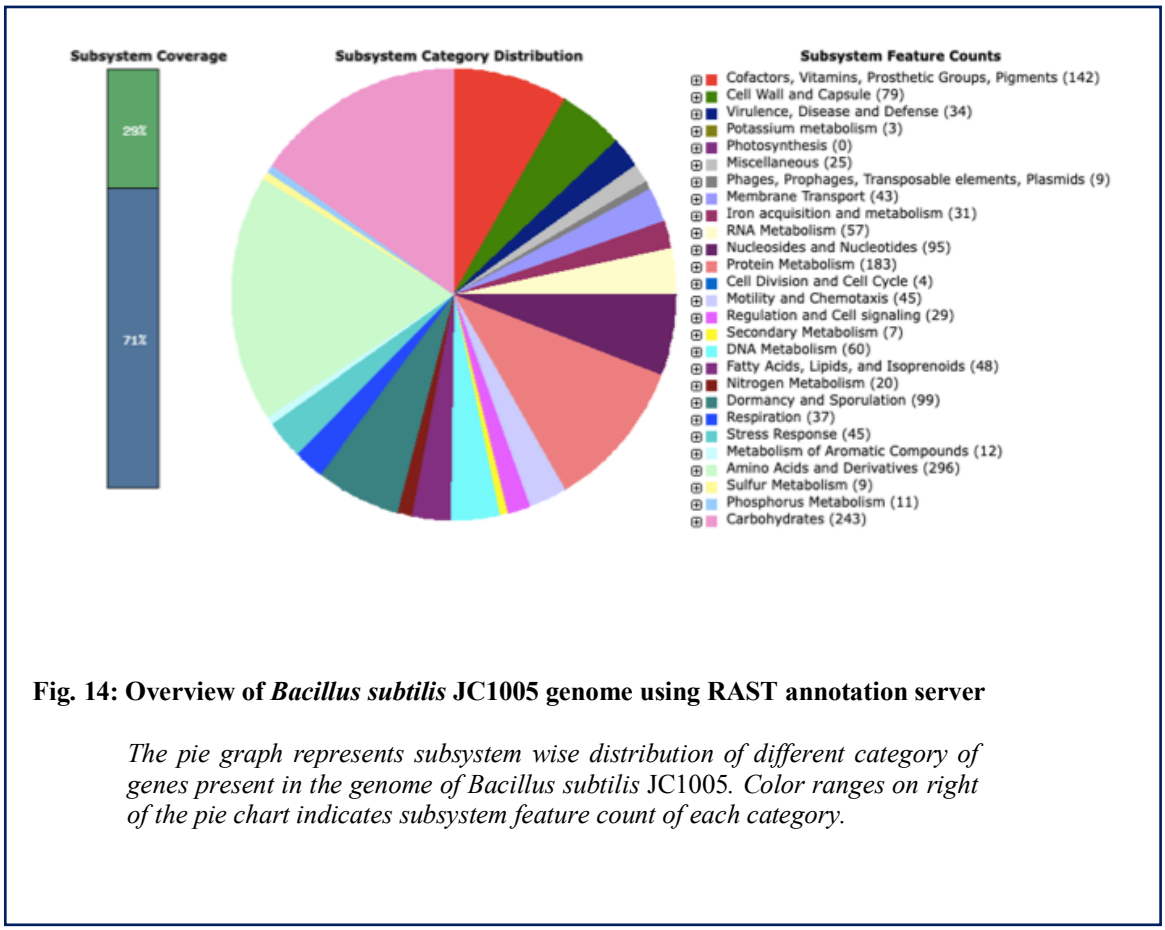


Fig. 14: Overview of *Bacillus subtilis* JC1005 genome using RAST annotation server

The pie graph represents subsystem wise distribution of different category of genes present in the genome of Bacillus subtilis JC1005*. Color ranges on right of the pie chart indicates subsystem feature count of each category.*

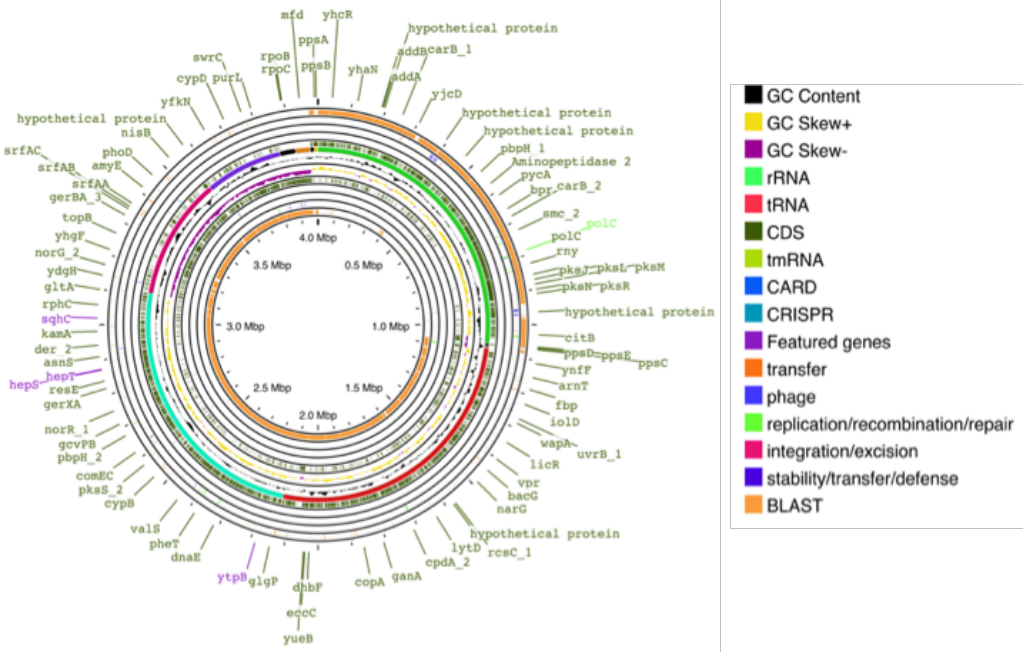


Fig. 15: Advance analysis and annotation of *Bacillus subtilis* JC1005 genome

Annotated genome of B. subtilis JC1005 visualized using CGView server's PROKSEE software for representing conserved and unique sequence features. The BLAST analysis was carried out against B. subtilis PY79 genome. Across the circular genome, selected genes and regions of interest are highlighted with different colors as represented by colored ranges on the right of circular map. Genes involved in sporulene biosynthesis are highlighted with purple color.

Genome analysis and comparison with *B. subtilis* PY79 (in which sporulenes were reported previously) revealed that, a total of three thousand eight hundred and forty-six and three thousand eight hundred and fifty genes were present in the genome of *B. subtilis* JC1005 and *B. subtilis* PY79 respectively. On comparing both the genomes through OrthoVenn it was observed that three thousand eight hundred and forty-five genes were present in common in both the genome whereas one and five elements were unique to *B. subtilis* PY79 and *B. subtilis* JC1005 respectively (Fig. 16, A). Also, the hepatprenyl diaphosphate synthase component I (HepS) and heptaprenyl diaphosphate synthase component II (HepT) of both the *Bacillus* were observed to share close identity (Fig. 16, B, C, D).

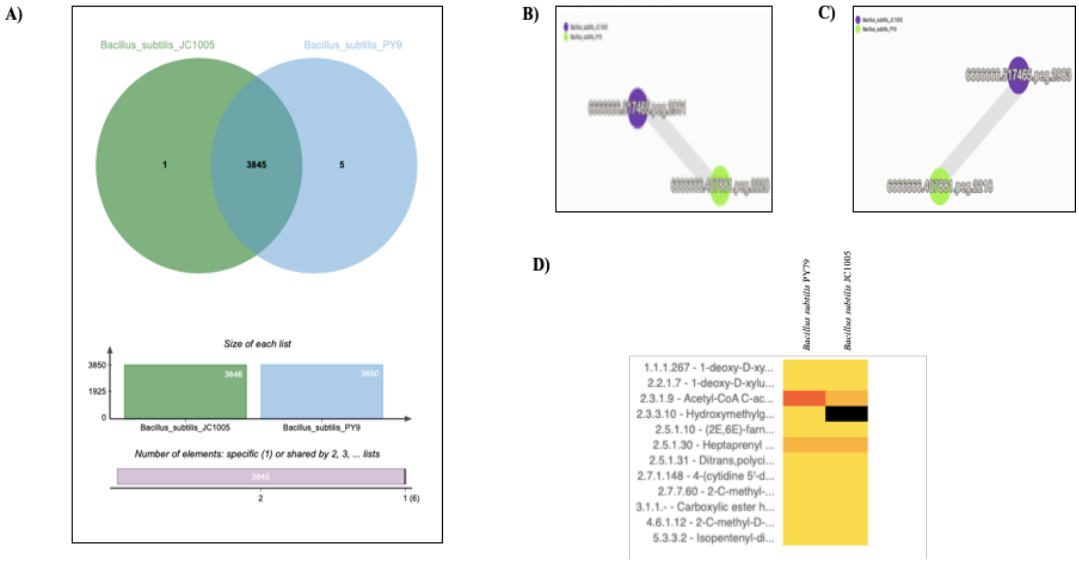


Fig. 16: Comparative analysis of *B. subtilis* PY79 and *B. subtilis* JC1005 at genomic level using OrthoVenn genome analysis tool

A) Venn diagram representing the common and unique genes present in both the genomes. Relatedness of B) Heptaprenyl diphosphate synthase component I (hepS) and C) Heptaprenyl diphosphate synthase component II (hepT) of both the Bacillus strains D) Heat map showing the presence of heptaprenyl diphosphate synthase in the genomes of both the Bacillus strains.

3.2.5 Sporulene characterization in *B. subtilis* JC1005

To identify sporulenes from the spores of *B. subtilis* JC1005, culture was allowed to sporulate in sporulating medium (Fig. 17, A) from which clean spore preparation was obtained (Fig. 17, B). When observed under confocal microscope, isolated spores were completely devoid of vegetative cells and suggested about autofluorescence property of spores of *B. subtilis* JC1005 (Fig. 17, C). Further, total lipids were extracted from the spores of *B. subtilis* JC1005 and partially purified fractions of lipid extract were run on two dimensional TLC (Fig. 18, A, B, C). Putative sporulene spots were scraped from the TLC plate, eluted and analysed for sporulenes through GC-MS. The MS fragmentation pattern from the silica elutes of fraction 1 showed featured characteristics of sporulenes (Fig. 19, A).

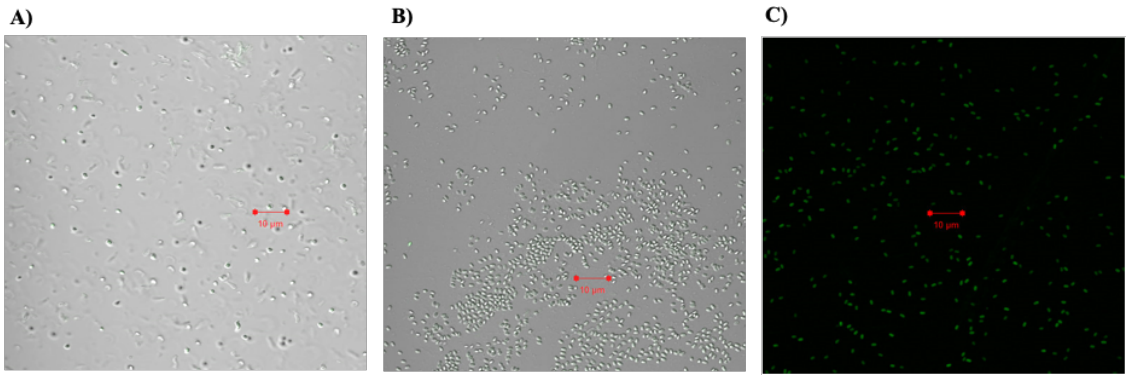


Fig. 17: Sporulated culture and clean spore preparation of *B. subtilis* JC1005 observed under confocal microscope

A) *Stationary phase culture of B. subtilis JC1005 representing vegetative cells and spores.* ***B)*** *Isolated spores of B. subtilis JC1005 devoid of vegetative cells* ***C)*** *Autofluorescent spores of B. subtilis JC1005 at 450 nm.*

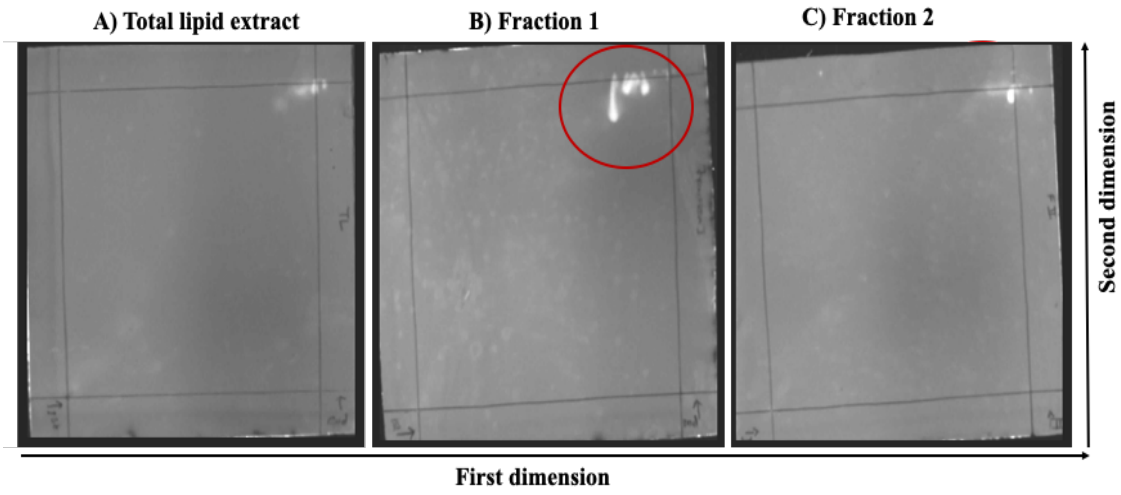


Fig. 18: Two-dimensional TLC of partially purified lipid fractions, extracted from the spore of *B. subtilis* JC1005

The samples were run using dichloromethane as solvent in both the dimensions. Probable sporulene spots were developed using 0.1 % of berberine chloride and observed under UV light as described in material and method section. Where, ***A)*** *Total lipid extract; no visible spots* ***B)*** *Fraction 1; three distinguishable spots* ***C)*** *Fraction 2; one faint spot.*

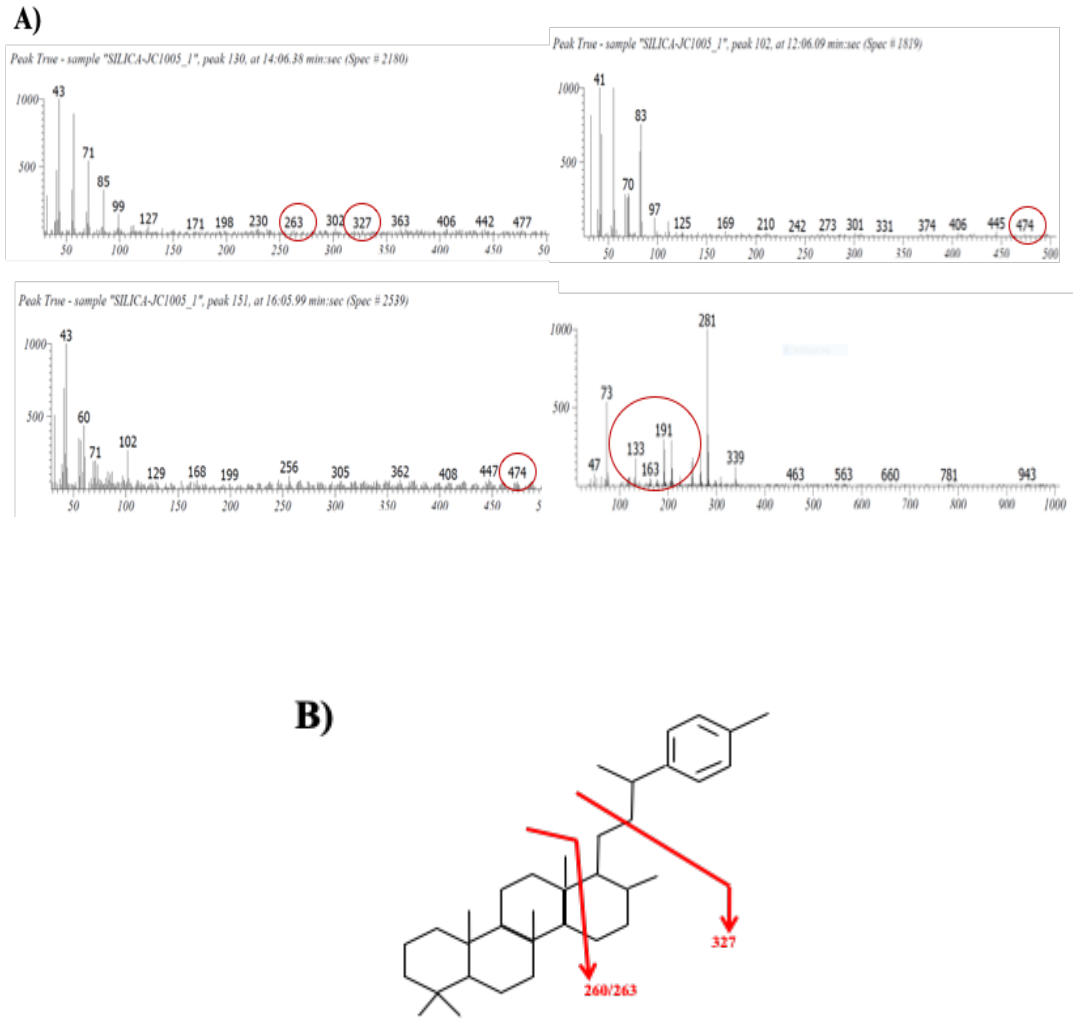


Fig. 19: GC-MS analysis revealed sporulene fragmentation patterns in silica elutes of fraction 1 (F1) of the total lipid extract of the spores of *B. subtilis* JC1005

A) *Selected-ion chromatogram of the m/z 474; molecular ion of sporulene and m/z 191, 327, 133, 163, 263; fragments characteristic for sporulenes.* ***B)*** *Fragmentation scheme that accounts for major fragments observed in the mass spectra of the sporulenes.*

Molecular ion peak of sporulene at *m/z* 474 along with additional MS fragmentation spectra at *m/z* 191, 133, 327, 263, 199, 260 were recorded (Fig. 19, A, B) thereby confirming the presence of sporulenes in the spores of *B. subtilis* JC1005.

3.3 Genome mining and *in silico* analysis of sporulene biosynthesis pathway genes in the members of class *Bacilli*

3.3.1 Class *Bacilli* members considered under study

Altogether one hundred and sixteen members of the family *Bacillaceae*, twenty-eight members of the family *Caryophanaceae*, twenty-one members of the family *Alicyclobacillaceae*, twenty-four members of the family *Paenibacillaceae*, six members of the family *Listeriaceae*, one member of the family *Pasteuriaceae*, eight members of the family *Sporolactobacillaceae*, nine members of the family *Staphylococcaceae*, which represented the order *Caryophanels* were collected for the study. Also, twenty-four members of *Lactobacillaceae*, twenty members of *Thermoactinomycetaceae*, fifteen members of the family *Carnobacteriaceae*, nine members of the family *Enterococcaceae*, nine members of the family *Streptococcaceae*, eight members of *Aerococcaceae* and six members of the family *Leuconostocaceae* which represented the order *Lactobacillales* were considered for study (Table 10).

3.3.2 Occurrence and distribution of sporulene biosynthesis pathway genes

Distribution of genes encoding putative proteins of sporulene biosynthesis pathway *viz.,* heptaprenyl diaphosphate synthase component I (*hepS*), heptaprenyl diaphosphate synthase component II (*hepT*), tetraprenyl-β-curcumene synthase (*ytpB*) and sporuleneol synthase (*sqhC*) was studied amongst the members of class *Bacilli*. Two-hundred-thirty-three members of the order *Caryophanales* and seventy-one members of the order *Lactobacillales* were screened by *in silico* analysis of collected genomes. Out of these, genomes of fifty-seven members belonging to sixteen genera of the family *Bacillacea*, one member of the genus *Jeotgalibacillus* of the family *Caryophanaceae*, four members belonging to three genera of the family *Paenibacillaceae*, two members belonging to two

genera of the family *Sporolactobacillaceae* showed the presence of all four genes encoding for putative proteins involved in sporulene biosynthesis (Fig. 20, A, B).

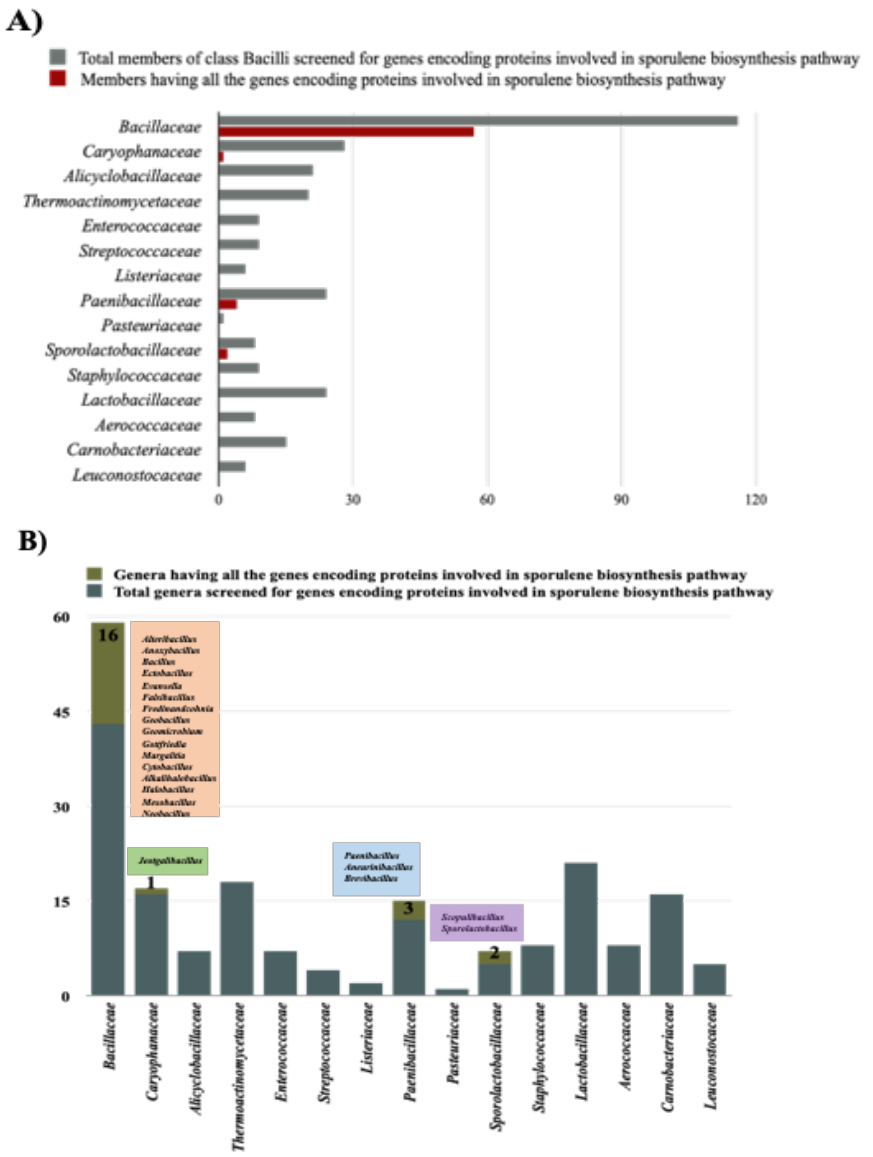


Fig. 20: Occurrence of genes encoding for proteins of sporulene biosynthesis pathway amongst the members of class *Bacilli* at family and genus level

A) Occurrence amongst the members of different families of the class Bacilli. Grey bars represent the total number of bacterial family members screened for sporulene biosynthesis pathway; Red bars indicate members with complete sporulene biosynthesis pathway genes in them. ***B)*** *occurrence amongst the different genera of the class Bacilli. Grey bars represent the genera that do not harbor all the pathway genes involved in sporulene biosynthesis. Green bars indicate the genera having all the sporulene biosynthesis pathway genes.*

Organisms with strain numbers	Putative proteins involved in sporulene biosynthesis pathway			
	HepS	**HepT**	**YtpB**	**SqhC**
	Family *Bacillaceae*			
Aeribacillus composti KCTC 33824	+ (WP_158640198)	+ (WP_063388586)	+ (WP_063386578)	–
Aeribacillus pallidus KCTC3564	+ (WP_066251950)	+ (WP_063388586)	+ (WP_157727915)	–
Alkalibacillus almallahensis CECT 8373	+ (WP_167262413)	–	–	–
Alkalibacillus haloalkaliphilus NBRC 103110	+ (WP_146814857)	–	–	–
Bacillus chagannorensis DSM 18086	+ (WP_051287607)	–	–	–
Alkalicoccus saliphilus 6AG	+ (WP_160058015)	+ (WP_107582765)	–	–
Alkalicoccus urumqiensis BZ-SZ-XJ18	+ (WP_181186048)	–	–	–
Alkalicoccus halolimnae BZ-SZ-XJ29	+ (WP_187254641)	+ (WP_147804793)	–	–
Alteribacillus bidgolensis DSM 25260	+ (WP_170031548)	+ (WP_091579649)	+ (WP_091581562)	+ (WP_091580195)
Alteribacillus iranensis DSM 23995	+ (WP_091656604)	+ (WP_091657830)	–	–
Alteribacillus persepolensis DSM 21632	–	+ (WP_091270906)	+ (WP_091272999)	+ (WP_091271519)
Amphibacillus marinus CGMCC 1	+ (WP_091493410)	–	–	–
Amphibacillus sediminis NBRC 103570	+ (WP_067836540)	–	+ (WP_067841554)	–
Anaerobacillus alkalidiazotrophicus DSM 22531	–	–	+ (WP_071389793)	+ (WP_071390328)
Anaerobacillus alkaliphilus B16-10	+ (WP_161568132)	+ (WP_129076289)	+ (WP_129080010)	–
Anaerobacillus arseniciselenatis DSM 15340	+ (WP_071311782)	+ (WP_071311778)	+ (WP_071313616	–
Anaerobacillus isosaccharinicus NB2006	+ (WP_071319402)	+ (WP_071319399)	+ (WP_071319535)	–
Anoxybacillus calidus DSM 25220	+ (WP_181536487)	+ (WP_181535751)	+ (WP_181537629)	+ (WP_181536973)
Anoxybacillus tepidamans DSM 16325	+ (WP_183251333)	+ (WP_183251126)	+ (WP_183253154)	–
Aquibacillus albus DSM 23711	+ (WP_204497268)	+ (WP_204497270)	+ (WP_204498466)	–
Aquibacillus halophilus B6B	+ (WP_153736295)	+ (WP_153736049)	+ (WP_153738393)	–
Aquibacillus sediminis BH258	+ (WP_138416962)	+ (WP_138416966)	+ (WP_138420313)	–

Aureibacillus halotolerans DSM 28697	+ (WP_124219675)	–	+ TDQ40291	–
Caldalkalibacillus mannanilyticus JCM 10596	+ (WP_025026757)	+ (WP_034680500)	–	–
Caldalkalibacillus thermarum CGMCC 1.4242	+ (WP_188623451)	+ (WP_188623449)	–	–
Caldibacillus debilis DSM 16016	+ (WP_169382662)	+ (WP_020154561)	+ (WP_081626284)	–
Calditerricola satsumensis JCM 14719	+ (WP_188816678)	–	+ (WP_054669393)	–
Calidifontibacillus erzurumensis P2	+ (WP_196237922)	+ (WP_173731499)	–	–
Cerasibacillus quisquiliarum NBRC 102429	+ (WP_146938196)	+ (WP_146938194)	–	–
Cerasibacillus terrae CC-CFT480	+ (WP_147665595)	+ (WP_147665855)	–	–
Compostibacillus humi CGMCC 1.12360	+ (WP_188390525)	+ (WP_188390523)	–	–
Domibacillus antri XD80	+ (WP_075397535)	+ (WP_075397533)	+ (WP_075398815)	–
Domibacillus indicus SD 111	+ (WP_046173725)	+ (WP_046173723)	+ (WP_046176168)	–
Ectobacillus panaciterrae DSM 19096	+ (WP_081415085)	+ (WP_028400485)	+ (WP_028399745)	+ (WP_028400097)
Evansella caseinilytica SP	+ (WP_090887948)	+ (WP_090887963)	+ (WP_175476057)	+ (WP_090888458)
Evansella cellulosilytica DSM 2522	+ (WP_013488463)	+ (WP_013488468)	+ (WP_013489893)	–
Falsibacillus pallidus DSM 25281	+ (WP_114745535)	+ (WP_114745314)	+ (WP_114745215)	–
Falsibacillus albus GY 10110	+ (WP_158598297)	+ (WP_121680753)	+ (WP_121678559)	+ (WP_121680618)
Fictibacillus aquaticus GDSW-R2A3	+ (WP_165766729)	+ (WP_094250486)	+ (WP_094252418)	–
Fictibacillus arsenicus Con a/3	+ (WP_171978929)	+ (WP_077364032)	+ (WP_077359391)	–
Filobacillus milosensis JCM 12288	+ (WP_194840813)	–	+ (WP_134341396)	–
Fredinandcohnia aciditolerans YN-1	+ (WP_165918267)	+ (WP_121448987)	+ (WP_121447452)	+ (WP_121448594)
Fredinandcohnia humi DSM 16318	+ (WP_057997424)	+ (WP_057997422)	+ (WP_057999353)	+ (WP_057997022)
Geobacillus subterraneus KCTC 3922	+ (WP_063167314)	+ (WP_063165462)	+ (WP_063164950)	+ (WP_063165717)
Geomicrobium halophilum DSM 21769	+ (WP_184402960)	+ (WP_184402964)	+ (WP_184404276)	–
Geomicrobium sediminis DSM 25540	+ (WP_204698244)	+ (WP_204698230)	+ (WP_204695942)	+ (WP_204699091)
Gottfriedia acidiceleris DSM 18954	+ (WP_167555238)	+ (WP_088010685)	+ (WP_088013581)	+ (WP_088011752)

Gottfriedia luciferensis DSM 18845	+ (WP_158095054)	+ (WP_088069207)	+ (WP_088066455)	+ (WP_088071848)
Gracilibacillus alcaliphilus DSM 102988	+ (WP_204667029)	+ (WP_204667030)	+ (WP_204667892)	–
Gracilibacillus halophilus YIM-C55.5	+ (WP_003466166)	+ (WP_003466161)	+ (WP_003467067)	–
Gracilibacillus orientalis CGMCC 1.4250	+ (WP_091483446)	+ (WP_091483448)	+ (WP_091484090)	–
Halalkalibacillus halophilus DSM 18494	+ (WP_027963377)	–	+ (WP_027964390)	–
Halalkalibacillus sediminis B3227	+ (WP_101330597)	–	+ (WP_202908623)	–
Halolactibacillus alkaliphilus NBRC 103919	–	–	–	–
Halolactibacillus miurensis NBRC 100873	–	–	–	–
Hydrogenibacillus schlegelii MA 48	+ (WP_066197744)	–	–	–
Lentibacillus amyloliquefaciens LAM0015	+ (WP_068442551)	+ (WP_068442544)	+ (WP_068448193)	–
Lentibacillus jeotgali Grbi	+ (WP_050901115)	+ (WP_010530820)	+ (WP_040912604)	–
Litchfieldia alkalitelluris DSM 16976	+ (WP_078546069)	+ (WP_078546073)	+ (WP_078544434)	–
Lottiidibacillus patelloidae SA5d-4	+ (WP_158217581)	+ (WP_094923799)	+ (WP_094926025)	–
Margalitia camelliae 7578-1	+ (WP_165786608)	+ (WP_101353280)	+ (WP_101354993)	+ (WP_101352689)
Margalitia shackletonii LMG 18435	+ (WP_055739435)	+ (WP_055739437)	+ (WP_055738970)	+ (WP_055740429)
**Bacillus atrophaeus* UCMB-5137	+ (AKL84947)	+ (AKL84945)	+ (AKL85663)	+ (AKL84767)
**Bacillus licheniformis* NCTC10341	+ (VEH78657)	+ (VEH78655)	+ (VEH80987)	+ (VEH78497)
Parageobacillus thermoglucosidasius DSM 2542	+ (ALF11316)	–	+ (ALF11869)	+ (ALF10934)
Virgibacillus dokdonensis DSM 16826	–	–	–	–
Bacillus megaterium NCTC10342	+ (SUV23016)	+ (SUV23018)	+ (WP_013059531)	+ (WP_016763969)
**Bacillus firmus* NCTC10335 *Cytobacillus firmus* ATCC 14575	+ (SUV07590)	+ (SUV07586)s	+ (SUV09482)	+ (SUV06113)
**Bacillus subtilis* NCIB 3610	+ (AQZ91176)	+ (AQZ91174)	+ (AQZ91934)	+ (AQZ90827)
Bacillus cereus NCTC2599	+ (SPT85215)	+ (SPT85210)	–	+ (SPT82560)

Bacillus subtilis NCTC3610	+ (SPU03415)	+ (SPU03424)	+ (SPU02257)	+ (SPU04151)
**Bacillus tequilensis* NCTC13306	+ SPU01610	+ SPU01608	+ SPU04834	+ SPT93464
Bacillus lentus NCTC4824	+ (SQI56540)	+ (SQI56542)	–	+ (SQI60668)
Geobacillus thermodenitrificans T12	+ (ARP43250)	+ (ARP43248)	–	+ (ARP43036)
Bacillus krulwichiae AM31D *Alkalihalobacillus krulwichiae* DSM 18225	+ (ARK30301)	+ (ARK30306)	+ (ARK31788)	–
Amphibacillus xylanus NBRC 15112	+ (BAM47371)	+ (BAM47372)	–	–
Bacillus beveridgei MLTeJB	+ (AOM82800)	+ (AOM82804)	–	–
Anoxybacillus amylolyticus DSM 15939	+ (ANB59615)	+ (ANB61337)	–	–
**Halobacillus halophilus* DSM 2266	+ (CCG45630)	+ (CCG45628)	+ (CCG46193)	+ (CCG45758)
**Bacillus amyloliquefaciens* DSM 7	+ (CBI43303)	+ (CBI43301)	+ (CBI43959)	+ (CBI43129)
Halobacillus karajensis DSM 14948	+ (SEH81404)	+ (SEH81451)	+ (SEI13868)	–
Parageobacillus caldoxylosilyticus NBRC 107762	+ (GAJ40213)	+ (GAJ40211)	–	+ (GAJ41568)
Bacillus methanolicus PB1	+ (EIJ78685)	+ (EIJ78682)	–	+ (EIJ78272)
Bacillus proteolyticus TD42	+ (OJE48705)	+ (OJE48707)	+ (OJE50047)	+ (OJE37724)
Bacillus pacificus EB422	+ (OJE27708)	+ (OJE27710)	+ (OJE22872)	+ (OJE28818)
Bacillus paranthracis Mn5	+ (OJE20915)	+ (OJE20913)	+ (OJE17563)	+ (OJE18183)
Bacillus paramycoides NH24A2	+ (OJD72780)	+ (OJD72778)	+ (OJD81274)	+ (OJD81591)
Bacillus nitratireducens 4049	+ (OJD52567)	+ (OJD52569)	+ (OJD54956)	+ (OJD39556)
Bacillus tropicus N24	+ (OJE40576)	+ (OJE40574)	+ (OJE42250)	+ (OJE40893)
Bacillus weihaiensis Alg07	+ (WP_083584315)	+ (WP_072579386)	+ (APH06955)	–
Bacillus gobiensis FJAT-4402	+ (ALC81422)	–	+ (ALC82083)	+ (ALC81302)
**Bacillus sporothermodurans* DSM 10599	+ (PTY86637)	+ (PTY86639)	+ (PTY83674)	+ (PTY83990)
Bacillus australimaris NH7I_1	+	+	+	+

	(KPN15420)	(KPN15418)	(KPN16035)	(KPN15279)
Bacillus campisalis SA2-6 *Mesobacillus campisalis* DSM 28801	+ (KKK37593)	+ (KKK37595)	+ (KKK38433)	+ (KKK37899)
Bacillus luti TD41	+ (OJE53240)	+ (OJE53238)	+ (OJE50888)	+ (OJE50184)
Bacillus clausii DSM 8716 *Alkalihalobacillus clausii* DSM 8716	+ (AST97893)	+ (AST97889)	+ (AST94481)	–
Bacillus cohnii DSM 6307	+ (AST92254)	+ (AST92252)	+ (AST92942)	+ (WP_066410632)
Bacillus nakamurai NRRL B-41091	+ (KXZ22006)	+ (KXZ22008)	+ (KXZ20247)	+ (KXZ18168)
Bacillus paralicheniformis KJ-16	+ (KRT87501)	+ (KRT87499)	+ (KRT88861)	+ (KRT87326)
Bacillus solani FJAT-18043 *Cytobacillus solani* DSM 29501	+ (KQL20275)	+ (KQL20273)	+ (KQL20905)	+ (KQL21916)
Bacillus murimartini LMG21005 *Alkalihalobacillus murimartini* LMG 21005	+ (KOO01297)	+ (KOO01745)	+ (KOO00559)	+ (KOO01615)
**Bacillus alcalophilus* ATCC 27647 *Alkalihalobacillus alcalophilus* ATCC 27647	+ (KGA99151)	+ (THG92032)	+ (KGA99066)	+ (KGA96998)
Bacillus foraminis CV53 *Mesobacillus foraminis* DSM 19613	+ (TCN23039)	+ (TCN23041)	+ (TCN24360)	+ (TCN27121)
Bacillus oleivorans JC228	+ (SNX68140)	+ (SNX68142)	+ (SNX71278)	+ (WP_097158550)
Bacillus swezeyi NRRL B-41294	+ (OMI32515)	+ (OMI32517)	+ (OMI30408)	+ (OMI25715)
Bacillus haynesii NRRL B-41327	+ (OMI26765)	+ (OMI26763)	+ (OMI27895)	+ (OMI26619)
Bacillus aryabhattai B8W22	+ (KJL04120)	+ (KJL04118)	+ (SDC57616)	+ (KJL06200)
Bacillus coagulans ATCC 7050	+ (AJH79551)	+ (AJH79279)	+ (SHE37035)	–
Bacillus gaemokensis KCTC 13318	+ (KYG36880)	+ (KYG36878)	+ (KYG39544)	+ (KYG28764)
Bacillus trypoxylicola KCTC 13244 *Alkalihalobacillus trypoxylicola* KCTC 13244	+ (KYG32855)	+ (KYG32858)	+ (KYG26644)	+ (KYG30397)
Bacillus vireti DSM 15602 *Neobacillus vireti* DSM 15602	+ (KLT17460)	+ (KLT17166)	+ (KLT15042)	+ (KLT17816)
Bacillus kochii BDGP4 *Cytobacillus kochii* DSM 23667	+ (ASV69191)	+ (ASV69193)	+ (ASV68599)	+ (ASV66456)
Bacillus vallismortis NBIF-001	+ (ARM28260)	+ (ARM28258)	+ (ARM29972)	+ (ARM28089)
Bacillus aquimaris SAMM	–	+ (OIU71236)	+ (OIU70923)	+ (OIU71457)
Bacillus xiamenensis VV3	+ (AOZ90235)	+ (AOZ90237)	+ (AOZ89627)	+ (AOZ90378)
Bacillus solimangrovi GH2-4	+ (WP_139125063)	+ (OEH93455)	+ (OEH92130)	+ (OEH93272)

Bacillus gibsonii FJAT-10019	+ (AOL30037)	+ (AOL32974)	+ (AOL30803)	+ (AOL29879)
Bacillus coahuilensis m2-6	+ (KUP07777)	+ (WP_010173107)	+ (KUP06332)	+ (KUP09831)
Bacillus enclensis SGD-1123	+ (KSU63474)	+ (KSU63472)	+ (KSU64062)	+ (KSU64394)
Bacillus plakortidis DSM 19153 *Alkalihalobacillus plakortidis* DSM 19153	+ (KQL51829)	+ (KQL51832)	+ (KQL56811)	+ (WP_055735413)
**Bacillus shackletonii* LMG 18435	+ (KQL53701)	+ (KQL53703)	+ (KQL53240)	+ (KQL54647)
Bacillus marisflavi JCM 11544	+ (KON91544)	+ (KON91546)	+ (KON91022)	–
Bacillus okhensis Kh10-101 *Alkalihalobacillus okhensis* DSM 23308	+ (KHF40345)	+ (WP_034628519)	+ (KHF39698)	–
Bacillus rhizosphaerae DSM 21911 *Alkalihalobacillus rhizosphaerae* DSM 21911	+ (SHL04854)	+ (SHL04943)	+ (SHL58683)	–
Bacillus persicus DSM 25386 *Mesobacillus persicus* DSM 25386	+ (SEM85123)	–	+ (SEM40370)	+ (SEM14480)
Bacillus caseinilyticus SP	+ (SDY86838)	+ (SDY87019)	+ (SDZ29761)	+ (SDY93193)
Bacillus lonarensis 25nlg *Alkalihalobacillus lonarensis* LMG 27974	+ (SDC12748)	+ (SDC12910)	+ (SDC26424)	–
Bacillus salsus IBRC-M10078	+ (SDP89357)	+ (SDP89344)	+ (SDP15314)	–
**Halobacillus faecis* NBRC 103569	+ (GEN53912)	+ (GEN53914)	+ (GEN54223)	+ (GEN52274)
**Bacillus subtilis* PY79	+ (AHA78150)	+ (AHA78148)	+ (AHA78968)	+ (AHA77980)
**Bacillus subtilis* JC1005	+ (WP_014480130)	+ (WP_003246013)	+ (WP_004399076)	+ (WP_032725974)
Exiguobacterium aurantiacum DSM 6208	–	–	–	–
Bacillus pasteurii NCTC 4822 *Sporosarcina pasteurii* NCTC4822	+ (SUJ02542)	+ (SUJ02561)	+ (WP_115360594)	–
Family *Caryophanaceae*				
Bhargavaea beijingensis CGMCC 1.6762	+ (SDE15668)	+ (SDE15613)	–	–
Bhargavaea cecembensis DSE10	+ (EMR07410)	+ (EMR07408)	–	–

Caryophanon latum DSM 14151	+ (WP_066465146)	+ (WP_083995406)	–	–
Caryophanon tenue DSM 14152	+ (WP_066548549)	–	–	–
Chryseomicrobium excrementi ET03	+ (WP_165775436)	–	–	–
Indiicoccus explosivorum S5-TSA-19	+ (WP_162287861)	–	–	–
Jeotgalibacillus campisalis SF-57	+ (KIL48065)	+ (KIL48063)	–	+ (KIL51040)
Jeotgalibacillus proteolyticus 22-7	+ (WP_167578572)	+ (WP_104056757)	+ (WP_104057225)	+ (WP_104057731)
Kurthia zopfii NCTC10597	+ (STX09306)	+ (STX09308)	–	–
Kurthia Trevisan NCTC10597				
Kurthia gibsonii NBRC 15534	+ (WP_170214692)	+ (WP_174795782)	–	–
Lysinibacillus sphaericus NCTC10338	+ (SUV16484)	+ (SUV16486)	–	–
Lysinibacillus varians NBRC 109424				
Lysinibacillus composti DSM 24785	+ (WP_124765010)	+ (WP_124765006)	–	–
Metalysinibacillus jejuensis N25	+ (WP_108306690)	+ (WP_108306688)	–	–
Metasolibacillus meyeri WS4626	+ (WP_107838558)	+ (WP_107838559)	–	–
Paenisporosarcina antarctica CGMCC	+ (WP_134209976)	+ (WP_134209974)	+ (WP_134211442)	–
Paenisporosarcina indica PN2	–	+ (WP_075617674)	+ (WP_075617894)	–
Planococcus massiliensis ES2	+ (CEG22597)	+ (CEG22599)	–	–
Planococcus halotolerans SCU63	–	+ (WP_112222694)	–	–
Planococcus citreus DSM 20549	+ (WP_121297581)	+ (WP_121297580)	–	–
Psychrobacillus soli NHI-2	+ (WP_142607235)	+ (WP_185907903)	–	–
Psychrobacillus glaciei PB01	+ (WP_151699639)	+ (WP_192797503)	–	+ (WP_151699828)
Rummeliibacillus pycnus DSM 15030	+ (WP_102692519)	+ (WP_211284590)	–	–
Rummeliibacillus suwonensis G20	+ (WP_186731377)	+ (WP_186731376)	–	–
Solibacillus isronensis B3W22	+ (EKB45986)	+ (EKB45984)	–	–
Solibacillus kalamii DSM 101595	+ (WP_087618203)	+ (WP_008404916)	–	–
Sporosarcina koreensis S-K12	+ (WP_040286733)	–	–	–
Sporosarcina pasteurii NCTC4822	+ (WP_115360666)	–	+ (WP_115360594)	–
Ureibacillus xyleni JC22	+ (WP_097074475)	+ (WP_097074473)	–	–

Family *Alicyclobacillaceae*				
Alicyclobacillus acidoterrestris ATCC 49025	+ (EPZ42661)	–	–	+ (AAT70691)
Alicyclobacillus acidiphilus NBRC 100859	+ (WP_067622754)	_	+ (WP_067623745)	+ (WP_067620273)
Alicyclobacillus acidocaldarius DSM 446	+ (WP_049763312)	–	+ (WP_012810357)	+ (WP_012811690)
Alicyclobacillus cellulosilyticus JCM 18487	+ (WP_188882722)	–	–	+ (WP_188880934)
Alicyclobacillus ferrooxydans TC-34	+ (WP_054968116)	–	+ (WP_054969563)	–
Alicyclobacillus herbarius DSM 13609	+ (WP_051344079)	–	+ (WP_051344014)	+ (WP_051344055)
Alicyclobacillus hesperidum DSM 12489	+ (WP_074693739)	–	+ (WP_052012358)	+ (WP_074691499)
Alicyclobacillus kakegawensis NBRC 103104	+ (WP_067933618)	–	+ (WP_067931853)	+ (WP_067933768)
Alicyclobacillus montanusUSBA-GBX-503	+ (WP_072875236)	–	+ (WP_072872913)	+ (WP_072873439)
Alicyclobacillus vulcanalis DSM 16176	+ (WP_076347604)	–	+ (WP_076346201)	+ (WP_076344177)
Alicyclobacillus sacchari DSM 17974	+ (WP_134158663)	–	+ (WP_208320843)	+ (WP_134158322)
Alicyclobacillus sendaiensis NBRC 100866	+ (WP_083515943)	–	+ (WP_062308885)	+ (WP_062306217)
Effusibacillus pohliae DSM 22757	+ (WP_083910637)	–	–	–
Kyrpidia spormannii EA-1	+ (WP_133121253)	–	–	–
Kyrpidia tusciae DSM 2912	+ (ADG06460)	–	–	–
Sulfobacillus acidophilus DSM 10332	+ (PSR21547)	–	–	–
Tumebacillus algifaecis THMBR28	+ (WP_157729480)	+ (WP_094238321)	–	–
Tumebacillus avium AR23208	+ (WP_1577220770)	–	–	
Tumebacillus permanentifrigoris DSM18773	+ (PWK06640)	+ (PWK06634)	+ (PWK06647)	–
Effusibacillus lacus DSM 27172	+ (TCS75281)	+ (TCS75288)	+ (TCS76843)	–
Sulfobacillus thermosulfidooxidans DSM 9293	+ (SMC05305)	–	–	+ (SMC02749)
Family *Thermoactinomycetaceae*				
Novibacillus thermophilus SG-1	+ (AQS56666)	+ (AQS57575)	+ (AQS55600)	–
Thermoactinomyces peptonophilus DSM 44666	+ (SHE43380)	+ (SHE43258)	–	+ (WP_073158315)
Seinonella peptonophila DSM 44666				
Desmospora active DSM 45169	+ (PTM58678)	+ (PTM58680)	–	+ (WP_107725084)
Moorella humiferrea DSM 23265	+ (WP_106005679)	+ (PRR73168)	+ (PRR73169)	–

Baia soyae DSM 46831	+ (WP_131847935)	–	–	–
Hazenella coriacea DSM 45707	+ (WP_131925697)	+ (WP_131925689)	–	+ (WP_131926838)
Kroppenstedtia eburnea DSM 45196	+ (WP_076522871)	–	–	–
Laceyella sediminis RHA1	+ (WP_022738460)	–	–	–
Lihuaxuella thermophila DSM 46701	+ (WP_089968429)	–	–	+ (WP_089973141)
Marininema halotolerans DSM 45789	+ (WP_091838582)	+ (WP_091838588)	–	+ (WP_176392116)
Melghirimyces algeriensis DSM 45474	+ (WP_185955992)	–	–	+ (WP_142503996)
Paludifilum halophilum DSM 102817	+ (WP_094263076)	–	–	–
Planifilum fulgidum DSM 44945	+ (WP_092035941)	–	–	–
Polycladomyces abyssicola JIR-001	+ (WP_212774950)	–	–	+ (WP_212775185)
Risungbinella massiliensis GD1	+ (WP_044640246)	–	–	–
Seinonella peptonophila DSM 44666	+ (WP_073151287)	–	–	+ (WP_073158315)
Shimazuella kribbensis DSM 45090	+ (WP_028775771)	–	–	–
Staphylospora marina SCSIO 07575	+ (WP_124727324)	–	–	+ (WP_124726867)
Thermoactinomyces daqus H-18	+ (WP_052154284)	–	–	–
Thermoactinomyces vulgaris DSM 43016	+ (WP_049719994)	–	–	+ (WP_121873932)
Family *Enterococcaceae*				
Enterococcus avium NCTC9938	+ (STP59974)	+ (STQ02738)	–	–
Enterococcus caccae ATCC 2215-02	+ (EOL45025)	+ (EOT60638)	–	–
Enterococcus columbae DSM 7374	+ (EOW84009)	–	–	–
*Pilibacter termitis*ATCC BAA-1030	+ (SJZ76563)	–	–	–
Vagococcus fluvialis bH819	+ (SLM86271)	+ (SLM84987)	–	–
Bavariicoccus seileri WCC 4188	–	–	–	–
Catellicoccus marimammalium M35/04/3	–	–	–	–
Melissococcus plutonius ATCC 35311	–	–	–	–
Tetragenococcus halophilus LMG 26042	–	–	–	–
Family *Streptococcaceae*				
Lactococcus fujiensis JCM 16395	+ (PCS00999)	+ (PCS00998)	–	–
Lactococcus chungangensis CAU 28	+ (PCS04213)	+ (PCS04212)	–	–

Lactococcus garvieae DSM 20684	–	+ (PCS02224)	–	–
Lactococcus lactis JCM 5805	+ (AIS03641)	+ (AIS03640)	–	–
Floricoccus penangensis HibF3	–	–	–	–
Floricoccus tropicus DF1	–	–	–	–
Lactovum miscens DSM 14925	–	–	–	–
Streptococcus acidominimus NCTC12957	–	–	–	–
Streptococcus entericus DSM 14446	–	–	–	–
Family *Listeriaceae*				
Listeria ivanovii subsp. Londoniensis NCTC12701	+ (VEH47138)	(VEH48260)	–	–
Listeria booriae FSL A5-0281	+ (KGL38003)	–	–	–
Listeria welshimeri NCTC11857	+ (SNV26688)	+ (SNV29940)	–	–
Listeria grayi NCTC 10815	+ (STY45645)	+ (STY44577)	–	–
Brochothrix campestris FSL F6-1037	+ (WP_035313457)	–	–	–
Brochothrix thermosphacta DSM 20171	+ (WP_029091197)	–	–	–
Family *Paenibacillaceae*				
**Paenibacillus macerans* NCTC6355	+ (SUA82584)	+ (SUA82589)	+ (SUA84524)	+ (SUA84562)
**Aneurinibacillus soli* CB4	+ (BAU28901)	+ (BAU28896)	+ (BAU28706)	+ (WP_096466269)
Paenibacillus thiaminolyticus NRRL B-4156	+ (QDM42996)	+ (QDM42991)	–	+ (QDM47467)
Paenibacillus polymyxa NCTC10343	+ (SUA70537)	+ (SUA70532)	+ (SUA68893)	–
Brevibacillus brevis NCTC2611	+ (VEF89691)	+ (VEF89686)	+ (WP_047073536)	+ (VEF88461)
**Brevibacillus laterosporus* DSM 25	+ (ATO50397)	+ (ATO50406)	+ (ATO49995)	+ (ATO51516)
Ammoniphilus oxalaticus RAOx-1	+ (WP_120188371)	+ (WP_120188375)	–	–
Ammoniphilus resinae DSM 24738	+ (WP_209811470)	+ (WP_209811467)	–	–
Aneurinibacillus thermoaerophilus L 420-91	+ (WP_057899306)	+ (WP_057899301)	+ (WP_091260622)	–
Brevibacillus fluminis JCM 15716	+ (WP_122920712)	–	+ (WP_122916057)	+ (WP_122918360)
Chengkuizengella marina YPA3-1-1	+ (WP_160647273)	+ (WP_160647278)	–	+ (WP_201763461)

Chengkuizengella sediminis	+ (WP_162035726)	+ (WP_162036753)	–	–
Cohnella algarum Pch-40	+ (WP_205432118)	–	+ (WP_080837228)	–
Cohnella endophytica M2MS4P-1	+ (WP_120974674)	+ (WP_120974670)	–	+ (WP_120975436)
Aneurinibacillus aneurinilyticus ATCC 12856	+ (ERI08368)	+ (ERI08363)	–	–
Fontibacillus phaseoli CECT 8333	+ (WP_114495155)	–	+ (WP_114496272)	–
Fontibacillus solani CECT 8693	+ (WP_182535240)	–	+ (WP_182536373)	–
Gorillibacterium massiliense G5	+ (WP_040951192)	–	+ (WP_052339709)	+ (WP_040949445)
Longirhabdus pacifica SCSIO 06110	+ (WP_128894446)	–	+ (WP_128894750)	+ (WP_128894047)
Paenibacillus amylolyticus FSL J3-0122	+ (OMF46010)	–	+ (OMF48277)	+ (OMF42105)
Saccharibacillus deserti KCTC 33693	+ (WP_172250428)	–	+ (WP_172253023)	–
Saccharibacillus endophyticus CCM 8702	+ (WP_172239951)	–	+ (WP_172243358)	–
Thermobacillus composti KWC4	+ (WP_015254739)	–	+ (WP_041854264)	–
Thermobacillus xylanilyticus XE	+ (WP_213484740)	–	+ (WP_213484799)	–
Xylanibacillus composti K13	+ (WP_213410146)	+ (WP_213410150)	+ (WP_213410847)	–
Family *Pasteuriaceae*				
Pasteuria penetrans RES148	+ (WP_149454122)	–	–	–
Family *Sporolactobacillaceae*				
Caenibacillus caldisaponilyticus B157	+ (WP_077616581)	+ (WP_077616583)	+ (WP_077614917)	
Pullulanibacillus camelliae CGMCC 1	+ (WP_188692114)	–	–	+ (WP_188688449)
Pullulanibacillus pueri DSM 100927	+ (WP_188496614)	–	–	+ (WP_188494793)
Scopulibacillus daqui DSM 28236	+ (WP_205003224)	+ (WP_205003220)	+ (WP_205004390)	+ (WP_205002112)
Scopulibacillus darangshiensis DSM 19377	+ (WP_132743248)	+ (WP_132743244)	+ (WP_207902953)	+ (WP_132746467)
Sporolactobacillus inulinus NBRC 13595	+ (WP_010023592)	–	+ (WP_010024995)	–
Sporolactobacillus terrae DSM 11697	+ (WP_028976314)	–	+ (WP_028976383)	–
Tuberibacillus calidus DSM 17572	+ (WP_027723764)	–	+ (WP_027725372)	–

Family *Staphylococcaceae*				
Abyssicoccus albus DSM 29158	–	–	–	–
Aliicoccus persicus IBRC-M10081	–	–	–	–
Jeotgalicoccus aerolatus DSM 22420	–	–	–	–
Jeotgalicoccus marinus DSM 19772	–	–	–	–
Macrococcus epidermidis 01/688	–	–	–	–
Mammaliicoccus lentus NCTC 12102	–	–	–	–
Nosocomiicoccus ampullae DSM 19163	–	–	–	–
Salinicoccus albus DSM 19776	–	–	–	–
Staphylococcus cohnii NCTC 11041	–	–	–	–
Family *Lactobacillaceae*				
Lactobacillus acidipiscis NBRC 102163	–	–	–	–
Ligilactobacillus acidipiscis NBRC 102163				
Acetilactobacillus jinshanensis HSLZ-75	–	–	–	–
Agrilactobacillus composti DSM 18527	–	–	–	–
Amylolactobacillus amylophilus DSM 20533	–	–	–	–
Apilactobacillus ozensis DSM 23829	–	–	–	–
Bombilactobacillus mellis Hon2	–	–	–	–
Companilactobacillus halodurans TMW 1	–	–	–	–
Dellaglioa algidus DSM 15638	–	–	–	–
Fructilactobacillus florum DSM 22689	–	–	–	–
Furfurilactobacillus rossiae DSM 15814	–	–	–	–
Holzapfelia floricola DSM 23037	–	–	–	–
Lacticaseibacillus casei NBRC 101979	–	–	–	–
Lactiplantibacillus garii FI11369	–	–	–	–
Lactobacillus acidophilus DSM 20079	–	–	–	–
Lapidilactobacillus achengensis 247-4	–	–	–	–
Lactobacillus curvatus JCM 1096	–	–	–	–
Lentilactobacillus hilgardii LMG 07934	–	–	–	–
Levilactobacillus brevis NCTC13768	–	–	–	–
Lactobacillus pontis DSM 8475	–	–	–	–

Lactobacillus aquaticus DSM 21051	–	–	–	–
Loigolactobacillus backii JCM 18665	–	–	–	–
Paucilactobacillus oligofermentans DSM 15707	–	–	–	–
Pediococcus ethanolidurans DSM 22301	–	–	–	–
Schleiferilactobacillus, harbinensis DSM 16991	–	–	–	–
Secundilactobacillus oryzae JCM 18671	–	–	–	–
Family *Aerococcaceae*				
Aerococcus christensenii CCUG28831	–	–	–	–
Abiotrophia defectiva ATCC 49176	–	–	–	–
Aerococcus urinae ATCC 51268	–	–	–	–
Dolosicoccus paucivorans DSM 15742	–	–	–	–
Eremococcus coleocola DSM 15696	–	–	–	–
Fundicoccus ignavus DSM 109652	–	–	–	–
Globicatella sanguinis NBRC 15551	–	–	–	–
Suicoccus acidiformans ZY16052	–	–	–	–
Family *Carnobacteriaceae*				
Alkalibacterium gilvum DSM 25751	–	–	–	–
Allofustis seminis DSM 15817	–	–	–	–
Alloiococcus otitis ATCC 51267	–	–	–	–
Atopobacter phocae ATCC BAA-285	–	–	–	–
Atopococcus tabaci DSM 17538	–	–	–	–
Atopostipes suicloacalis DSM 15692	–	–	–	–
Carnobacterium jeotgali MS3	–	–	–	–
Desemzia incerta DSM 20581	–	–	–	–
Dolosigranulum pigrum KPL1914	–	–	–	–
Granulicatella elegans ATCC 700633	–	–	–	–
Isobaculum melis DSM 13760	–	–	–	–
Jeotgalibaca dankookensis KCCM 90229	–	–	–	–
Lacticigenium napthae DSM 19658	–	–	–	–
Lacticigenium napthae DSM 19658	–	–	–	–
Marinilactibacillus piezotolerans DSM 16108	–	–	–	–
Pisciglobus halotolerans DSM 27630	–	–	–	–
Trichococcus alkaliphilus B5	–	–	–	–

Family *Leuconostocaceae*				
Convivina intestine DSM 28795	_	_	_	_
Fructilactobacillus fructivorans KCTC 3543	_	_	_	_
Leuconostoc carnosum DSM 5576	_	_	_	_
Oenococcus kitaharae DSM 17330	_	_	_	_
Weissella bombi DSM 28794	_	_	_	_
Weissella oryzae SG25	_	_	_	_

Table 10. Occurrence of putative proteins of sporulene biosynthesis pathway, amongst class *Bacilli* members

Members of different families of class Bacilli screened for the presence of putative genes encoding proteins of sporulene biosynthesis pathway. Accession numbers of protein sequences used for screening and identification sporulene synthesis genes are enlisted along with the name of organisms. Where, HepS, Heptaprenyl diphosphate synthase component I; HepT, Heptaprenyl diphosphate synthase component II; YtpB, Tetraprenyl beta curcumene synthase; and SqhC, Squalene hopene cyclase/Sporulenol synthase respectively. +, present; _, absent.

From the annotated phylogenetic tree (Fig. 21), it was observed that *hepS*, *hepT*, *ytpB* and *sqhC* were present in majority of the screened members of the family *Bacillaceae* and uniform distribution was observed amongst all the screened members of the genus *Bacillus*. Whereas, families of the order *Caryophanaceae* such as *Alicyclobacillaceae*, *Thermoactinomycetaceae*, *Listeriaceae* and *Pasteuriaceae* along with the order *Lactobacillales* i.e members of the family *Enterococcaceae* and the family *Streptococcaceae* represented an uneven distribution of the pathway genes (Fig. 21). In dept analysis showed that, contrary to the irregular distribution of *ytpB* and *sqhC* genes all the members of the order *Caryophanales* invariably harboured *hepS* and *hepT* genes. However, the members of family *Aerococcaceae*, *Carnobacteriaceae, Lactobacillaceae*, *Leuconostocaceae* and *Staphylococcaceae* showed complete absence of the pathway genes. The sporulene biosynthesis pathway genes were not identified in the members of the order *Lactobacillales* with exception of few members of the family *Streptococcaceae* and *Enterococcaceae* which showed the presence of only *hepT* and *hepS* gene in their genomes.

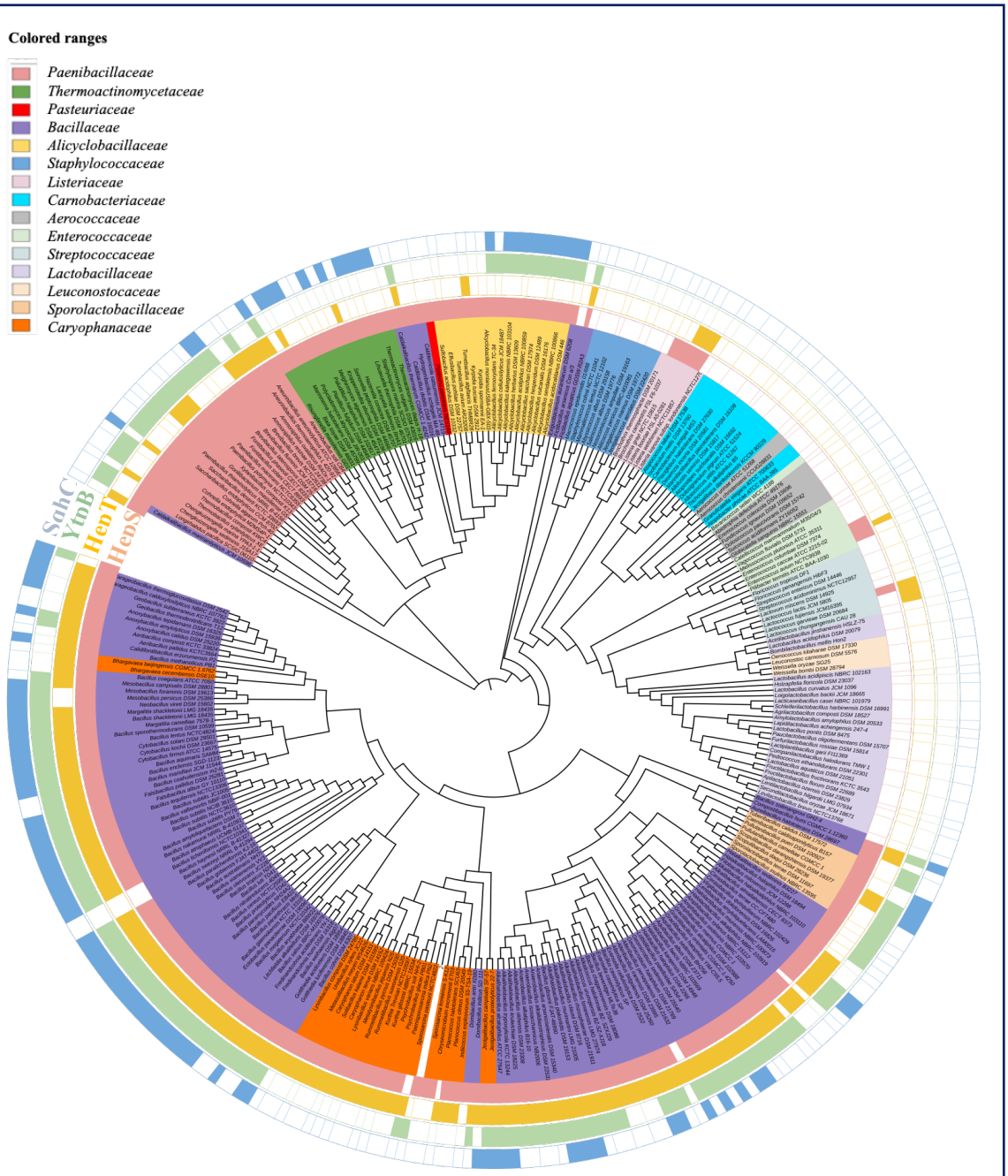


Fig. 21: Occurrence and distribution of genes encoding proteins involved in sporulene biosynthesis pathway among the screened members of different families of class *Bacilli*

The neighbour-joining tree was drawn using MEGA 7 software and annotated using iTOL. HepS, Hepatprenyl diphosphate synthase component I; HepT, Hepatprenyl diphosphate synthase component II; YtpB, Tetraprenyl-beta-curcumene synthase; SqhC, Sporulenol synthase/ Squalene hopene cyclase. Different colors of the innermost circle represent different families of class Bacilli.

3.3.3 Synteny of sporulene biosynthesis pathway genes

Gene arrangement study of sporulene biosynthesis pathway genes amongst the genus *Bacillus* members through SynTax revealed that, the gene encoding for heptaprenyl diphosphate synthase component I (*hepS*) and II (*hepT*) were adjacently positioned along with *menG*/*H* gene (Fig. 22, A, B). Gene encoding for Tetraprenyl-β-curcumene synthase (*ytqB*) was preceded by *ytqA* and proceeded by *ytpB* gene (Fig. 22, C). Whereas, the gene encoding the for key enzyme squalene hopene cyclase (*sqhC*) was situated between *sodF* and *dnaS* gene (Fig. 22, D). The observed syntenies indicated constant and homogeneous arrangement of sporulene biosynthesis genes amongst the genus *Bacillus* members, whereas a distinct arrangement of genes was observed for the family *Bacillaceae* member with absence of few genes (data not shown).

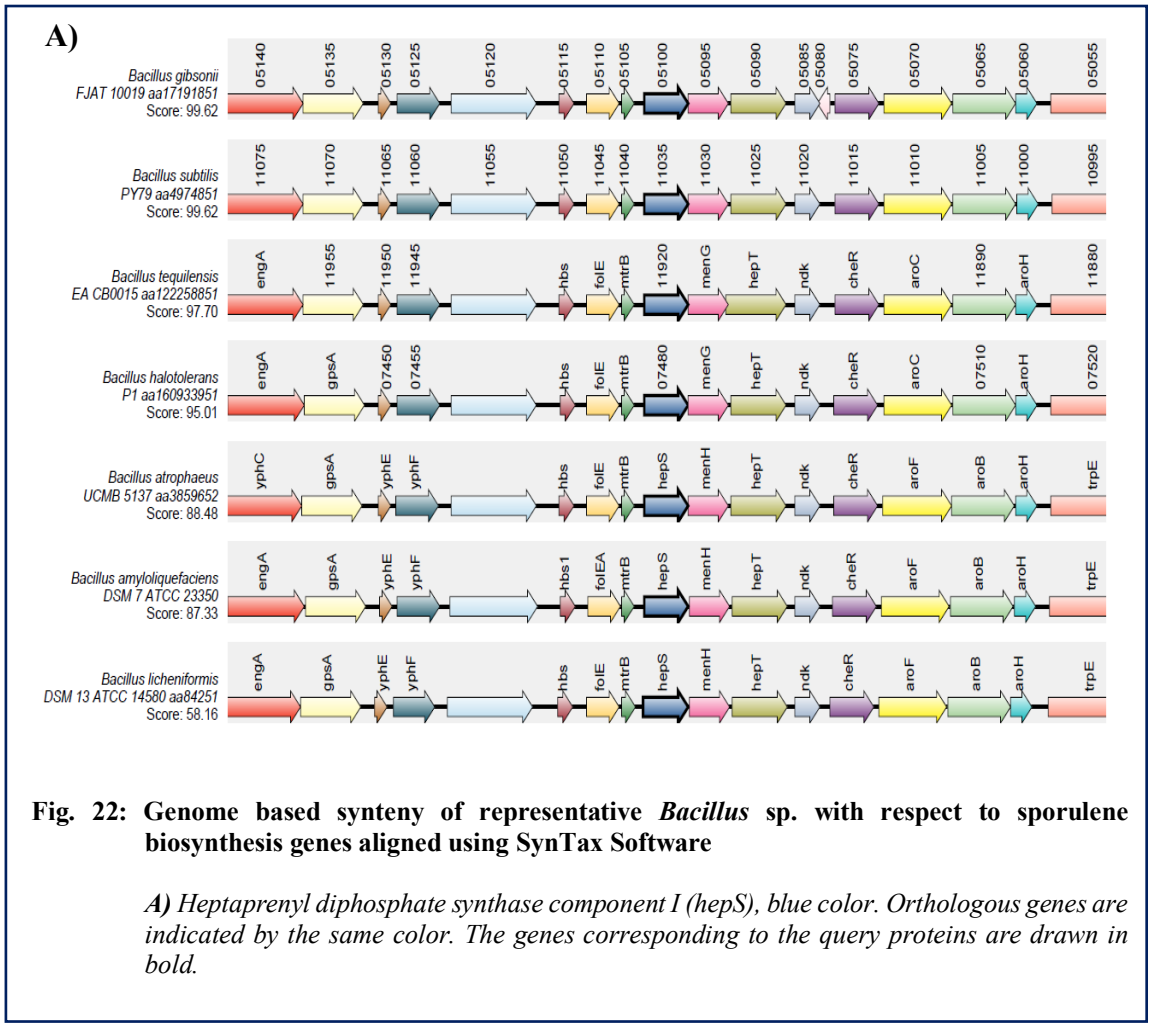


Fig. 22: Genome based synteny of representative *Bacillus* sp. with respect to sporulene biosynthesis genes aligned using SynTax Software

A) *Heptaprenyl diphosphate synthase component I (hepS), blue color. Orthologous genes are indicated by the same color. The genes corresponding to the query proteins are drawn in bold.*

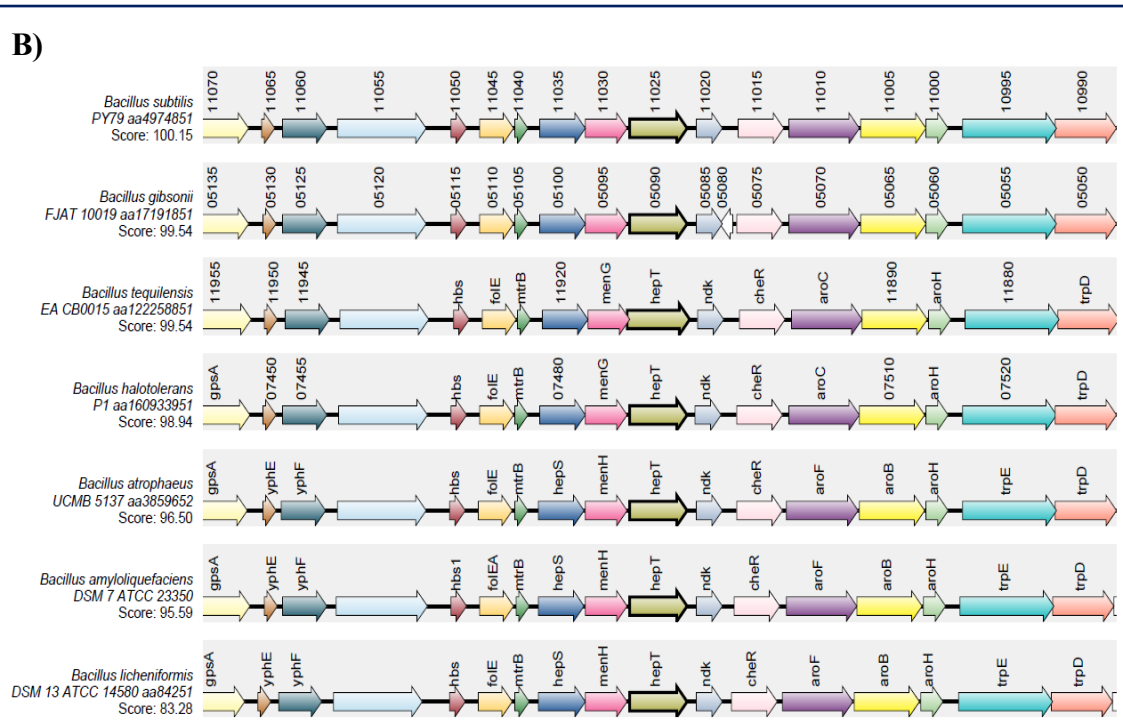


Fig. 22: Genome based synteny of representative *Bacillus* sp. with respect to sporulene biosynthesis genes aligned using SynTax Software

B) *Heptaprenyl diphosphate synthase component II (hepT), olive color. Orthologous genes are indicated by the same color. The genes corresponding to the query proteins are drawn in bold.*

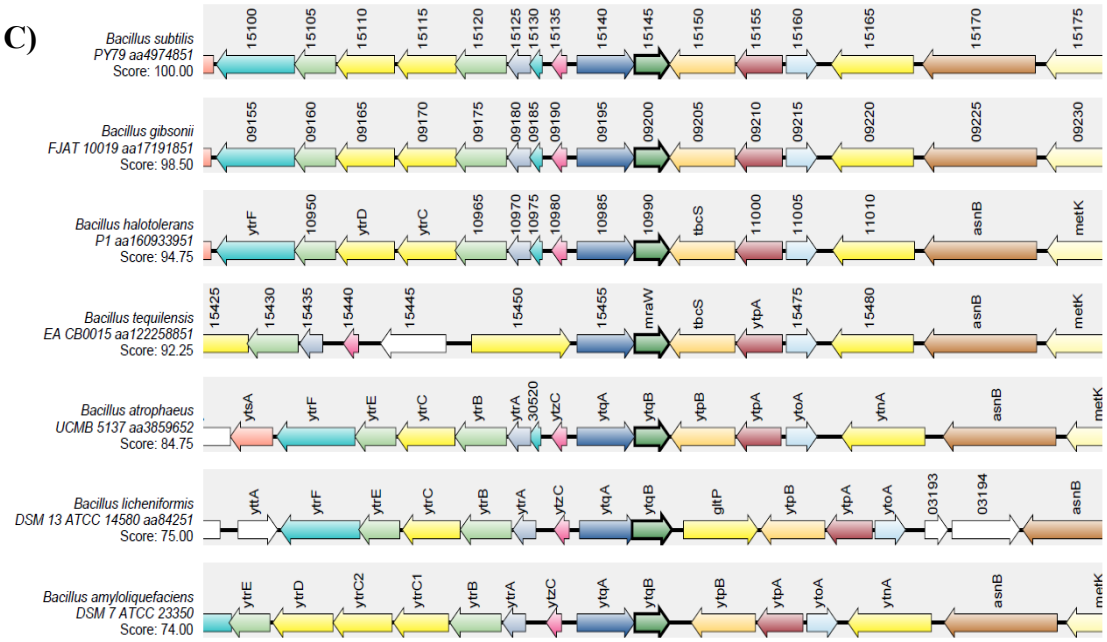


Fig. 22: Genome based synteny of representative *Bacillus* sp. with respect to sporulene biosynthesis genes aligned using SynTax Software

C) *Tetraprenyl-Beta- curcumene synthase (ytpB), green color. Orthologous genes are indicated by the same color. The genes corresponding to the query proteins are drawn in bold.*

D)

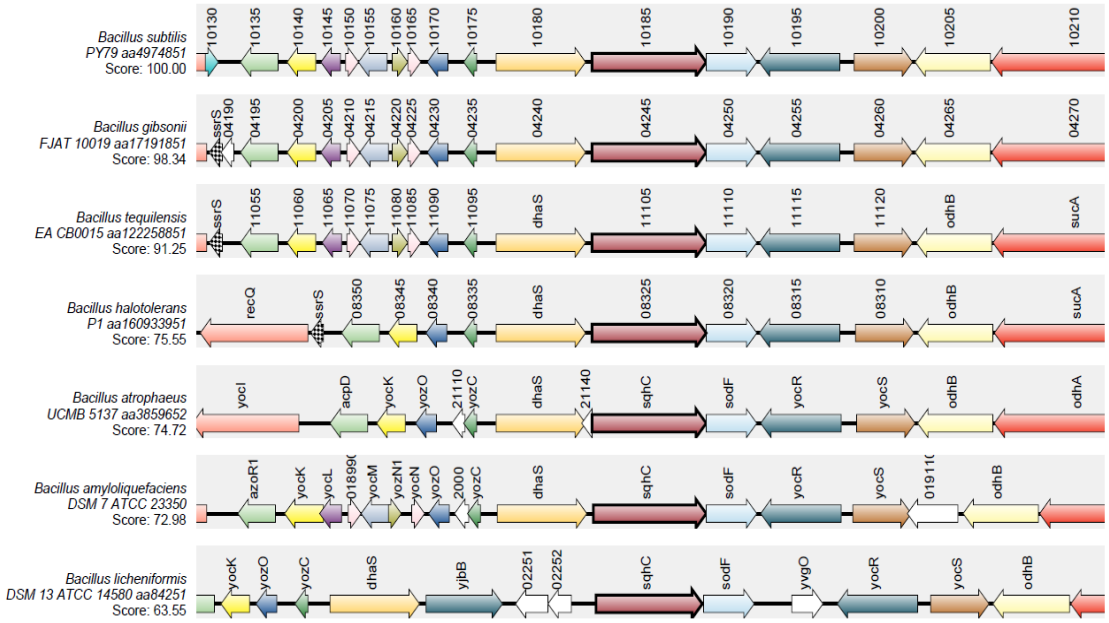


Fig. 22: Genome based synteny of representative *Bacillus* sp. with respect to sporulene biosynthesis genes aligned using SynTax Software

D) *Sporulenol synthase/ Squalene hopene cyclase (sqhC), pink color. Orthologous genes are indicated by the same color. The genes corresponding to the query proteins are drawn in bold.*

3.3.4 Amino acid identity of putative sporulene biosynthesis proteins

In order to study the homologies of putative proteins of sporulene biosynthesis pathway amongst the genus *Bacillus* and the family *Bacillaceae* members, multiple sequence alignment was performed. Putative tetraprenyl-β-curcumene synthase (YtpB) protein of the family *Bacillaceae* members showed many conserved amino acid residues. However, a completely conserved domain of 16 amino acids (positioned from 81 to 96) was observed for the genus *Bacillus* members (Fig. R23, A).

A)

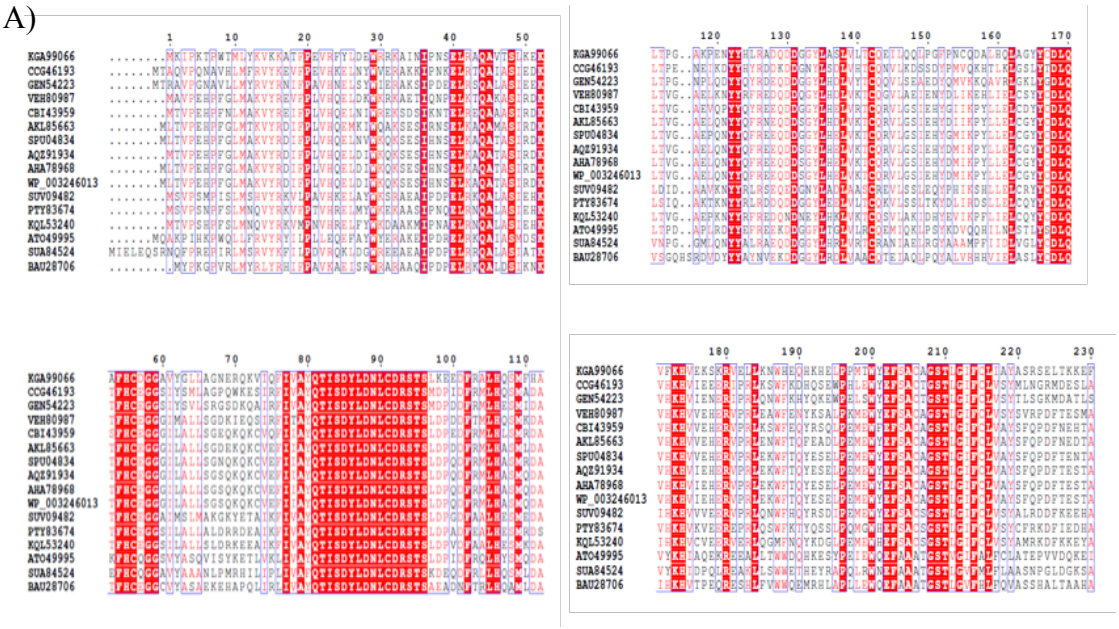


Fig. 23: A) Multiple sequence alignment of putative tetraprenyl-beta-curcumene synthase proteins (YtpB) with its homolog's from different *Bacillus* members

Amino acid residues of YtpB protein which are highly conserved amongst the compared Bacillus members are highlighted with red background color

B)

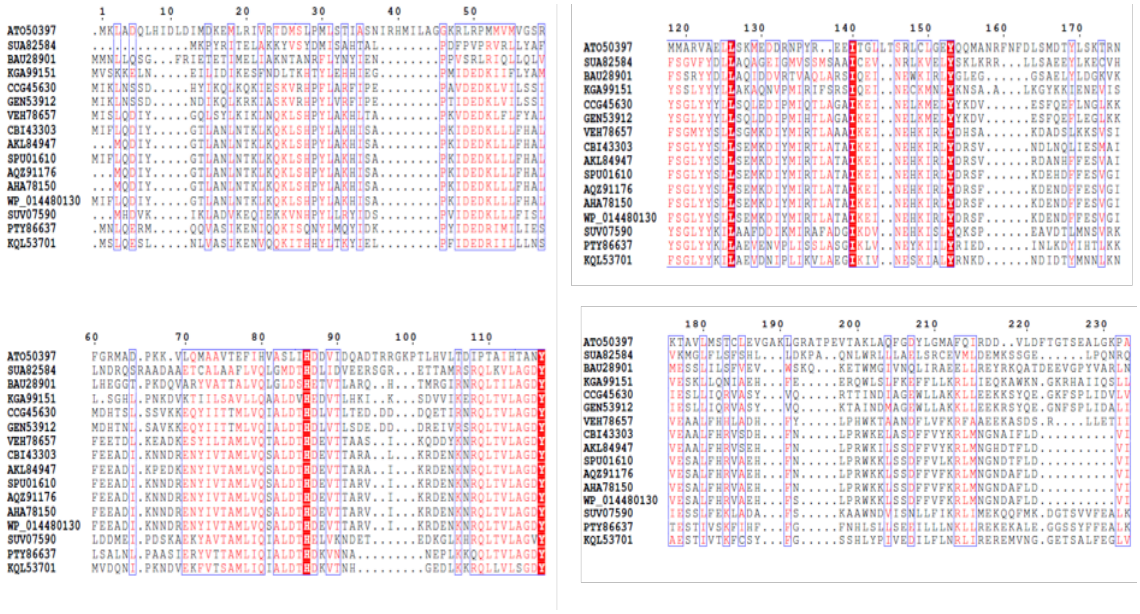
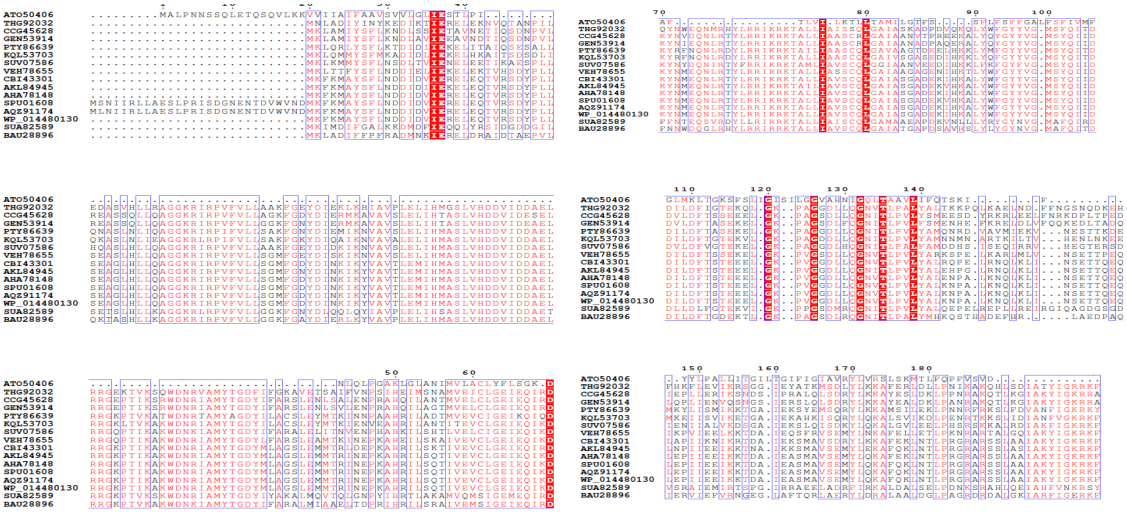


Fig. 23: B) Multiple sequence alignment of putative heptaprenyl diphosphate synthase component I (HepS) with its homolog's from different *Bacillus* members

Amino acid residues of HepS protein which are highly conserved amongst the compared Bacillus members are highlighted with red background color.

C)



D)

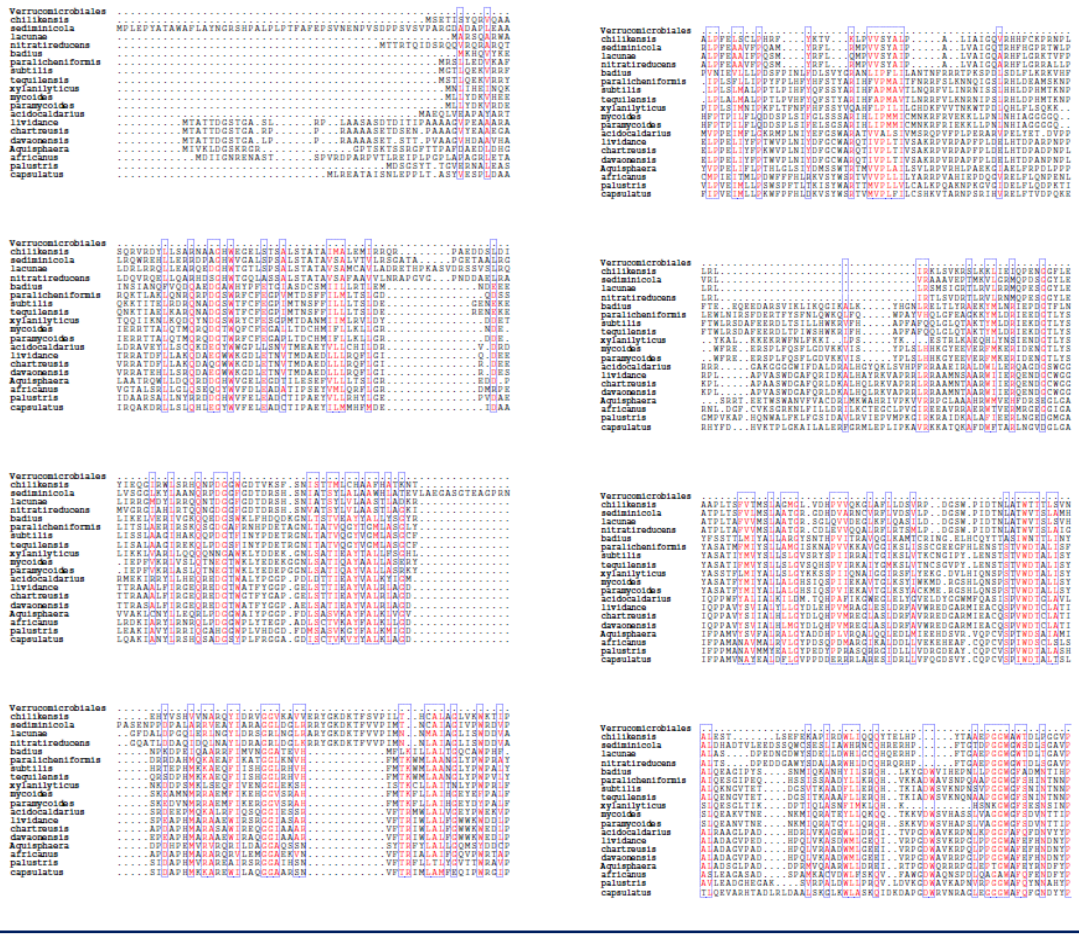


Fig. 23: C) Multiple sequence alignment of putative heptaprenyl diphosphate synthase component II (HepT) with its homolog's from different *Bacillus* members

Amino acid residues of HepT protein which are highly conserved amongst the compared Bacillus members are highlighted with red background color.

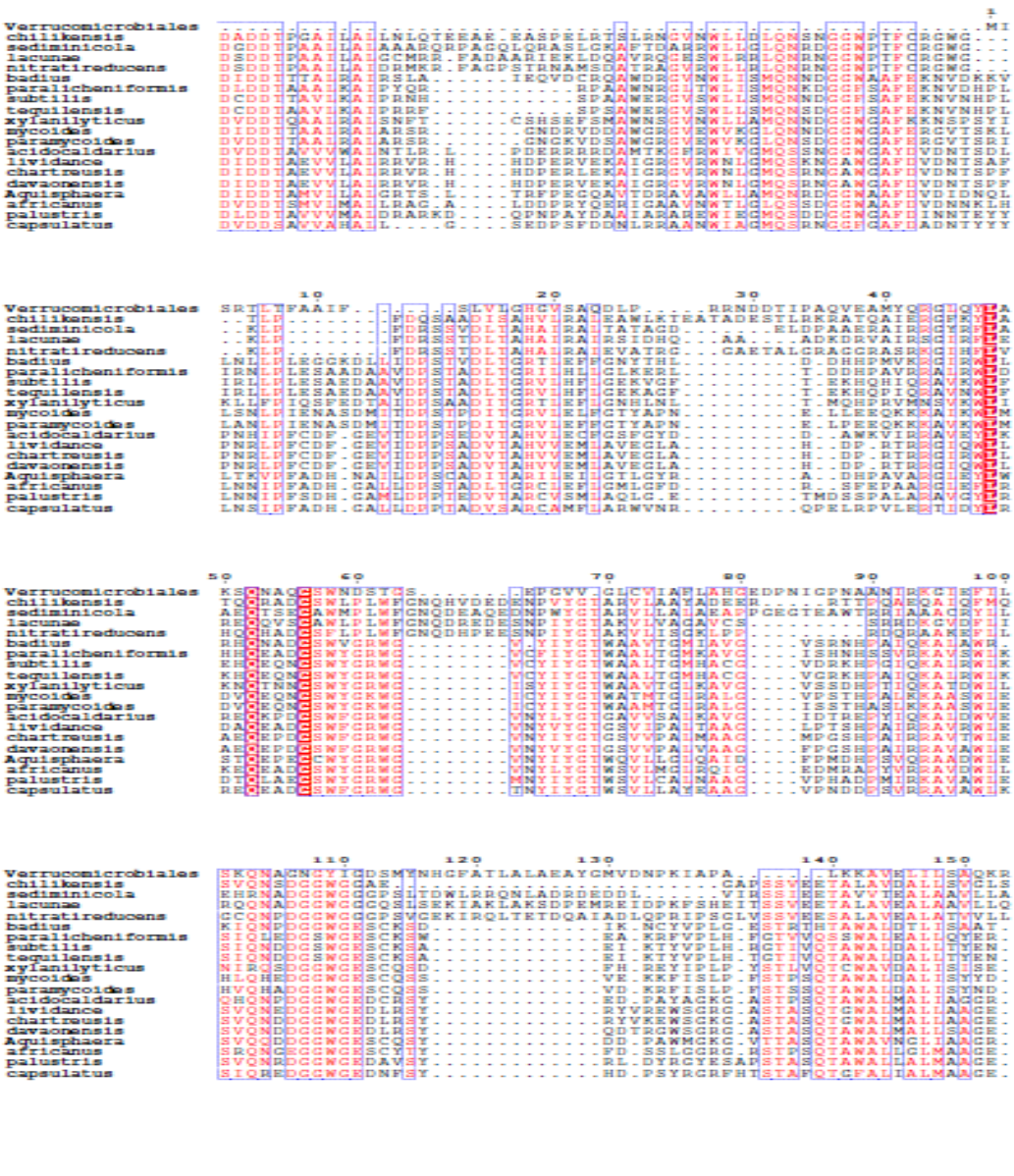


Fig. 23: D) Multiple sequence alignment of putative sporulenol synthase (SqhC) with its homolog's from different *Bacillus* members

Amino acid residues of SqhC protein which are highly conserved amongst the compared Bacillus members are highlighted with red background color.

Despite having dynamic amino acid compositions, putative HepS, HepT, and SqhC proteins shared few conserved amino acid residues in the members of the family *Bacillaceae* and the members of the genus *Bacillus* (Fig. 23 B, C, D) with almost 50% amino acid sequence homology.

3.3.5 Interaction network and co-occurrence study of sporulene biosynthesis genes

Gene interaction and co-occurrence studies for the genes involved in sporulene biosynthesis pathway were performed using SMART software. When observed at phylogenetic level, the genes of sporulene biosynthesis pathway showed high frequency of co-occurrence amongst the family *Bacillaceae* members with maximum sequence conservation (Fig. 24).

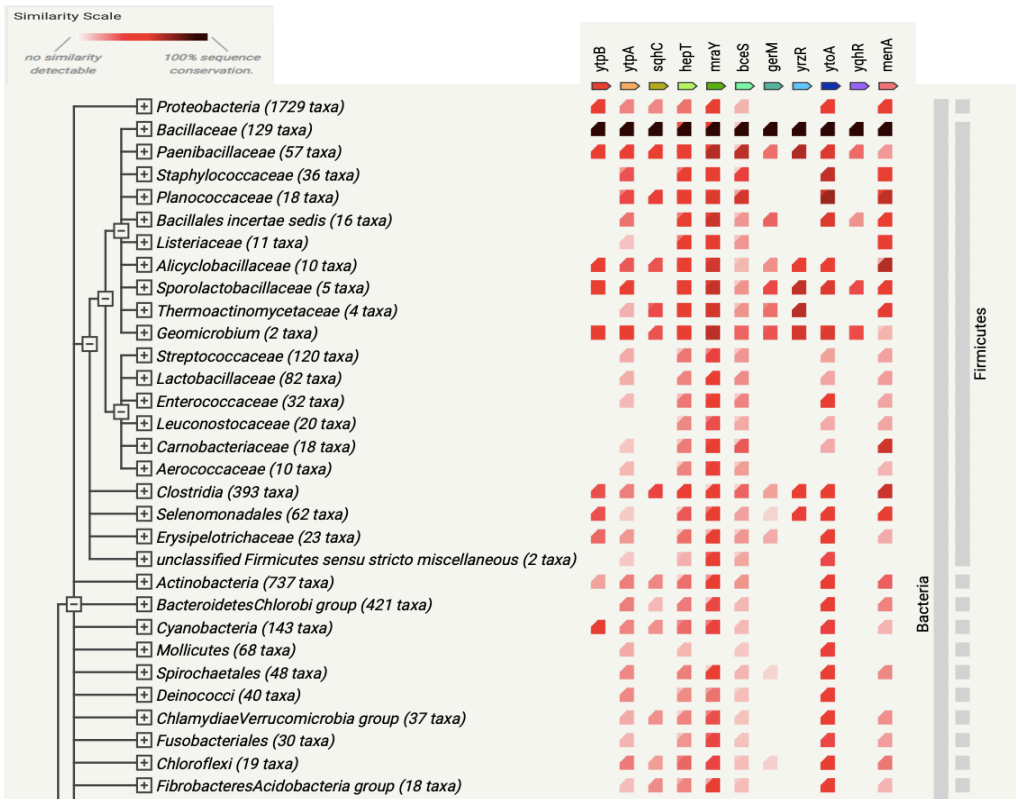


Fig. 24: Co-occurrence of sporulene biosynthesis pathway genes at phylogenetic level amongst family *Bacillaceae* members

The phylogenetic tree for co-occurrence was constructed through SMART, a (Simple Modular Architecture Research Tool). The co-occurrence study at phylogenetic level showed that all the genes of sporulene biosynthesis pathway co-occur with maximum sequence conservation amongst the family Bacillaceae members. The genes ytpB, hepT and sqhC were found conserved within the family Bacillaceae.

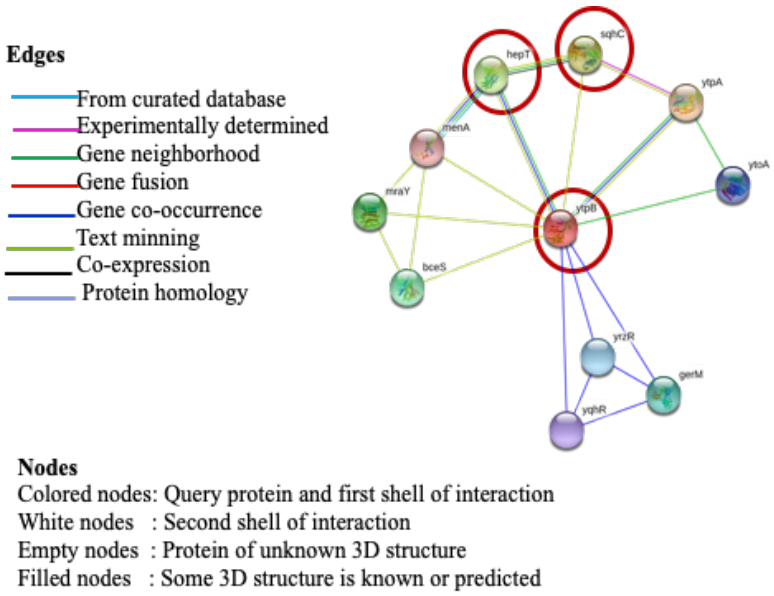


Fig. 25: String network constructed based on putative proteins of sporulene biosynthesis pathway

The putative proteins of sporulene biosynthesis are highlighted with red circle. Each node represents the proteins produced by a single, protein encoding gene locus. Edges represents protein-protein association. The association projects towards the joint contribution of a shared function of the genes responsible for these proteins; this does not necessarily mean they are physically binding each other.

However, studies based on the string network constructed with help of amino acid sequences of putative proteins of sporulene biosynthesis pathway (Fig. 25), briefed about their possible functional interactions. Detailed study of the interaction network deduced that, *hepT* and *sqhC* genes co-express and share gene neighborhood whereas *hepT* and *ytpB* genes also share neighborhood and co-occur, as observed in their synteny analysis (Fig. 22). Additionally, all these genes were found interacting through the text mining and literature studies.

3.4 Insights into peroxide toxicity to *Bacillus* spores and characterization of protective activity of sporulenes in *B. subtilis*

3.4.1 Bacterial strains used under the study

B. subtilis strains used in this study were received as gratis from Dr. Tanja Bosak, Massachusetts Institute of Technology, Cambridge, USA and Dr. Tushar Lodha, National Centre for Microbial Resource, Pune, India. The two strain use were *B. subtilis* PY79, a wild type strain (WT) and *B. subtilis* TB10 a deletion mutant (*ΔsqhC*), where a long flanking homology segment carrying Tet resistance cassette was inserted to inactivate *sqhC-sodF* operon (Bosak et al. 2008).

3.4.2 Cellular responses of WT and *ΔsqhC* mutant *B. subtilis* to different stress conditions

3.4.2.1 Growth studies of WT and *ΔsqhC* mutant *B. subtilis*

To study the physiological response of sporulene mutant to different stressors, growth studies were conducted for WT and *ΔsqhC* mutant of *B. subtilis* under different stress conditions as described in material and methods. Under control condition both strains attended similar growth in nutrient broth as well as in sporulation medium with doubling time of ~25 min (Fig. 26, A, B). Also, no significant difference was observed in the growth patterns of WT and *ΔsqhC* mutant with ethanol and butanol treatment (Fig 27, A, B). However under H_2O_2 stress, the *ΔsqhC* mutant showed differential behaviour when compared to WT. More prominent changes were observed in the growth pattern of WT and *ΔsqhC* mutant in sporulation medium as compared to the growth in nutrient broth medium (Fig. 28, A, B). The growth of *ΔsqhC* mutant was observed to be stifled by H_2O_2 treatment, from late log phase to stationary phase (Fig. 28, B).

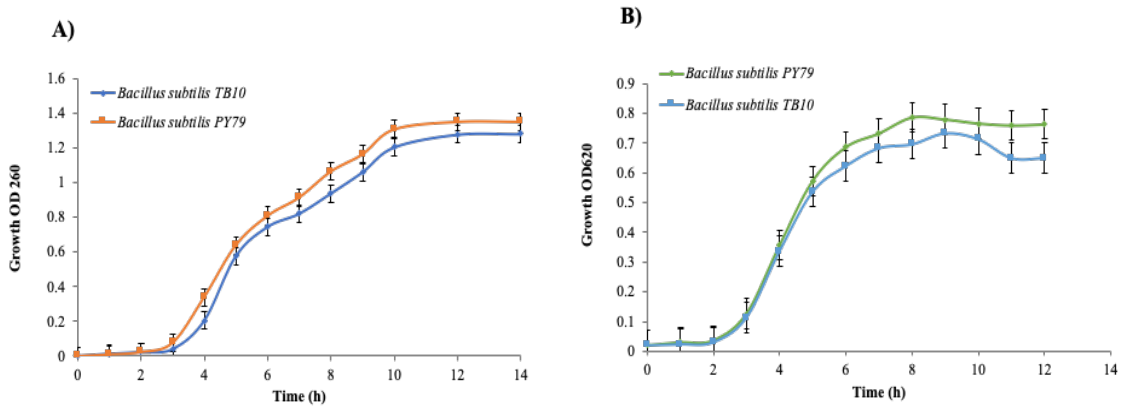


Fig. 26: Growth studies of WT and *ΔsqhC* mutant *B. subtilis in two different media*

Growth kinetics of WT, B. subtilis PY79 (diamonds) and ΔsqhC mutant, B. subtilis TB10 (squares) at OD620 = 0.5 in control condition A) Nutrient broth medium B) Sporulation medium. The experiment was conducted in biological triplicates.

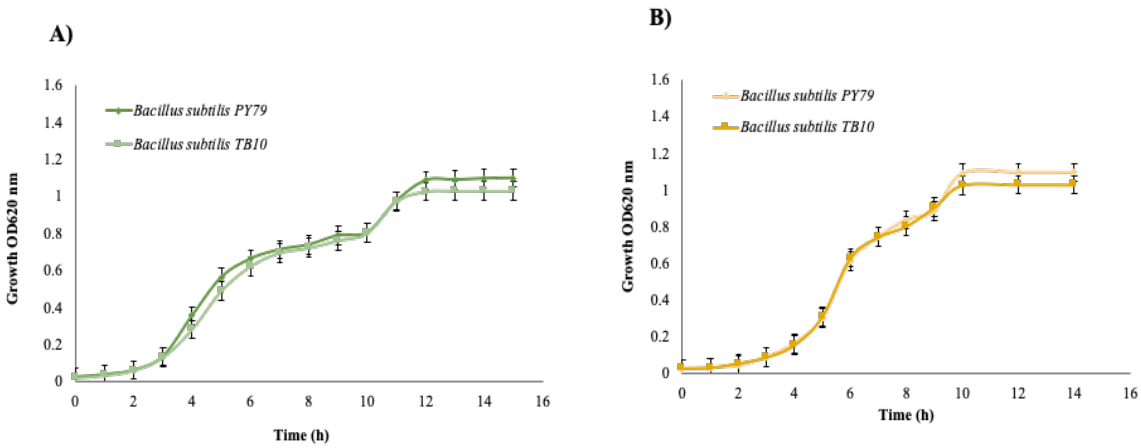


Fig. 27: Growth kinetics of WT *B. subtilis* PY79 (diamonds) and *ΔsqhC* mutant *B. subtilis* TB10 (squares) in nutrient broth medium

The growth studies were conducted for WT and sporulene mutant B. subtilis under different stress conditions A) 5 % Ethanol B) 5 % Butanol. Where, stress was induced at OD620 = 0.5. The experiment was conducted in biological triplicates.

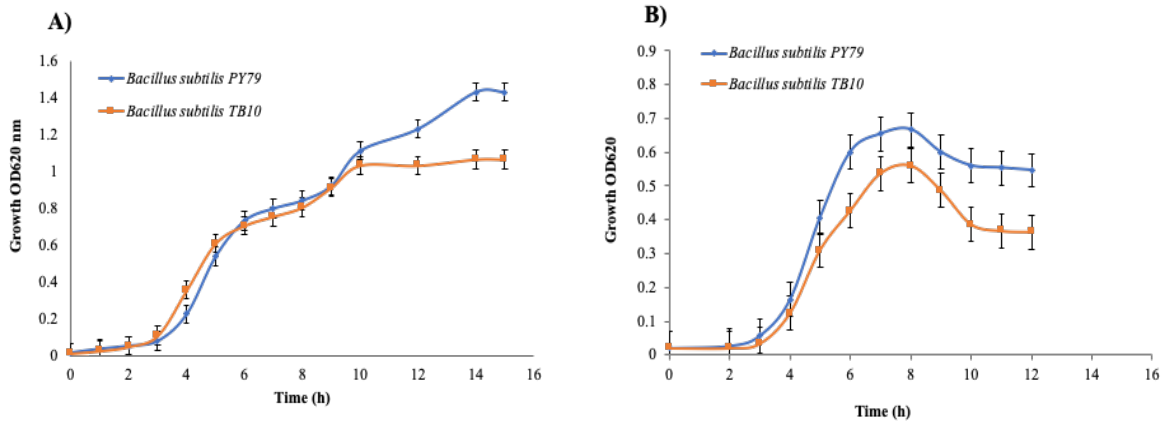


Fig. 28: Growth kinetics of WT, *B. subtilis* PY79 (diamonds) and *ΔsqhC* mutant, *B. subtilis* TB10 (squares) under H_2O_2 stress in two different media

The growth studies WT and sporulene mutant B. subtilis conducted in A) Nutrient broth B) Sporulation medium. Where, 0.005 % of H_2O_2 stress was induced at OD620=0.5. The experiment was conducted in biological triplicates.

3.4.2.2 Real time expressions analysis of the genes involved in sporulene biosynthesis pathway

Time dependent quantification of *hepS*, *hepT*, *sqhC* and *ytpB* genes was carried out in order to study the response of sporulene biosynthesis pathway genes to H_2O_2 stress. Log_2 fold changes were calculated and expression of each gene was analysed at 0, 15, 30 and 60 min under treated and untreated (control) condition. The basal level of expressions were nearly similar and constant for all the pathway genes under control condition, where *recA* was used as housekeeping gene. Interestingly for H_2O_2 treated condition, except for the 0 min, a gradual upregulation of pathway genes was observed with the increasing time points (Fig. 29, A, B, C, D). Especially after 30 min of induction of H_2O_2 stress, all the genes showed a statistically significant increase (2 fold) in expression (Fig. 29, C) as compared to expression of genes at other time points. Indicating the activated sporulene biosynthesis pathway as a result of H_2O_2 exposure. However, 60 min. after the induction stress, the

expression of pathway genes under treated condition became almost equivalent to gradually increased basal level expression of genes under control condition (Fig. 29, C).

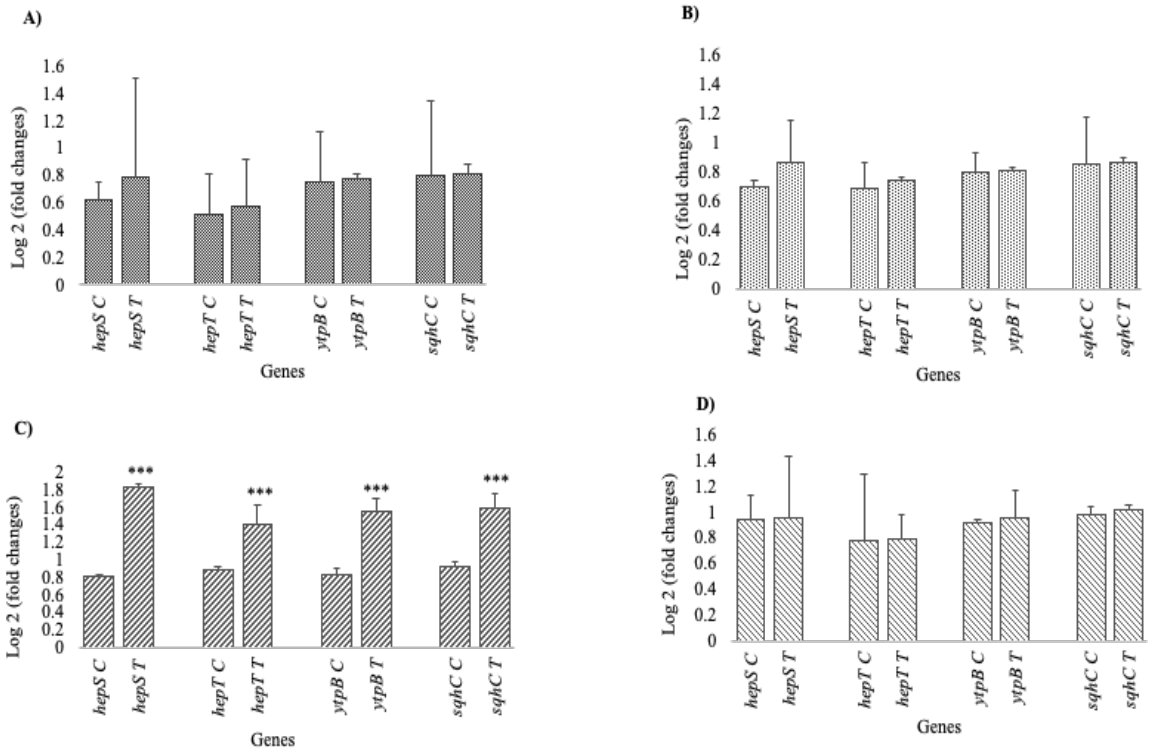


Fig. 29: qRT-PCR analysis of the genes encoding for putative proteins of sporulene biosynthesis pathway

*Real time analysis was carried out to study the expression of genes encoding putative proteins of sporulene biosynthesis pathway viz. hepS, hepT, ytpB and sqhC at different time points after exposure to hydrogen peroxide (0.005%). **A)** 0 min **B)** 15 min **C)** 30 min **D)**60 min. The experiment was performed in biological triplicates. C, Control; T, Treated. The asterisks indicates statistically significant upregulated in the expression of genes under peroxide stress as determined by ANOVA ($p < 0.05$) w. t., control.*

3.4.3 Responses of WT and sporulene deficient spores to different stress conditions with special reference to hydrogen peroxide (H_2O_2) toxicity

B. subtilis TB10, a *ΔsqhC* mutant of *B. subtilis* PY79 (WT) produced spores lacking sporulenes (Bosak et al. 2008). The response of these sporulene deficient spores to H_2O_2

was analysed in comparison to spores harbouring sporulenes with different experimental strategies.

3.4.3.1 Assessment of cultivability of spore of WT and *ΔsqhC* mutant *B. subtilis*

The cultivability of WT and *ΔsqhC* mutant spores exposed to different stress conditions was determined with the help of spore germination and plate count assay. A marginal difference was observed between the germination rates of WT and *ΔsqhC* mutant spores under control condition (Fig. 30 A, B). However, the cultivability of *ΔsqhC* mutant spores was affected severely under 0.05 % hydrogen peroxide, 70 % ethanol, 50 % butanol, 0.5 % glutaraldehyde and 0.05 % sodium hypochloride stress (Fig. 30, A) when compared to WT spores.

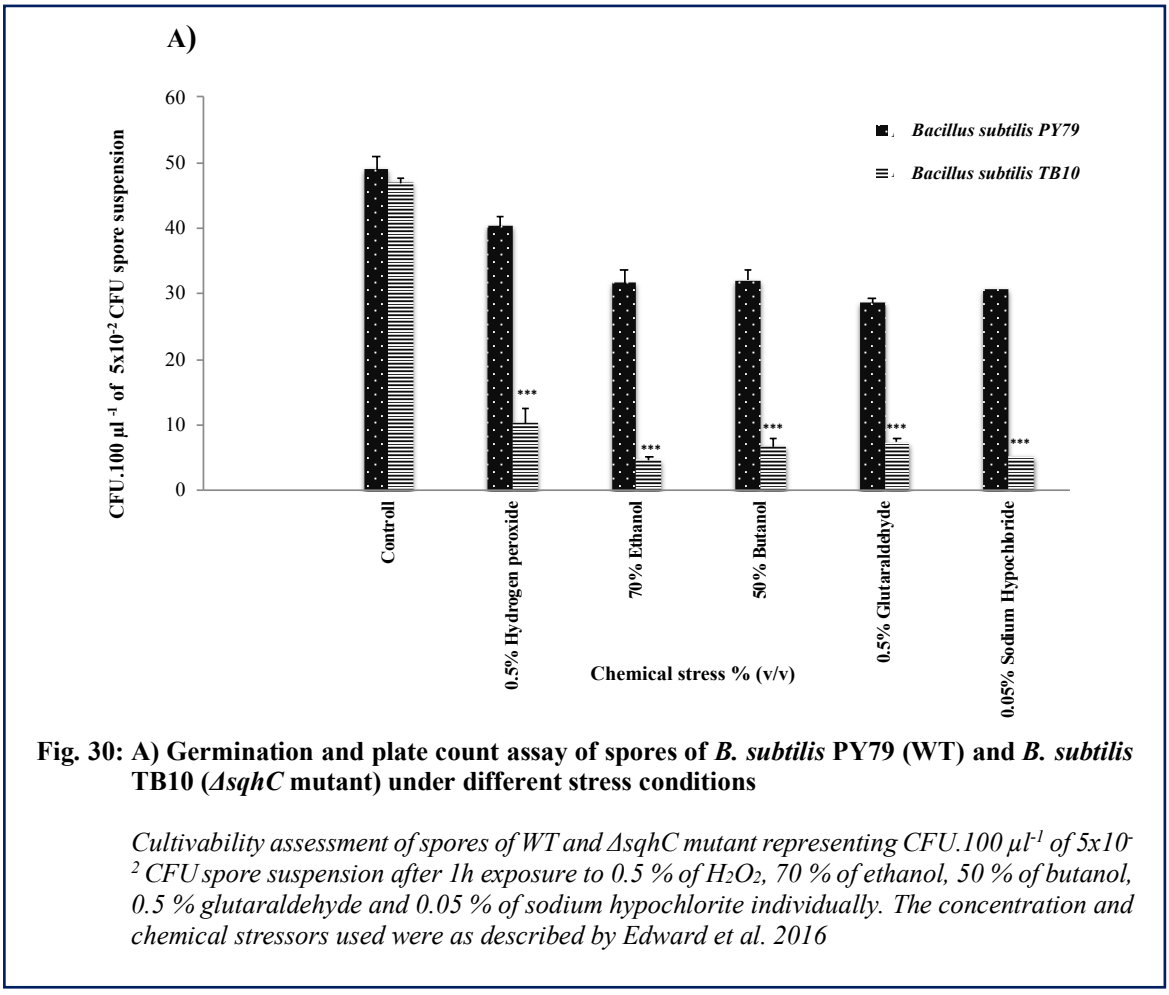


Fig. 30: A) Germination and plate count assay of spores of *B. subtilis* PY79 (WT) and *B. subtilis* TB10 (*ΔsqhC* mutant) under different stress conditions

Cultivability assessment of spores of WT and ΔsqhC mutant representing CFU.100 µl^{-1} of 5x10^{-2} CFU spore suspension after 1h exposure to 0.5 % of H_2O_2, 70 % of ethanol, 50 % of butanol, 0.5 % glutaraldehyde and 0.05 % of sodium hypochlorite individually. The concentration and chemical stressors used were as described by Edward et al. 2016

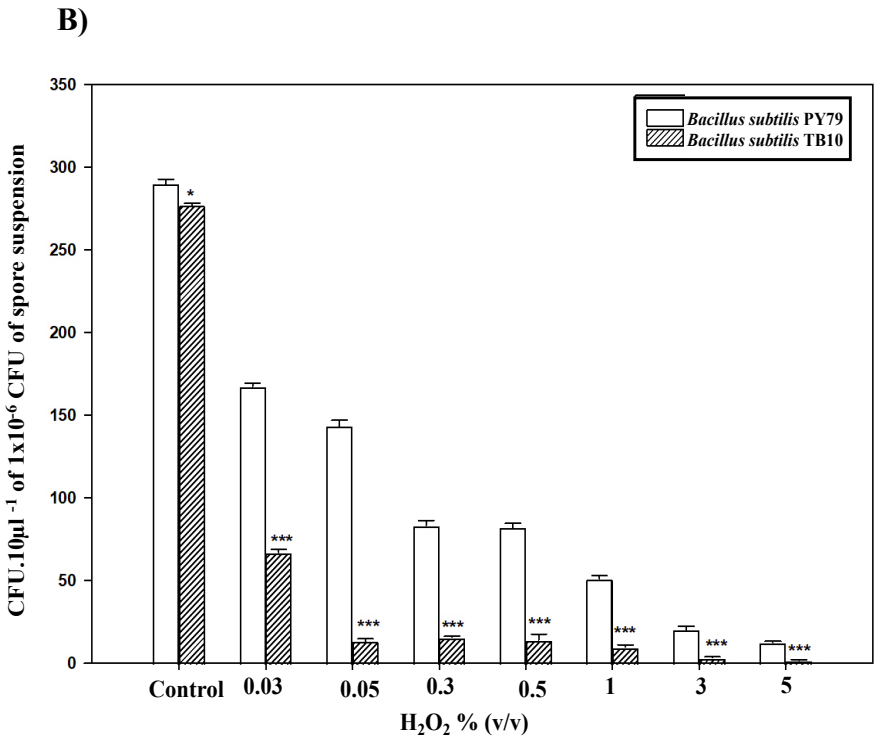


Fig. 30: B) Germination and plate count assay of spores of *B. subtilis* PY79 (WT) and *B. subtilis* TB10 (*ΔsqhC* mutant) under different concentration of H_2O_2

Cultivability assessment of spores of WT and ΔsqhC mutant representing CFU.10 μl^{-1} of $1x10^{-6}$ CFU/ml spore suspension after 1 hr exposure to different concentrations of H_2O_2 (v/v). The asterisks indicates significant reduction in cultivability of H_2O_2 treated mutant spores as determined by ANOVA ($p < 0.05$) w. t., wild type spore. The experiment was performed in biological triplicates.

Overall, the sporulene mutant spores were impacted more by H_2O_2, as compared to other stress conditions. Thus, the cultivability of *ΔsqhC* mutant spore was analysed at different concentrations of H_2O_2. Where, the cultivability of both WT and *ΔsqhC* mutant spores was observed to decrease consistently with the increasing concentrations of H_2O_2. Notably, at all the concentrations of H_2O_2, a statistically significant difference (ANOVA; $P<0.001$) was observed between colony forming units of spores of WT and *ΔsqhC* mutant (Fig. 30, B) making it evident that viability of sporulene deficient spore was affected more that WT spores. The *ΔsqhC* mutant's spore viability was compromised maximum at 0.05 % (v/v) concentration of H_2O_2 resulting in almost 80 % reduction in cultivability with respect to WT spores.

3.4.3.2 Microscopic analysis of spores of WT and *ΔsqhC* mutant *B. subtilis* exposed to H_2O_2

The affected cultivability of mutant spores on H_2O_2 treatment prompted us to analyse what was happening at the structural level. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to examine the morphological changes at surface and ultrastructural level in the spores of WT and sporulene mutant of *B. subtilis*, under oxidative (H_2O_2) stress.

3.4.3.2.1 Surface level morphological alterations of spores

When observed under SEM, the spores of both the WT and *ΔsqhC* mutant showed smooth outer surface appearance with oval and plump shape (Fig. 31, A, B) under untreated (control) condition.

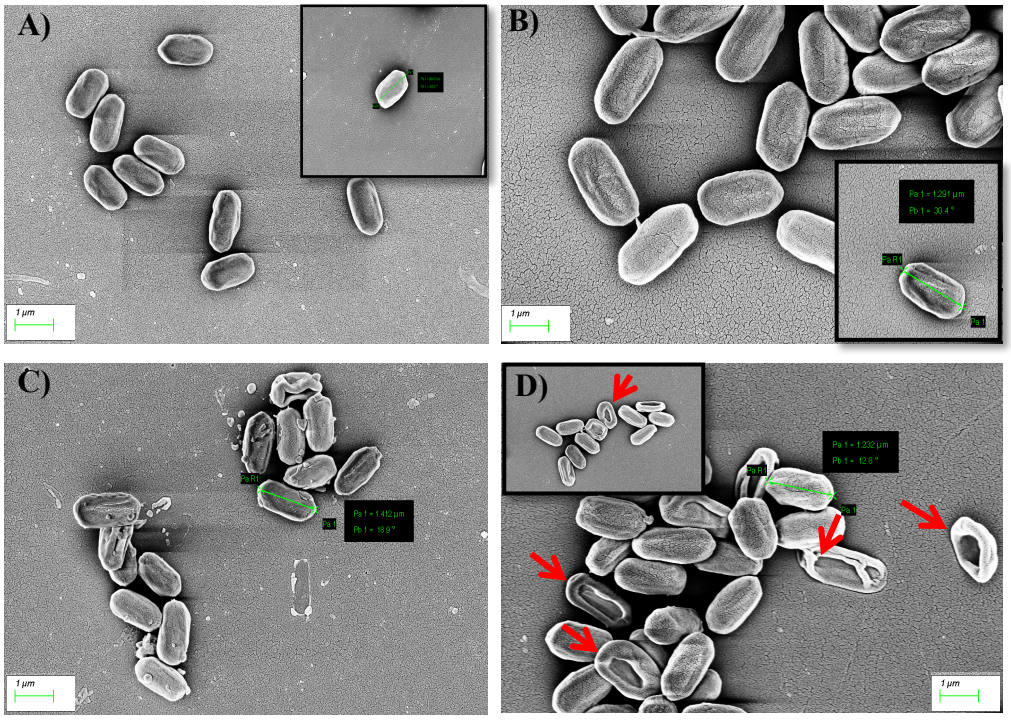


Fig. 31: Morphological alteration and surface level changes of spores of WT, *B. subtilis* PY79 and *ΔsqhC* mutant *B. subtilis* TB10 observed under scanning electron microscope

Scanning electron microscopy (SEM) of spores with and without 0.05 % (v/v) of H_2O_2 treatment ***A)*** *Untreated spores of B. subtilis PY79;* ***B)*** *Untreated spores of B. subtilis TB10;* ***C)*** *Treated spores of B. subtilis PY79;* ***D)*** *Treated spores of B. subtilis TB10 . Arrows indicate withered, deflated, crateriform spore shapes.*

Unlike WT spores, most of the affected *ΔsqhC* mutant spores showed deflated and withered morphology after exposure to H_2O_2. Where, majority (~50 %) of the peroxide treated spores of *ΔsqhC* mutant showed noticeable crateriform like structures with prominent acclivities and declivities (Fig. 31, C, D).

3.4.3.2.2 Ultrastructural changes of spores

For better understanding the morphological changes observed in SEM, thin sections of spores were observed under TEM.

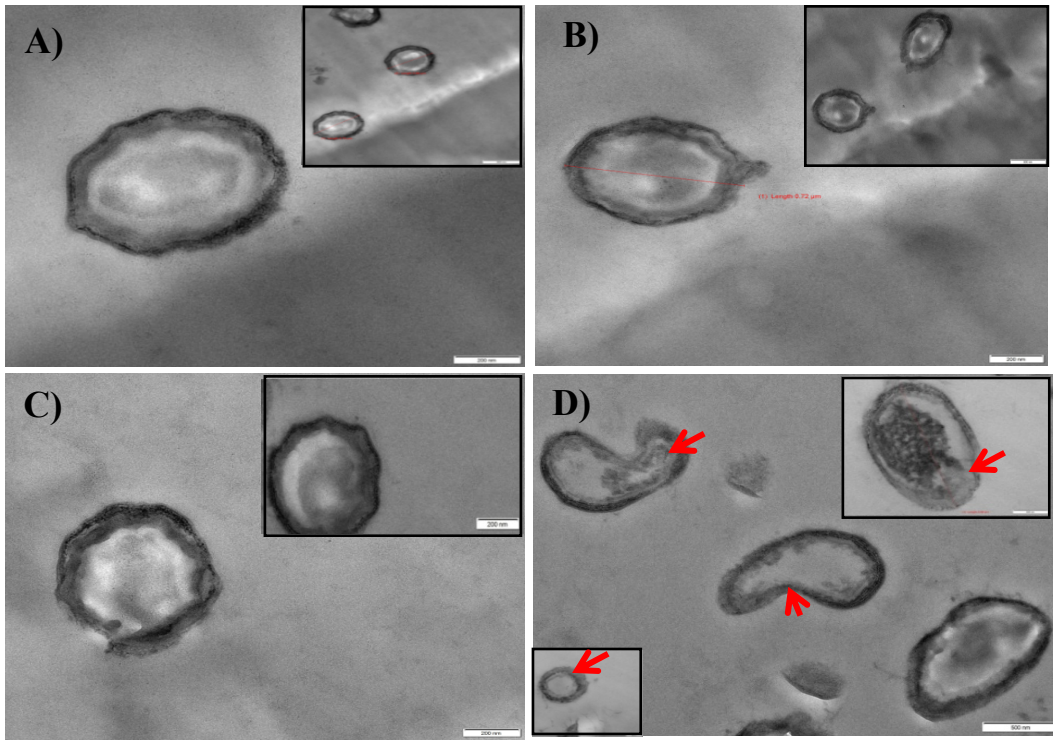


Fig. 32: Ultrastructural changes of spores of WT, *B. subtilis* PY79 and *ΔsqhC* mutant *B. subtilis* TB10 observed under transmission electron microscope

Transmission electron microscopy (TEM) photomicrographs of B. subtilis PY79 and B. subtilis TB10 spores before and after peroxide stress. Untreated spores of ***A)*** *B. subtilis PY79,* ***B)*** *B. subtilis TB10; Spores treated with 0.05% (v/v) of H_2O_2* ***C)*** *B. subtilis PY79,* ***D)*** *B. subtilis TB10. Arrows indicates possible damage to core, inner membrane and collapsed spore structures.*

The ultrastructural images of treated and untreated spores of WT and *ΔsqhC* mutant were in line with the observation of SEM. Untreated spores of both strains showed regular arrangements of inner spore structure with intact inner membranes (Fig. 32, A, B). After peroxide treatment, deformed spore structures (Fig. 32, D) were observed for sporulene mutant, contrary to WT spores (Fig. 32, C). The detailed examination of spore cross sections revealed possible damage to inner membrane structures (Fig. 32, D).

3.4.3.3 Loss of dipicolinic acid through leaky spore membrane

To assess if there were any changes in dipicolinic acid (DPA) content of spores of WT and *ΔsqhC* mutant due to H_2O_2 treatment, DPA was extracted and quantified using HPLC. The total DPA content of spores of *B. subtilis* PY79 (WT) was slightly higher (0.47 $\mu g.mg^{-1}$) than *ΔsqhC* mutant *B. subtilis* TB10 (0.34 $\mu g.mg^{-1}$) (Fig. 33, A, B). On germination of spores without H_2O_2 treatment, almost equivalent amount of DPA was released by the spores of WT (0.215 $\mu g.mg^{-1}$) and *ΔsqhC* mutant (0.172 $\mu g.mg^{-1}$) *B. subtilis* . However a marginal but significant difference was observed in the amount of DPA retained by spores after germination. Spores of *ΔsqhC* mutant retained significantly lower content of DPA (0.155 $\mu g.mg^{-1}$) than WT spore (0.192 $\mu g.mg^{-1}$) (Fig. 33, C). Contrarily, when treated with H_2O_2, a statistically significant difference (ANOVA; $P<0.05$) was observed in the amount of DPA released and retained between the two strains. Significantly lower content of DPA was released (TIT, 0.044 $\mu g.mg^{-1}$) and retained (T2T,0.015 $\mu g.mg^{-1}$) by *ΔsqhC* mutant spores as compared to the content of DPA released (P1T. 0.084 $\mu g.mg^{-1}$) and retained (P2T, 0.041 $\mu g.mg^{-1}$) by WT spores (Fig. 33, D). It clearly indicated the untimely loss of DPA through leaky spore membranes resulted from H_2O_2 toxicity

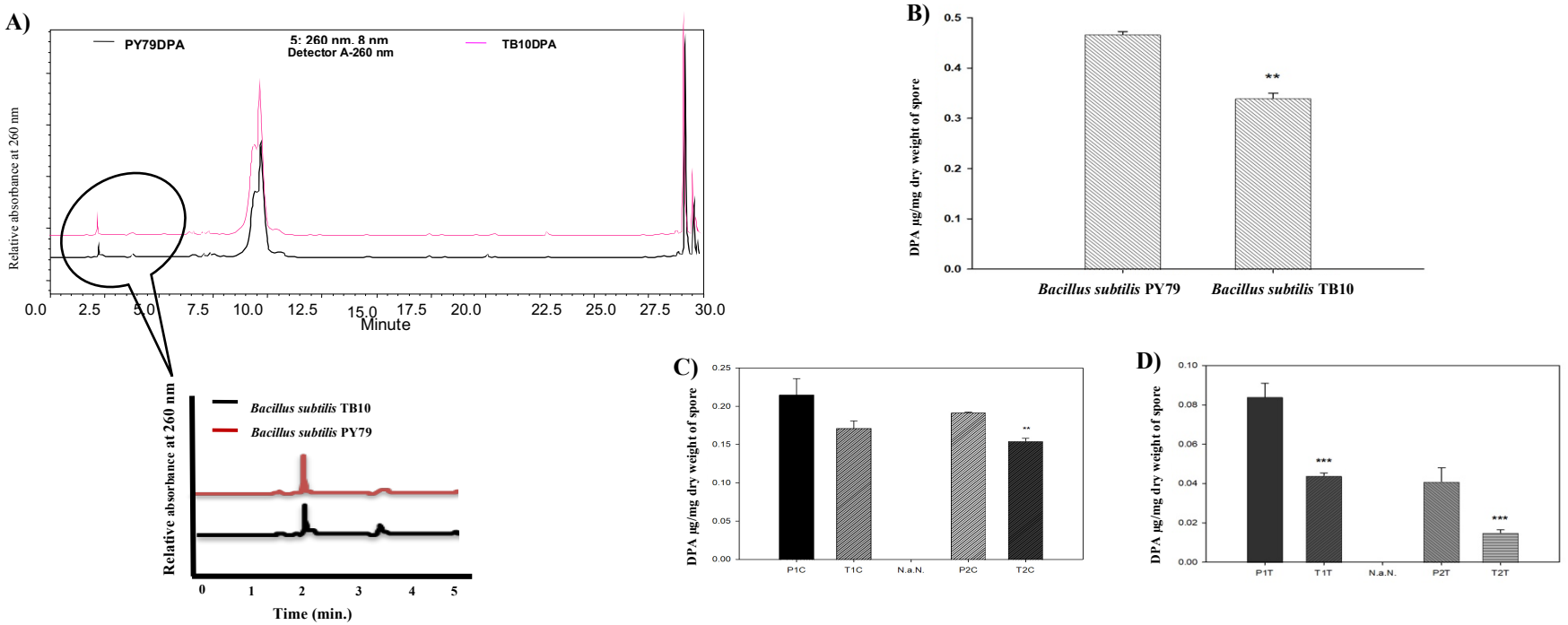


Fig. 33: Dipicolinic acid (DPA) quantification of spores of *B. subtilis* PY79 (WT) and *B. subtilis* TB10 (*ΔsqhC* mutant)

*DPA was quantified for spores of WT and ΔsqhC mutant using HPLC analysis **A)** HPLC chromatogram of DPA content of B. subtilis PY79 and B. subtilis TB10, recorded with the PDA detector at 260 nm wavelength. DPA was detected as a clear, baseline-separated peak at about 2.25 min retention time in each case. **B)** DPA content in µg/mg of dry weight of spores of B. subtilis PY79 and B. subtilis TB10. The content of DPA released and retained on spore germination **C)** before and **D)** after treatment with 0.05 % (v/v) of H_2O_2. Where, P, B. subtilis PY79 (WT); T, B. subtilis TB10 (ΔSqhC mutant); 1, DPA released; 2, DPA remained/retained; C, Control; T, treated. The asterisks indicate significant difference in DPA content w.t., wild type as calculated by ANOVA ($p < 0.05$).*

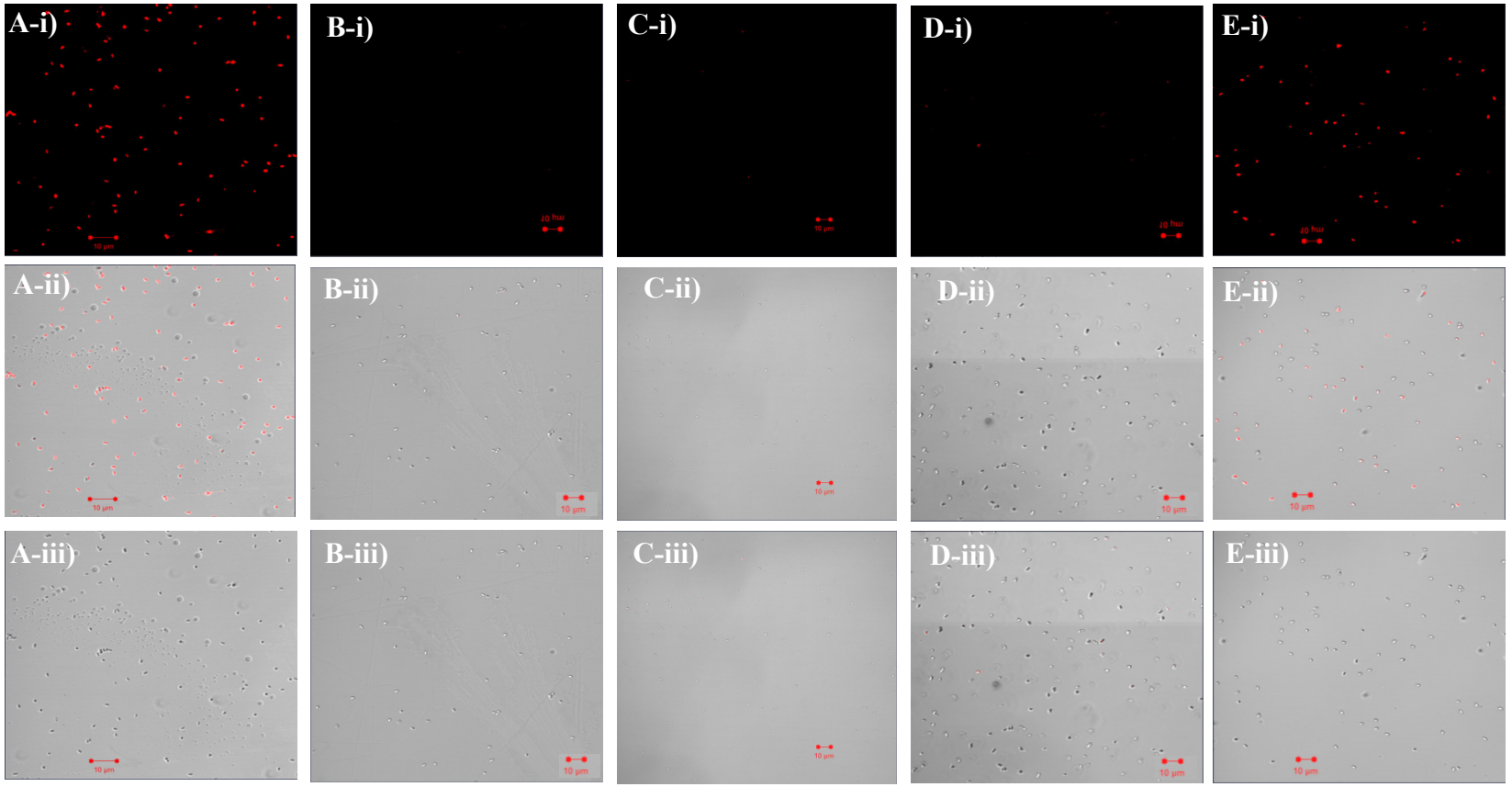


Fig. 34: Propidium iodide staining and confocal microscopy for assessment of compromised membrane permeability of spores of *B. subtilis* PY79 (WT) and *ΔsqhC* mutant *B. subtilis* TB10 with and without H_2O_2 treatment

Propidium iodide (PI) stained confocal microscopic images of spores of B. subtilis PY79 and B. subtilis TB10 before and after 0.05 % (v/v) H_2O_2 treatment. ***A)-(i), (ii), (iii)*** *Positive control (autoclaved spores)* ***B)-(i), (ii), (iii)*** *B. subtilis PY79 untreated spores* ***C)-(i), (ii), (iii)*** *B. subtilis TB10 untreated spores* ***D)-(i), (ii), (iii)*** *B. subtilis PY79 treated spores* ***E)-(i), (ii), (iii)*** *B. subtilis TB10 treated spores. The red fluorescence was observed in the range of 560-620 nm.*

3.4.3.4 Compromised membrane permeability of sporulene deficient spores

Compromised membrane permeability of sporulene deficient spore as hypothesised from to untimely loss of DPA and observations of SEM and TEM was finally confirmed with viability staining using propidium iodide (PI). The membrane damage was checked under confocal laser microscope for untreated, treated and autoclaved spores of WT and *ΔsqhC* mutant *B. subtilis*, which were stained with PI. It was discovered that almost all of the autoclaved spores were stained with PI (Fig. 34, A-i, A-ii, A-iii) indicating that the entire spore population had complete membrane damage. Whereas, the untreated spores of WT and *ΔsqhC* mutant remained unstained (Fig. 34, B-i, B-ii, B-iii; C-i, C-ii, C-iii) and negligible uptake of dye was observed for WT spores, after H_2O_2 treatment (Fig. 34, D-i, D-ii, D-iii). Nonetheless, the spores of *ΔsqhC* mutant exposed H_2O_2, showed high frequency of PI infiltration as evidenced by high degree of red fluorescence (Fig. 34, E-i, E-ii, E-iii). Quantitatively, 100 % of autoclaved spores were stained indicative of the complete membrane damage. Whereas, ~50-75 % of the total spore population took up the stain in case of sporulene mutant. It confirmed that the permeability barrier of *ΔsqhC* mutant spore was highly compromised due to H_2O_2 toxicity, albeit nor as severely as autoclaved spores.

3.4.4 Reorganization of spore fatty acids

For the spores produced by WT and *ΔsqhC* mutant *B. subtilis*, under H_2O_2 treated and untreated conditions, fatty acids were assorted into six categories (Table 11, Fig. 35, A, B) and relative abundance of each group to the total fatty acid content was examined. Under untreated conditions, the production of different categories of fatty acids by the spores of WT and *ΔsqhC* mutant was essentially identical (Table 11). Nevertheless,

branched and straight chain fatty acid contents in H_2O_2 treated spores of WT and *ΔsqhC* mutant strains differed significantly.

Fatty acids	***B. subtilis* PY79 (Control)**	***B. subtilis* PY79 (Treated)**	***B. subtilis* TB10 (Control)**	***B. subtilis* TB10 (Treated)**
Saturated fatty acids				
$C_{12:0}$	0.3	2.8	0.6	1.9
iso-$C_{14:0}$	2.6	1.7	2.1	2.1
$C_{14:0}$	0.9	1.7	0.7	1.4
iso-$C_{15:0}$	23.2	13.3	21.5	16.9
iso-$C_{15:0}$	1.2	-	-	-
anteiso-$C_{15:0}$	39.7	23.5	37.4	30.5
$C_{16:0}$ 2 OH	2.2	5.9	7.9	8.2
iso-$C_{16:0}$	4.2	3.8	4.4	3.6
$C_{16:0}$	4.4	10.3	3.6	6.8
iso-$C_{17:0}$	5.6	3.9	5.6	4.5
anteiso-$C_{17:0}$	9.2	11.5	8.3	7.2
$C_{18:0}$	0.4	-	0.7	1.6
$C_{19:0}$	-	6.5	0.8	2.9
Unsaturated fatty acids				
$C_{18:3}$ ω6C (6,9,12)	1.0	6.3	3.1	4.8
$C_{18:1}$ ω9C	1.0	2.9	1.0	3.9
Sum In Feature 4 ($C_{17:1}$ iso I/anteiso B)	-	4.9	0.7	2.9
Sum In Feature 5 ($C_{18:0}$ ante/$C_{18:2}$ ω6,9C)	-	1.7	-	2.4
Sum In Feature 8 ($C_{18:1}$ ω7C/ $C_{18:1}$ ω6C)	1.7	-	1.7	0.5

Table 11. Fatty acid composition of spores of WT and *ΔsqhC* mutant *B. subtilis*

Total fatty acid content of spores of B. subtilis PY79 and its ΔsqhC mutant B. subtilis TB10. The spores were produced with (treated) and without (control) 0.005% (v/v) H_2O_2 treatment to the cells at OD_{600} = 0.5 nm.

For *ΔsqhC* mutant spores, branched chain fatty acid content was found to be higher and straight chain fatty acid content to be relatively lower. Interestingly, this increase in branched chain was amongst saturated fatty acids. Altogether, the *ΔsqhC* mutant spores produced with H_2O_2 stress appeared to have higher percentages of saturated and *iso* fatty acids. Whereas, lower percentage of unsaturated, *anteiso* and unsaturated branched chain fatty acids was recorded for sporulene mutant spores produced under H_2O_2 toxicity (Fig. 35, Table 11).

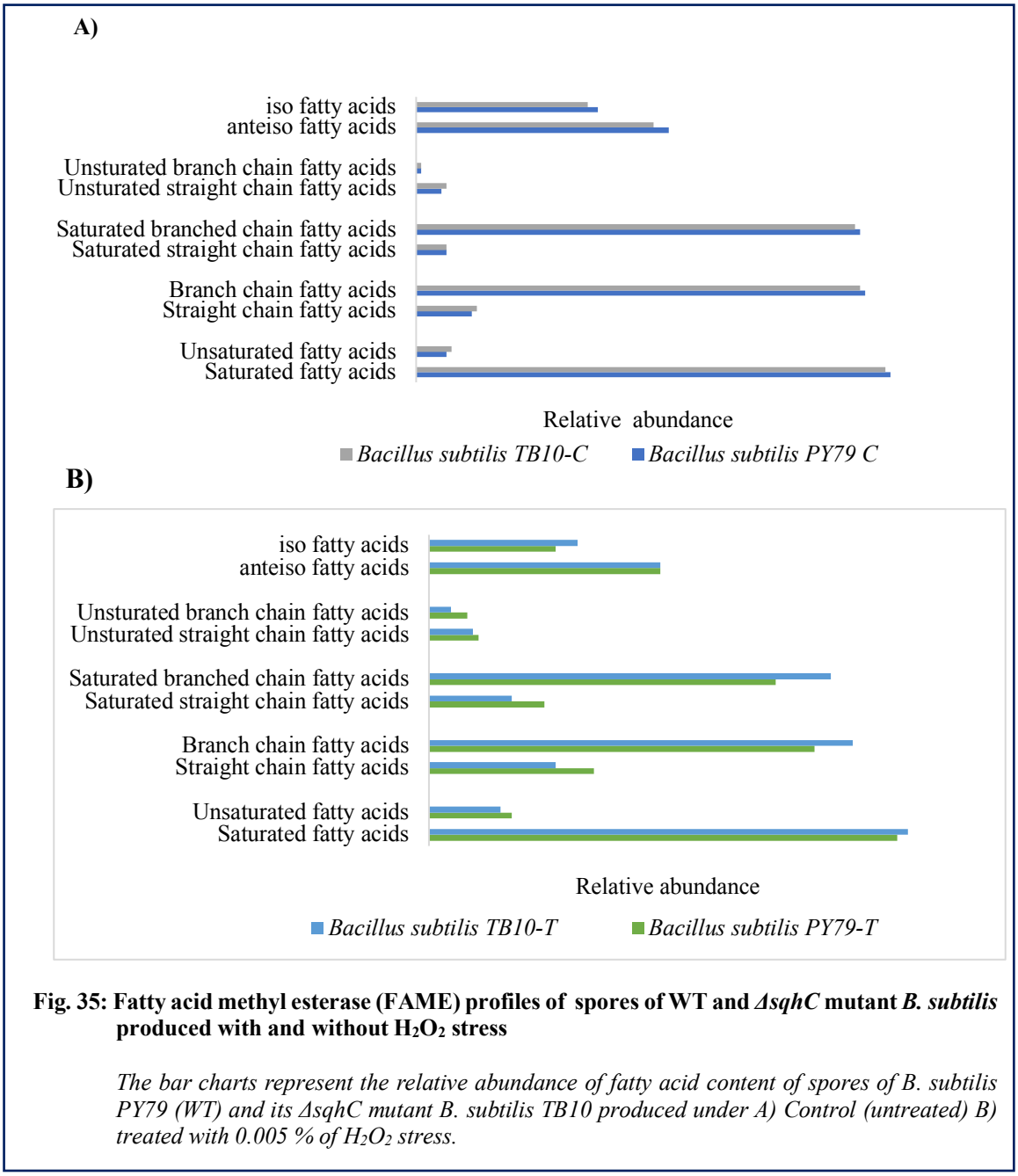


Fig. 35: Fatty acid methyl esterase (FAME) profiles of spores of WT and *ΔsqhC* mutant *B. subtilis* produced with and without H_2O_2 stress

The bar charts represent the relative abundance of fatty acid content of spores of B. subtilis PY79 (WT) and its ΔsqhC mutant B. subtilis TB10 produced under A) Control (untreated) B) treated with 0.005 % of H_2O_2 stress.

3.4.5 Protein profiling of spores

The gene ontology database (GO) (http://geneontology.org) was used to classify the proteins isolated from the spores of treated and untreated WT and *ΔsqhC* mutant *B. subtilis*, while the KEGG database (https://www.genome.jp) was used to determine the subcategories (Table S1, A, B, C, D). Seven major categories were identified for spore proteins *viz.*, molecular function, metabolic pathway, transport, stress response, sporulation specific, uncharacterised proteins and others. These were further subcategorised as DNA repair, DNA replication, transcription factors, basic transport and secretion system, chemotaxis and flagellar movement, kinases and transferases, C/N metabolism, amino acid metabolism, nucleotide metabolism, TCA cycle and cell wall synthesis (Fig. 36 A, B, C, D). Each of the four sets had varying protein profiles. According to our study, when we compared the sporulation-specific proteins of all four samples, unlikely to FAME profiles, the protein profile of spore formed under oxidative stress did not show any significant alterations. Nevertheless, the presence of a few unique proteins only in *ΔsqhC* mutant spore produced under H_2O_2 treatment cannot be neglected. Sporulation killing factor (SkfC), germination factors KB (GerKB), YlaJ and YhnC; morphogenetic proteins SafK and GroEL which were known for their auxiliary role in sporulation, spore germination and spore morphogenesis respectively, under stress condition (Allenby et al. 2006, Bagyan et al. 1998, Johnson and Moir. 2017, Lin and Rye et al. 2006, Ozin et al. 2001, Ross and Abel-Santos 2010, Zheng et al. 2016) were found in mutant spores produced on peroxide treatment.

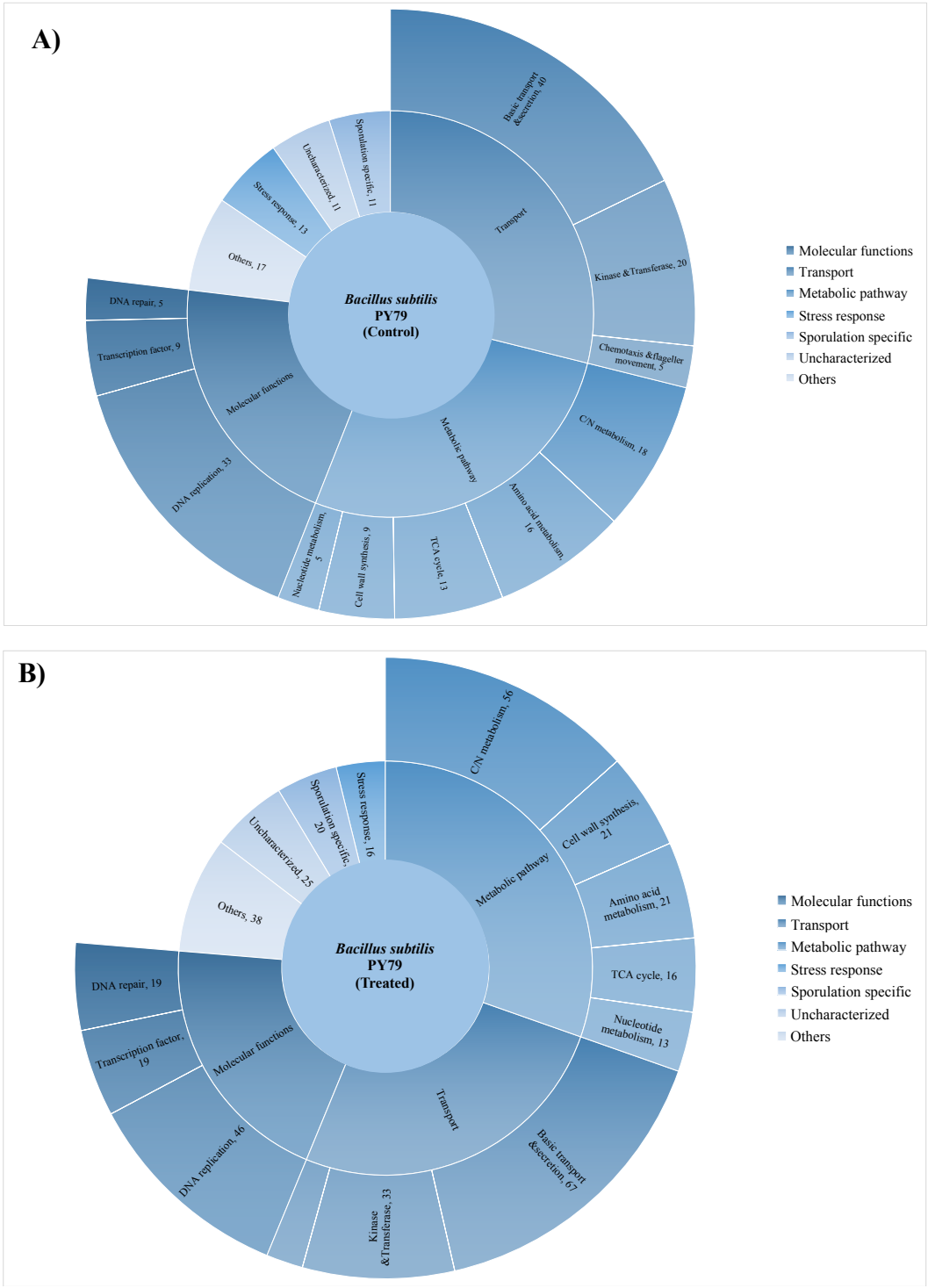
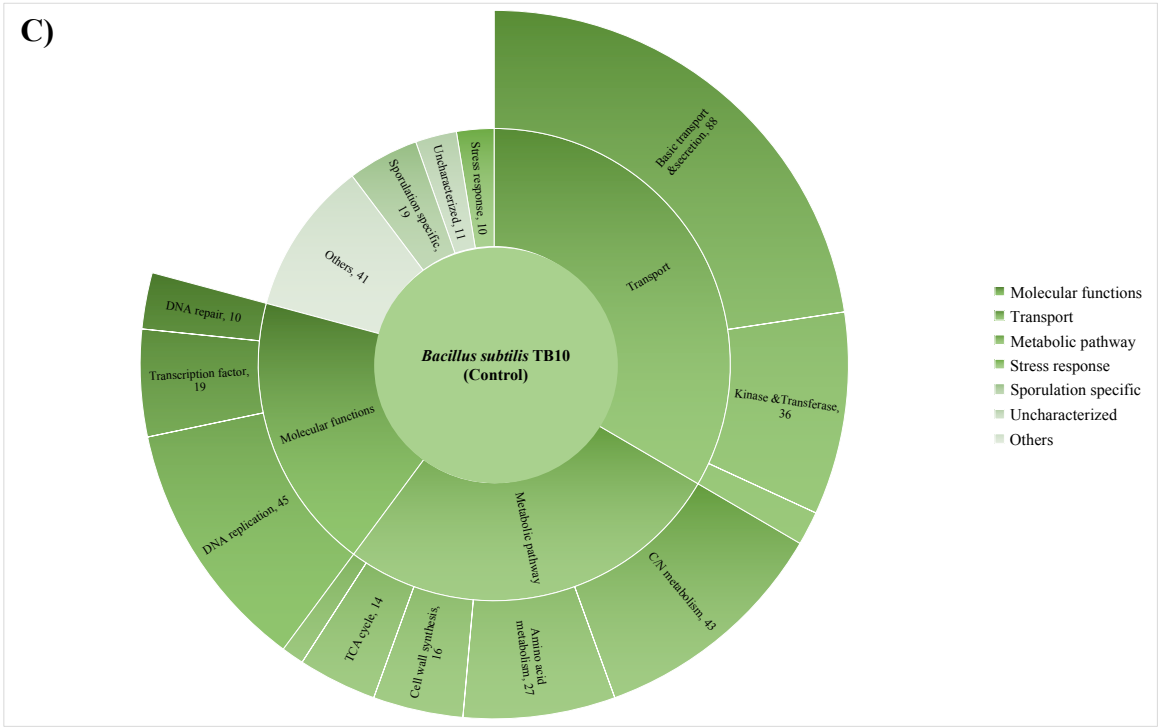


Fig. 36: Protein profiles of spores of *B. subtilis* PY79 (WT) and *ΔsqhC* mutant *B. subtilis* TB10 produced with and without induction of H_2O_2 stress

Protein profile of spore produced with and without 0.005 % (v/v) H_2O_2 treatment where, ***A)*** *B. subtilis PY79 (Control);* ***B)*** *B. subtilis PY79 (Treated). The proteins were separated* through nano-LC MS, identified through UniProt and categorized with help of KEGG and GO database.



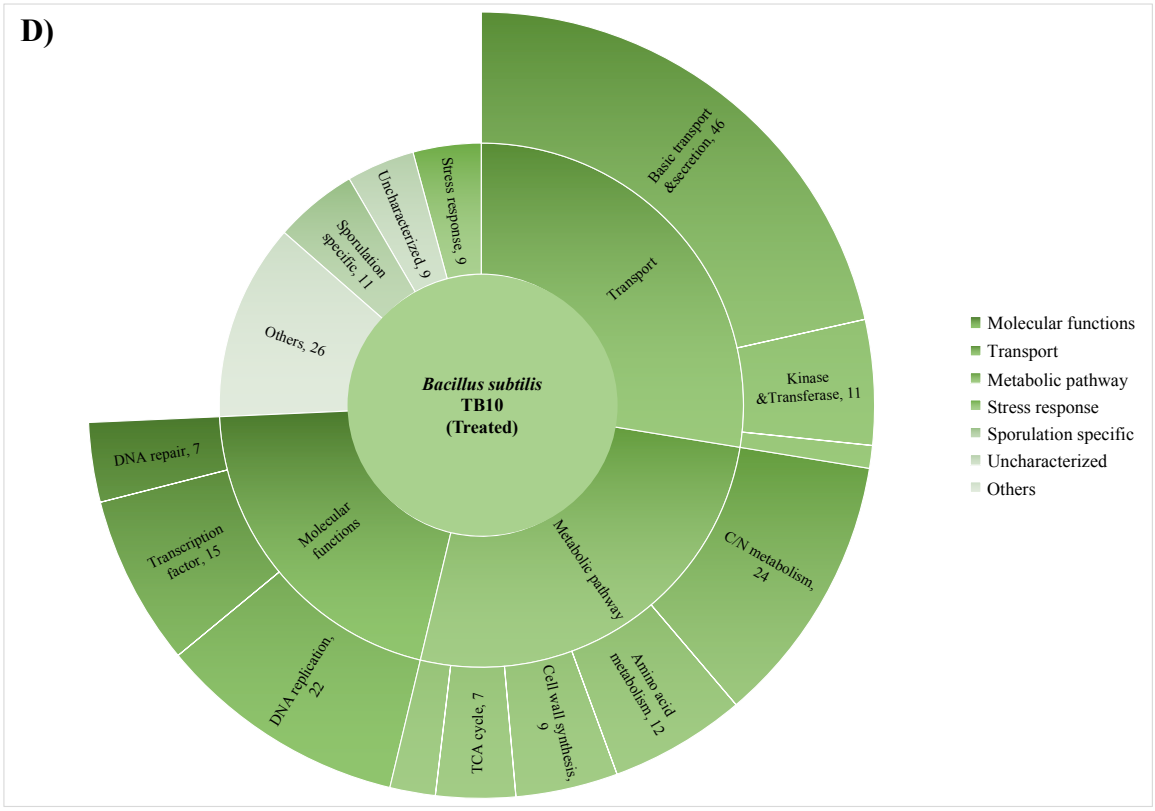


Fig. 36: Protein profiles of spores of *B. subtilis* PY79 (WT) and *ΔsqhC* mutant *B. subtilis* TB10 produced with and without induction of H_2O_2 stress

Protein profile of spore produced with and without 0.005 % (v/v) H_2O_2 treatment where, ***C)*** *B. subtilis TB10 (Control);* ***D)*** *B. subtilis TB10 (Treated). The proteins were separated* through nano-LC MS, identified through UniProt and categorized with help of KEGG and GO database.

Discussion

4. DISCUSSION

One of the most basic questions in microbiology is how bacteria deal with unfavorable conditions and the approaches they use to remain alive under adverse circumstances. Bacterial populations expand exponentially in an ideal environment where substrates and nutrients are readily accessible and metabolic waste products are continuously eliminated. Unraveling cellular activities during this stage of bacterial life has long been the focus of research groups in classical microbial physiology (Forchhammer 2021). However, in the majority of natural habitats, bacteria are exposed to unfavorable conditions that limit their development or put their viability in jeopardy. In abiotic situations, bacterial viability may be threatened by harmful substances, sever nutrient deficiencies, toxic compound accumulation or adverse physiochemical environment settings (Haruta and Kanno 2015). Additionally, the nearby species pose a threat to bacterial survival by acting as predators or competition for nutrients and niche. These selection pressures have been present throughout the whole evolutionary process of bacteria. As a result, they developed sophisticated coping mechanisms to deal with these difficulties. These mechanisms include, biofilm formation (Fazeli-Nsab et al. 2022), VBNC state (Ramamurthy et al. 2014), cyst and endospore formation (Haruta and Kanno 2015, Tan and Ramamurthi 2014). Apart from this, cellular membranes with unique components and associated cell envelop stress responses also support bacterial cell against various stresses and ensures their survival under unfavorable conditions (Willdigg and Helmann 2021).

Cellular membranes are predominantly made up of proteins and lipids and are of great significance for maintaining cell growth and integrity. Hopanoids, are triterpenoid lipids which act as sterol surrogate in bacterial cell membrane (Lodha et al. 2015). Recent advancements in lipid identification and quantification have aided our understanding of

hopanoid production in variety of genetically tractable hopanoid producing bacteria. However, with all the extensive research on hopanoids of different bacterial groups (Belin et al. 2018, Lodha et al. 2015, Rohmer 1984, Sáenz et al. 2015) there are very few studies available on hopanoids of *Planctomycetota* member (Damsté et al. 2004, Kharbush et al. 2018, Rivas-Marin et al. 2019), leaving a lot to explore. Thus, the present study tries to add to the existing hopanoid diversity of *Planctomycetota* members with a curiosity to identify unidentified hopanoids associated with this group of bacteria.

Similarly, sporulenes are triterpenoid lipids discovered from the endospores of *B. subtilis,* they resemble hopanoids and said to have protective role against oxidative stress (Bosak et al. 2008). Unlike hopanoids the studies on distribution and function of sporulenes are limited, offering rich apportunity of discovery in this area. Research available till date, revealed association of sporulenes only with the varying strains of *B. subtilis* (Bosak et al. 2008, Sato et al. 2013, Takigawa et al. 2010). Therefore, we started the present study with *in silico* analysis of the class *Bacilli* members for sporulene biosynthesis pathway genes. Further, working with *B. subtilis* PY79 (WT) and its *ΔsqhC* mutant *B. subtilis* TB10, we observed that the vegetative cells as well as spores of the sporulene mutant *B.subtilis* were more sensitive to different stress conditions especially to H_2O_2 as compare to WT. Based on spore cultivability, microscopic observations, spore molecular composition and molecular analysis, the present study provides additional findings on sporulenes as one of the strategy used by *Bacillus* sp. to defray the toxicity of H_2O_2 and gives better characterization of protective activity of sporulenes.

4.1 Hopanoid diversity of the *Planctomycetota* isolates

4.1.1 Ground for the selection of *Planctomycetota* members under this study

Planctomycetota members have long been under-studied in the field of microbial taxonomy and systematics when compared to other well studied bacteria. Currently the area

is beeming with novel discoveries of the phylum *Planctomycetota* members holding interesting biological characteristics (Wiegand et al. 2020). Therefore, we considered that exploring *Planctomycetota* members for characterization and understandig of unconventional hopanoids could be an interesting approach for spotting innovation in the field of hopanoid diversity. Most of the members of the phylum *Planctomycetota* are associated with the aquatic habitats (marine or fresh water) (Wagner and Horn 2006). Bacteria featuring in low O_2 conditions are major hopanoid contributors in marine sediments (Kharbush et al. 2016). There are instances where, less studied anammox bacteria of phylum *Planctomycetota* which are strictly anaerobic were reported to produce hopanoids (van Niftrik and Jetten 2012). Therefore, the present study attempted to understand the hopanoid diversity of the *Planctomycetota* members isolated from sediments of Chilika lagoon (marine water) and rhizospheric soil of Loktak lake (fresh water), a unique habitat (Lhingjakim et al. 2022).

4.1.2 Leads on genetic capacity of *Planctomycetota* isolates to synthesize hopanoids and sporulenes

Hopanoids are widely distributed among many bacteria and are considered as taxonomic biomarkers (Ourisson and Albrecht 1992). In order to investigate the chemotaxonomic significance of hopanoids in *Planctmycetota* members, their genomes were mined for genes encoding putative proteins of hopanoid biosynthesis pathway. Screening the genomes of five lab isolates of *Planctomycetota* members had revealed that all the screened organisms posses the genetic capacity to synthesize hopanoids and its derivatives (Table 6 and table 7). *P. rhizosphaerae* JC665^T and *G. chilikensis* JC646^T represented the complete hopanoid biosynthesis machinery in them indicating their potential to synthesize most of the commonly known hopanoids and derivatives (Lodha et al. 2015, Rhomer et al. 1984). Aside from the genes involved in hopanoid production, some

of the genes are involved in hopanoid regulation or shipment of hopanoids to the right location. One such gene *hpnN*, encoding a RND-like transporter involved in the localization of hopanoids to the outer membrane (Doughty et al. 2011) could not be identified from the genomes of *Planctmycetota* strains under this study. However, other three member *viz, R. sediminicola* JC651^{T}, "*R. lacunae* JC635$^{T"}$ and "*R. nitratireducens*" JC645^{T} shared similar hopanoid biosynthesis machinery where, only the genes required for synthesis of basic hopanoid skeleton were present (Table 7) (Lodha et al. 2015).

Our results based on genome mining for hopanoid biosynthesis pathway (Table 6 and Table 7) suggested that, variation in the hopanoid biosynthesis machinery of the *Planctomycetota* members colud be related with their phylogenetic affiliation. The *Planctomycetota* members belonging to different genus may harbour different hopanoid biosynthesis assembly. Except for the *P. rhizosphaerae* JC665^{T} all the other strains were isolated from same geographical location which were sourced from sediments of Chilika lagoon (Table 6) but we could not deduce any significant corelation between source of isolation and the hopanoids associated. Thus the association of hopanoids to *Planctomycetota* members appear to be organism specific rather than niche specific however a genome screening data, based on a larger sample size would yeild better information in this aspect. Unlike hopanoids, the sporulene biosynthesis machinary was not identified in *Planctomycetota* members. Till date, sporulenes were discovered only from the spore forming *B. subtilis* strains (Bosak et al. 2008, Kontnik et al. 2008) while, there were no reports of spore forming members among the phylum *Planctomycetota,* the absence of spore formers hints towards the absence of sporulenes in phylum *Planctomycetota* members.

4.1.3 Diversity of squalene hopene cyclases (SHCs)

In the process of extraction of genes encoding for SHCs from *Planctomycetota* genomes, multiple copies of the genes encoding for SHC protein were recorded amongst the mined *Planctomycetota* isolates. Similar was observed in case of squalene-hopene cyclase (*shc*) gene in δ-Proteobacteria, which inevitably harbour two copies of the *shc* gene per genome, each of which is said to have distinct phylogenetic origin (Pearson et al. 2007). Whereas, majority of the hopanoid producing taxa (other than *Planctomycetota* members) only have one copy of *shc* (Pearson et al. 2009).

The genes endoding for SHCs exibit majorly a vertical pattern of inheritance and rare lateral gene transfer (LGT), thus the phylogenetic trees based on this cyclase are mostly identicle to trees based on 16S rRNA genes sequences (Frickey and Kannenberg, 2009 Pearson et al. 2007) defining their evolutionary relatedness. In the present study, three distinct clades fromed by three bacterial groups *viz.*, well studied hopanoid producing members, *Planctomycetota* isolates and genus *Bacillus* member (Fig. 9) indicated phylogenetic variation of these groups of bacteria (based on SHC sequences) in context of hopanogenesis. Interestingly, SHC of *P. rhizosphaerae* JC665^{T} grouped with the clade formed by known hopanoid producing bacteria, in accordance to a study on the environmental distribution of hopanoid biosynthetic genes which stated that the *shc* genes in *Planctomycetes* are diverse in nature (Pearson et al. 2009). However, the existence of the *shc* gene in a specific species is invariably associated with the identification of hopanoids in that species (Härtner et al. 2005, Perzl et al. 1998, Rohmer et al. 1984). It hinted about the definate hopanogenesis in *P. rhizosphaerae* JC665^{T} that was further investigated from its endometabolome.

In the resulted phylogenetic tree (NJ), the probable SHCs of genus *Bacillus* members formed a separate clade (Fig. 9). As studied by Bosak et al. (2008), the putative

squalene hopene cyclase of *B. subtilis* shared 90 % of its amino acid (AA) sequence with the SHCs from a few other *Bacillus* species, none of which were known for synthesizing hopanoids, however it only shared 30-50 % AA identity with many closely related SHCs associated to hopanoid-producing bacteria. It led to the interpretation that, putative SHCs of the genus *Bacillus* members were not similar to hopanoid producing SHCs but it is a distinct cyclase called sporulenol synthase (SqhC), a key enzyme involved in the formation of sporulenes which is a similar triterpenoid of hopanoids (Kontnik et al. 2008) and thus the separate clade. Unlike to these *Bacillus* sp., *A. acidocaldarius* DSM 446^T being a hopanoid producing *Bacillus*, cladded along with hopanoid producing members (Poralla et al. 1984, Siedenburg and Jendrossek 2011).

4.1.4 Cataloging of hopanoids of *P. rhizosphaerae* JC665^T

The ability to produce hopanoids appeared to be an uncommon physiological characteristic among rare bacterial taxa, occurring less frequently than previous research revealed (Rohmer et al. 1984). Till date, members of *Planctomycetota* were associated with the synthesis of diploptene, diplopterol, bacteriohopanetetrol and composite-BHPs. The synthesis of C_{27} hopanoid ketone is unique characteristics of *Planctomycetota* members which can be used as a biomarker (Damsté et al. 2004) although how it is synthesised is still unknown. In this study, the *Planctomycetota* isolate *P. rhizosphaerae* JC665^T was observed to operates a non-canonical hopanoid biosynthesis pathway (Fig. 10) with synthesis of a few unique hopanoids. Along with the previously reported hopanoids (Damsté et al. 2004) it produced two unususal pentacyclic triterpenoids of hopane series as hop-2-ene and 28-Nor-17a(H)-hopane (Table 8). Hop-21-ene is unlikely to be found in bacterial hydrocarbon fractions analysed by GC-MS on a column injector at low temperature, making it a peculiar characteristic of *P. rhizosphaerae* JC665^T. It is most likely to be derived from the dehydration of diplopterol and/or the isomerization of

diploptene (Pale-Grosdemange et al. 1998). Whereas, 28-Nor-17a(H)-hopane is a structure which could not be directly derived from squalene by cyclase activity thus we suspect the presence of intermediated hopane compounds and enzymes in the rout of synthesis, which remained unidentified in this study. 28-Nor-17a(H)-hopane is a hopanoid resembling geohopanoids which generally occur in C_{31} 17a, 21b(H) configurations (French et al. 2012) and known for their thermostable nature (Peters and Moldowan1991). Thus the association of hop-21-ene and 28-Nor-17a(H)-hopane could be considered as special characteristic of *P. rhizosphaerae* JC665^T, as these two hopanoid compounds are not common to the bacterial hopanoids (biohopanoids). In addition, few hopanoid like fragmentation patterns were observed (Table 8) in the mass spectra of *P. rhizosphaerae* JC665^T endo-metabolome, suggesting the possibility of some more hopanoids which remained unidentified; mostly due to their non-availability in the hopanoid library.

4.2 Isolation and cataloguing of potential sporulene producing *Bacillus* sp. and insights into sporulenes of *Bacillus subtilis* JC1005

4.2.1 Rationale for selection of sample, source and enrichment medium

Phylum *Bacillota* members are known for their spore forming ability under adverse environmental conditions (Tan and Ramamurthi 2014). Mostly, members of the genus *Bacillus* were believed to reside in soil, but studies have shown that the soil may only serve as a reservoir for spores of *Bacillus sp.* (Hong et al. 2009). Due to their expansive physiology and spore forming abilities, *Bacillus* sp. have been able to colonise practically in all natural habitats like soil, sediments, air, water, lake and fodder as well as harsh environments including hot springs, salt marshes, thermal acid waters and sub-Antarctic soils (Claus and Berkeley 1986, Gardener 2004, Nicholson 2002). Considering the spore forming ability of the genus *Bacillus* members and their survival ability even in harsh environments, the samples used for isolation of *Bacillus* sp. were sourced from water

starved soil, dry sand, hot spring water, sediments and sponges. *Bacillus* sp. being Gram-stain-positive bacteria, the enrichment medium used for isolations were supplemented with 0.01 % sodium azide to inhibit the growth of Gram-stain-negative bacteria and 0.01 % cycloheximide as an antifungal agent (Dal Pizzol et al. 2021, Snyder and Lichstein 1940) followed by pasteurization treatment which helped in selective isolation of spore forming *Bacillus* sp.

4.2.2 *Bacillus* sp. cultured in laboratory and association with sporulene biosynthesis

Most of the *Bacillus* strains isolated in the laboratory showed 100 % sequence identity with previously reported *Bacillus* sp. (Table 9) representing the extensive studies available on *Bacillus* members (Cihan et al. 2012). Six of the fifteen *Bacillus* strains isolated, shared closest 16S rRNA gene sequence identity with *B. tequilensis* KCTC 13622^{T}. Interestingly all these strains were isolated from various geographical locations of Tamil Nadu suggesting that, the niches and associated sources of the said region harbor rich diversity of *B. tequilensis*, a close phylogenetic neighbor of *B. subtilis*. Whereas *Bacillus* strain JC1013, based on its 98.7 % 16S rRNA gene identity to *M. selenatarsenatis* SF-1^{T}, it was proposed as novel strain of genus *Mesobacillus* i.e *M. aurantius* JC1013^{T} with characteristic features of high salt tolerance (7 %) and production of carotenoid like orange pigment (Rai et al. 2020). The genome mining results (Table 9) indicated that, all the phylogenetically closest strains of lab-isolates of genus *Bacillus* members invariably possess the potential sporulene biosynthesis pathway stating that these strains are possible sporulene producers. Apart from these *Bacillus* strains, two novel bacterial isolates i.e *C. candidae* JC507^{T} and *P. aeridis* JC501^{T}, screened for sporulene biosynthesis pathway genes didn't show the association of pathway with them (Indu et al. 2020, Rai et al. 2020) suggesting that synthesis of sporulene is not a common phenomenon but is associated with specific group of bacteria especially spore producers as previously reported by Bosak et al.

(2008). Although some of the phylum *Bacillota* members were reported to harbor few genes involved in the synthesis of hopanoids (Hippchen et al. 1981, Poralla et al. 1984) we could not identify association of these genes with the *Bacillus* genomes screened under this study.

4.2.3 Diversity of sporulenol synthase (SqhC's)

The phylogenetic relatedness of hopanoid and sporulene producing bacteria was studied with a neighbor joining tree (NJ) (Fig. 13) constructed based on SqhC's of probable sporulene producing *Bacillus* members and SHC's of hopanoid producing members. Although, HHpred an advanced protein structure prediction method (So¨ding et al. 2005) grouped SqhC with SHC (Bosak et al. 2008); two major clades of bacteria, formed based on differences of SqhC and SHC amino acid sequence, proclaimed them as two separate cyclase. A strong electrophilic motif rich in aspartic acid (DXDD motif) is particularly preserved in the SqhC from *B. subtilis* (Wendt et al. 1997). However, cyclization of hopanoid rings involves the activity of phenylalanine F601 (Merkofer et al. 1999, Wendt et al. 1997) and F605 residues which forms a part of SHC's (Fischer and Pearson 2007) but not of SqhC's. Therefore, the two cyclase can be considered as highly similar but not identical as they involve in two distinct reactions, catalyzed by different groups of bacteria as previously suggested by Bosak et al. (2008). This justified the two separated clades formed by *Bacillus* members and hopanoid producing bacteria in the NJ tree (Fig. 13). Further considering the previous studies on sporulenes (Bosak et al. 2008, Kontnik et al. 2008), majorly in *B. subtilis* PY79 and close phylogeny of *B. subtilis* JC1005 with it (Fig. 13), the screening for sporulenes at genotypic and phenotypic level was started with *B. subtilis* JC1005.

4.2.4 Genomic and phenotypic insight into sporulenes of *B. subtilis* JC1005

As per 16S rRNA gene and whole genome sequence identities, strain JC1005 was identified as *B. subtilis*. In accordance with the phylogenetic relatedness of *B. subtilis* PY79 and *B. subtilis* JC1005 (Fig. 13) the genome comparison indicated high similarity between both the strains (Fig. 16). Parallelly, the genome annotation and genome mining of *B. subtilis* JC1005 revealed the presence of complete genetic machinery of sporulene biosynthesis (Fig. 14 and Fig. 15) similar to *B. subtilis* PY79 (Takigawa et al. 2010). Since, all the genes encoding for putative proteins of sporulene biosynthesis were present in *B. subtilis* JC1005 genome, we presumed that the bacterium must be producing sporulenes which were screened from the lipid extract of isolated spores of *B. subtilis* JC1005. As anticipated from the genomic information, the GC-MS analysis of partially purified lipid extract showed MS fragmentation similar to featured characteristics exhibited by tetracyclic scalarane of sporulenes (Fig. 19) (Kontnik et al. 2008). The molecular ion peak at *m/z* 474 represented a common molecular mass of 474 possessed by all three types of sporulenes (A, B and C) indicative of molecular formula $C_{35}H_{54}$. However, other significant fractions observed were indicative of probable aromatic side chain moiety in the structure of sporulenes (Kontnik et al. 2008). Overall results suggested that the presence of complete sporulene biosynthetic pathway in an organism (at genomic level) is associated with the presence of sporulenes at phenotypic level.

Microscopic observations of spore revealed that, the lysozyme treatment and detergent washes used to obtain clean spore preparation essentially lysed and eliminated the vegetative cells and isolated spores exhibited autofluorescence (Fig. 17). However, as per the previous studies, this autofluorescence property observed in *B. subtilis* spores is a common phenomenon observed due to many factor such as, nicotinamide adenine

dinucleotide (NADH), riboflavin content, few amino acids, flavin compounds and other carotenoid like pigments (Laflamme et al. 2004, Müllerová et al. 2022).

4.3 Genetic potential of sporulene biosynthesis amongst the class *Bacilli* members

Well-established classical techniques are employed to extract and identify wide range of secondary metabolites, produced by bacteria. The increased bacterial discoveries and upscale conventional techniques used for *in vitro* analysis of bacterial metabolites, made it challenging and time-consuming process (Baltz 2019, Chen et al. 2019). Therefore, classical methods were replaced with more cost-effective and rapid *in silico* methods of gathering such information; as a result, the identification of novel biosynthetic pathways today mainly relies on genome sequencing and microbial genome mining for unexplored biosynthetic gene clusters (BGCs) (Tracanna et al. 2017).

An organism is considered to become more complex during the process of evolution, however it has been observed that it often grows and survives by simplifying its scheduled life and reducing the complexity with new adaptations (Wolf and Koonin 2013). The strategies of this evolutionary adaptation include loss or gain of the pathway or related genes once they are no farther required because of access to the resulting compound from an external resource (Browne et al. 2021, Fani and Fondi 2009). Otherwise, in response to environmental stress, the organism prefers to establish new metabolic cascades (Copley et al. 2000, Wang et al. 2019). Sporulenes, have been reported to attribute to one such response mechanism of *B. subtilis* endospore towards oxidative stress (Checinska et al. 2012, Bosak et al. 2008). Thus, keeping in mind the recent bioinformatic advances for identifying new metabolites, novelty of sporulene molecule and its understudied distribution in the domain bacteria, we tried to understand the potential of class *Bacilli* member to synthesis sporulenes with the help of genome mining study.

4.3.1 Why class *Bacilli*?

Spore formation is a distinctive characteristic of the members of the phylum *Bacillota* (Galperin 2016). However, the phylum *Bacillota* includes both sporogenic and asporogenic members (Onyenwoke et al. 2004), where most of the spore-forming members belong to class *Bacilli* (Gopal et al. 2015). Keeping in mind, the association of sporulenes with bacterial endospore (Kontnik et al. 2008), here we postulated that this pathway is more likely to be associated to the class *Bacilli* members and accordingly the study was conducted to understand the theoretical distribution of sporulene biosynthesis pathway amongst the class *Bacilli* members.

4.3.2 Genome based diversity and abundance of sporulene biosynthesis pathway genes

Genome mining study (Table 10) revealed that, the two components (*hepS and hepT*) of the first enzyme heptaprenyl diphosphate synthase were consistently present in most of the screened members of the order *Caryophanales*, the family *Enterococcaceae* and *Streptococcaceae* irrespective of the presence or absence of other enzymes involved in the biosynthesis of sporulenes (Fig. 21). The two components of heptaprenyl diphosphate synthase constitute a composite enzyme that brings about the condensation of IPP and FPP into heptaprenyl diphosphate (Sato et al. 2013, Takigawa et al. 2010). Apart from sporulene biosynthesis, heptaprenyl diphosphate can be channelized into other metabolic pathways which includes terpenoid backbone biosynthesis and biosynthesis of secondary metabolites (Davis and Croteau 2000). Here, we speculate that the organisms with only *hepS* and *hepT* genes and no other genes encoding for putative proteins of sporulene biosynthesis, must be channelizing this enzyme components in other terpenoid biosynthesis pathways. The terpenoid cyclase enzyme, although not evenly distributed amongst the class *Bacilli* members, it was consistent amongst the family *Bacillaceae* and highly conserved amongst the genus *Bacillus* members indicating its commitment to sporulene synthesis with the

family *Bacillaceae* and the genus *Bacillus* members (Smita et al. 2023). It appeared that, unlike members of the order *Caryophanales,* none of the order *Lactobacillales* members were identified with all the sporulene biosynthesis genes in them (Fig. 20), which could be corelated with the non-spore-forming nature of the order *Lactobacillales* members (Galperin 2016). Previously, it was hypothesised that the property of spore formation could be eliminated several times in different lineages of the phylum *Bacillota,* in order to adapt to life in nutrient-rich conditions (Bate et al. 2014, Hutchison et al. 2014, Onyenwoke et al. 2004). Apparently, the loss of spore forming property in the *Lactobacillales* members resulted in the loss of sporulene biosynthesis phenomenon. Whereas, the order *Caryophanales* members of the family *Bacillaceae*, *Caryophanaceae*, *Paenibacillaceae* and *Sporolactobacillaceae* harboring all the pathway proteins could be linked with the sporulating nature of these members (Galperin 2016, Smita et al. 2023). It strikes as, the capacity to endure challenging environmental conditions by endospores having sporulenes is conditional and such conditions remain unexplored.

4.3.3 Conserved nature of sporulene pathway genes and possible functional-interactions

The highly conserved nature of tetraprenyl-β-curcumene synthase enzyme (YtpB protein) as observed through multiple sequence alignment (Fig. 23) suggested that, likely to the key enzyme sporulenol synthase (SqhC), the protein YtpB can also act as highlighting characteristic of bacterial members having sporulene biosynthesis capacity. Whereas, amino acid variability of the enzymes HepS, HepT and SqhC indicated their dynamic nature and possible involvement in wide variety of functions (Davis and Croteau 2000; for SqhC refer section 4.1.3 and 4.2.3). Also, not so conserved nature of these proteins amongst family *Bacillacea*e members, hinted towards uncertainty of some members of the family to contribute to sporulene synthesis. On the other side, gene

arrangements through SyntTax accessed all the completely sequenced prokaryotic genomes, of which class *Bacilli* members (includes family *Bacillaceae* and the genus *Bacillus*) were used to analyze syntenies of genes involved in sporulene biosynthesis. Besides the conserved nature of YtpB protein, the alike orthology of all the sporulene pathway genes were exclusive and endemic to the genus *Bacillus* members.

The conserved gene order and co-occurrence of sporulene biosynthetic gene cluster amongst the genus *Bacillus* members (Fig. 22 and Fig. 24) was in accordance with the proposed functional interaction between genes (Bosak et al. 2008, Oberto 2013). Syntenies amongst the genus *Bacillus* members unraveled that genes *sqhC* and *sodF* were located together (Fig. 22D) as also observed in a two-operon system of *B. subtilis* (Bosak et al. 2008). Generally, the genes situated in same operon are functionally related or share common function and the gene expression occurs hand-in-hand for such genes (Mihelčić et al. 2019). Protein product of *sodF* gene dissect the superoxide into hydrogen peroxide and oxygen (McCord and Fridovich 1969) thus plays a part in oxidative stress related system. The first hint about the proposed role of sporulene in alleviating oxidative stress, thus came from role of superoxide dismutase (*sodF*) situated after *sqhC* gene (Bosak et al. 2008). Further, the STRING network (Fig. 25) has validated the functional interaction of these genes based on co-occurrence (Szklarczyk et al. 2023), co-expression (van Dam et a. 2018, Anand et al. 2016) and literature mining confirming their involvement in sporulene biosynthesis.

Overall, *in-silico* analysis of genomes stated that, sporulenes are not prevalent to diverse bacterial taxa but are associated only with the spore forming members. In conclusion, this study provides an unclouded picture of the conserved and endemic nature of the sporulene biosynthesis among members of the genus *Bacillus*; nevertheless, experimental validation is required to substantiate the computer-based analysis. The

conserved nature and unequivocal distribution of pathway genes within the genus *Bacillus* members advocate that sporulenes can form a core property of the genus *Bacillus*. However, *in vitro* screening for these C_{35} triterpenoids in endospore of the genus *Bacillus* members might give improved idea, if sporulenes can be endorsed as a signature molecule for the genus *Bacillus*.

4.4 Insights into peroxide toxicity: sporulenes aids in resistance of endospore of *B. subtilis* to hydrogen peroxide

Hydrogen peroxide based products are being utilised more frequently in a variety of environmental applications, including water treatment, as well as in the medical, food, and industrial sectors (Alcalá--Delgado et al. 2018, Jia et al. 2022, Lin et al. 2018, Mamaye et al. 2022, McEvoy and Rowan 2019, Zhao et al. 2021). On the other hand, a huge amount of H_2O_2 is being produced as a consequence of photoactivation of hazardous polycyclic aromatic hydrocarbons (PAH), UV filters in cosmetic waste, chromophoric and non-chromophoric dissolved organic matter (CDOM), creating a serious threat to microbial communities (Clark et al. 2009, Lesser 2006, Pelletier et al. 2006, Sánchez-Quiles and Tovar-Sánchez 2014). The ecological risks posed by H_2O_2 as to how it affects fish, macroinvertebrates, zooplankton and animals was well documented (Burson et al. 2014, Matthijs et al. 2012, Reichwaldt et al. 2012). However, the influence of H_2O_2 toxicity on microbial ecology and responses of microorganisms to it are less understood (Glaeser et al. 2014, Lin et al. 2018). One of the most common mechanism of such a stress response possessed by microorganisms is formation of spores (Brun and Shimkets 2000). In general, bacterial cells are more vulnerable to cytotoxicity induced by H_2O_2 (Boateng et al. 2011). Contrary to vegetative cells, bacterial spores can withstand considerable number of stress factors like moist and dry heat, toxic chemicals, UV as well as gamma radiation and oxidising agents (Chen et al. 2006, Nicholson et al. 2000). Though, majority of the spores

are killed with the use of appropriate concentration of H_2O_2, many spores remain tolerant and viable (Linley et al. 2012, Murdoch et al. 2016). The mechanism of such resistance possessed by the bacterial endospores is less understood. The present study addresses this question, majorly focusing on sporulenes as these are unique to the bacterial endospores. In this study, two strains of *B. subtilis* (strain PY79 [WT] and strain TB10 [sporulene deficient mutant/*ΔsqhC* mutant]) were used to understand the interplay between sporulenes and H_2O_2 resistance of endospores. Our results based on spore cultivability, microscopic observations, spore macromolecular composition and molecular analysis suggests that the toxic effects of H_2O_2 can be more profound to sporulene deficient spores, as sporulenes bolsters the inner membrane of the spores thereby protecting it from peroxide toxicity.

4.4.1 Altered cellular responses of Δ*sqhC* mutant *B. subtilis* under stress

Majority of the studies till date have claimed that sporulenes were exclusively present in endospore and have a possible role in alleviating the oxidative stress (Bosak et al. 2008, Kontnik et al. 2008). However, this study speculated the association of sporulenes with vegetative cells of *B. subtilis*. Here the affected growth of *ΔsqhC* mutant under H_2O_2 stress (Fig. 28B), led to the speculation that, the induction of sporulene synthesis due to H_2O_2 stress could be helping WT cells to sustain better growth unlike *ΔsqhC* mutant *B. subtilis*. This observation was further supported by real time analysis, which indicated the activation and upregulation of sporulene biosynthesis pathway genes on encounter with H_2O_2 in WT *B. subtilis* PY79 (Fig. 29C). Therefore, in addition to the previous reports of synthesis of sporulenes at the onset of sporulation due to activation of σ^E (Bosak et al. 2008, Eichenberger et al. 2003); our growth analysis and expression studies suggested that synthesis of sporulenes could also happen in exponentially growing cells as a consequence of oxidative stress induction. Although, how this induction actually occurs and what are the factors involved remains unknown. Overall, the cellular responses from the present

study were in accordance with the previous research on C_{35} terpenes from *B. subtilis* KSM6-10 (Takigawa et al. 2010), which suggested the association of sporulenes with both vegetative cells and spores. These findings suggested that, though not an obligate requirement but sporulenes might be important to vegetative cells in order to sustain normal cellular growth under H_2O_2 stress. Whereas, it seemed that the other stressors (ethanol and butanol) and used concentrations were gentle enough, and did not cause any substantial change in the growth pattern of both WT and *ΔsqhC* mutant *B. subtilis* (Fig. 27, A, B).

4.4.2 Increased sensitivity of sporulene deficient spores to various stress conditions

Assessment of cultivability revealed that, the spores of WT as well as *ΔsqhC* mutant *B. subtilis* were differently sensitive to various stress applied under this study, however in all the stress conditions the cultivability of *ΔsqhC* mutant spore was affected more than WT spores (Fig. 30A, B). As compared to other stress conditions used, spores of both strains were more sensitive to H_2O_2 stress (Fig. 30A) mostly due to the strong oxidizing activity of H_2O_2 (Checinska et al. 2012). The loss in cultivability of WT spores could be reasoned with the already known factors such as spore enzyme inactivation (Palop et al. 1996, Palop et al. 1998), spore coat degeneration (King and Gould 1969) and unusually low wet density of spore after peroxide treatment (Melly et al. 2002). Moreover higher susceptibility of *ΔsqhC* mutant spores to H_2O_2 suggested that, in addition to the above listed reasons for loss in cultivability, absence of sporulenes could be considered as one promising reason for the increased loss of cultivability of *ΔsqhC* mutant spores. Further, in order to understand the molecular mechanism of the loss in cultivability of sporulene deficient spore, microscopic analysis were conducted.

4.4.3 Microscopic observations anticipated morphological alteration of *ΔsqhC* mutant spores exposed to H_2O_2

The extraordinary resistance property of spores is accredited to its unique structural components like relatively impermeable inner membrane, spore coat (Driks and Stelow 2000, Nicholson et al. 2000) and the biochemical composition of these components (Checinska et al. 2012). However, our microscopic observations unravelled the physical damage to sporulene deficient spore of *B. subtilis* exposed to H_2O_2, unlikely to WT *B. subtilis* spores. The surface level and ultrastructural changes observed through SEM and TEM indicated deformed spore structure and possible membrane damage to H_2O_2 treated *ΔsqhC* mutant spores (Fig. 31D and Fig. 32D). It is established that, inner membrane of *B. subtilis* spores remains unaffected after H_2O_2 exposure, preventing the leakage of DPA and protecting the spore core encapsulated by membrane (Melly et al. 2002). The intact spore membrane prevents the attack of many sporicidal factors on germination receptors and disallow their entry to the spore core, which in turn preserves the viability of spores (Driks 1999, Driks and Setlow 2000, Paidhungat et al. 2000). Perhaps our microscopic observations anticipated that, in case of sporulene deficient spores the possible damage to inner membrane due to H_2O_2 toxicity allowed for its easy access to germination receptors on inner membrane resulting in their inactivation (Moir and Smith 1990, Sakae et al. 1995) and rendering the spores incapable of germination thus the loss of cultivability as observed in plate count assay (Fig. 30B). The observed membrane damage was eventually supported by loss of DPA from spore core.

4.4.4 H_2O_2 toxicity resulted in leaky membrane of spores of *ΔsqhC* mutant *B. subtilis*

The DPA quantification explained that, spores of WT *B. subtilis* had slightly higher DPA content than spores of *ΔsqhC* mutant (Fig. 33, A, B). According to our experimental

strategy and microscopic observations, there was no variations in weight and size of spores that could have been a contributing factor for difference in DPA content (Fichtel et al. 2007). Thus, this slight disparity in DPA content might be attributed to the difference in spore volumes (Kort et al. 2005). The germination of WT and *ΔsqhC* mutant spores under control condition suggested no significant difference in the amount of DPA released but slight difference was observed in the amount of DPA retained (Fig. 33, C, D). This difference mostly accounts for the difference in DPA content of WT and *ΔsqhC* as observed in (Fig. 33, A, B). Whereas on germination of H_2O_2 treated *ΔsqhC* mutant spores, a profound decrease in the amount of DPA released and retained was observed contrary to WT spores (Fig. 33, C, D); clearly indicating the untimely loss of DPA which could happen through leaky spore membranes as also suggested by microscopic observations. Given that the amount of DPA released is often proportional to germination rates of spore (Woese and Morowitz 1958), the lowered release of DPA cannot be ignored as one of the factor responsible for affected germination rates and reduced cultivability of H_2O_2 treated *ΔsqhC* mutant spores (Fig. 30B).

The leaky membrane of H_2O_2 treated *ΔsqhC* mutant spores was finally confirmed through PI staining. Being a membrane impermeable dye, PI stains nucleic acid only when membrane is compromised (Mathys et al. 2007, Rao et al. 2016, Reineke et al. 2013); in accordance, all the autoclaved spores were stained with PI (Fig. 34, A-i, A-ii, A-iii) indicative of complete membrane damage of entire spore population. It led to a conclusion that, on account of its severity autoclaving destroyed nearly all the spores by disrupting spore inner membrane (Coleman et al. 2007, Zhang et al. 2007). Whereas, untreated spores of WT and *ΔsqhC* mutant remained unstained (Fig. R34, B-i, B-ii, B-iii; C-i, C-ii, C-iii) most probably due to the inability of PI to percolate through intact membrane (Laflamme

et al. 2004). Negligible uptake of PI was observed for H_2O_2 treated WT spores (Fig. 34, D-i, D-ii, D-iii) contrary to high PI fluorescence of H_2O_2 treated *ΔsqhC* mutant spores (Fig. 34, E-i, E-ii, E-iii), that gave hint of relatively unaffected membrane of WT spores and confirmed the highly compromised permeability barrier of *ΔsqhC* mutant spores due to H_2O_2 toxicity, albeit nor as severely as autoclaved spores.

4.4.5 Pre-exposure of *ΔsqhC* mutant *B. subtilis* to H_2O_2 led to refurbished spore composition

Maintaining membrane homeostasis is crucial for cell survival thus bacteria rapidly evolved their ability to modify membrane lipids (Zhang and Rock 2008). The sporulene deficient spores were challenged by H_2O_2 stress, therefore to make up for sporulene deficit and retain membrane strength, *ΔsqhC* mutant *B. subtilis* appeared to have refurbished the fatty acids composition of its spore on pre-exposure to H_2O_2 (Table 11, Fig. 35, A, B). It was evidenced by an increased level of saturated and *iso* fatty acids for tightly stacked and compact membranes (Zang and Rock 2008) and decreased level of *cis*-unsaturated fatty acids which increases the permeability of membrane (Gennis 2013). However, considering the crucial role of proteins in maintaining spore structure (Rabi et al. 2018), the presence of some specific proteins (Fig. 36 A, B, C, D, Table S1 A, B, C,D) known for their auxiliary role in sporulation, spore germination and spore morphogenesis under stress condition (Allenby et al. 2006, Bagyan et al. 1998, Johnson and Moir. 2017, Lin and Rye 2006, Ozin et al. 2001, Ross and Abel-Santos 2010, Zheng et al. 2016) suggested the altered protein composition of *ΔsqhC* mutant *B. subtilis* spores, as a response to H_2O_2 toxicity.

It is evident from this study that, sporulenes probably insulate the endospore core and protect it from oxidizing agents like H_2O_2 and thus forms a major driving force in considering it as a critical factor in spore DNA protection. Sporulenes may offer this

protection in two possible ways; first, by forming a defensive barrier by integrating in membrane (of cell or spore) in an arrangement that effectively mimic bacteriohopanetetrol and second, by forming a chemical barricade in the form of O_2 scavengers that could be resulting from oxidation of its cyclohexadiene to a phenyl moiety (Kontnik et al 2008). Thus, apart from the usual factors that help in spore resistance to peroxide toxicity, sporulenes play an explicit role in mitigating the cytotoxic and genotoxic effects of H_2O_2 and gives an extra tier of protection to endospores of *B. subtilis*.

Major findings

MAJOR FINDINGS

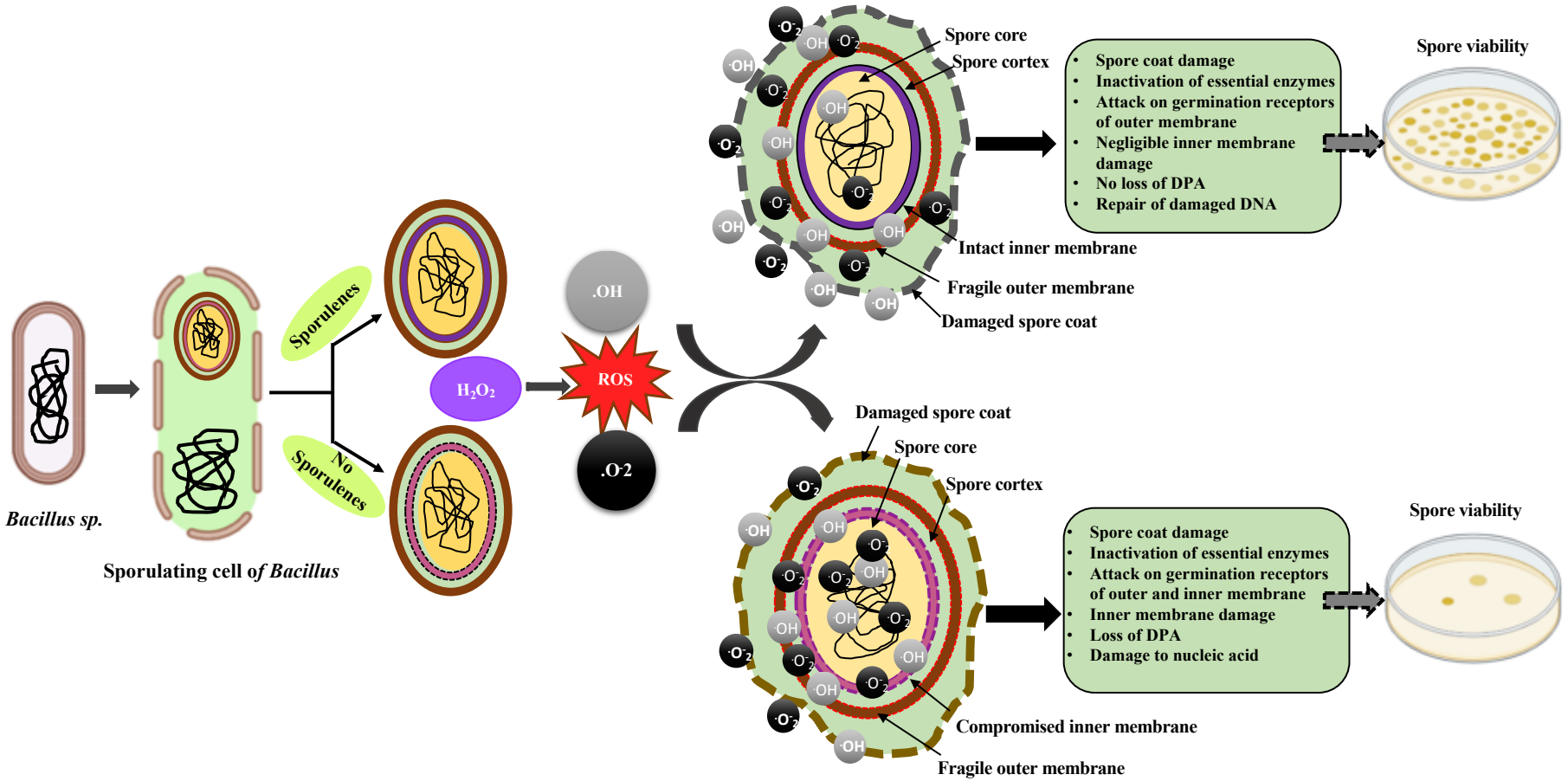
- The presence of hopanoids (but not sporulenes) among the screened members of the *Planctomycetota* in this study indicates their probable wide distribution among other taxa of this phylum and other phyla.
- A non-canonical hopanoid biosynthetic pathway was discovered among a few *Planctomycetota* members.
- Sporulenes are widely distributed among the members of the genus *Bacillus*.
- *In silico* analysis revealed that, the genes encoding probable proteins (HepS, HepT, YtpB, SqhC) for the biogenesis of sporulenes are restricted to the families (*Bacillaceae*, *Caryophanaceae*, *Paenibacillaceae, Sporolactobacillacea*) of the order *Caryophanales* but not among the *Lactobacillales.*
- The enzyme terpenoid cyclase encoded by gene *ytpB* is highly conserved amongst the members of the genus *Bacillus;* gene *ytpB* is in synteny with *hepS, hepT* and *sqhC.*
- Sporulene deficiency results in endospore damage due to the synergistic effect of more than one factor such as:
  - Peroxide induced damage to the inner membrane
  - Loss of DPA through leaky membrane
  - Consequent inactivation of germination receptors
  - Genotoxicity to spore genetic material
- The pre-encounter of sporulene deficient mutant *B. subtilis* with peroxide toxicity led to refurbishing of composition of its spores, as a part of spore defence system.
- Sporulenes form an integral parts of the endospore membrane and add an extra tier of resistance to *Bacillus* endospores thus play an explicit role in mitigating the damages associated with peroxide stress.

- The presence of sporulenes also in the vegetative cells probably indicates their additional role in overcoming stress, particularly from H_2O_2 used in this study.

OUTCOME OF THE STUDY

- In summary, the genome mining study conveys that, sporulenes can be considered as a signature-biomolecules of the genus *Bacillus* owning to its endemic nature; however not all spore producers are sporulene producers.
- Peroxide toxicity is one of the major factors which influences the survivability of vegetative cells and spores, this study for the first time demonstrated extensive evidences that, sporulenes play a pivotal role in the survival of bacterial endospores. Our study provides reference for "sporulenes" as a new found response of *Bacillus* endospores to peroxide toxicity rooted directly from the natural sources, disinfectants or indirectly formed as the consequential product of many other environmental pollutants and hazardous chemicals.

Quick glance


Bacillus sp.
Sporulating cell of Bacillus
Sporulenes
No Sporulenes
H2O2
.OH
ROS
.O-2
Spore core
Spore cortex
Intact inner membrane
Fragile outer membrane
Damaged spore coat
Spore coat damage
Inactivation of essential enzymes
Attack on germination receptors of outer membrane
Negligible inner membrane damage
No loss of DPA
Repair of damaged DNA
Spore viability
Damaged spore coat
Spore core
Spore cortex
Compromised inner membrane
Fragile outer membrane
Spore coat damage
Inactivation of essential enzymes
Attack on germination receptors of outer and inner membrane
Inner membrane damage
Loss of DPA
Damage to nucleic acid
Spore viability

Supplementary data

Accession	Entry	Description
Molecular functions		
DNA Replication		
A0A6M3Z6D7	A0A6M3Z6D7_BACSU	Methionine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=metG PE=3 SV=1
A0A6M3Z6P2	A0A6M3Z6P2_BACSU	DNA-directed RNA polymerase subunit beta' OS=Bacillus subtilis (strain 168) OX=224308 GN=rpoC PE=3 SV=1
A0A6M3ZAK9	A0A6M3ZAK9_BACSU	ATP-dependent DNA helicase RecG OS=Bacillus subtilis (strain 168) OX=224308 GN=recG PE=3 SV=1
A0A6M3ZBW7	A0A6M3ZBW7_BACSU	DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=recQ PE=3 SV=1
A0A6M3ZDE4	A0A6M3ZDE4_BACSU	ATP-dependent DNA helicase RecQ OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13785 PE=4 SV=1
A0A6M3ZEW6	A0A6M3ZEW6_BACSU	Threonine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=thrS PE=3 SV=1
A0A6M3ZFD9	A0A6M3ZFD9_BACSU	DNA polymerase III subunit alpha OS=Bacillus subtilis (strain 168) OX=224308 GN=dnaE PE=3 SV=1
A0A6M3ZH20	A0A6M3ZH20_BACSU	Arginine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=argS PE=3 SV=1
A0A6M3ZIY8	A0A6M3ZIY8_BACSU	Exodeoxyribonuclease 7 large subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=xseA PE=3 SV=1
A0A6M4JKZ0	A0A6M4JKZ0_BACSU	DNA polymerase I OS=Bacillus subtilis (strain 168) OX=224308 GN=polA PE=3 SV=1
Q45493	RNJ1_BACSU	Ribonuclease J1 OS=Bacillus subtilis (strain 168) OX=224308 GN=rnjA PE=1 SV=1
A0A6M3Z837	A0A6M3Z837_BACSU	Ribonuclease YeeF family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03880 PE=4 SV=1
A0A6M3Z8A8	A0A6M3Z8A8_BACSU	DNA relaxase NicK OS=Bacillus subtilis (strain 168) OX=224308 GN=nicK PE=3 SV=1
A0A6M3ZAM9	A0A6M3ZAM9_BACSU	16S rRNA m5C967 methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=rsmB PE=3 SV=1
A0A6M3ZBT0	A0A6M3ZBT0_BACSU	LysR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10200 PE=3 SV=1
A0A6M3ZC49	A0A6M3ZC49_BACSU	DNA-directed DNA polymerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10985 PE=4 SV=1
A0A6M3ZCT6	A0A6M3ZCT6_BACSU	Serine-type integrase SprA OS=Bacillus subtilis (strain 168) OX=224308 GN=sprA PE=4 SV=1
A0A6M3ZDQ1	A0A6M3ZDQ1_BACSU	DEAD/DEAH box helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13375 PE=4 SV=1
A0A6M3ZGR7	A0A6M3ZGR7_BACSU	GTPase Der OS=Bacillus subtilis (strain 168) OX=224308 GN=engA PE=3 SV=1
A0A6M3ZHU5	A0A6M3ZHU5_BACSU	Ribonuclease YxiD OS=Bacillus subtilis (strain 168) OX=224308 GN=yxiD PE=4 SV=1
A0A6M3ZK18	A0A6M3ZK18_BACSU	4Fe-4S dicluster domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21325 PE=4 SV=1
A0A6M3ZKA6	A0A6M3ZKA6_BACSU	M48 family metalloprotease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22150 PE=4 SV=1
A0A6M4JIN7	A0A6M4JIN7_BACSU	DNA topoisomerase 4 subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=parC PE=3 SV=1
A0A6M4JJ92	A0A6M4JJ92_BACSU	PIN/TRAM domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_00590 PE=3 SV=1
A0A6M4JJB7	A0A6M4JJB7_BACSU	DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11310 PE=3 SV=1
A0A6M4JLU8	A0A6M4JLU8_BACSU	DNA topoisomerase 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=topA PE=3 SV=1
A0A6M4JLW0	A0A6M4JLW0_BACSU	ATP-dependent DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13325 PE=4 SV=1
A0A6M4JNB0	A0A6M4JNB0_BACSU	Ribonucleoside-diphosphate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10885 PE=3 SV=1
A0A6M4JPX6	A0A6M4JPX6_BACSU	Ribonuclease YeeF family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20790 PE=4 SV=1
A0A6M4JJK9	A0A6M4JJK9_BACSU	tRNA (N(6)-L-threonylcarbamoyladenosine(37)-C(2))-methylthiotransferase MtaB OS=Bacillus subtilis (strain 168) OX=224308 GN=mtaB PE=4 SV=1
A0A6M4JD30	A0A6M4JD30_BACSU	Acyl--CoA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02395 PE=4 SV=1
A3F305	A3F305_BACSU	ATP-dependent helicase (Fragment) OS=Bacillus subtilis (strain 168) OX=224308 GN=dinG PE=4 SV=1
A0A6M3Z7G6	A0A6M3Z7G6_BACSU	tRNA uridine(34) hydroxylase OS=Bacillus subtilis (strain 168) OX=224308 GN=trhO PE=3 SV=1
DNA Repair		

A0A6M3ZB00	A0A6M3ZB00_BACSU	DNA mismatch repair protein MutS OS=Bacillus subtilis (strain 168) OX=224308 GN=mutS PE=3 SV=1
A0A6M3ZEJ8	A0A6M3ZEJ8_BACSU	Single-stranded-DNA-specific exonuclease RecJ OS=Bacillus subtilis (strain 168) OX=224308 GN=recJ PE=3 SV=1
A0A6M4JDS6	A0A6M4JDS6_BACSU	Class I SAM-dependent DNA methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03850 PE=4 SV=1
A0A6M4JGL3	A0A6M4JGL3_BACSU	DNA repair/recombination ATPase SbcE OS=Bacillus subtilis (strain 168) OX=224308 GN=sbcE PE=4 SV=1
A0A6M4JJK7	A0A6M4JJK7_BACSU	Type II toxin-antitoxin system toxin ribonuclease YqcG OS=Bacillus subtilis (strain 168) OX=224308 GN=yqcG PE=4 SV=1

Transcription regulator

A0A6M3Z7Z6	A0A6M3Z7Z6_BACSU	Glucitol operon transcriptional regulator GutR OS=Bacillus subtilis (strain 168) OX=224308 GN=gutR PE=4 SV=1
A0A6M3ZDU4	A0A6M3ZDU4_BACSU	Sigma-54-dependent Fis family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14340 PE=4 SV=1
A0A6M3ZEE9	A0A6M3ZEE9_BACSU	Elongation factor Ts OS=Bacillus subtilis (strain 168) OX=224308 GN=tsf PE=3 SV=1
A0A6M3ZHF7	A0A6M3ZHF7_BACSU	Transcriptional regulator LicR OS=Bacillus subtilis (strain 168) OX=224308 GN=licR PE=4 SV=1
A0A6M3ZKS3	A0A6M3ZKS3_BACSU	Helix-turn-helix transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05990 PE=4 SV=1
A0A6M4JCE0	A0A6M4JCE0_BACSU	Anti-sigma-W factor RsiW OS=Bacillus subtilis (strain 168) OX=224308 GN=rsiW PE=3 SV=1
A0A6M4JFI2	A0A6M4JFI2_BACSU	Anti-sigma-I factor RsgI OS=Bacillus subtilis (strain 168) OX=224308 GN=rsgI PE=4 SV=1
A0A6M4JE45	A0A6M4JE45_BACSU	Transcriptional regulator YesS OS=Bacillus subtilis (strain 168) OX=224308 GN=yesS PE=4 SV=1
A0A6M4JR12	A0A6M4JR12_BACSU	TetR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22690 PE=4 SV=1

Transport and chemotaxis and flageller movement

Basic transport & secretion system

A0A6M3Z760	A0A6M3Z760_BACSU	Zinc ABC transporter permease ZnuB OS=Bacillus subtilis (strain 168) OX=224308 GN=znuB PE=3 SV=1
A0A6M3ZAU0	A0A6M3ZAU0_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07885 PE=4 SV=1
A0A6M3ZDM8	A0A6M3ZDM8_BACSU	Arginine ABC transporter substrate-binding protein ArtP OS=Bacillus subtilis (strain 168) OX=224308 GN=artP PE=3 SV=1
A0A6M3ZFE7	A0A6M3ZFE7_BACSU	Carbohydrate ABC transporter substrate-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17570 PE=4 SV=1
A0A6M3ZKF2	A0A6M3ZKF2_BACSU	Amino acid ABC transporter substrate-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22515 PE=3 SV=1
A0A6M4JCP7	A0A6M4JCP7_BACSU	Transporter substrate-binding domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01975 PE=3 SV=1
A0A6M4JE53	A0A6M4JE53_BACSU	Sugar ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04035 PE=3 SV=1
A0A6M4JN68	A0A6M4JN68_BACSU	Bacitracin ABC transporter permease BceB OS=Bacillus subtilis (strain 168) OX=224308 GN=bceB PE=3 SV=1
P42178	NARJ_BACSU	Probable nitrate reductase molybdenum cofactor assembly chaperone NarJ OS=Bacillus subtilis (strain 168) OX=224308 GN=narJ PE=3 SV=1
A0A6M3Z7K9	A0A6M3Z7K9_BACSU	Potassium transporter KimA OS=Bacillus subtilis (strain 168) OX=224308 GN=kimA PE=4 SV=1
A0A6M3ZAE5	A0A6M3ZAE5_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07735 PE=4 SV=1
A0A6M3ZAG9	A0A6M3ZAG9_BACSU	Dipicolinic acid transporter SpoVV OS=Bacillus subtilis (strain 168) OX=224308 GN=spoVV PE=4 SV=1
A0A6M3ZBM8	A0A6M3ZBM8_BACSU	MATE family efflux transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09975 PE=4 SV=1
A0A6M3ZCF4	A0A6M3ZCF4_BACSU	YfcC family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01770 PE=4 SV=1
A0A6M3ZE21	A0A6M3ZE21_BACSU	Na/Pi symporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15010 PE=4 SV=1
A0A6M3ZE94	A0A6M3ZE94_BACSU	Branched-chain amino acid transport system carrier protein OS=Bacillus subtilis (strain 168) OX=224308 GN=brnQ PE=3 SV=1
A0A6M3ZF35	A0A6M3ZF35_BACSU	Diguanylate cyclase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17265 PE=4 SV=1
A0A6M3ZFI4	A0A6M3ZFI4_BACSU	ABC transporter substrate-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17735 PE=4 SV=1
A0A6M3ZG80	A0A6M3ZG80_BACSU	Type VII secretion protein EssC OS=Bacillus subtilis (strain 168) OX=224308 GN=essC PE=4 SV=1

A0A6M3ZHG6	A0A6M3ZHG6_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21350 PE=4 SV=1
A0A6M3ZHQ2	A0A6M3ZHQ2_BACSU	Citrate/malate transporter CimH OS=Bacillus subtilis (strain 168) OX=224308 GN=cimH PE=3 SV=1
A0A6M3ZHU7	A0A6M3ZHU7_BACSU	Gluconate permease GntP OS=Bacillus subtilis (strain 168) OX=224308 GN=gntP PE=4 SV=1
A0A6M3ZI39	A0A6M3ZI39_BACSU	Metallophosphoesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22885 PE=4 SV=1
A0A6M3ZI65	A0A6M3ZI65_BACSU	Efflux RND transporter permease subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03825 PE=4 SV=1
A0A6M3ZK31	A0A6M3ZK31_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21435 PE=4 SV=1
A0A6M3ZKM1	A0A6M3ZKM1_BACSU	Nucleoside permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18680 PE=3 SV=1
A0A6M3ZLA5	A0A6M3ZLA5_BACSU	Allantoin permease OS=Bacillus subtilis (strain 168) OX=224308 GN=pucI PE=3 SV=1
A0A6M4JCE4	A0A6M4JCE4_BACSU	Probable inorganic carbon transporter subunit DabA OS=Bacillus subtilis (strain 168) OX=224308 GN=dabA PE=3 SV=1
A0A6M4JCS5	A0A6M4JCS5_BACSU	Dicarboxylate/amino acid:cation symporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01410 PE=3 SV=1
A0A6M4JDP1	A0A6M4JDP1_BACSU	Arsenical pump membrane protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03055 PE=3 SV=1
A0A6M4JG53	A0A6M4JG53_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04730 PE=4 SV=1
A0A6M4JHK0	A0A6M4JHK0_BACSU	MATE family efflux transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10590 PE=4 SV=1
A0A6M4JKY1	A0A6M4JKY1_BACSU	HlyC/CorC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18225 PE=3 SV=1
A0A6M4JL52	A0A6M4JL52_BACSU	Type VII secretion protein EssB OS=Bacillus subtilis (strain 168) OX=224308 GN=essB PE=3 SV=1
A0A6M4JLE6	A0A6M4JLE6_BACSU	Diguanylate cyclase DgcK OS=Bacillus subtilis (strain 168) OX=224308 GN=dgcK PE=4 SV=1
A0A6M4JMS1	A0A6M4JMS1_BACSU	AI-2E family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18145 PE=3 SV=1
A0A6M4JP02	A0A6M4JP02_BACSU	Type VII secretion protein EsaA OS=Bacillus subtilis (strain 168) OX=224308 GN=esaA PE=3 SV=1
A0A6M4JPT1	A0A6M4JPT1_BACSU	Membrane-bound protein LytA OS=Bacillus subtilis (strain 168) OX=224308 GN=lytA PE=4 SV=1
A0A6M3ZBH5	A0A6M3ZBH5_BACSU	KAP NTPase domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10335 PE=4 SV=1
A0A6M3Z709	A0A6M3Z709_BACSU	Glucarate transporter GudP OS=Bacillus subtilis (strain 168) OX=224308 GN=gudP PE=4 SV=1
Chemotaxis & flageller movement		
A0A6M3ZBG8	A0A6M3ZBG8_BACSU	Chemotaxis protein CheA OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08960 PE=4 SV=1
A0A6M3ZG40	A0A6M3ZG40_BACSU	Flagellar motor switch protein FliG OS=Bacillus subtilis (strain 168) OX=224308 GN=fliG PE=3 SV=1
A0A6M4JGD4	A0A6M4JGD4_BACSU	Flagellar protein export ATPase FliI OS=Bacillus subtilis (strain 168) OX=224308 GN=fliI PE=4 SV=1
A0A6M4JLK3	A0A6M4JLK3_BACSU	Methyl-accepting chemotaxis protein TlpB OS=Bacillus subtilis (strain 168) OX=224308 GN=tlpB PE=4 SV=1
A0A6M4JQ01	A0A6M4JQ01_BACSU	Flagellar basal body protein OS=Bacillus subtilis (strain 168) OX=224308 GN=flhP PE=3 SV=1
Kinase & tranferases		
A0A6M3Z7K0	A0A6M3Z7K0_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=dctS PE=4 SV=1
A0A6M3Z7S5	A0A6M3Z7S5_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=ydfH PE=4 SV=1
A0A6M3ZF44	A0A6M3ZF44_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=phoR PE=4 SV=1
A0A6M3ZFF8	A0A6M3ZFF8_BACSU	Phosphoenolpyruvate-protein phosphotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=ptsP PE=3 SV=1
A0A6M3ZJQ8	A0A6M3ZJQ8_BACSU	Signal transduction histidine-protein kinase/phosphatase DegS OS=Bacillus subtilis (strain 168) OX=224308 GN=degS PE=4 SV=1
A0A6M4JHL1	A0A6M4JHL1_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinD PE=4 SV=1
A0A6M4JR07	A0A6M4JR07_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=walK PE=4 SV=1
A0A6M3Z9B4	A0A6M3Z9B4_BACSU	GNAT family N-acetyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06070 PE=4 SV=1
A0A6M3ZH15	A0A6M3ZH15_BACSU	Glycosyl transferase family 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10580 PE=4 SV=1
A0A6M4JF26	A0A6M4JF26_BACSU	Macrolide 2'-phosphotransferase MphK OS=Bacillus subtilis (strain 168) OX=224308 GN=mphK PE=4 SV=1

A0A6M4JK44	A0A6M4JK44_BACSU	Phosphatidylglycerophosphatase A OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13150 PE=4 SV=1
A0A6M4JP41	A0A6M4JP41_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yvrG PE=4 SV=1
A0A6M4JPS2	A0A6M4JPS2_BACSU	Glycosyltransferase family 2 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20520 PE=4 SV=1
A0A6M3Z8H2	A0A6M3Z8H2_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yflR PE=4 SV=1
A0A6M3ZEN5	A0A6M3ZEN5_BACSU	Acetylglutamate kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=argB PE=3 SV=1
A0A6M3ZHQ0	A0A6M3ZHQ0_BACSU	Galactokinase OS=Bacillus subtilis (strain 168) OX=224308 GN=galK PE=3 SV=1
A0A6M4JGW5	A0A6M4JGW5_BACSU	Glycerol kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=glpK PE=3 SV=1
A0A6M4JP01	A0A6M4JP01_BACSU	Nicotinate phosphoribosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18470 PE=3 SV=1
P37564	COAX_BACSU	Type III pantothenate kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=coaX PE=1 SV=3
A0A6M3Z962	A0A6M3Z962_BACSU	Serine/threonine protein kinase PrkA OS=Bacillus subtilis (strain 168) OX=224308 GN=prkA PE=4 SV=1

Metabolic pathway

C/ N & lipid metabolism

A0A6M3Z8J1	A0A6M3Z8J1_BACSU	Alpha,alpha-phosphotrehalase OS=Bacillus subtilis (strain 168) OX=224308 GN=treC PE=3 SV=1
A0A6M3Z9T4	A0A6M3Z9T4_BACSU	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Bacillus subtilis (strain 168) OX=224308 GN=fabF PE=3 SV=1
A0A6M3ZAG6	A0A6M3ZAG6_BACSU	Pyruvate carboxylase OS=Bacillus subtilis (strain 168) OX=224308 GN=pyc PE=4 SV=1
A0A6M3ZB65	A0A6M3ZB65_BACSU	1-deoxy-D-xylulose 5-phosphate reductoisomerase OS=Bacillus subtilis (strain 168) OX=224308 GN=dxr PE=3 SV=1
A0A6M3ZBF4	A0A6M3ZBF4_BACSU	Glycoside hydrolase family 43 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09540 PE=3 SV=1
A0A6M3ZBR6	A0A6M3ZBR6_BACSU	Xylose isomerase OS=Bacillus subtilis (strain 168) OX=224308 GN=xylA PE=3 SV=1
A0A6M3ZLA7	A0A6M3ZLA7_BACSU	Cardiolipin synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=cls PE=3 SV=1
A0A6M3ZIC8	A0A6M3ZIC8_BACSU	Aminodeoxychorismate lyase OS=Bacillus subtilis (strain 168) OX=224308 GN=pabC PE=3 SV=1
A0A6M3ZK40	A0A6M3ZK40_BACSU	Agmatinase OS=Bacillus subtilis (strain 168) OX=224308 GN=speB PE=3 SV=1
A0A6M4JEW2	A0A6M4JEW2_BACSU	Beta-N-acetylhexosaminidase OS=Bacillus subtilis (strain 168) OX=224308 GN=nagZ PE=3 SV=1
A0A6M4JG47	A0A6M4JG47_BACSU	Carbamoyl-phosphate synthase large chain OS=Bacillus subtilis (strain 168) OX=224308 GN=carB PE=3 SV=1
A0A6M4JKL7	A0A6M4JKL7_BACSU	1-deoxy-D-xylulose-5-phosphate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=dxs PE=3 SV=1
A0A6M4JLF6	A0A6M4JLF6_BACSU	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=menD PE=3 SV=1
A0A6M4JMI0	A0A6M4JMI0_BACSU	Glycolate oxidase subunit GlcD OS=Bacillus subtilis (strain 168) OX=224308 GN=glcD PE=4 SV=1
A0A6M4JNS0	A0A6M4JNS0_BACSU	Alpha-glucosidase/alpha-galactosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17585 PE=3 SV=1
A0A6M3ZJI5	A0A6M3ZJI5_BACSU	Biofilm exopolysaccharide biosynthesis protein EpsG OS=Bacillus subtilis (strain 168) OX=224308 GN=epsG PE=4 SV=1
A0A6M4JIX2	A0A6M4JIX2_BACSU	M20/M25/M40 family metallo-hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14185 PE=4 SV=1
O31682	YKVQ_BACSU	Putative glycosylase YkvQ OS=Bacillus subtilis (strain 168) OX=224308 GN=ykvQ PE=3 SV=1

Amino acid metabolism

A0A6M3Z794	A0A6M3Z794_BACSU	Homocysteine S-methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=mmuM PE=4 SV=1
A0A6M3Z893	A0A6M3Z893_BACSU	Alkaline phosphatase PhoB OS=Bacillus subtilis (strain 168) OX=224308 GN=phoB PE=3 SV=1
A0A6M3Z9L3	A0A6M3Z9L3_BACSU	Carbamoyl-phosphate synthase large chain OS=Bacillus subtilis (strain 168) OX=224308 GN=carB PE=3 SV=1
A0A6M3Z9P9	A0A6M3Z9P9_BACSU	Glycine oxidase ThiO OS=Bacillus subtilis (strain 168) OX=224308 GN=thiO PE=4 SV=1
A0A6M3Z9S5	A0A6M3Z9S5_BACSU	Carbamoyl-phosphate synthase small chain OS=Bacillus subtilis (strain 168) OX=224308 GN=carA PE=3 SV=1
A0A6M3ZBP5	A0A6M3ZBP5_BACSU	Glutathione hydrolase proenzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=ggt PE=3 SV=1

A0A6M3ZFZ8	A0A6M3ZFZ8_BACSU	Homoserine dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18720 PE=3 SV=1
A0A6M4JC62	A0A6M4JC62_BACSU	Glutamate--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=gltX PE=3 SV=1
A0A6M4JEL1	A0A6M4JEL1_BACSU	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Bacillus subtilis (strain 168) OX=224308 GN=glmS PE=3 SV=1
A0A6M4JEP9	A0A6M4JEP9_BACSU	Cysteine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=cysS PE=3 SV=1
A0A6M4JHZ8	A0A6M4JHZ8_BACSU	Adenosine 5'-phosphosulfate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=cysH PE=3 SV=1
A0A6M4JIH4	A0A6M4JIH4_BACSU	Asparagine synthase (Glutamine-hydrolyzing) OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=3 SV=1
A0A6M4JLB2	A0A6M4JLB2_BACSU	Glutamate-1-semialdehyde 2,1-aminomutase OS=Bacillus subtilis (strain 168) OX=224308 GN=hemL PE=3 SV=1
P54420	ASNB_BACSU	Asparagine synthetase [glutamine-hydrolyzing] 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=1 SV=2
A0A6M3ZCJ5	A0A6M3ZCJ5_BACSU	L-lysine 2,3-aminomutase OS=Bacillus subtilis (strain 168) OX=224308 GN=ablA PE=3 SV=1
A0A6M4JGE4	A0A6M4JGE4_BACSU	Protein-glutamate methylesterase/protein-glutamine glutaminase OS=Bacillus subtilis (strain 168) OX=224308 GN=cheB PE=3 SV=1
Nucleotide metabolism		
A0A6M4JFL8	A0A6M4JFL8_BACSU	GMP synthase [glutamine-hydrolyzing] OS=Bacillus subtilis (strain 168) OX=224308 GN=guaA PE=3 SV=1
A0A6M4JG22	A0A6M4JG22_BACSU	Multifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase/5'-nucleotidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04385 PE=3 SV=1
A0A6M4JKT5	A0A6M4JKT5_BACSU	Bifunctional purine biosynthesis protein PurH OS=Bacillus subtilis (strain 168) OX=224308 GN=purH PE=3 SV=1
A0A6M3ZDC6	A0A6M3ZDC6_BACSU	Glycosyltransferase family 39 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07120 PE=4 SV=1
A0A6M3ZFM2	A0A6M3ZFM2_BACSU	Glycosyltransferase family 4 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17880 PE=3 SV=1
Central metabolism (TCA/electron transport)		
A0A6M3ZBL3	A0A6M3ZBL3_BACSU	2-oxoglutarate dehydrogenase E1 component OS=Bacillus subtilis (strain 168) OX=224308 GN=sucA PE=3 SV=1
A0A6M3ZBL7	A0A6M3ZBL7_BACSU	Bifunctional cytochrome P450/NADPH--P450 reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04105 PE=3 SV=1
A0A6M3ZEC0	A0A6M3ZEC0_BACSU	Bifunctional cytochrome P450/NADPH--P450 reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15965 PE=3 SV=1
A0A6M3ZGY7	A0A6M3ZGY7_BACSU	Bifunctional glyoxylate/hydroxypyruvate reductase B OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20000 PE=3 SV=1
A0A6M4JMD9	A0A6M4JMD9_BACSU	NADP-dependent malic enzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=maeB PE=3 SV=1
O34720	YJGC_BACSU	Probable oxidoreductase YjgC OS=Bacillus subtilis (strain 168) OX=224308 GN=yjgC PE=3 SV=1
A0A6M3ZLS6	A0A6M3ZLS6_BACSU	LLM class flavin-dependent oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22525 PE=3 SV=1
A0A6M4JM60	A0A6M4JM60_BACSU	Cytochrome P450 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15725 PE=3 SV=1
P35162	RESC_BACSU	Cytochrome c biogenesis protein ResC OS=Bacillus subtilis (strain 168) OX=224308 GN=resC PE=1 SV=2
A0A6M4JKP5	A0A6M4JKP5_BACSU	Cytochrome c biogenesis protein CcsA OS=Bacillus subtilis (strain 168) OX=224308 GN=ccsA PE=4 SV=1
A0A6M4JKS6	A0A6M4JKS6_BACSU	Acyl-CoA dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14365 PE=3 SV=1
A0A6M4JKR8	A0A6M4JKR8_BACSU	Cytochrome ubiquinol oxidase subunit I OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17795 PE=3 SV=1
A0A6M4JQI7	A0A6M4JQI7_BACSU	LLM class flavin-dependent oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21795 PE=4 SV=1
Cell wall synthesis		
A0A6M3ZI35	A0A6M3ZI35_BACSU	Glutamate racemase OS=Bacillus subtilis (strain 168) OX=224308 GN=racE PE=3 SV=1
A0A6M3Z8T2	A0A6M3Z8T2_BACSU	Lipoteichoic acid synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=ltaS PE=3 SV=1
A0A6M3ZG90	A0A6M3ZG90_BACSU	LTA synthase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19310 PE=3 SV=1
A0A6M3ZAJ3	A0A6M3ZAJ3_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08320 PE=3 SV=1

A0A6M3ZL66	A0A6M3ZL66_BACSU	Teichoic acid glycerol-phosphate primase OS=Bacillus subtilis (strain 168) OX=224308 GN=tagB PE=3 SV=1
A0A6M4JBY5	A0A6M4JBY5_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_00075 PE=3 SV=1
A0A6M4JIN4	A0A6M4JIN4_BACSU	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=murF PE=3 SV=1
A0A6M3ZEE3	A0A6M3ZEE3_BACSU	Transglycosylase SLT domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15330 PE=4 SV=1
A0A6M3ZHH5	A0A6M3ZHH5_BACSU	Protein DltD OS=Bacillus subtilis (strain 168) OX=224308 GN=dltD PE=3 SV=1

Uncharacterized protein

A0A6M3Z7Y8	A0A6M3Z7Y8_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03165 PE=4 SV=1
A0A6M3Z8V1	A0A6M3Z8V1_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05150 PE=4 SV=1
A0A6M3Z9I5	A0A6M3Z9I5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06580 PE=4 SV=1
A0A6M3ZCP1	A0A6M3ZCP1_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11460 PE=4 SV=1
A0A6M3ZEC5	A0A6M3ZEC5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05615 PE=4 SV=1
A0A6M4JFW5	A0A6M4JFW5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04025 PE=4 SV=1
A0A6M4JGJ2	A0A6M4JGJ2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09265 PE=4 SV=1
A0A6M4JNN7	A0A6M4JNN7_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14545 PE=4 SV=1
A0A6M4JPL9	A0A6M4JPL9_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20085 PE=4 SV=1
O34320	FADG_BACSU	Uncharacterized protein FadG OS=Bacillus subtilis (strain 168) OX=224308 GN=fadG PE=2 SV=2
A0A6M4JJS5	A0A6M4JJS5_BACSU	Uncharacterized methyltransferase HIR78_16030 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16030 PE=3 SV=1

Stress resistance proteins

A0A6M3ZAD4	A0A6M3ZAD4_BACSU	Lipoprotein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08015 PE=4 SV=1
A0A6M3ZB47	A0A6M3ZB47_BACSU	Damage-inducible protein DinB OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03225 PE=3 SV=1
A0A6M4JEP2	A0A6M4JEP2_BACSU	Fatty-acid peroxygenase OS=Bacillus subtilis (strain 168) OX=224308 GN=cypC PE=4 SV=1
A0A6M4JQY7	A0A6M4JQY7_BACSU	General stress protein 30 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22785 PE=4 SV=1
A0A6M3ZKR2	A0A6M3ZKR2_BACSU	Secretion stress-responsive two-component system response regulator CssR OS=Bacillus subtilis (strain 168) OX=224308 GN=cssR PE=4 SV=1
A0A6M3ZLF9	A0A6M3ZLF9_BACSU	Penicillin-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21500 PE=4 SV=1
A0A6M4JLN6	A0A6M4JLN6_BACSU	Penicillin-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18335 PE=4 SV=1
A0A6M3ZDQ8	A0A6M3ZDQ8_BACSU	PBP1A family penicillin-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13425 PE=4 SV=1
A0A6M3ZGR5	A0A6M3ZGR5_BACSU	Pyrroline-5-carboxylate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=proC PE=3 SV=1
A0A6M3Z7P4	A0A6M3Z7P4_BACSU	Manganese catalase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02530 PE=3 SV=1
A0A6M3Z8S4	A0A6M3Z8S4_BACSU	Thioredoxin-dependent thiol peroxidase OS=Bacillus subtilis (strain 168) OX=224308 GN=bcp PE=4 SV=1
A0A6M3ZHX8	A0A6M3ZHX8_BACSU	Alkyl hydroperoxide reductase subunit F OS=Bacillus subtilis (strain 168) OX=224308 GN=ahpF PE=3 SV=1
A0A6M3ZIP2	A0A6M3ZIP2_BACSU	YpdA family putative bacillithiol disulfide reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=ypdA PE=4 SV=1

Sporulation specific proteins

A0A6M3ZB79	A0A6M3ZB79_BACSU	DNA translocase SpoIIIE OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIIE PE=3 SV=1
A0A6M3ZDR8	A0A6M3ZDR8_BACSU	Stage III sporulation protein AG OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIIAG PE=4 SV=1

A0A6M3ZFX0	A0A6M3ZFX0_BACSU	Spore maturation glycosyltransferase CgeD OS=Bacillus subtilis (strain 168) OX=224308 GN=cgeD PE=4 SV=1
A0A6M4JEJ9	A0A6M4JEJ9_BACSU	Stage II sporulation protein E OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIE PE=4 SV=1
A0A6M4JKT8	A0A6M4JKT8_BACSU	Spore coat protein CotSA OS=Bacillus subtilis (strain 168) OX=224308 GN=cotSA PE=3 SV=1
A0A6M4JMB5	A0A6M4JMB5_BACSU	Stage III sporulation protein AF OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIIAF PE=4 SV=1
P07788	COTA_BACSU	Laccase OS=Bacillus subtilis (strain 168) OX=224308 GN=cotA PE=1 SV=4
P13801	SP2G_BACSU	Sporulation sigma-E factor-processing peptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIGA PE=1 SV=1
P35149	SP4A_BACSU	Stage IV sporulation protein A OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIVA PE=1 SV=1
A0A6M3ZH01	A0A6M3ZH01_BACSU	Sporulenol synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=sqhC PE=3 SV=1
A0A6M3Z8L3	A0A6M3Z8L3_BACSU	YfhO family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04765 PE=4 SV=1

Others

A0A6M3ZFA8	A0A6M3ZFA8_BACSU	Cell shape-determining protein MreC OS=Bacillus subtilis (strain 168) OX=224308 GN=mreC PE=3 SV=1
A0A6M3ZLZ5	A0A6M3ZLZ5_BACSU	YitT family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_23215 PE=4 SV=1
A0A6M4JEA8	A0A6M4JEA8_BACSU	DUF1343 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01050 PE=4 SV=1
A0A6M4JI00	A0A6M4JI00_BACSU	Prophage_tail domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11535 PE=4 SV=1
A0A6M4JJ90	A0A6M4JJ90_BACSU	EAL domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07400 PE=4 SV=1
A0A6M4JKV0	A0A6M4JKV0_BACSU	Prophage_tail domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12895 PE=4 SV=1
A0A6M4JN94	A0A6M4JN94_BACSU	Tetratricopeptide repeat protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13560 PE=4 SV=1
A0A6M4JPA1	A0A6M4JPA1_BACSU	Zinc ribbon domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19575 PE=4 SV=1
A0A6M4JQU4	A0A6M4JQU4_BACSU	Sulfur-containing aminoacid acetyltransferase SnaB OS=Bacillus subtilis (strain 168) OX=224308 GN=snaB PE=4 SV=1
A0A6M4JR44	A0A6M4JR44_BACSU	DUF2705 family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_23145 PE=4 SV=1
A0A6M4JR88	A0A6M4JR88_BACSU	Thioredoxin domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_23265 PE=4 SV=1
A0A6M4JR91	A0A6M4JR91_BACSU	Recombinase domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03840 PE=4 SV=1
O31856	RSBRC_BACSU	RsbT co-antagonist protein RsbRC OS=Bacillus subtilis (strain 168) OX=224308 GN=rsbRC PE=1 SV=1
A0A6M3ZA04	A0A6M3ZA04_BACSU	Phage-like element PBSX protein XkdV OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07045 PE=4 SV=1
A0A6M4JFE2	A0A6M4JFE2_BACSU	Surfactin non-ribosomal peptide synthetase SrfAC OS=Bacillus subtilis (strain 168) OX=224308 GN=srfAC PE=3 SV=1
A0A6M3ZC29	A0A6M3ZC29_BACSU	DUF817 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10195 PE=4 SV=1
A0A6M3ZD22	A0A6M3ZD22_BACSU	Probable disulfide formation protein OS=Bacillus subtilis (strain 168) OX=224308 GN=bdbC PE=3 SV=1

Table S1 A) Protein profiles of spores of *Bacillus subtilis* PY79 produced under control condition

Accession	Entry	Description
Molecular functions		
DNA Replication		
A0A6M3Z6N2	A0A6M3Z6N2_BACSU	DNA-directed RNA polymerase subunit beta OS=Bacillus subtilis (strain 168) OX=224308 GN=rpoB PE=3 SV=1
A0A6M3Z6P2	A0A6M3Z6P2_BACSU	DNA-directed RNA polymerase subunit beta' OS=Bacillus subtilis (strain 168) OX=224308 GN=rpoC PE=3 SV=1
A0A6M3Z765	A0A6M3Z765_BACSU	50S ribosomal protein L7/L12 OS=Bacillus subtilis (strain 168) OX=224308 GN=rplL PE=3 SV=1
A0A6M3Z9W8	A0A6M3Z9W8_BACSU	DNA-directed RNA polymerase subunit alpha OS=Bacillus subtilis (strain 168) OX=224308 GN=rpoA PE=3 SV=1
A0A6M3ZA14	A0A6M3ZA14_BACSU	NHEJ DNA polymerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07390 PE=4 SV=1
A0A6M3ZAK9	A0A6M3ZAK9_BACSU	ATP-dependent DNA helicase RecG OS=Bacillus subtilis (strain 168) OX=224308 GN=recG PE=3 SV=1
A0A6M3ZAS8	A0A6M3ZAS8_BACSU	DNA polymerase III PolC-type OS=Bacillus subtilis (strain 168) OX=224308 GN=polC PE=3 SV=1
A0A6M3ZBA6	A0A6M3ZBA6_BACSU	Primosomal protein N' OS=Bacillus subtilis (strain 168) OX=224308 GN=priA PE=3 SV=1
A0A6M3ZBW7	A0A6M3ZBW7_BACSU	DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=recQ PE=3 SV=1
A0A6M3ZEJ9	A0A6M3ZEJ9_BACSU	Histidine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=hisS PE=3 SV=1
A0A6M3ZEU4	A0A6M3ZEU4_BACSU	Phenylalanine--tRNA ligase alpha subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=pheS PE=3 SV=1
A0A6M3ZEW6	A0A6M3ZEW6_BACSU	Threonine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=thrS PE=3 SV=1
A0A6M3ZFA6	A0A6M3ZFA6_BACSU	Probable tRNA sulfurtransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=thiI PE=3 SV=1
A0A6M3ZIG2	A0A6M3ZIG2_BACSU	Exodeoxyribonuclease III OS=Bacillus subtilis (strain 168) OX=224308 GN=xth PE=3 SV=1
A0A6M3ZKU9	A0A6M3ZKU9_BACSU	Ribonuclease R OS=Bacillus subtilis (strain 168) OX=224308 GN=rnr PE=3 SV=1
A0A6M4JBU0	A0A6M4JBU0_BACSU	DNA polymerase III subunit gamma/tau OS=Bacillus subtilis (strain 168) OX=224308 GN=dnaX PE=3 SV=1
A0A6M4JGU2	A0A6M4JGU2_BACSU	Polyribonucleotide nucleotidyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=pnp PE=3 SV=1
O31774	RNY_BACSU	Ribonuclease Y OS=Bacillus subtilis (strain 168) OX=224308 GN=rny PE=1 SV=1
O31875	NRDEB_BACSU	Ribonucleoside-diphosphate reductase NrdEB subunit alpha OS=Bacillus subtilis (strain 168) OX=224308 GN=nrdEB PE=3 SV=2
A0A6M3Z7G6	A0A6M3Z7G6_BACSU	tRNA uridine(34) hydroxylase OS=Bacillus subtilis (strain 168) OX=224308 GN=trhO PE=3 SV=1
A0A6M3Z837	A0A6M3Z837_BACSU	Ribonuclease YeeF family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03880 PE=4 SV=1
A0A6M3Z855	A0A6M3Z855_BACSU	DNA topoisomerase 3 OS=Bacillus subtilis (strain 168) OX=224308 GN=topB PE=3 SV=1
A0A6M3Z8K8	A0A6M3Z8K8_BACSU	23S rRNA (Uracil(1939)-C(5))-methyltransferase RlmD OS=Bacillus subtilis (strain 168) OX=224308 GN=rlmD PE=3 SV=1
A0A6M3ZAX9	A0A6M3ZAX9_BACSU	Chromosome partition protein Smc OS=Bacillus subtilis (strain 168) OX=224308 GN=smc PE=3 SV=1
A0A6M3ZC49	A0A6M3ZC49_BACSU	DNA-directed DNA polymerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10985 PE=4 SV=1
A0A6M3ZCT6	A0A6M3ZCT6_BACSU	Serine-type integrase SprA OS=Bacillus subtilis (strain 168) OX=224308 GN=sprA PE=4 SV=1
A0A6M3ZDS1	A0A6M3ZDS1_BACSU	DNA polymerase IV OS=Bacillus subtilis (strain 168) OX=224308 GN=dinB PE=3 SV=1
A0A6M3ZEB6	A0A6M3ZEB6_BACSU	DNA internalization-related competence protein ComEC/Rec2 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15095 PE=4 SV=1
A0A6M3ZGR7	A0A6M3ZGR7_BACSU	GTPase Der OS=Bacillus subtilis (strain 168) OX=224308 GN=engA PE=3 SV=1
A0A6M4JEP0	A0A6M4JEP0_BACSU	ATP-dependent helicase/deoxyribonuclease subunit B OS=Bacillus subtilis (strain 168) OX=224308 GN=addB PE=3 SV=1
A0A6M4JF53	A0A6M4JF53_BACSU	tRNA pseudouridine synthase A OS=Bacillus subtilis (strain 168) OX=224308 GN=truA PE=3 SV=1
A0A6M4JIC0	A0A6M4JIC0_BACSU	5'-3' exonuclease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13245 PE=4 SV=1
A0A6M4JIN7	A0A6M4JIN7_BACSU	DNA topoisomerase 4 subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=parC PE=3 SV=1
A0A6M4JJ59	A0A6M4JJ59_BACSU	MoxR family ATPase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03630 PE=4 SV=1

A0A6M4JJK9	A0A6M4JJK9_BACSU	tRNA (N(6)-L-threonylcarbamoyladenosine(37)-C(2))-methylthiotransferase MtaB OS=Bacillus subtilis (strain 168) OX=224308 GN=mtaB PE=4 SV=1
A0A6M4JLU8	A0A6M4JLU8_BACSU	DNA topoisomerase 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=topA PE=3 SV=1
A0A6M4JMP9	A0A6M4JMP9_BACSU	DNA translocase SftA OS=Bacillus subtilis (strain 168) OX=224308 GN=sftA PE=3 SV=1
A0A6M4JN52	A0A6M4JN52_BACSU	Ribonuclease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18870 PE=4 SV=1
A0A6M4JNB0	A0A6M4JNB0_BACSU	Ribonucleoside-diphosphate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10885 PE=3 SV=1
A0A6M4JPZ1	A0A6M4JPZ1_BACSU	DEAD/DEAH box helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20845 PE=4 SV=1
A0A6M4JQ73	A0A6M4JQ73_BACSU	Chromosome-anchoring protein RacA OS=Bacillus subtilis (strain 168) OX=224308 GN=racA PE=3 SV=1
A3F305	A3F305_BACSU	ATP-dependent helicase (Fragment) OS=Bacillus subtilis (strain 168) OX=224308 GN=dinG PE=4 SV=1
P37476	FTSH_BACSU	ATP-dependent zinc metalloprotease FtsH OS=Bacillus subtilis (strain 168) OX=224308 GN=ftsH PE=1 SV=1
P05648	DNAA_BACSU	Chromosomal replication initiator protein DnaA OS=Bacillus subtilis (strain 168) OX=224308 GN=dnaA PE=1 SV=1
A0A6M4JMC3	A0A6M4JMC3_BACSU	DEAD/DEAH box helicase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14585 PE=4 SV=1
P23478	ADDA_BACSU	ATP-dependent helicase/nuclease subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=addA PE=1 SV=2
DNA repair		
A0A6M4JGT2	A0A6M4JGT2_BACSU	Exonuclease subunit SbcC OS=Bacillus subtilis (strain 168) OX=224308 GN=sbcC PE=4 SV=1
A0A6M4JGV6	A0A6M4JGV6_BACSU	Endonuclease YhcR OS=Bacillus subtilis (strain 168) OX=224308 GN=yhcR PE=4 SV=1
A0A6M4JGL3	A0A6M4JGL3_BACSU	DNA repair/recombination ATPase SbcE OS=Bacillus subtilis (strain 168) OX=224308 GN=sbcE PE=4 SV=1
A0A6M3Z9X4	A0A6M3Z9X4_BACSU	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=metE PE=3 SV=1
A0A6M3ZB00	A0A6M3ZB00_BACSU	DNA mismatch repair protein MutS OS=Bacillus subtilis (strain 168) OX=224308 GN=mutS PE=3 SV=1
A0A6M3ZEJ8	A0A6M3ZEJ8_BACSU	Single-stranded-DNA-specific exonuclease RecJ OS=Bacillus subtilis (strain 168) OX=224308 GN=recJ PE=3 SV=1
A0A6M3ZEY8	A0A6M3ZEY8_BACSU	Endonuclease MutS2 OS=Bacillus subtilis (strain 168) OX=224308 GN=mutS2 PE=3 SV=1
A0A6M3ZGE7	A0A6M3ZGE7_BACSU	UvrABC system protein A OS=Bacillus subtilis (strain 168) OX=224308 GN=uvrA PE=3 SV=1
A0A6M3ZHZ7	A0A6M3ZHZ7_BACSU	Single-stranded-DNA-specific exonuclease RecJ OS=Bacillus subtilis (strain 168) OX=224308 GN=recJ PE=3 SV=1
A0A6M4JE64	A0A6M4JE64_BACSU	Transcription-repair-coupling factor OS=Bacillus subtilis (strain 168) OX=224308 GN=mfd PE=3 SV=1
A0A6M4JJA3	A0A6M4JJA3_BACSU	Chaperone protein DnaK OS=Bacillus subtilis (strain 168) OX=224308 GN=dnaK PE=2 SV=1
A0A6M4JLQ4	A0A6M4JLQ4_BACSU	ATP-dependent RecD-like DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=recD2 PE=3 SV=1
A0A6M4JPL4	A0A6M4JPL4_BACSU	UvrABC system protein B OS=Bacillus subtilis (strain 168) OX=224308 GN=uvrB PE=3 SV=1
A0A6M3ZAM7	A0A6M3ZAM7_BACSU	Probable dual-specificity RNA methyltransferase RlmN OS=Bacillus subtilis (strain 168) OX=224308 GN=rlmN PE=3 SV=1
A0A6M3ZCZ6	A0A6M3ZCZ6_BACSU	Class I SAM-dependent RNA methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13340 PE=4 SV=1
A0A6M4JDS6	A0A6M4JDS6_BACSU	Class I SAM-dependent DNA methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03850 PE=4 SV=1
A0A6M4JEY3	A0A6M4JEY3_BACSU	DNA repair protein RadA OS=Bacillus subtilis (strain 168) OX=224308 GN=radA PE=3 SV=1
A0A6M3Z8K8	A0A6M3Z8K8_BACSU	23S rRNA (Uracil(1939)-C(5))-methyltransferase RlmD OS=Bacillus subtilis (strain 168) OX=224308 GN=rlmD PE=3 SV=1
A0A6M4JK56	A0A6M4JK56_BACSU	CCA-adding enzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=cca PE=3 SV=1
Transcription regulator		
A0A6M3Z7Q5	A0A6M3Z7Q5_BACSU	Response regulator aspartate phosphatase RapJ OS=Bacillus subtilis (strain 168) OX=224308 GN=rapJ PE=3 SV=1
A0A6M3Z813	A0A6M3Z813_BACSU	Transcriptional repressor MtlR OS=Bacillus subtilis (strain 168) OX=224308 GN=mtlR PE=4 SV=1
A0A6M3Z972	A0A6M3Z972_BACSU	NTD biosynthesis operon transcriptional regulator NtdR OS=Bacillus subtilis (strain 168) OX=224308 GN=ntdR PE=4 SV=1
A0A6M3ZAG3	A0A6M3ZAG3_BACSU	Transcriptional regulator CcpC OS=Bacillus subtilis (strain 168) OX=224308 GN=ccpC PE=3 SV=1

A0A6M3ZFD6	A0A6M3ZFD6_BACSU	AraC family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17510 PE=4 SV=1
A0A6M3ZHI4	A0A6M3ZHI4_BACSU	PucR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22170 PE=4 SV=1
A0A6M3ZJE4	A0A6M3ZJE4_BACSU	LysR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15670 PE=3 SV=1
A0A6M3ZJP2	A0A6M3ZJP2_BACSU	Ribosome hibernation promoting factor OS=Bacillus subtilis (strain 168) OX=224308 GN=hpf PE=3 SV=1
A0A6M4JCC8	A0A6M4JCC8_BACSU	Transcriptional regulator Btr OS=Bacillus subtilis (strain 168) OX=224308 GN=btr PE=4 SV=1
A0A6M4JD43	A0A6M4JD43_BACSU	RNA-binding transcriptional accessory protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02715 PE=4 SV=1
A0A6M4JE44	A0A6M4JE44_BACSU	Acetoin dehydrogenase operon transcriptional activator AcoR OS=Bacillus subtilis (strain 168) OX=224308 GN=acoR PE=4 SV=1
A0A6M4JE45	A0A6M4JE45_BACSU	Transcriptional regulator YesS OS=Bacillus subtilis (strain 168) OX=224308 GN=yesS PE=4 SV=1
A0A6M4JFI2	A0A6M4JFI2_BACSU	Anti-sigma-I factor RsgI OS=Bacillus subtilis (strain 168) OX=224308 GN=rsgI PE=4 SV=1
A0A6M4JIX0	A0A6M4JIX0_BACSU	Anti-sigma-X factor RsiX OS=Bacillus subtilis (strain 168) OX=224308 GN=rsiX PE=4 SV=1
A0A6M4JJH8	A0A6M4JJH8_BACSU	Sigma-70 family RNA polymerase sigma factor OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15435 PE=4 SV=1
A0A6M4JR12	A0A6M4JR12_BACSU	TetR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22690 PE=4 SV=1
A0A6M4JF07	A0A6M4JF07_BACSU	EIICB-Mtl OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02290 PE=4 SV=1
P05655	SACB_BACSU	Levansucrase OS=Bacillus subtilis (strain 168) OX=224308 GN=sacB PE=1 SV=1
A0A6M4JFH9	A0A6M4JFH9_BACSU	Transcriptional regulator GabR OS=Bacillus subtilis (strain 168) OX=224308 GN=gabR PE=3 SV=1

Transport and chemotaxis and flageller movement

Basic transport & secretion system

A0A6M3Z8L5	A0A6M3Z8L5_BACSU	PTS maltose transporter subunit IIBC OS=Bacillus subtilis (strain 168) OX=224308 GN=malP PE=4 SV=1
A0A6M3Z8S0	A0A6M3Z8S0_BACSU	Aliphatic sulfonate ABC transporter substrate-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04980 PE=3 SV=1
A0A6M3Z8V3	A0A6M3Z8V3_BACSU	Fe(3+)-citrate ABC transporter substrate-binding protein YfmC OS=Bacillus subtilis (strain 168) OX=224308 GN=yfmC PE=4 SV=1
A0A6M3Z8Z2	A0A6M3Z8Z2_BACSU	Multidrug ABC transporter ATP-binding protein BmrC OS=Bacillus subtilis (strain 168) OX=224308 GN=bmrC PE=4 SV=1
A0A6M3Z9L5	A0A6M3Z9L5_BACSU	PTS mannose transporter subunit IIABC OS=Bacillus subtilis (strain 168) OX=224308 GN=manP PE=4 SV=1
A0A6M3ZAM2	A0A6M3ZAM2_BACSU	PTS transporter subunit EIIA OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07925 PE=4 SV=1
A0A6M3ZAN6	A0A6M3ZAN6_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07890 PE=4 SV=1
A0A6M3ZFC5	A0A6M3ZFC5_BACSU	Manganese ABC transporter permease MntD OS=Bacillus subtilis (strain 168) OX=224308 GN=mntD PE=3 SV=1
A0A6M3ZGR2	A0A6M3ZGR2_BACSU	Protein translocase subunit SecA OS=Bacillus subtilis (strain 168) OX=224308 GN=secA PE=3 SV=1
A0A6M3ZGS1	A0A6M3ZGS1_BACSU	Arabinogalactan oligomer ABC transporter permease GanP OS=Bacillus subtilis (strain 168) OX=224308 GN=ganP PE=3 SV=1
A0A6M3ZGX4	A0A6M3ZGX4_BACSU	Multidrug resistance ABC transporter ATP-binding protein/permease BmrA OS=Bacillus subtilis (strain 168) OX=224308 GN=bmrA PE=4 SV=1
A0A6M3ZH04	A0A6M3ZH04_BACSU	Choline ABC transporter permease OpuBB OS=Bacillus subtilis (strain 168) OX=224308 GN=opuBB PE=3 SV=1
A0A6M3ZHU6	A0A6M3ZHU6_BACSU	Thiol reductant ABC exporter subunit CydC OS=Bacillus subtilis (strain 168) OX=224308 GN=cydC PE=4 SV=1
A0A6M3ZIV6	A0A6M3ZIV6_BACSU	ABC transporter permease YxdM OS=Bacillus subtilis (strain 168) OX=224308 GN=yxdM PE=3 SV=1
A0A6M4JE55	A0A6M4JE55_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04570 PE=4 SV=1
A0A6M4JEH2	A0A6M4JEH2_BACSU	Energy-coupling factor transporter transmembrane protein EcfT OS=Bacillus subtilis (strain 168) OX=224308 GN=ecfT PE=3 SV=1
A0A6M4JFL2	A0A6M4JFL2_BACSU	Alanine:cation symporter family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01455 PE=3 SV=1
A0A6M4JGW2	A0A6M4JGW2_BACSU	Oligopeptide ABC transporter permease AppC OS=Bacillus subtilis (strain 168) OX=224308 GN=appC PE=3 SV=1
A0A6M4JHB4	A0A6M4JHB4_BACSU	ATP-binding cassette domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07185 PE=3 SV=1
A0A6M4JJE0	A0A6M4JJE0_BACSU	PTS glucose transporter subunit IIABC OS=Bacillus subtilis (strain 168) OX=224308 GN=ptsG PE=4 SV=1

A0A6M4JN68	A0A6M4JN68_BACSU	Bacitracin ABC transporter permease BceB OS=Bacillus subtilis (strain 168) OX=224308 GN=bceB PE=3 SV=1
A0A6M4JP91	A0A6M4JP91_BACSU	Betaine/proline/choline family ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19500 PE=3 SV=1
A0A6M3Z6T6	A0A6M3Z6T6_BACSU	Iron ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01030 PE=3 SV=1
A0A6M3Z804	A0A6M3Z804_BACSU	EamA family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02965 PE=3 SV=1
A0A6M3Z835	A0A6M3Z835_BACSU	ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02565 PE=4 SV=1
A0A6M3Z8Q6	A0A6M3Z8Q6_BACSU	ABC-F family ATP-binding cassette domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04165 PE=4 SV=1
A0A6M3Z8T8	A0A6M3Z8T8_BACSU	L-cystine transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05135 PE=3 SV=1
A0A6M3Z9U7	A0A6M3Z9U7_BACSU	Serine/threonine exchanger OS=Bacillus subtilis (strain 168) OX=224308 GN=steT PE=4 SV=1
A0A6M3ZAR6	A0A6M3ZAR6_BACSU	ATP-dependent protease ATPase subunit HslU OS=Bacillus subtilis (strain 168) OX=224308 GN=hslU PE=3 SV=1
A0A6M3ZBN7	A0A6M3ZBN7_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04210 PE=4 SV=1
A0A6M3ZBZ5	A0A6M3ZBZ5_BACSU	Bile acid:sodium symporter family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10545 PE=4 SV=1
A0A6M3ZF08	A0A6M3ZF08_BACSU	Type II toxin-antitoxin system toxin ribonuclease YobL OS=Bacillus subtilis (strain 168) OX=224308 GN=yobL PE=4 SV=1
A0A6M3ZF92	A0A6M3ZF92_BACSU	Alanine:cation symporter family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16320 PE=3 SV=1
A0A6M3ZG28	A0A6M3ZG28_BACSU	Na+/H+ antiporter subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18390 PE=3 SV=1
A0A6M3ZI09	A0A6M3ZI09_BACSU	Citrate/sodium symporter CitN OS=Bacillus subtilis (strain 168) OX=224308 GN=citN PE=4 SV=1
A0A6M3ZI39	A0A6M3ZI39_BACSU	Metallophosphoesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22885 PE=4 SV=1
A0A6M3ZIB4	A0A6M3ZIB4_BACSU	Purine/pyrimidine permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21715 PE=3 SV=1
A0A6M3ZIV2	A0A6M3ZIV2_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20950 PE=4 SV=1
A0A6M3ZIW5	A0A6M3ZIW5_BACSU	Ferrous ion permease EfeU OS=Bacillus subtilis (strain 168) OX=224308 GN=efeU PE=3 SV=1
A0A6M3ZJ63	A0A6M3ZJ63_BACSU	ATP-binding cassette domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19200 PE=4 SV=1
A0A6M4JCE4	A0A6M4JCE4_BACSU	Probable inorganic carbon transporter subunit DabA OS=Bacillus subtilis (strain 168) OX=224308 GN=dabA PE=3 SV=1
A0A6M4JEZ0	A0A6M4JEZ0_BACSU	DMT family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01345 PE=3 SV=1
A0A6M4JF57	A0A6M4JF57_BACSU	DegV family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06130 PE=4 SV=1
A0A6M4JG64	A0A6M4JG64_BACSU	Iron ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04690 PE=3 SV=1
A0A6M4JGA7	A0A6M4JGA7_BACSU	Carbohydrate ABC transporter substrate-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03965 PE=4 SV=1
A0A6M4JGZ8	A0A6M4JGZ8_BACSU	HAMP domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10110 PE=4 SV=1
A0A6M4JH75	A0A6M4JH75_BACSU	Iron export ABC transporter permease subunit FetB OS=Bacillus subtilis (strain 168) OX=224308 GN=fetB PE=3 SV=1
A0A6M4JHA0	A0A6M4JHA0_BACSU	ABC-F family ATP-binding cassette domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04190 PE=4 SV=1
A0A6M4JHK0	A0A6M4JHK0_BACSU	MATE family efflux transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10590 PE=4 SV=1
A0A6M4JIG0	A0A6M4JIG0_BACSU	Peptide MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02125 PE=3 SV=1
A0A6M4JIL0	A0A6M4JIL0_BACSU	Calcium-translocating P-type ATPase, SERCA-type OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08565 PE=4 SV=1
A0A6M4JJH3	A0A6M4JJH3_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14805 PE=4 SV=1
A0A6M4JJK7	A0A6M4JJK7_BACSU	Type II toxin-antitoxin system toxin ribonuclease YqcG OS=Bacillus subtilis (strain 168) OX=224308 GN=yqcG PE=4 SV=1
A0A6M4JKX8	A0A6M4JKX8_BACSU	HlyC/CorC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14670 PE=3 SV=1
A0A6M4JLG2	A0A6M4JLG2_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19060 PE=3 SV=1
A0A6M4JMH5	A0A6M4JMH5_BACSU	TspO/MBR family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17905 PE=3 SV=1
A0A6M4JMQ1	A0A6M4JMQ1_BACSU	CitMHS family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15800 PE=4 SV=1

A0A6M4JP02	A0A6M4JP02_BACSU	Type VII secretion protein EsaA OS=Bacillus subtilis (strain 168) OX=224308 GN=esaA PE=3 SV=1
A0A6M4JP37	A0A6M4JP37_BACSU	Iron ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19205 PE=3 SV=1
A0A6M4JQ31	A0A6M4JQ31_BACSU	AEC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21250 PE=3 SV=1
A0A6M4JRB4	A0A6M4JRB4_BACSU	Tetracycline efflux MFS transporter Tet(L) OS=Bacillus subtilis (strain 168) OX=224308 GN=tet(L) PE=4 SV=1
A0A6M4JRB8	A0A6M4JRB8_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06180 PE=4 SV=1
A0A6M3Z803	A0A6M3Z803_BACSU	Two-component system response regulator YclJ OS=Bacillus subtilis (strain 168) OX=224308 GN=yclJ PE=4 SV=1
A0A6M4JEZ4	A0A6M4JEZ4_BACSU	Amino acid permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01835 PE=4 SV=1
A0A6M4JFK2	A0A6M4JFK2_BACSU	Metallophosphoesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07375 PE=4 SV=1
A0A6M4JR32	A0A6M4JR32_BACSU	Cyclic-di-AMP phosphodiesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=gdpP PE=3 SV=1
A0A6M3ZI65	A0A6M3ZI65_BACSU	Efflux RND transporter permease subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03825 PE=4 SV=1
Chemotaxis & flageller movement		
A0A6M3ZAR0	A0A6M3ZAR0_BACSU	Chemotaxis protein CheV OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07725 PE=4 SV=1
A0A6M3ZAT0	A0A6M3ZAT0_BACSU	Flagellar basal body rod protein FlgB OS=Bacillus subtilis (strain 168) OX=224308 GN=flgB PE=3 SV=1
A0A6M3ZBG8	A0A6M3ZBG8_BACSU	Chemotaxis protein CheA OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08960 PE=4 SV=1
A0A6M4JGR4	A0A6M4JGR4_BACSU	Flagellar biosynthesis protein FlhA OS=Bacillus subtilis (strain 168) OX=224308 GN=flhA PE=3 SV=1
A0A6M4JIN9	A0A6M4JIN9_BACSU	Flagellar hook-length control protein FliK OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08875 PE=3 SV=1
A0A6M4JLK3	A0A6M4JLK3_BACSU	Methyl-accepting chemotaxis protein TlpB OS=Bacillus subtilis (strain 168) OX=224308 GN=tlpB PE=4 SV=1
A0A6M4JMS8	A0A6M4JMS8_BACSU	Methyl-accepting chemotaxis protein McpB OS=Bacillus subtilis (strain 168) OX=224308 GN=mcpB PE=4 SV=1
A0A6M4JMY2	A0A6M4JMY2_BACSU	Methyl-accepting chemotaxis protein TlpA OS=Bacillus subtilis (strain 168) OX=224308 GN=tlpA PE=4 SV=1
Kinase & tranferases		
A0A6M3Z7K0	A0A6M3Z7K0_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=dctS PE=4 SV=1
A0A6M3Z8H2	A0A6M3Z8H2_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yflR PE=4 SV=1
A0A6M3Z8M0	A0A6M3Z8M0_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yesM PE=4 SV=1
A0A6M3ZAL1	A0A6M3ZAL1_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinE PE=4 SV=1
A0A6M3ZBG3	A0A6M3ZBG3_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=desK PE=4 SV=1
A0A6M3ZFU0	A0A6M3ZFU0_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18435 PE=4 SV=1
A0A6M3ZG86	A0A6M3ZG86_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=maeL PE=4 SV=1
A0A6M4JG44	A0A6M4JG44_BACSU	Phosphatidylglycerol lysyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=mprF PE=3 SV=1
A0A6M4JHL1	A0A6M4JHL1_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinD PE=4 SV=1
A0A6M4JP28	A0A6M4JP28_BACSU	Sensor histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=liaS PE=4 SV=1
A0A6M4JR07	A0A6M4JR07_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=walK PE=4 SV=1
A0A6M3Z8H8	A0A6M3Z8H8_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=lnrJ PE=4 SV=1
A0A6M3ZBS1	A0A6M3ZBS1_BACSU	Protein-arginine kinase activator protein McsA OS=Bacillus subtilis (strain 168) OX=224308 GN=mcsA PE=4 SV=1
A0A6M3ZEI0	A0A6M3ZEI0_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yrkQ PE=4 SV=1
A0A6M3ZH24	A0A6M3ZH24_BACSU	Phosphatidylglycerol--prolipoprotein diacylglyceryl transferase OS=Bacillus subtilis (strain 168) OX=224308 GN=lgt PE=3 SV=1
A0A6M3ZH60	A0A6M3ZH60_BACSU	Aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19775 PE=3 SV=1
A0A6M3ZID7	A0A6M3ZID7_BACSU	Aminoglycoside N(3)-acetyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13040 PE=3 SV=1
A0A6M4JFG2	A0A6M4JFG2_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=ykoH PE=4 SV=1

A0A6M4JM24	A0A6M4JM24_BACSU	Queuine tRNA-ribosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=tgt PE=3 SV=1
A0A6M4JNB7	A0A6M4JNB7_BACSU	Phosphatidylglycerophosphatase A OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18730 PE=4 SV=1
A0A6M4JPS2	A0A6M4JPS2_BACSU	Glycosyltransferase family 2 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20520 PE=4 SV=1
A0A6M3Z6K0	A0A6M3Z6K0_BACSU	Serine/threonine protein kinase PrkT OS=Bacillus subtilis (strain 168) OX=224308 GN=prkT PE=4 SV=1
A0A6M3ZAY7	A0A6M3ZAY7_BACSU	Aspartokinase OS=Bacillus subtilis (strain 168) OX=224308 GN=dapG PE=3 SV=1
A0A6M3ZFG4	A0A6M3ZFG4_BACSU	Acetate kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=ackA PE=3 SV=1
A0A6M4JIQ6	A0A6M4JIQ6_BACSU	Bifunctional hydroxymethylpyrimidine kinase/phosphomethylpyrimidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=thiD PE=3 SV=1
A0A6M4JJD7	A0A6M4JJD7_BACSU	Alpha-glucosidase/alpha-galactosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04045 PE=3 SV=1
A0A6M4JKH1	A0A6M4JKH1_BACSU	Pantothenate kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=coaA PE=3 SV=1
A0A6M4JPK3	A0A6M4JPK3_BACSU	Phosphotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20260 PE=4 SV=1
A0A6M4JQ13	A0A6M4JQ13_BACSU	Serine hydroxymethyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=glyA PE=3 SV=1
P39211	XYLB_BACSU	Xylulose kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=xylB PE=3 SV=2
A0A6M3Z7Z7	A0A6M3Z7Z7_BACSU	GNAT family N-acetyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03210 PE=4 SV=1
A0A6M4JKL9	A0A6M4JKL9_BACSU	Glycosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03270 PE=4 SV=1
A0A6M3ZHK1	A0A6M3ZHK1_BACSU	DegT/DnrJ/EryC1/StrS aminotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21690 PE=3 SV=1

Metabolic pathway

C/ N & lipid metabolism

A0A6M3Z7D1	A0A6M3Z7D1_BACSU	Assimilatory nitrate reductase catalytic subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=nasC PE=4 SV=1
A0A6M3Z8A5	A0A6M3Z8A5_BACSU	6-phospho-beta-glucosidase GmuD OS=Bacillus subtilis (strain 168) OX=224308 GN=gmuD PE=3 SV=1
A0A6M3Z8G0	A0A6M3Z8G0_BACSU	Dihydrolipoyl dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=acoL PE=3 SV=1
A0A6M3Z9T4	A0A6M3Z9T4_BACSU	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Bacillus subtilis (strain 168) OX=224308 GN=fabF PE=3 SV=1
A0A6M3ZB03	A0A6M3ZB03_BACSU	Malonyl CoA-acyl carrier protein transacylase OS=Bacillus subtilis (strain 168) OX=224308 GN=fabD PE=3 SV=1
A0A6M3ZBJ9	A0A6M3ZBJ9_BACSU	Pyridoxal 5'-phosphate synthase subunit PdxS OS=Bacillus subtilis (strain 168) OX=224308 GN=pdxS PE=3 SV=1
A0A6M3ZGD9	A0A6M3ZGD9_BACSU	Urate oxidase OS=Bacillus subtilis (strain 168) OX=224308 GN=pucL PE=4 SV=1
A0A6M3ZGJ9	A0A6M3ZGJ9_BACSU	Beta-N-acetylglucosaminidase LytD OS=Bacillus subtilis (strain 168) OX=224308 GN=lytD PE=4 SV=1
A0A6M3ZGQ2	A0A6M3ZGQ2_BACSU	3-phosphoshikimate 1-carboxyvinyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=aroA PE=3 SV=1
A0A6M3ZKG5	A0A6M3ZKG5_BACSU	Inosose dehydratase OS=Bacillus subtilis (strain 168) OX=224308 GN=iolE PE=3 SV=1
A0A6M4JES8	A0A6M4JES8_BACSU	Glucose-6-phosphate 3-dehydrogenase NdtC OS=Bacillus subtilis (strain 168) OX=224308 GN=ntdC PE=4 SV=1
A0A6M4JFC1	A0A6M4JFC1_BACSU	Assimilatory nitrate reductase electron transfer subunit NasB OS=Bacillus subtilis (strain 168) OX=224308 GN=nasB PE=4 SV=1
A0A6M4JG05	A0A6M4JG05_BACSU	Nitric oxide synthase oxygenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04285 PE=3 SV=1
A0A6M4JG47	A0A6M4JG47_BACSU	Carbamoyl-phosphate synthase large chain OS=Bacillus subtilis (strain 168) OX=224308 GN=carB PE=3 SV=1
A0A6M4JGV3	A0A6M4JGV3_BACSU	Alpha-L-arabinofuranosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=xynD PE=3 SV=1
A0A6M4JI83	A0A6M4JI83_BACSU	Alpha-glucosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01680 PE=4 SV=1
A0A6M4JKK3	A0A6M4JKK3_BACSU	Alpha-N-arabinofuranosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16795 PE=3 SV=1
A0A6M4JKP6	A0A6M4JKP6_BACSU	Phospholipase YtpA OS=Bacillus subtilis (strain 168) OX=224308 GN=ytpA PE=4 SV=1
A0A6M4JL50	A0A6M4JL50_BACSU	Acetate--CoA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=acsA PE=4 SV=1
A0A6M4JLF6	A0A6M4JLF6_BACSU	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=menD PE=3 SV=1

A0A6M4JLN8	A0A6M4JLN8_BACSU	Carbamoyl-phosphate synthase small chain OS=Bacillus subtilis (strain 168) OX=224308 GN=carA PE=3 SV=1
A0A6M4JLV8	A0A6M4JLV8_BACSU	Lipoyl synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=lipA PE=3 SV=1
A0A6M4JM99	A0A6M4JM99_BACSU	UDP-glucose 6-dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17870 PE=3 SV=1
A0A6M4JMW5	A0A6M4JMW5_BACSU	Alpha-1,4 glucan phosphorylase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17910 PE=3 SV=1
A0A6M4JMX6	A0A6M4JMX6_BACSU	Undecaprenyl-diphosphatase OS=Bacillus subtilis (strain 168) OX=224308 GN=uppP PE=3 SV=1
A0A6M4JN15	A0A6M4JN15_BACSU	3-hydroxyacyl-CoA dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19030 PE=4 SV=1
A0A6M4JNS0	A0A6M4JNS0_BACSU	Alpha-glucosidase/alpha-galactosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17585 PE=3 SV=1
A0A6M4JP71	A0A6M4JP71_BACSU	Scyllo-inositol 2-dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=iolW PE=4 SV=1
A0A6M4JPI8	A0A6M4JPI8_BACSU	N-acetylglucosamine-6-phosphate deacetylase OS=Bacillus subtilis (strain 168) OX=224308 GN=nagA PE=3 SV=1
A0A6M4JPV2	A0A6M4JPV2_BACSU	UDP-glucose 6-dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=uglF PE=3 SV=1
A0A6M4JQW6	A0A6M4JQW6_BACSU	6-phospho-5-dehydro-2-deoxy-D-gluconate aldolase OS=Bacillus subtilis (strain 168) OX=224308 GN=iolJ PE=4 SV=1
A0A6M4JR43	A0A6M4JR43_BACSU	6-phosphogluconate dehydrogenase, decarboxylating OS=Bacillus subtilis (strain 168) OX=224308 GN=gnd PE=3 SV=1
A0A6M4JR71	A0A6M4JR71_BACSU	Adenylosuccinate synthetase OS=Bacillus subtilis (strain 168) OX=224308 GN=purA PE=3 SV=1
A3F3D1	A3F3D1_BACSU	Anthranilate synthase component 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=trpE PE=3 SV=1
P13484	TAGE_BACSU	Poly(glycerol-phosphate) alpha-glucosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=tagE PE=1 SV=1
P18159	PGCA_BACSU	Phosphoglucomutase OS=Bacillus subtilis (strain 168) OX=224308 GN=pgcA PE=1 SV=3
A0A6M3Z802	A0A6M3Z802_BACSU	GABA permease OS=Bacillus subtilis (strain 168) OX=224308 GN=gabP PE=4 SV=1
A0A6M3Z8U9	A0A6M3Z8U9_BACSU	Rhamnogalacturonan acetylesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04015 PE=4 SV=1
A0A6M3Z9F7	A0A6M3Z9F7_BACSU	Aldo/keto reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05330 PE=4 SV=1
A0A6M3Z9J1	A0A6M3Z9J1_BACSU	Enoyl-CoA hydratase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05495 PE=3 SV=1
A0A6M3ZBI3	A0A6M3ZBI3_BACSU	Nitric oxide reductase activation protein NorD OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10560 PE=4 SV=1
A0A6M3ZGE8	A0A6M3ZGE8_BACSU	Acyl-CoA dehydrogenase FadE OS=Bacillus subtilis (strain 168) OX=224308 GN=fadE PE=3 SV=1
A0A6M3ZHN2	A0A6M3ZHN2_BACSU	M20/M25/M40 family metallo-hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21630 PE=4 SV=1
A0A6M3ZIW3	A0A6M3ZIW3_BACSU	Bifunctional aldolase/short-chain dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18175 PE=4 SV=1
A0A6M3ZLP2	A0A6M3ZLP2_BACSU	Polysaccharide deacetylase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22160 PE=4 SV=1
A0A6M4JC95	A0A6M4JC95_BACSU	Polysaccharide biosynthesis protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_00345 PE=4 SV=1
A0A6M4JGX6	A0A6M4JGX6_BACSU	[Acyl-carrier-protein] S-malonyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=fabD PE=4 SV=1
A0A6M3ZA09	A0A6M3ZA09_BACSU	Mannonate dehydratase OS=Bacillus subtilis (strain 168) OX=224308 GN=uxuA PE=3 SV=1
A0A6M3Z8C9	A0A6M3Z8C9_BACSU	Rhamnogalacturonan acetylesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03990 PE=4 SV=1
A0A6M3ZCJ6	A0A6M3ZCJ6_BACSU	Long-chain-fatty-acid--CoA ligase LcfB OS=Bacillus subtilis (strain 168) OX=224308 GN=lcfB PE=3 SV=1
A0A6M3ZDD7	A0A6M3ZDD7_BACSU	6-phosphogluconolactonase OS=Bacillus subtilis (strain 168) OX=224308 GN=pgl PE=3 SV=1
A0A6M3ZDJ3	A0A6M3ZDJ3_BACSU	Isopentenyl-diphosphate delta-isomerase OS=Bacillus subtilis (strain 168) OX=224308 GN=fni PE=3 SV=1
A0A6M3ZH93	A0A6M3ZH93_BACSU	Nitrate reductase (quinone) OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21380 PE=3 SV=1
A0A6M3ZH93	A0A6M3ZH93_BACSU	Nitrate reductase (quinone) OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21380 PE=3 SV=1
A0A6M4JQ85	A0A6M4JQ85_BACSU	Nitrate reductase subunit beta OS=Bacillus subtilis (strain 168) OX=224308 GN=narH PE=4 SV=1
A0A6M3ZHA0	A0A6M3ZHA0_BACSU	Galactose-1-phosphate uridylyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=galT PE=3 SV=1

Amino acid metabolism

A0A6M3Z6D7	A0A6M3Z6D7_BACSU	Methionine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=metG PE=3 SV=1
A0A6M3Z9P9	A0A6M3Z9P9_BACSU	Glycine oxidase ThiO OS=Bacillus subtilis (strain 168) OX=224308 GN=thiO PE=4 SV=1
A0A6M3ZBC2	A0A6M3ZBC2_BACSU	Glutamate synthase large subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=gltB PE=3 SV=1
A0A6M3ZFB7	A0A6M3ZFB7_BACSU	Glutamate-1-semialdehyde 2,1-aminomutase OS=Bacillus subtilis (strain 168) OX=224308 GN=hemL PE=3 SV=1
A0A6M3ZGI8	A0A6M3ZGI8_BACSU	Homoserine O-acetyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=metA PE=3 SV=1
A0A6M3ZGN0	A0A6M3ZGN0_BACSU	Glutathione hydrolase proenzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=ggt PE=3 SV=1
A0A6M3ZI13	A0A6M3ZI13_BACSU	Asparagine synthase (Glutamine-hydrolyzing) OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=3 SV=1
A0A6M4JEL1	A0A6M4JEL1_BACSU	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Bacillus subtilis (strain 168) OX=224308 GN=glmS PE=3 SV=1
A0A6M4JIH4	A0A6M4JIH4_BACSU	Asparagine synthase (Glutamine-hydrolyzing) OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=3 SV=1
A0A6M4JLA6	A0A6M4JLA6_BACSU	Leucine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=leuS PE=3 SV=1
A0A6M4JPI5	A0A6M4JPI5_BACSU	Histidine biosynthesis bifunctional protein HisIE OS=Bacillus subtilis (strain 168) OX=224308 GN=hisI PE=3 SV=1
A0A6M4JQA6	A0A6M4JQA6_BACSU	Threonine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=thrS PE=3 SV=1
P19406	PPB4_BACSU	Alkaline phosphatase 4 OS=Bacillus subtilis (strain 168) OX=224308 GN=phoA PE=1 SV=4
P54420	ASNB_BACSU	Asparagine synthetase [glutamine-hydrolyzing] 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=1 SV=2
A0A6M3ZAN5	A0A6M3ZAN5_BACSU	Non-specific serine/threonine protein kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=prkC PE=4 SV=1
A0A6M3ZDB3	A0A6M3ZDB3_BACSU	Dihydroxy-acid dehydratase OS=Bacillus subtilis (strain 168) OX=224308 GN=ilvD PE=3 SV=1
A0A6M3ZDW6	A0A6M3ZDW6_BACSU	Glutamate dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=gudB PE=3 SV=1
A0A6M3ZEC8	A0A6M3ZEC8_BACSU	O-acetylserine dependent cystathionine beta-synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16020 PE=4 SV=1
A0A6M3ZEJ7	A0A6M3ZEJ7_BACSU	Farnesyl diphosphate phosphatase OS=Bacillus subtilis (strain 168) OX=224308 GN=farP PE=4 SV=1
A0A6M4JMZ9	A0A6M4JMZ9_BACSU	Gamma-polyglutamate hydrolase PghZ OS=Bacillus subtilis (strain 168) OX=224308 GN=pghZ PE=4 SV=1
A0A6M3ZHM6	A0A6M3ZHM6_BACSU	Branched-chain-amino-acid aminotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22035 PE=3 SV=1
Nucleotide metabolism		
A0A6M3Z814	A0A6M3Z814_BACSU	Phosphoribosylformylglycinamidine cyclo-ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=purM PE=3 SV=1
A0A6M3Z8J7	A0A6M3Z8J7_BACSU	Phosphoribosylformylglycinamidine synthase subunit PurL OS=Bacillus subtilis (strain 168) OX=224308 GN=purL PE=3 SV=1
A0A6M3Z8R4	A0A6M3Z8R4_BACSU	Adenine deaminase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03750 PE=3 SV=1
A0A6M3ZET1	A0A6M3ZET1_BACSU	Bifunctional folylpolyglutamate synthase/dihydrofolate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16460 PE=3 SV=1
A0A6M3ZJM5	A0A6M3ZJM5_BACSU	(p)ppGpp synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=relA PE=3 SV=1
A0A6M4JEI9	A0A6M4JEI9_BACSU	Phosphomethylpyrimidine synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=thiC PE=3 SV=1
A0A6M4JG22	A0A6M4JG22_BACSU	Multifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase/5'-nucleotidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04385 PE=3 SV=1
A0A6M4JGM5	A0A6M4JGM5_BACSU	AMP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05755 PE=4 SV=1
A0A6M4JGM8	A0A6M4JGM8_BACSU	Ribonucleoside-diphosphate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=nrdE PE=3 SV=1
A0A6M4JKT5	A0A6M4JKT5_BACSU	Bifunctional purine biosynthesis protein PurH OS=Bacillus subtilis (strain 168) OX=224308 GN=purH PE=3 SV=1
A0A6M4JLE8	A0A6M4JLE8_BACSU	Adenine deaminase OS=Bacillus subtilis (strain 168) OX=224308 GN=ade PE=3 SV=1
A0A6M4JNX5	A0A6M4JNX5_BACSU	Bifunctional oligoribonuclease/PAP phosphatase NrnA OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17065 PE=4 SV=1
A0A6M4JP08	A0A6M4JP08_BACSU	Xanthine dehydrogenase accessory protein PucB OS=Bacillus subtilis (strain 168) OX=224308 GN=pucB PE=4 SV=1
Central metabolism(TCA/electron transport)		
A0A6M3ZAP4	A0A6M3ZAP4_BACSU	D-malate dehydrogenase [decarboxylating] OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02305 PE=3 SV=1

A0A6M3ZBL3	A0A6M3ZBL3_BACSU	2-oxoglutarate dehydrogenase E1 component OS=Bacillus subtilis (strain 168) OX=224308 GN=sucA PE=3 SV=1
A0A6M3ZBL7	A0A6M3ZBL7_BACSU	Bifunctional cytochrome P450/NADPH--P450 reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04105 PE=3 SV=1
A0A6M3ZF07	A0A6M3ZF07_BACSU	Quinolinate synthase A OS=Bacillus subtilis (strain 168) OX=224308 GN=nadA PE=3 SV=1
A0A6M4JH04	A0A6M4JH04_BACSU	Coproporphyrinogen III oxidase OS=Bacillus subtilis (strain 168) OX=224308 GN=hemY PE=3 SV=1
A0A6M4JH12	A0A6M4JH12_BACSU	Coproporphyrinogen III oxidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05475 PE=4 SV=1
A0A6M4JH25	A0A6M4JH25_BACSU	Protoheme IX farnesyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=cyoE PE=3 SV=1
O34720	YJGC_BACSU	Probable oxidoreductase YjgC OS=Bacillus subtilis (strain 168) OX=224308 GN=yjgC PE=3 SV=1
A0A6M3ZFM1	A0A6M3ZFM1_BACSU	LLM class flavin-dependent oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17105 PE=4 SV=1
A0A6M3ZI44	A0A6M3ZI44_BACSU	MmgE/PrpD family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22495 PE=3 SV=1
A0A6M4JI32	A0A6M4JI32_BACSU	NAD(P)-dependent oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07695 PE=4 SV=1
A0A6M4JIK5	A0A6M4JIK5_BACSU	Aldo/keto reductase family oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02385 PE=4 SV=1
A0A6M4JKI9	A0A6M4JKI9_BACSU	Cytochrome c biogenesis protein ResB OS=Bacillus subtilis (strain 168) OX=224308 GN=resB PE=4 SV=1
A0A6M4JKP0	A0A6M4JKP0_BACSU	Phosphoenolpyruvate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=ppsA PE=4 SV=1
A0A6M3ZEC0	A0A6M3ZEC0_BACSU	Bifunctional cytochrome P450/NADPH--P450 reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15965 PE=3 SV=1
A0A6M4JMD9	A0A6M4JMD9_BACSU	NADP-dependent malic enzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=maeB PE=3 SV=1

Cell wall synthesis

A0A6M3Z8Q4	A0A6M3Z8Q4_BACSU	Beta-galactosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=yesZ PE=3 SV=1
A0A6M3Z8T2	A0A6M3Z8T2_BACSU	Lipoteichoic acid synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=ltaS PE=3 SV=1
A0A6M3ZAJ3	A0A6M3ZAJ3_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08320 PE=3 SV=1
A0A6M3ZG90	A0A6M3ZG90_BACSU	LTA synthase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19310 PE=3 SV=1
A0A6M3ZHY9	A0A6M3ZHY9_BACSU	Aryl-phospho-beta-d-glucosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=bglH PE=3 SV=1
A0A6M4JJR6	A0A6M4JJR6_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08325 PE=3 SV=1
A0A6M4JPR3	A0A6M4JPR3_BACSU	Undecaprenyl/decaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20435 PE=4 SV=1
A0A6M3Z993	A0A6M3Z993_BACSU	Cell wall-associated protease WprA OS=Bacillus subtilis (strain 168) OX=224308 GN=wprA PE=3 SV=1
A0A6M3ZEE3	A0A6M3ZEE3_BACSU	Transglycosylase SLT domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15330 PE=4 SV=1
A0A6M3ZHM4	A0A6M3ZHM4_BACSU	Teichoic acid biosynthesis protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20580 PE=4 SV=1
O34798	PDAC_BACSU	Peptidoglycan-N-acetylmuramic acid deacetylase PdaC OS=Bacillus subtilis (strain 168) OX=224308 GN=pdaC PE=1 SV=1
A0A6M3Z8B5	A0A6M3Z8B5_BACSU	Transglycosylase SLT domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02860 PE=3 SV=1
A0A6M4JE15	A0A6M4JE15_BACSU	Peptidylprolyl isomerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_00430 PE=4 SV=1
A0A6M4JHU0	A0A6M4JHU0_BACSU	Aminopeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07950 PE=3 SV=1
O31605	PEPF_BACSU	Oligoendopeptidase F homolog OS=Bacillus subtilis (strain 168) OX=224308 GN=yjbG PE=3 SV=2
A0A6M3ZDD9	A0A6M3ZDD9_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13865 PE=3 SV=1
A0A6M3ZHH5	A0A6M3ZHH5_BACSU	Protein DltD OS=Bacillus subtilis (strain 168) OX=224308 GN=dltD PE=3 SV=1
A0A6M4JIR1	A0A6M4JIR1_BACSU	LysM peptidoglycan-binding domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13775 PE=4 SV=1
A0A6M3ZH02	A0A6M3ZH02_BACSU	LTD domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20185 PE=4 SV=1
A0A6M4JNK6	A0A6M4JNK6_BACSU	Endolytic murein transglycosylase OS=Bacillus subtilis (strain 168) OX=224308 GN=mltG PE=3 SV=1
P55179	PEPT_BACSU	Peptidase T OS=Bacillus subtilis (strain 168) OX=224308 GN=pepT PE=3 SV=1

Uncharacterized protein

A0A6M3Z7N5	A0A6M3Z7N5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02465 PE=4 SV=1
A0A6M3Z8U3	A0A6M3Z8U3_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05095 PE=4 SV=1
A0A6M3Z8V2	A0A6M3Z8V2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05140 PE=4 SV=1
A0A6M3ZBS8	A0A6M3ZBS8_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09615 PE=4 SV=1
A0A6M3ZC19	A0A6M3ZC19_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11415 PE=4 SV=1
A0A6M3ZC58	A0A6M3ZC58_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10940 PE=4 SV=1
A0A6M3ZCE0	A0A6M3ZCE0_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11550 PE=4 SV=1
A0A6M3ZCM7	A0A6M3ZCM7_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11410 PE=4 SV=1
A0A6M3ZCQ5	A0A6M3ZCQ5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12865 PE=4 SV=1
A0A6M3ZCT8	A0A6M3ZCT8_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12715 PE=4 SV=1
A0A6M3ZF64	A0A6M3ZF64_BACSU	Unsaturated rhamnogalacturonyl hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=rmgQ PE=4 SV=1
A0A6M3ZI73	A0A6M3ZI73_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21505 PE=4 SV=1
A0A6M4JE22	A0A6M4JE22_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03885 PE=4 SV=1
A0A6M4JFW5	A0A6M4JFW5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04025 PE=4 SV=1
A0A6M4JH27	A0A6M4JH27_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10270 PE=4 SV=1
A0A6M4JHG6	A0A6M4JHG6_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04540 PE=4 SV=1
A0A6M4JIL6	A0A6M4JIL6_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08775 PE=4 SV=1
A0A6M4JJC7	A0A6M4JJC7_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11365 PE=4 SV=1
A0A6M4JJH9	A0A6M4JJH9_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11405 PE=4 SV=1
A0A6M4JJP6	A0A6M4JJP6_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15290 PE=4 SV=1
A0A6M4JK58	A0A6M4JK58_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11075 PE=4 SV=1
A0A6M4JNZ2	A0A6M4JNZ2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17885 PE=4 SV=1
A0A6M3ZIJ9	A0A6M3ZIJ9_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11540 PE=4 SV=1
A0A6M3ZIM2	A0A6M3ZIM2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12900 PE=4 SV=1
A0A6M3ZIT4	A0A6M3ZIT4_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11470 PE=4 SV=1
A0A2K4Z9K2	A0A2K4Z9K2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=BSU_21639 PE=4 SV=1

Stress resistance/response proteins

A0A6M3Z979	A0A6M3Z979_BACSU	Penicillin-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05630 PE=4 SV=1
A0A6M3Z7S3	A0A6M3Z7S3_BACSU	Zinc metallochaperone ZinU OS=Bacillus subtilis (strain 168) OX=224308 GN=zinU PE=3 SV=1
A0A6M3Z8S4	A0A6M3Z8S4_BACSU	Thioredoxin-dependent thiol peroxidase OS=Bacillus subtilis (strain 168) OX=224308 GN=bcp PE=4 SV=1
A0A6M3ZB88	A0A6M3ZB88_BACSU	Beta-lactamase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09210 PE=4 SV=1
A0A6M3ZEE2	A0A6M3ZEE2_BACSU	Heme chaperone HemW OS=Bacillus subtilis (strain 168) OX=224308 GN=hemW PE=3 SV=1
A0A6M3ZEL7	A0A6M3ZEL7_BACSU	Chaperone protein DnaJ OS=Bacillus subtilis (strain 168) OX=224308 GN=dnaJ PE=3 SV=1
A0A6M4JGI4	A0A6M4JGI4_BACSU	Foldase protein PrsA OS=Bacillus subtilis (strain 168) OX=224308 GN=prsA PE=3 SV=1
A0A6M4JGX0	A0A6M4JGX0_BACSU	SdpA family antimicrobial peptide system protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06105 PE=4 SV=1

A0A6M4JK71	A0A6M4JK71_BACSU	GlsB/YeaQ/YmgE family stress response membrane protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02500 PE=3 SV=1
A0A6M3ZJU8	A0A6M3ZJU8_BACSU	Carbon starvation protein A OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16790 PE=3 SV=1
A0A6M3ZKH0	A0A6M3ZKH0_BACSU	Chaperone protein HtpG OS=Bacillus subtilis (strain 168) OX=224308 GN=htpG PE=3 SV=1
A0A6M4JF46	A0A6M4JF46_BACSU	Ureidoglycolate dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=allD PE=4 SV=1
A0A6M4JQY7	A0A6M4JQY7_BACSU	General stress protein 30 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22785 PE=4 SV=1
A0A6M3ZF30	A0A6M3ZF30_BACSU	Lon protease OS=Bacillus subtilis (strain 168) OX=224308 GN=lon PE=2 SV=1
A0A6M3ZF63	A0A6M3ZF63_BACSU	Flavohemoprotein OS=Bacillus subtilis (strain 168) OX=224308 GN=hmpA PE=3 SV=1
A0A6M3Z752	A0A6M3Z752_BACSU	ATP-dependent Clp protease ATP-binding subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_00575 PE=3 SV=1

Sporulation specific proteins

A0A6M3Z6S1	A0A6M3Z6S1_BACSU	Spore cortex biosynthesis protein YabQ OS=Bacillus subtilis (strain 168) OX=224308 GN=yabQ PE=4 SV=1
A0A6M3Z910	A0A6M3Z910_BACSU	Endospore germination permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04345 PE=3 SV=1
A0A6M3ZEK5	A0A6M3ZEK5_BACSU	Stage II sporulation protein SpoIIB OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIB PE=4 SV=1
A0A6M3ZG80	A0A6M3ZG80_BACSU	Type VII secretion protein EssC OS=Bacillus subtilis (strain 168) OX=224308 GN=essC PE=4 SV=1
A0A6M3ZH01	A0A6M3ZH01_BACSU	Sporulenol synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=sqhC PE=3 SV=1
A0A6M3ZJ55	A0A6M3ZJ55_BACSU	Sporulation protein YqfD OS=Bacillus subtilis (strain 168) OX=224308 GN=yqfD PE=4 SV=1
A0A6M3ZM43	A0A6M3ZM43_BACSU	Sporulation sigma-E factor-processing peptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIGA PE=3 SV=1
A0A6M4JC46	A0A6M4JC46_BACSU	LysM peptidoglycan-binding domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_00110 PE=4 SV=1
A0A6M4JEJ9	A0A6M4JEJ9_BACSU	Stage II sporulation protein E OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIE PE=4 SV=1
A0A6M4JJI5	A0A6M4JJI5_BACSU	Sporulation killing factor system radical SAM maturase OS=Bacillus subtilis (strain 168) OX=224308 GN=skfB PE=4 SV=1
A0A6M4JKH4	A0A6M4JKH4_BACSU	Stage V sporulation protein B OS=Bacillus subtilis (strain 168) OX=224308 GN=spoVB PE=4 SV=1
A0A6M4JLK4	A0A6M4JLK4_BACSU	YheC/YheD family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05450 PE=4 SV=1
A0A6M4JMX1	A0A6M4JMX1_BACSU	Spore coat morphogenetic protein SpoVID OS=Bacillus subtilis (strain 168) OX=224308 GN=spoVID PE=4 SV=1
A0A6M4JQ07	A0A6M4JQ07_BACSU	Stage II sporulation protein spoIIQ OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIQ PE=4 SV=1
A0A6M4JQ30	A0A6M4JQ30_BACSU	Stage II sporulation protein R OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIR PE=4 SV=1
A0A6M4JR62	A0A6M4JR62_BACSU	Sporulation initiation inhibitor protein Soj OS=Bacillus subtilis (strain 168) OX=224308 GN=soj PE=4 SV=1
P37875	SP5R_BACSU	Stage V sporulation protein R OS=Bacillus subtilis (strain 168) OX=224308 GN=spoVR PE=4 SV=1
A0A6M3ZFA1	A0A6M3ZFA1_BACSU	Sporulation initiation phosphotransferase Sop0B OS=Bacillus subtilis (strain 168) OX=224308 GN=spo0B PE=4 SV=1
A0A6M3Z8L3	A0A6M3Z8L3_BACSU	YfhO family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04765 PE=4 SV=1

Others

A0A6M4JH86	A0A6M4JH86_BACSU	Non-ribosomal plipastatin synthetase PpsE OS=Bacillus subtilis (strain 168) OX=224308 GN=ppsE PE=3 SV=1
A7XMX8	A7XMX8_BACSU	PpsD (Fragment) OS=Bacillus subtilis (strain 168) OX=224308 GN=ppsD PE=4 SV=1
A0A6M3Z6Z9	A0A6M3Z6Z9_BACSU	Serine protease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01360 PE=3 SV=1
A0A6M3Z822	A0A6M3Z822_BACSU	Lipoprotein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02445 PE=4 SV=1
A0A6M3ZBH5	A0A6M3ZBH5_BACSU	KAP NTPase domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10335 PE=4 SV=1
A0A6M3ZE64	A0A6M3ZE64_BACSU	HXXEE domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15585 PE=4 SV=1
A0A6M3ZE99	A0A6M3ZE99_BACSU	PBSX family phage terminase large subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15420 PE=4 SV=1

A0A6M3ZEW0	A0A6M3ZEW0_BACSU	U32 family peptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16060 PE=4 SV=1
A0A6M3ZEY6	A0A6M3ZEY6_BACSU	DUF1049 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16220 PE=4 SV=1
A0A6M3ZFA2	A0A6M3ZFA2_BACSU	DedA family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07405 PE=4 SV=1
A0A6M3ZGH9	A0A6M3ZGH9_BACSU	Divergent PAP2 family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18620 PE=4 SV=1
A0A6M3ZHZ2	A0A6M3ZHZ2_BACSU	DUF2326 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22875 PE=4 SV=1
A0A6M3ZI55	A0A6M3ZI55_BACSU	CvpA family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16725 PE=4 SV=1
A0A6M3ZIX3	A0A6M3ZIX3_BACSU	DUF2627 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14345 PE=4 SV=1
A0A6M3ZJ10	A0A6M3ZJ10_BACSU	3D domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12345 PE=4 SV=1
A0A6M3ZLZ5	A0A6M3ZLZ5_BACSU	YitT family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_23215 PE=4 SV=1
A0A6M4JDN3	A0A6M4JDN3_BACSU	DUF4129 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03640 PE=4 SV=1
A0A6M4JF49	A0A6M4JF49_BACSU	DUF4901 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01655 PE=4 SV=1
A0A6M4JI41	A0A6M4JI41_BACSU	Rqc2 homolog RqcH OS=Bacillus subtilis (strain 168) OX=224308 GN=rqcH PE=1 SV=1
A0A6M4JJZ0	A0A6M4JJZ0_BACSU	Phage tail protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12890 PE=4 SV=1
A0A6M4JKH6	A0A6M4JKH6_BACSU	YtxH domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17330 PE=4 SV=1
A0A6M4JL47	A0A6M4JL47_BACSU	Phage_Mu_F domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15410 PE=4 SV=1
A0A6M4JMM8	A0A6M4JMM8_BACSU	DUF2953 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17195 PE=4 SV=1
A0A6M4JMR1	A0A6M4JMR1_BACSU	S9 family peptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18705 PE=3 SV=1
A0A6M4JNF4	A0A6M4JNF4_BACSU	Right-handed parallel beta-helix repeat-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12405 PE=4 SV=1
A0A6M4JNW4	A0A6M4JNW4_BACSU	YutD family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18745 PE=4 SV=1
A0A6M4JPA1	A0A6M4JPA1_BACSU	Zinc ribbon domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19575 PE=4 SV=1
A0A6M4JPE4	A0A6M4JPE4_BACSU	DUF3231 family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19910 PE=4 SV=1
A0A6M4JPT8	A0A6M4JPT8_BACSU	DUF2642 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20715 PE=4 SV=1
A0A6M4JQA0	A0A6M4JQA0_BACSU	YwiC-like family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21390 PE=4 SV=1
A0A6M4JQB0	A0A6M4JQB0_BACSU	Antilisterial bacteriocin subtilosin biosynthesis protein AlbD OS=Bacillus subtilis (strain 168) OX=224308 GN=albD PE=4 SV=1
P17865	FTSZ_BACSU	Cell division protein FtsZ OS=Bacillus subtilis (strain 168) OX=224308 GN=ftsZ PE=1 SV=3
A0A6M4JFE2	A0A6M4JFE2_BACSU	Surfactin non-ribosomal peptide synthetase SrfAC OS=Bacillus subtilis (strain 168) OX=224308 GN=srfAC PE=3 SV=1
A0A6M3ZG81	A0A6M3ZG81_BACSU	Wzz domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19840 PE=4 SV=1
A0A6M3ZIF9	A0A6M3ZIF9_BACSU	Minor protease Epr OS=Bacillus subtilis (strain 168) OX=224308 GN=epr PE=3 SV=1
A0A6M3ZK18	A0A6M3ZK18_BACSU	4Fe-4S dicluster domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21325 PE=4 SV=1
P29141	SUBV_BACSU	Minor extracellular protease vpr OS=Bacillus subtilis (strain 168) OX=224308 GN=vpr PE=1 SV=1
A0A6M3ZMB6	A0A6M3ZMB6_BACSU	Insulinase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21445 PE=4 SV=1

Table S1 B) Protein profiles of spores of *Bacillus subtilis* PY79 produced under H_2O_2 treated condition.

Accession	Entry	Description
Molecular functions		
DNA Replication		
A0A6M3Z6N2	A0A6M3Z6N2_BACSU	DNA-directed RNA polymerase subunit beta OS=Bacillus subtilis (strain 168) OX=224308 GN=rpoB PE=3 SV=1
A0A6M3Z765	A0A6M3Z765_BACSU	50S ribosomal protein L7/L12 OS=Bacillus subtilis (strain 168) OX=224308 GN=rplL PE=3 SV=1
A0A6M3Z778	A0A6M3Z778_BACSU	50S ribosomal protein L3 OS=Bacillus subtilis (strain 168) OX=224308 GN=rplC PE=3 SV=1
A0A6M3Z852	A0A6M3Z852_BACSU	DNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=ligA PE=3 SV=1
A0A6M3ZAS8	A0A6M3ZAS8_BACSU	DNA polymerase III PolC-type OS=Bacillus subtilis (strain 168) OX=224308 GN=polC PE=3 SV=1
A0A6M3ZAU7	A0A6M3ZAU7_BACSU	Ribonuclease J OS=Bacillus subtilis (strain 168) OX=224308 GN=rnj PE=3 SV=1
A0A6M3ZBW7	A0A6M3ZBW7_BACSU	DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=recQ PE=3 SV=1
A0A6M3ZH20	A0A6M3ZH20_BACSU	Arginine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=argS PE=3 SV=1
A0A6M3ZHQ1	A0A6M3ZHQ1_BACSU	Uracil-DNA glycosylase OS=Bacillus subtilis (strain 168) OX=224308 GN=ung PE=3 SV=1
A0A6M3ZIS2	A0A6M3ZIS2_BACSU	Valine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=valS PE=3 SV=1
A0A6M3ZM76	A0A6M3ZM76_BACSU	Asparagine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=asnS PE=3 SV=1
A0A6M4JC51	A0A6M4JC51_BACSU	Lysine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=lysS PE=3 SV=1
A0A6M4JI35	A0A6M4JI35_BACSU	DNA-directed DNA polymerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12350 PE=4 SV=1
A0A6M4JKJ5	A0A6M4JKJ5_BACSU	Phenylalanine--tRNA ligase beta subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=pheT PE=3 SV=1
A0A6M4JKZ0	A0A6M4JKZ0_BACSU	DNA polymerase I OS=Bacillus subtilis (strain 168) OX=224308 GN=polA PE=3 SV=1
A0A6M4JLA6	A0A6M4JLA6_BACSU	Leucine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=leuS PE=3 SV=1
A0A6M4JMA0	A0A6M4JMA0_BACSU	Alanine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=alaS PE=3 SV=1
A0A6M4JNA5	A0A6M4JNA5_BACSU	30S ribosomal protein S1 OS=Bacillus subtilis (strain 168) OX=224308 GN=rpsA PE=4 SV=1
O34580	PCRA_BACSU	ATP-dependent DNA helicase PcrA OS=Bacillus subtilis (strain 168) OX=224308 GN=pcrA PE=1 SV=1
P96614	CSHA_BACSU	DEAD-box ATP-dependent RNA helicase CshA OS=Bacillus subtilis (strain 168) OX=224308 GN=cshA PE=1 SV=2
A0A6M3ZAQ9	A0A6M3ZAQ9_BACSU	Ribosome biogenesis GTPase A OS=Bacillus subtilis (strain 168) OX=224308 GN=ylqF PE=3 SV=1
A0A6M3ZAX9	A0A6M3ZAX9_BACSU	Chromosome partition protein Smc OS=Bacillus subtilis (strain 168) OX=224308 GN=smc PE=3 SV=1
A0A6M3ZC49	A0A6M3ZC49_BACSU	DNA-directed DNA polymerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10985 PE=4 SV=1
A0A6M3ZCT6	A0A6M3ZCT6_BACSU	Serine-type integrase SprA OS=Bacillus subtilis (strain 168) OX=224308 GN=sprA PE=4 SV=1
A0A6M3ZDQ1	A0A6M3ZDQ1_BACSU	DEAD/DEAH box helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13375 PE=4 SV=1
A0A6M3ZEB8	A0A6M3ZEB8_BACSU	Ribosome biogenesis GTPase YqeH OS=Bacillus subtilis (strain 168) OX=224308 GN=yqeH PE=4 SV=1
A0A6M3ZGN4	A0A6M3ZGN4_BACSU	3'-5' exonuclease DinG OS=Bacillus subtilis (strain 168) OX=224308 GN=dinG PE=3 SV=1
A0A6M3ZGR7	A0A6M3ZGR7_BACSU	GTPase Der OS=Bacillus subtilis (strain 168) OX=224308 GN=engA PE=3 SV=1
A0A6M3ZHU5	A0A6M3ZHU5_BACSU	Ribonuclease YxiD OS=Bacillus subtilis (strain 168) OX=224308 GN=yxiD PE=4 SV=1
A0A6M4JEP0	A0A6M4JEP0_BACSU	ATP-dependent helicase/deoxyribonuclease subunit B OS=Bacillus subtilis (strain 168) OX=224308 GN=addB PE=3 SV=1
A0A6M4JG55	A0A6M4JG55_BACSU	DNA-entry nuclease OS=Bacillus subtilis (strain 168) OX=224308 GN=nucA PE=4 SV=1
A0A6M4JGV6	A0A6M4JGV6_BACSU	Endonuclease YhcR OS=Bacillus subtilis (strain 168) OX=224308 GN=yhcR PE=4 SV=1
A0A6M4JIN7	A0A6M4JIN7_BACSU	DNA topoisomerase 4 subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=parC PE=3 SV=1
A0A6M4JIP4	A0A6M4JIP4_BACSU	Oligoribonuclease NrnB OS=Bacillus subtilis (strain 168) OX=224308 GN=nrnB PE=4 SV=1
A0A6M4JJ14	A0A6M4JJ14_BACSU	DNA gyrase subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=gyrA PE=3 SV=1

A0A6M4JJ59	A0A6M4JJ59_BACSU	MoxR family ATPase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03630 PE=4 SV=1
A0A6M4JJK9	A0A6M4JJK9_BACSU	tRNA (N(6)-L-threonylcarbamoyladenosine(37)-C(2))-methylthiotransferase MtaB OS=Bacillus subtilis (strain 168) OX=224308 GN=mtaB PE=4 SV=1
A0A6M4JJN2	A0A6M4JJN2_BACSU	50S ribosomal subunit assembly factor BipA OS=Bacillus subtilis (strain 168) OX=224308 GN=typA PE=3 SV=1
A0A6M4JLU8	A0A6M4JLU8_BACSU	DNA topoisomerase 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=topA PE=3 SV=1
A0A6M4JMC3	A0A6M4JMC3_BACSU	DEAD/DEAH box helicase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14585 PE=4 SV=1
A0A6M4JMP9	A0A6M4JMP9_BACSU	DNA translocase SftA OS=Bacillus subtilis (strain 168) OX=224308 GN=sftA PE=3 SV=1
A0A6M4JNG9	A0A6M4JNG9_BACSU	Ribosomal protein L11 methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=prmA PE=3 SV=1
A0A6M4JR11	A0A6M4JR11_BACSU	MBL fold metallo-hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22985 PE=4 SV=1
A0A6M3Z7G6	A0A6M3Z7G6_BACSU	tRNA uridine(34) hydroxylase OS=Bacillus subtilis (strain 168) OX=224308 GN=trhO PE=3 SV=1
A0A6M3ZF32	A0A6M3ZF32_BACSU	Replication initiation membrane attachment protein DnaB OS=Bacillus subtilis (strain 168) OX=224308 GN=dnaB PE=4 SV=1
DNA repair		
A0A6M3ZEJ0	A0A6M3ZEJ0_BACSU	DNA mismatch repair protein MutL OS=Bacillus subtilis (strain 168) OX=224308 GN=mutL PE=3 SV=1
A0A6M4JLQ4	A0A6M4JLQ4_BACSU	ATP-dependent RecD-like DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=recD2 PE=3 SV=1
A0A6M4JN18	A0A6M4JN18_BACSU	
A0A6M4JPL4	A0A6M4JPL4_BACSU	UvrABC system protein B OS=Bacillus subtilis (strain 168) OX=224308 GN=uvrB PE=3 SV=1
A0A6M3ZFN4	A0A6M3ZFN4_BACSU	UV damage repair protein UvrX OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11585 PE=3 SV=1
A0A6M3ZFN8	A0A6M3ZFN8_BACSU	S-adenosyl-L-methionine-dependent methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08080 PE=3 SV=1
A0A6M4JFJ5	A0A6M4JFJ5_BACSU	8-oxo-dGTP diphosphatase MutT OS=Bacillus subtilis (strain 168) OX=224308 GN=mutT PE=3 SV=1
A0A6M4JFK2	A0A6M4JFK2_BACSU	Metallophosphoesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07375 PE=4 SV=1
A0A6M4JGL3	A0A6M4JGL3_BACSU	DNA repair/recombination ATPase SbcE OS=Bacillus subtilis (strain 168) OX=224308 GN=sbcE PE=4 SV=1
A0A6M3Z6L7	A0A6M3Z6L7_BACSU	Ribosomal RNA small subunit methyltransferase I OS=Bacillus subtilis (strain 168) OX=224308 GN=rsmI PE=3 SV=1
Transcription regulator		
A0A6M4JE64	A0A6M4JE64_BACSU	Transcription-repair-coupling factor OS=Bacillus subtilis (strain 168) OX=224308 GN=mfd PE=3 SV=1
A0A6M3Z6X2	A0A6M3Z6X2_BACSU	Elongation factor Tu OS=Bacillus subtilis (strain 168) OX=224308 GN=tuf PE=3 SV=1
A0A6M3Z881	A0A6M3Z881_BACSU	MarR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03230 PE=4 SV=1
A0A6M3Z8Y6	A0A6M3Z8Y6_BACSU	MerR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04175 PE=4 SV=1
A0A6M3Z8Y6	A0A6M3Z8Y6_BACSU	MerR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04175 PE=4 SV=1
A0A6M3ZBI5	A0A6M3ZBI5_BACSU	Translation initiation factor IF-2 OS=Bacillus subtilis (strain 168) OX=224308 GN=infB PE=3 SV=1
A0A6M3ZEE9	A0A6M3ZEE9_BACSU	Elongation factor Ts OS=Bacillus subtilis (strain 168) OX=224308 GN=tsf PE=3 SV=1
A0A6M3ZJ61	A0A6M3ZJ61_BACSU	
A0A6M3ZJ61	A0A6M3ZJ61_BACSU	Bifunctional metallophosphatase/5'-nucleotidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18775 PE=3 SV=1
A0A6M4JCC8	A0A6M4JCC8_BACSU	Transcriptional regulator Btr OS=Bacillus subtilis (strain 168) OX=224308 GN=btr PE=4 SV=1
A0A6M4JE44	A0A6M4JE44_BACSU	Acetoin dehydrogenase operon transcriptional activator AcoR OS=Bacillus subtilis (strain 168) OX=224308 GN=acoR PE=4 SV=1
A0A6M4JE45	A0A6M4JE45_BACSU	Transcriptional regulator YesS OS=Bacillus subtilis (strain 168) OX=224308 GN=yesS PE=4 SV=1
A0A6M4JFI2	A0A6M4JFI2_BACSU	Anti-sigma-I factor RsgI OS=Bacillus subtilis (strain 168) OX=224308 GN=rsgI PE=4 SV=1
A0A6M4JFS1	A0A6M4JFS1_BACSU	HTH psq-type domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01615 PE=4 SV=1
A0A6M4JI24	A0A6M4JI24_BACSU	LacI family DNA-binding transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07650 PE=4 SV=1
A0A6M4JJQ3	A0A6M4JJQ3_BACSU	Transcriptional regulator LevR OS=Bacillus subtilis (strain 168) OX=224308 GN=levR PE=4 SV=1

A0A6M4JKY0	A0A6M4JKY0_BACSU	Elongation factor 4 OS=Bacillus subtilis (strain 168) OX=224308 GN=lepA PE=3 SV=1
A0A6M4JR56	A0A6M4JR56_BACSU	Transcriptional regulator HypR OS=Bacillus subtilis (strain 168) OX=224308 GN=hypR PE=4 SV=1
A0A6M3Z813	A0A6M3Z813_BACSU	Transcriptional repressor MtlR OS=Bacillus subtilis (strain 168) OX=224308 GN=mtlR PE=4 SV=1

Transport and chemotaxis and flageller assembly

Basic transport & secretion system

A0A6M3Z6Z8	A0A6M3Z6Z8_BACSU	PTS transporter subunit EIIC OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01415 PE=4 SV=1
A0A6M3Z760	A0A6M3Z760_BACSU	Zinc ABC transporter permease ZnuB OS=Bacillus subtilis (strain 168) OX=224308 GN=znuB PE=3 SV=1
A0A6M3Z7Y1	A0A6M3Z7Y1_BACSU	MMPL family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03115 PE=4 SV=1
A0A6M3Z8Z5	A0A6M3Z8Z5_BACSU	Iron ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04230 PE=3 SV=1
A0A6M3Z9N1	A0A6M3Z9N1_BACSU	Oligopeptide ABC transporter ATP-binding protein OppD OS=Bacillus subtilis (strain 168) OX=224308 GN=oppD PE=3 SV=1
A0A6M3ZDF8	A0A6M3ZDF8_BACSU	HMP/thiamine ABC transporter permease ThiX OS=Bacillus subtilis (strain 168) OX=224308 GN=thiX PE=4 SV=1
A0A6M3ZFM0	A0A6M3ZFM0_BACSU	NAD-dependent malic enzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17385 PE=3 SV=1
A0A6M3ZGQ4	A0A6M3ZGQ4_BACSU	Glycine betaine/carnitine/choline/choline sulfate ABC transporter permease OpuCD OS=Bacillus subtilis (strain 168) OX=224308 GN=opuCD PE=3 SV=1
A0A6M3ZGR2	A0A6M3ZGR2_BACSU	Protein translocase subunit SecA OS=Bacillus subtilis (strain 168) OX=224308 GN=secA PE=3 SV=1
A0A6M3ZHX5	A0A6M3ZHX5_BACSU	Thiol reductant ABC exporter subunit CydD OS=Bacillus subtilis (strain 168) OX=224308 GN=cydD PE=4 SV=1
A0A6M3ZKF2	A0A6M3ZKF2_BACSU	Amino acid ABC transporter substrate-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22515 PE=3 SV=1
A0A6M3ZLW3	A0A6M3ZLW3_BACSU	ATP-binding cassette domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22850 PE=4 SV=1
A0A6M4JCI0	A0A6M4JCI0_BACSU	Sodium ABC transporter permease NatB OS=Bacillus subtilis (strain 168) OX=224308 GN=natB PE=4 SV=1
A0A6M4JCJ2	A0A6M4JCJ2_BACSU	Zinc ABC transporter substrate-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01685 PE=3 SV=1
A0A6M4JFX9	A0A6M4JFX9_BACSU	PTS transporter subunit EIIC OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04320 PE=4 SV=1
A0A6M4JHB5	A0A6M4JHB5_BACSU	Oligopeptide ABC transporter permease AppB OS=Bacillus subtilis (strain 168) OX=224308 GN=appB PE=3 SV=1
A0A6M4JHD5	A0A6M4JHD5_BACSU	Oligopeptide ABC transporter ATP-binding protein AppF OS=Bacillus subtilis (strain 168) OX=224308 GN=appF PE=3 SV=1
A0A6M4JMG3	A0A6M4JMG3_BACSU	Two-component system response regulator MaeM OS=Bacillus subtilis (strain 168) OX=224308 GN=maeM PE=4 SV=1
A0A6M4JN68	A0A6M4JN68_BACSU	Bacitracin ABC transporter permease BceB OS=Bacillus subtilis (strain 168) OX=224308 GN=bceB PE=3 SV=1
A0A6M4JP81	A0A6M4JP81_BACSU	Protein-export membrane protein SecG OS=Bacillus subtilis (strain 168) OX=224308 GN=secG PE=3 SV=1
A0A6M4JQE9	A0A6M4JQE9_BACSU	PTS system sucrose transporter subunit IIBC OS=Bacillus subtilis (strain 168) OX=224308 GN=scrA PE=4 SV=1
A0A6M4JQQ2	A0A6M4JQQ2_BACSU	PTS beta-glucoside transporter subunit IIABC OS=Bacillus subtilis (strain 168) OX=224308 GN=bglP PE=4 SV=1
A0A6M3Z6T7	A0A6M3Z6T7_BACSU	Amino acid permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01275 PE=4 SV=1
A0A6M3Z766	A0A6M3Z766_BACSU	Niacin permease NiaP OS=Bacillus subtilis (strain 168) OX=224308 GN=niaP PE=4 SV=1
A0A6M3Z770	A0A6M3Z770_BACSU	L-lactate permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01810 PE=3 SV=1
A0A6M3Z784	A0A6M3Z784_BACSU	Lincomycin efflux MFS transporter Lmr(B) OS=Bacillus subtilis (strain 168) OX=224308 GN=lmr(B) PE=3 SV=1
A0A6M3Z7V3	A0A6M3Z7V3_BACSU	Amino acid permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03220 PE=4 SV=1
A0A6M3Z7Z4	A0A6M3Z7Z4_BACSU	Two pore domain potassium channel family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03565 PE=4 SV=1
A0A6M3Z802	A0A6M3Z802_BACSU	GABA permease OS=Bacillus subtilis (strain 168) OX=224308 GN=gabP PE=4 SV=1
A0A6M3Z835	A0A6M3Z835_BACSU	ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02565 PE=4 SV=1
A0A6M3Z870	A0A6M3Z870_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02560 PE=4 SV=1
A0A6M3Z8Q6	A0A6M3Z8Q6_BACSU	ABC-F family ATP-binding cassette domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04165 PE=4 SV=1

A0A6M3Z8Y4	A0A6M3Z8Y4_BACSU	HlyC/CorC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05355 PE=3 SV=1
A0A6M3Z902	A0A6M3Z902_BACSU	Iron ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04685 PE=3 SV=1
A0A6M3Z946	A0A6M3Z946_BACSU	Sodium:solute symporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05800 PE=3 SV=1
A0A6M3Z958	A0A6M3Z958_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04805 PE=4 SV=1
A0A6M3ZA02	A0A6M3ZA02_BACSU	Magnesium transporter MgtE OS=Bacillus subtilis (strain 168) OX=224308 GN=mgtE PE=3 SV=1
A0A6M3ZA91	A0A6M3ZA91_BACSU	Efflux RND transporter periplasmic adaptor subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07900 PE=3 SV=1
A0A6M3ZBM8	A0A6M3ZBM8_BACSU	MATE family efflux transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09975 PE=4 SV=1
A0A6M3ZE73	A0A6M3ZE73_BACSU	Multidrug efflux MFS transporter Blt OS=Bacillus subtilis (strain 168) OX=224308 GN=blt PE=3 SV=1
A0A6M3ZF08	A0A6M3ZF08_BACSU	Type II toxin-antitoxin system toxin ribonuclease YobL OS=Bacillus subtilis (strain 168) OX=224308 GN=yobL PE=4 SV=1
A0A6M3ZF92	A0A6M3ZF92_BACSU	Alanine:cation symporter family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16320 PE=3 SV=1
A0A6M3ZFS2	A0A6M3ZFS2_BACSU	Multidrug efflux MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18330 PE=4 SV=1
A0A6M3ZFY6	A0A6M3ZFY6_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19190 PE=4 SV=1
A0A6M3ZFZ3	A0A6M3ZFZ3_BACSU	Na+/H+ antiporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19340 PE=3 SV=1
A0A6M3ZG28	A0A6M3ZG28_BACSU	Na+/H+ antiporter subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18390 PE=3 SV=1
A0A6M3ZG73	A0A6M3ZG73_BACSU	Na+/H+ antiporter family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18610 PE=4 SV=1
A0A6M3ZG80	A0A6M3ZG80_BACSU	Type VII secretion protein EssC OS=Bacillus subtilis (strain 168) OX=224308 GN=essC PE=4 SV=1
A0A6M3ZG83	A0A6M3ZG83_BACSU	ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19260 PE=4 SV=1
A0A6M3ZGD3	A0A6M3ZGD3_BACSU	Multidrug efflux SMR transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19415 PE=3 SV=1
A0A6M3ZGK6	A0A6M3ZGK6_BACSU	Efflux RND transporter periplasmic adaptor subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19270 PE=4 SV=1
A0A6M3ZGN3	A0A6M3ZGN3_BACSU	Cadmium-translocating P-type ATPase OS=Bacillus subtilis (strain 168) OX=224308 GN=cadA PE=3 SV=1
A0A6M3ZHI5	A0A6M3ZHI5_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22080 PE=4 SV=1
A0A6M3ZI65	A0A6M3ZI65_BACSU	Efflux RND transporter permease subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03825 PE=4 SV=1
A0A6M3ZIB4	A0A6M3ZIB4_BACSU	Purine/pyrimidine permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21715 PE=3 SV=1
A0A6M3ZIW5	A0A6M3ZIW5_BACSU	Ferrous ion permease EfeU OS=Bacillus subtilis (strain 168) OX=224308 GN=efeU PE=3 SV=1
A0A6M4JCE4	A0A6M4JCE4_BACSU	Probable inorganic carbon transporter subunit DabA OS=Bacillus subtilis (strain 168) OX=224308 GN=dabA PE=3 SV=1
A0A6M4JDN4	A0A6M4JDN4_BACSU	PLP-dependent aminotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03005 PE=3 SV=1
A0A6M4JEA9	A0A6M4JEA9_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04395 PE=4 SV=1
A0A6M4JEZ4	A0A6M4JEZ4_BACSU	Amino acid permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01835 PE=4 SV=1
A0A6M4JFE9	A0A6M4JFE9_BACSU	Mannose transport/utilization transcriptional regulator ManR OS=Bacillus subtilis (strain 168) OX=224308 GN=manR PE=4 SV=1
A0A6M4JG53	A0A6M4JG53_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04730 PE=4 SV=1
A0A6M4JGZ8	A0A6M4JGZ8_BACSU	HAMP domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10110 PE=4 SV=1
A0A6M4JHK0	A0A6M4JHK0_BACSU	MATE family efflux transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10590 PE=4 SV=1
A0A6M4JIG0	A0A6M4JIG0_BACSU	Peptide MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02125 PE=3 SV=1
A0A6M4JJ69	A0A6M4JJ69_BACSU	PDZ domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10670 PE=3 SV=1
A0A6M4JJH3	A0A6M4JJH3_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14805 PE=4 SV=1
A0A6M4JJU0	A0A6M4JJU0_BACSU	Uracil permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08480 PE=3 SV=1
A0A6M4JJU8	A0A6M4JJU8_BACSU	Sulfate permease OS=Bacillus subtilis (strain 168) OX=224308 GN=cysP PE=4 SV=1

A0A6M4JLG2	A0A6M4JLG2_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19060 PE=3 SV=1
A0A6M4JLI3	A0A6M4JLI3_BACSU	HlyC/CorC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05340 PE=3 SV=1
A0A6M4JMS1	A0A6M4JMS1_BACSU	AI-2E family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18145 PE=3 SV=1
A0A6M4JMV3	A0A6M4JMV3_BACSU	Na(+)/H(+) antiporter subunit C OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18400 PE=3 SV=1
A0A6M4JNI8	A0A6M4JNI8_BACSU	Probable queuosine precursor transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13230 PE=3 SV=1
A0A6M4JNZ4	A0A6M4JNZ4_BACSU	Ktr system potassium transporter KtrB OS=Bacillus subtilis (strain 168) OX=224308 GN=ktrB PE=4 SV=1
A0A6M4JP02	A0A6M4JP02_BACSU	Type VII secretion protein EsaA OS=Bacillus subtilis (strain 168) OX=224308 GN=esaA PE=3 SV=1
A0A6M4JQ31	A0A6M4JQ31_BACSU	AEC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21250 PE=3 SV=1
A0A6M4JQB7	A0A6M4JQB7_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21530 PE=3 SV=1
A0A6M4JQG2	A0A6M4JQG2_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21920 PE=4 SV=1
A0A6M4JRE8	A0A6M4JRE8_BACSU	Sodium/proline symporter OS=Bacillus subtilis (strain 168) OX=224308 GN=putP PE=3 SV=1
A0A6M4JRR6	A0A6M4JRR6_BACSU	MOP flippase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20470 PE=4 SV=1
A0A6M3ZAZ1	A0A6M3ZAZ1_BACSU	VirB4-like ATPase ConE OS=Bacillus subtilis (strain 168) OX=224308 GN=conE PE=4 SV=1
A0A6M3ZHB1	A0A6M3ZHB1_BACSU	Cyclic-di-AMP phosphodiesterase PgpH OS=Bacillus subtilis (strain 168) OX=224308 GN=pgpH PE=4 SV=1
A0A6M3ZHR8	A0A6M3ZHR8_BACSU	HD domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21545 PE=4 SV=1
A0A6M3ZJ63	A0A6M3ZJ63_BACSU	ATP-binding cassette domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19200 PE=4 SV=1
A0A6M4JEQ1	A0A6M4JEQ1_BACSU	Two-component system response regulator YhcZ OS=Bacillus subtilis (strain 168) OX=224308 GN=yhcZ PE=4 SV=1
A0A6M4JKX8	A0A6M4JKX8_BACSU	HlyC/CorC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14670 PE=3 SV=1
P42178	NARJ_BACSU	Probable nitrate reductase molybdenum cofactor assembly chaperone NarJ OS=Bacillus subtilis (strain 168) OX=224308 GN=narJ PE=3 SV=1
Chemotaxis & flageller movement		
A0A6M3Z866	A0A6M3Z866_BACSU	Extracellular solute-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04030 PE=4 SV=1
A0A6M3ZIA9	A0A6M3ZIA9_BACSU	Flagellar basal-body rod protein FlgC OS=Bacillus subtilis (strain 168) OX=224308 GN=flgC PE=3 SV=1
A0A6M3ZJE1	A0A6M3ZJE1_BACSU	Methyl-accepting chemotaxis protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19480 PE=4 SV=1
A0A6M4JMS8	A0A6M4JMS8_BACSU	Methyl-accepting chemotaxis protein McpB OS=Bacillus subtilis (strain 168) OX=224308 GN=mcpB PE=4 SV=1
A0A6M4JMY2	A0A6M4JMY2_BACSU	Methyl-accepting chemotaxis protein TlpA OS=Bacillus subtilis (strain 168) OX=224308 GN=tlpA PE=4 SV=1
A0A6M3ZGX5	A0A6M3ZGX5_BACSU	N-acetylmuramoyl-L-alanine amidase LytC OS=Bacillus subtilis (strain 168) OX=224308 GN=lytC PE=4 SV=1
Kinase & tranferases		
A0A6M3Z8H2	A0A6M3Z8H2_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yflR PE=4 SV=1
A0A6M3Z8M0	A0A6M3Z8M0_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yesM PE=4 SV=1
A0A6M3ZAL1	A0A6M3ZAL1_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinE PE=4 SV=1
A0A6M3ZBG3	A0A6M3ZBG3_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=desK PE=4 SV=1
A0A6M3ZG20	A0A6M3ZG20_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinB PE=4 SV=1
A0A6M3ZG86	A0A6M3ZG86_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=maeL PE=4 SV=1
A0A6M3ZHS7	A0A6M3ZHS7_BACSU	Teichoic acid D-alanyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=dltB PE=3 SV=1
A0A6M3ZIN3	A0A6M3ZIN3_BACSU	Two-component sensor histidine kinase BceS OS=Bacillus subtilis (strain 168) OX=224308 GN=bceS PE=4 SV=1
A0A6M4JG44	A0A6M4JG44_BACSU	Phosphatidylglycerol lysyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=mprF PE=3 SV=1
A0A6M4JHL1	A0A6M4JHL1_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinD PE=4 SV=1
A0A6M4JJL3	A0A6M4JJL3_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinC PE=4 SV=1

A0A6M4JNC2	A0A6M4JNC2_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=resE PE=4 SV=1
P39211	XYLB_BACSU	Xylulose kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=xylB PE=3 SV=2
A0A6M3Z8D7	A0A6M3Z8D7_BACSU	PLP-dependent aminotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02975 PE=3 SV=1
A0A6M3Z962	A0A6M3Z962_BACSU	Serine/threonine protein kinase PrkA OS=Bacillus subtilis (strain 168) OX=224308 GN=prkA PE=4 SV=1
A0A6M3Z988	A0A6M3Z988_BACSU	PLP-dependent aminotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06015 PE=3 SV=1
A0A6M3ZAB7	A0A6M3ZAB7_BACSU	Glycosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07365 PE=3 SV=1
A0A6M3ZFM2	A0A6M3ZFM2_BACSU	Glycosyltransferase family 4 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17880 PE=3 SV=1
A0A6M3ZHH9	A0A6M3ZHH9_BACSU	Peptidoglycan glycosyltransferase RodA OS=Bacillus subtilis (strain 168) OX=224308 GN=rodA PE=3 SV=1
A0A6M4JGX6	A0A6M4JGX6_BACSU	[Acyl-carrier-protein] S-malonyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=fabD PE=4 SV=1
A0A6M4JJP7	A0A6M4JJP7_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=ycbM PE=4 SV=1
A0A6M4JK14	A0A6M4JK14_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=yclK PE=4 SV=1
A0A6M4JM70	A0A6M4JM70_BACSU	GNAT family N-acetyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14115 PE=4 SV=1
A0A6M4JPX7	A0A6M4JPX7_BACSU	Non-specific protein-tyrosine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=ptkA PE=3 SV=1
A0A6M4JQD6	A0A6M4JQD6_BACSU	Glycosyltransferase family 2 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21735 PE=4 SV=1
A0A6M3Z8A9	A0A6M3Z8A9_BACSU	Diacylglycerol kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03830 PE=3 SV=1
A0A6M4JFR7	A0A6M4JFR7_BACSU	Amidophosphoribosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=purF PE=3 SV=1
A0A6M4JGW5	A0A6M4JGW5_BACSU	Glycerol kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=glpK PE=3 SV=1
A0A6M4JHC5	A0A6M4JHC5_BACSU	Glutamate 5-kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=proB PE=3 SV=1
A0A6M4JK02	A0A6M4JK02_BACSU	Aminotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05350 PE=3 SV=1
A0A6M4JPK3	A0A6M4JPK3_BACSU	Phosphotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20260 PE=4 SV=1
Q45539	CSBB_BACSU	Putative glycosyltransferase CsbB OS=Bacillus subtilis (strain 168) OX=224308 GN=csbB PE=2 SV=1
A0A6M3ZBA3	A0A6M3ZBA3_BACSU	Sugar kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10070 PE=4 SV=1
A0A6M3ZIY7	A0A6M3ZIY7_BACSU	Aminotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18285 PE=3 SV=1
A0A6M4JHW5	A0A6M4JHW5_BACSU	Glycosyltransferase family 2 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07385 PE=4 SV=1
A0A6M4JKL9	A0A6M4JKL9_BACSU	Glycosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03270 PE=4 SV=1

Metabolic pathway

C/ N & lipid metabolism

A0A6M3Z7D1	A0A6M3Z7D1_BACSU	Assimilatory nitrate reductase catalytic subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=nasC PE=4 SV=1
A0A6M3Z9C7	A0A6M3Z9C7_BACSU	Ornithine carbamoyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=argF PE=3 SV=1
A0A6M3Z9L3	A0A6M3Z9L3_BACSU	Carbamoyl-phosphate synthase large chain OS=Bacillus subtilis (strain 168) OX=224308 GN=carB PE=3 SV=1
A0A6M3ZB03	A0A6M3ZB03_BACSU	Malonyl CoA-acyl carrier protein transacylase OS=Bacillus subtilis (strain 168) OX=224308 GN=fabD PE=3 SV=1
A0A6M3ZBD1	A0A6M3ZBD1_BACSU	2-hydroxyacid dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10075 PE=3 SV=1
A0A6M3ZC25	A0A6M3ZC25_BACSU	Enoyl-[acyl-carrier-protein] reductase FabL OS=Bacillus subtilis (strain 168) OX=224308 GN=fabL PE=4 SV=1
A0A6M3ZDL7	A0A6M3ZDL7_BACSU	6-phosphogluconate dehydrogenase, decarboxylating OS=Bacillus subtilis (strain 168) OX=224308 GN=gndA PE=3 SV=1
A0A6M3ZED6	A0A6M3ZED6_BACSU	Levanase OS=Bacillus subtilis (strain 168) OX=224308 GN=sacC PE=3 SV=1
A0A6M3ZF01	A0A6M3ZF01_BACSU	L-ribulose-5-phosphate 4-epimerase OS=Bacillus subtilis (strain 168) OX=224308 GN=araD PE=3 SV=1
A0A6M3ZFX3	A0A6M3ZFX3_BACSU	Glycogen synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=glgA PE=3 SV=1
A0A6M3ZG89	A0A6M3ZG89_BACSU	(2,3-dihydroxybenzoyl)adenylate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18580 PE=4 SV=1

A0A6M3ZGM1	A0A6M3ZGM1_BACSU	Assimilatory sulfite reductase (NADPH) flavoprotein subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19350 PE=4 SV=1
A0A6M3ZHW5	A0A6M3ZHW5_BACSU	Catalase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22285 PE=3 SV=1
A0A6M3ZHY9	A0A6M3ZHY9_BACSU	Aryl-phospho-beta-d-glucosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=bglH PE=3 SV=1
A0A6M3ZIA5	A0A6M3ZIA5_BACSU	Malate dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=mdh PE=3 SV=1
A0A6M4JH25	A0A6M4JH25_BACSU	Protoheme IX farnesyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=cyoE PE=3 SV=1
A0A6M4JJN1	A0A6M4JJN1_BACSU	6-phospho-alpha-glucosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04555 PE=3 SV=1
A0A6M4JKK3	A0A6M4JKK3_BACSU	Alpha-N-arabinofuranosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16795 PE=3 SV=1
A0A6M4JKQ5	A0A6M4JKQ5_BACSU	3-isopropylmalate dehydratase large subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=leuC PE=3 SV=1
A0A6M4JN96	A0A6M4JN96_BACSU	Glucose-6-phosphate isomerase OS=Bacillus subtilis (strain 168) OX=224308 GN=pgi PE=3 SV=1
A0A6M4JNX9	A0A6M4JNX9_BACSU	Acyl--CoA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17220 PE=4 SV=1
A0A6M4JP24	A0A6M4JP24_BACSU	Fumarate hydratase class II OS=Bacillus subtilis (strain 168) OX=224308 GN=fumC PE=3 SV=1
A0A6M4JPA8	A0A6M4JPA8_BACSU	Triosephosphate isomerase OS=Bacillus subtilis (strain 168) OX=224308 GN=tpiA PE=3 SV=1
A0A6M4JPE5	A0A6M4JPE5_BACSU	2,6-beta-fructan 6-levanbiohydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=levB PE=3 SV=1
A0A6M4JPH5	A0A6M4JPH5_BACSU	Maltogenic alpha-amylase OS=Bacillus subtilis (strain 168) OX=224308 GN=mdxD PE=4 SV=1
A0A6M4JPR3	A0A6M4JPR3_BACSU	Undecaprenyl/decaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20435 PE=4 SV=1
A0A6M4JQE7	A0A6M4JQE7_BACSU	Aldehyde dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21725 PE=3 SV=1
A0A6M4JR50	A0A6M4JR50_BACSU	6-phospho-beta-glucosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22825 PE=3 SV=1
A0A6M4JRM0	A0A6M4JRM0_BACSU	Formate dehydrogenase subunit alpha OS=Bacillus subtilis (strain 168) OX=224308 GN=fdhF PE=3 SV=1
A0A6M3Z746	A0A6M3Z746_BACSU	Enantioselective carboxylesterase CesB OS=Bacillus subtilis (strain 168) OX=224308 GN=cesB PE=4 SV=1
A0A6M3ZF64	A0A6M3ZF64_BACSU	Unsaturated rhamnogalacturonyl hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=rmgQ PE=4 SV=1
A0A6M3ZJU8	A0A6M3ZJU8_BACSU	Carbon starvation protein A OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16790 PE=3 SV=1
A0A6M4JM36	A0A6M4JM36_BACSU	Polyketide biosynthesis malonyl-ACP decarboxylase PksF OS=Bacillus subtilis (strain 168) OX=224308 GN=pksF PE=3 SV=1
A0A6M4JNA0	A0A6M4JNA0_BACSU	Alpha/beta hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10650 PE=4 SV=1
A0A6M3ZHA0	A0A6M3ZHA0_BACSU	Galactose-1-phosphate uridylyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=galT PE=3 SV=1
A0A6M4JGU3	A0A6M4JGU3_BACSU	Fumarylacetoacetate hydrolase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05950 PE=4 SV=1
A0A6M4JKS6	A0A6M4JKS6_BACSU	Acyl-CoA dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14365 PE=3 SV=1
A0A6M3ZCJ6	A0A6M3ZCJ6_BACSU	Long-chain-fatty-acid--CoA ligase LcfB OS=Bacillus subtilis (strain 168) OX=224308 GN=lcfB PE=3 SV=1
A0A6M3ZH93	A0A6M3ZH93_BACSU	Nitrate reductase (quinone) OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21380 PE=3 SV=1
A0A6M4JIC1	A0A6M4JIC1_BACSU	4-hydroxy-tetrahydrodipicolinate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=dapA PE=3 SV=1
A0A6M4JK59	A0A6M4JK59_BACSU	Long-chain-fatty-acid--CoA ligase LcfA OS=Bacillus subtilis (strain 168) OX=224308 GN=lcfA PE=4 SV=1
P00691	AMY_BACSU	Alpha-amylase OS=Bacillus subtilis (strain 168) OX=224308 GN=amyE PE=1 SV=2
A0A6M4JKS6	A0A6M4JKS6_BACSU	Acyl-CoA dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14365 PE=3 SV=1
Amino acid metabolism		
A0A6M3Z6D7	A0A6M3Z6D7_BACSU	Methionine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=metG PE=3 SV=1
A0A6M3Z814	A0A6M3Z814_BACSU	Phosphoribosylformylglycinamidine cyclo-ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=purM PE=3 SV=1
A0A6M3Z8V8	A0A6M3Z8V8_BACSU	Aspartate aminotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05195 PE=3 SV=1
A0A6M3Z920	A0A6M3Z920_BACSU	Phosphoserine aminotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=serC PE=3 SV=1

A0A6M3Z9X4	A0A6M3Z9X4_BACSU	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=metE PE=3 SV=1
A0A6M3ZBC2	A0A6M3ZBC2_BACSU	Glutamate synthase large subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=gltB PE=3 SV=1
A0A6M3ZBL3	A0A6M3ZBL3_BACSU	2-oxoglutarate dehydrogenase E1 component OS=Bacillus subtilis (strain 168) OX=224308 GN=sucA PE=3 SV=1
A0A6M3ZBP5	A0A6M3ZBP5_BACSU	Glutathione hydrolase proenzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=ggt PE=3 SV=1
A0A6M3ZEA7	A0A6M3ZEA7_BACSU	Glutathione-dependent formaldehyde dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15860 PE=3 SV=1
A0A6M3ZF59	A0A6M3ZF59_BACSU	Cysteine synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=cysK PE=3 SV=1
A0A6M3ZFZ8	A0A6M3ZFZ8_BACSU	Homoserine dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18720 PE=3 SV=1
A0A6M3ZG60	A0A6M3ZG60_BACSU	Alanine dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=ald PE=3 SV=1
A0A6M3ZI13	A0A6M3ZI13_BACSU	Asparagine synthase (Glutamine-hydrolyzing) OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=3 SV=1
A0A6M4JEL1	A0A6M4JEL1_BACSU	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Bacillus subtilis (strain 168) OX=224308 GN=glmS PE=3 SV=1
A0A6M4JL79	A0A6M4JL79_BACSU	Dipeptidase PepV OS=Bacillus subtilis (strain 168) OX=224308 GN=pepV PE=4 SV=1
A0A6M4JM76	A0A6M4JM76_BACSU	Probable D-serine dehydratase OS=Bacillus subtilis (strain 168) OX=224308 GN=dsdA PE=3 SV=1
A0A6M4JPH8	A0A6M4JPH8_BACSU	Histidinol dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=hisD PE=3 SV=1
A0A6M4JRI7	A0A6M4JRI7_BACSU	Cystathionine gamma-synthase/O-acetylhomoserine thiolyase OS=Bacillus subtilis (strain 168) OX=224308 GN=metI PE=3 SV=1
A0A6M4JRL8	A0A6M4JRL8_BACSU	Choline dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=gbsB PE=4 SV=1
A3F3C7	A3F3C7_BACSU	Tryptophan synthase beta chain OS=Bacillus subtilis (strain 168) OX=224308 GN=trpB PE=3 SV=1
O30509	GATB_BACSU	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B OS=Bacillus subtilis (strain 168) OX=224308 GN=gatB PE=1 SV=2
P54420	ASNB_BACSU	Asparagine synthetase [glutamine-hydrolyzing] 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=1 SV=2
A0A6M3Z9U7	A0A6M3Z9U7_BACSU	Serine/threonine exchanger OS=Bacillus subtilis (strain 168) OX=224308 GN=steT PE=4 SV=1
A0A6M3ZAN3	A0A6M3ZAN3_BACSU	Gamma-glutamylcyclotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07975 PE=3 SV=1
A0A6M4JGK6	A0A6M4JGK6_BACSU	Poly-gamma-glutamate hydrolase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09375 PE=4 SV=1
A0A6M4JMR1	A0A6M4JMR1_BACSU	S9 family peptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18705 PE=3 SV=1
A0A6M3Z9F9	A0A6M3Z9F9_BACSU	N-acetyl amino acid acetylase SndC OS=Bacillus subtilis (strain 168) OX=224308 GN=sndC PE=4 SV=1
Nucleotide metabolism		
A0A6M3ZJM5	A0A6M3ZJM5_BACSU	(p)ppGpp synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=relA PE=3 SV=1
A0A6M4JEI9	A0A6M4JEI9_BACSU	Phosphomethylpyrimidine synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=thiC PE=3 SV=1
A0A6M4JG22	A0A6M4JG22_BACSU	Multifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase/5'-nucleotidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04385 PE=3 SV=1
O31875	NRDEB_BACSU	Ribonucleoside-diphosphate reductase NrdEB subunit alpha OS=Bacillus subtilis (strain 168) OX=224308 GN=nrdEB PE=3 SV=2
Central metabolism(TCA/electron transport)		
A0A6M3ZBL7	A0A6M3ZBL7_BACSU	Bifunctional cytochrome P450/NADPH--P450 reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04105 PE=3 SV=1
A0A6M3ZEC0	A0A6M3ZEC0_BACSU	Bifunctional cytochrome P450/NADPH--P450 reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15965 PE=3 SV=1
A0A6M4JJZ2	A0A6M4JJZ2_BACSU	Quinolinate phosphoribosyltransferase [decarboxylating] OS=Bacillus subtilis (strain 168) OX=224308 GN=nadC PE=3 SV=1
A0A6M4JNE1	A0A6M4JNE1_BACSU	Pyrroline-5-carboxylate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=proC PE=3 SV=1
A0A6M4JNX1	A0A6M4JNX1_BACSU	Dephospho-CoA kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=coaE PE=3 SV=1
A0A6M4JNY5	A0A6M4JNY5_BACSU	Biotin biosynthesis cytochrome P450 OS=Bacillus subtilis (strain 168) OX=224308 GN=bioI PE=3 SV=1
A0A6M4JP58	A0A6M4JP58_BACSU	Sulfite reductase [NADPH] hemoprotein beta-component OS=Bacillus subtilis (strain 168) OX=224308 GN=cysI PE=3 SV=1
A0A6M3ZAF6	A0A6M3ZAF6_BACSU	Cytochrome c oxidase assembly factor CtaG OS=Bacillus subtilis (strain 168) OX=224308 GN=ctaG PE=4 SV=1

A0A6M3ZB16	A0A6M3ZB16_BACSU	Cytochrome P450 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09355 PE=3 SV=1
A0A6M3ZD81	A0A6M3ZD81_BACSU	Cytochrome c biogenesis protein ResC OS=Bacillus subtilis (strain 168) OX=224308 GN=resC PE=4 SV=1
A0A6M3ZDZ2	A0A6M3ZDZ2_BACSU	Heme A synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=ctaA PE=3 SV=1
A0A6M4JMD9	A0A6M4JMD9_BACSU	NADP-dependent malic enzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=maeB PE=3 SV=1
A0A6M4JQL6	A0A6M4JQL6_BACSU	Cytochrome d ubiquinol oxidase subunit II OS=Bacillus subtilis (strain 168) OX=224308 GN=cydB PE=3 SV=1
P09339	ACNA_BACSU	Aconitate hydratase A OS=Bacillus subtilis (strain 168) OX=224308 GN=citB PE=1 SV=4
Cell wall synthesis		
A0A6M3Z8T2	A0A6M3Z8T2_BACSU	Lipoteichoic acid synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=ltaS PE=3 SV=1
A0A6M3ZID1	A0A6M3ZID1_BACSU	LTA synthase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04110 PE=3 SV=1
A0A6M4JME5	A0A6M4JME5_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14800 PE=3 SV=1
A0A6M3Z9U6	A0A6M3Z9U6_BACSU	Gamma-D-glutamyl-L-lysine dipeptidyl-peptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=eepC PE=3 SV=1
A0A6M3ZEE3	A0A6M3ZEE3_BACSU	Transglycosylase SLT domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15330 PE=4 SV=1
A0A6M3ZIM4	A0A6M3ZIM4_BACSU	Polyisoprenyl-teichoic acid--peptidoglycan teichoic acid transferase TagU OS=Bacillus subtilis (strain 168) OX=224308 GN=lytR PE=3 SV=1
A0A6M3ZJI5	A0A6M3ZJI5_BACSU	Biofilm exopolysaccharide biosynthesis protein EpsG OS=Bacillus subtilis (strain 168) OX=224308 GN=epsG PE=4 SV=1
A0A6M4JK47	A0A6M4JK47_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02380 PE=3 SV=1
A0A6M4JLM2	A0A6M4JLM2_BACSU	Bacillopeptidase F OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08390 PE=3 SV=1
O34851	LDC_BACSU	Probable murein peptide carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=ykfA PE=2 SV=2
A0A6M3ZA38	A0A6M3ZA38_BACSU	Di/tri-peptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=papB PE=4 SV=1
A0A6M3ZAD6	A0A6M3ZAD6_BACSU	Endopeptidase La OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08260 PE=3 SV=1
A0A6M4JFF8	A0A6M4JFF8_BACSU	Peptidoglycan-N-acetylmuramic acid deacetylase PdaC OS=Bacillus subtilis (strain 168) OX=224308 GN=pdaC PE=4 SV=1
A0A6M3ZH02	A0A6M3ZH02_BACSU	LTD domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20185 PE=4 SV=1
A0A6M3ZM39	A0A6M3ZM39_BACSU	Transglycosylase SLT domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07005 PE=4 SV=1
A0A6M4JIR1	A0A6M4JIR1_BACSU	LysM peptidoglycan-binding domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13775 PE=4 SV=1
Uncharacterized protein		
A0A6M3Z7Y8	A0A6M3Z7Y8_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03165 PE=4 SV=1
A0A6M3Z9Z3	A0A6M3Z9Z3_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07380 PE=4 SV=1
A0A6M3ZBR5	A0A6M3ZBR5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10210 PE=4 SV=1
A0A6M3ZBY2	A0A6M3ZBY2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11200 PE=4 SV=1
A0A6M3ZD43	A0A6M3ZD43_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11505 PE=4 SV=1
A0A6M3ZIT4	A0A6M3ZIT4_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11470 PE=4 SV=1
A0A6M4JFW5	A0A6M4JFW5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04025 PE=4 SV=1
A0A6M4JPI6	A0A6M4JPI6_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20155 PE=4 SV=1
A0A6M4JRK5	A0A6M4JRK5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10265 PE=4 SV=1
P45917	YQBA_BACSU	Uncharacterized protein YqbA OS=Bacillus subtilis (strain 168) OX=224308 GN=yqbA PE=3 SV=1
P54722	YFIF_BACSU	Uncharacterized HTH-type transcriptional regulator YfiF OS=Bacillus subtilis (strain 168) OX=224308 GN=yfiF PE=4 SV=1
Stress resistance/response proteins		

A0A6M3ZDQ8	A0A6M3ZDQ8_BACSU	PBP1A family penicillin-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13425 PE=4 SV=1
A0A6M3ZAB0	A0A6M3ZAB0_BACSU	Autolysin OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06870 PE=4 SV=1
A0A6M3ZHM5	A0A6M3ZHM5_BACSU	Azaleucine resistance protein AzlC OS=Bacillus subtilis (strain 168) OX=224308 GN=azlC PE=3 SV=1
A0A6M3ZAZ2	A0A6M3ZAZ2_BACSU	Putative competence-damage inducible protein OS=Bacillus subtilis (strain 168) OX=224308 GN=cinA PE=3 SV=1
A0A6M4JQQ1	A0A6M4JQQ1_BACSU	Catalase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22075 PE=3 SV=1
A0A6M3Z7P4	A0A6M3Z7P4_BACSU	Manganese catalase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02530 PE=3 SV=1
A0A6M3Z9L6	A0A6M3Z9L6_BACSU	Monooxygenase YjiB OS=Bacillus subtilis (strain 168) OX=224308 GN=yjiB PE=3 SV=1
A0A6M3ZFN9	A0A6M3ZFN9_BACSU	Gfo/Idh/MocA family oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17490 PE=4 SV=1
A0A6M4JLT0	A0A6M4JLT0_BACSU	Monooxygenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05825 PE=4 SV=1
O34720	YJGC_BACSU	Probable oxidoreductase YjgC OS=Bacillus subtilis (strain 168) OX=224308 GN=yjgC PE=3 SV=1

Sporulation specific proteins

A0A6M3ZAY4	A0A6M3ZAY4_BACSU	YhcN/YlaJ family sporulation lipoprotein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08135 PE=4 SV=1
A0A6M3ZB79	A0A6M3ZB79_BACSU	DNA translocase SpoIIIE OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIIE PE=3 SV=1
A0A6M3ZHC5	A0A6M3ZHC5_BACSU	Germination protease OS=Bacillus subtilis (strain 168) OX=224308 GN=gpr PE=3 SV=1
A0A6M4JDY4	A0A6M4JDY4_BACSU	Spore germination protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04360 PE=3 SV=1
A0A6M4JEX2	A0A6M4JEX2_BACSU	Spore germination protein GerKA OS=Bacillus subtilis (strain 168) OX=224308 GN=gerKA PE=3 SV=1
A0A6M4JGB8	A0A6M4JGB8_BACSU	Spore coat protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05155 PE=3 SV=1
A0A6M4JGP6	A0A6M4JGP6_BACSU	Spore coat protein CotU OS=Bacillus subtilis (strain 168) OX=224308 GN=cotU PE=4 SV=1
A0A6M4JIW0	A0A6M4JIW0_BACSU	Adapter protein MecA OS=Bacillus subtilis (strain 168) OX=224308 GN=mecA PE=3 SV=1
A0A6M4JJI5	A0A6M4JJI5_BACSU	Sporulation killing factor system radical SAM maturase OS=Bacillus subtilis (strain 168) OX=224308 GN=skfB PE=4 SV=1
A0A6M4JJI9	A0A6M4JJI9_BACSU	Ger(X)C family spore germination protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04355 PE=3 SV=1
A0A6M4JMX1	A0A6M4JMX1_BACSU	Spore coat morphogenetic protein SpoVID OS=Bacillus subtilis (strain 168) OX=224308 GN=spoVID PE=4 SV=1
A0A6M4JMX4	A0A6M4JMX4_BACSU	Endopeptidase La OS=Bacillus subtilis (strain 168) OX=224308 GN=lonB PE=3 SV=1
A0A6M4JR62	A0A6M4JR62_BACSU	Sporulation initiation inhibitor protein Soj OS=Bacillus subtilis (strain 168) OX=224308 GN=soj PE=4 SV=1
O34894	EZRA_BACSU	Septation ring formation regulator EzrA OS=Bacillus subtilis (strain 168) OX=224308 GN=ezrA PE=1 SV=1
P35149	SP4A_BACSU	Stage IV sporulation protein A OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIVA PE=1 SV=1
P37575	SP2B_BACSU	Stage II sporulation protein B OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIB PE=1 SV=1
A0A6M3ZJ27	A0A6M3ZJ27_BACSU	Sporulation transcriptional activator AdeR OS=Bacillus subtilis (strain 168) OX=224308 GN=adeR PE=3 SV=1
A0A6M3Z7Q5	A0A6M3Z7Q5_BACSU	Response regulator aspartate phosphatase RapJ OS=Bacillus subtilis (strain 168) OX=224308 GN=rapJ PE=3 SV=1
A0A6M3ZE41	A0A6M3ZE41_BACSU	Rhomboid protease GluP OS=Bacillus subtilis (strain 168) OX=224308 GN=glpG PE=4 SV=1

Others

A0A6M4JH86	A0A6M4JH86_BACSU	Non-ribosomal plipastatin synthetase PpsE OS=Bacillus subtilis (strain 168) OX=224308 GN=ppsE PE=3 SV=1
A0A6M4JLH4	A0A6M4JLH4_BACSU	Cysteine desulfurase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16155 PE=3 SV=1
A0A6M3Z8Q5	A0A6M3Z8Q5_BACSU	Amidase domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05030 PE=4 SV=1
A0A6M3Z9J5	A0A6M3Z9J5_BACSU	HTTM domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06100 PE=4 SV=1
A0A6M3Z9K2	A0A6M3Z9K2_BACSU	DUF1641 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06780 PE=4 SV=1
A0A6M3ZC38	A0A6M3ZC38_BACSU	LXG domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11620 PE=4 SV=1

A0A6M3ZDR1	A0A6M3ZDR1_BACSU	EAL domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07770 PE=4 SV=1
A0A6M3ZE83	A0A6M3ZE83_BACSU	Small-conductance mechanosensitive channel protein MscY OS=Bacillus subtilis (strain 168) OX=224308 GN=mscY PE=3 SV=1
A0A6M3ZEQ1	A0A6M3ZEQ1_BACSU	Trigger factor OS=Bacillus subtilis (strain 168) OX=224308 GN=tig PE=3 SV=1
A0A6M3ZFT3	A0A6M3ZFT3_BACSU	YtkA domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17770 PE=4 SV=1
A0A6M3ZGF4	A0A6M3ZGF4_BACSU	Carboxy-terminal processing protease CtpB OS=Bacillus subtilis (strain 168) OX=224308 GN=ctpB PE=3 SV=1
A0A6M3ZHG5	A0A6M3ZHG5_BACSU	TIGR02206 family membrane protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21970 PE=4 SV=1
A0A6M3ZHQ9	A0A6M3ZHQ9_BACSU	Holin-like protein CidA OS=Bacillus subtilis (strain 168) OX=224308 GN=cidA PE=3 SV=1
A0A6M3ZHZ2	A0A6M3ZHZ2_BACSU	DUF2326 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22875 PE=4 SV=1
A0A6M3ZK18	A0A6M3ZK18_BACSU	4Fe-4S dicluster domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21325 PE=4 SV=1
A0A6M3ZLZ5	A0A6M3ZLZ5_BACSU	YitT family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_23215 PE=4 SV=1
A0A6M4JEA8	A0A6M4JEA8_BACSU	DUF1343 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01050 PE=4 SV=1
A0A6M4JEX1	A0A6M4JEX1_BACSU	CdaA regulatory protein CdaR OS=Bacillus subtilis (strain 168) OX=224308 GN=cdaR PE=4 SV=1
A0A6M4JF49	A0A6M4JF49_BACSU	DUF4901 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01655 PE=4 SV=1
A0A6M4JGL2	A0A6M4JGL2_BACSU	UPF0060 membrane protein HIR78_04525 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04525 PE=3 SV=1
A0A6M4JI00	A0A6M4JI00_BACSU	Prophage_tail domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11535 PE=4 SV=1
A0A6M4JIA3	A0A6M4JIA3_BACSU	UPF0637 protein HIR78_08060 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08060 PE=3 SV=1
A0A6M4JJ90	A0A6M4JJ90_BACSU	EAL domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07400 PE=4 SV=1
A0A6M4JJS7	A0A6M4JJS7_BACSU	DUF1275 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10125 PE=4 SV=1
A0A6M4JKH6	A0A6M4JKH6_BACSU	YtxH domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17330 PE=4 SV=1
A0A6M4JM88	A0A6M4JM88_BACSU	YndM family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09690 PE=4 SV=1
A0A6M4JMA5	A0A6M4JMA5_BACSU	Tetratricopeptide repeat protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16145 PE=4 SV=1
A0A6M4JMV4	A0A6M4JMV4_BACSU	Herpes_ori_bp domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11330 PE=4 SV=1
A0A6M4JN58	A0A6M4JN58_BACSU	Extracellular solute-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17515 PE=4 SV=1
A0A6M4JN83	A0A6M4JN83_BACSU	YppE family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13400 PE=4 SV=1
A0A6M4JN94	A0A6M4JN94_BACSU	Tetratricopeptide repeat protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13560 PE=4 SV=1
A0A6M4JPN1	A0A6M4JPN1_BACSU	YitT family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20305 PE=4 SV=1
A0A6M4JQ24	A0A6M4JQ24_BACSU	DUF1565 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20940 PE=4 SV=1
P29141	SUBV_BACSU	Minor extracellular protease vpr OS=Bacillus subtilis (strain 168) OX=224308 GN=vpr PE=1 SV=1
P32437	YVYE_BACSU	IMPACT family member YvyE OS=Bacillus subtilis (strain 168) OX=224308 GN=yvyE PE=3 SV=2
P50863	APBC_BACSU	Iron-sulfur cluster carrier protein OS=Bacillus subtilis (strain 168) OX=224308 GN=salA PE=2 SV=1
A0A6M3ZMB6	A0A6M3ZMB6_BACSU	Insulinase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21445 PE=4 SV=1
A0A6M3ZBH5	A0A6M3ZBH5_BACSU	KAP NTPase domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10335 PE=4 SV=1
A0A6M3ZFG6	A0A6M3ZFG6_BACSU	Molybdenum cofactor biosynthesis protein B OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17165 PE=3 SV=1
A0A6M3ZIN0	A0A6M3ZIN0_BACSU	Type III polyketide synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13275 PE=3 SV=1
A0A6M4JHK3	A0A6M4JHK3_BACSU	PhzF family phenazine biosynthesis protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04695 PE=4 SV=1

Table S1 C) Protein profiles of spores of *Bacillus subtilis* TB10 produced under control condition

Accession	Entry	Description
Molecular functions		
DNA Replication		
A0A6M3Z6P2	A0A6M3Z6P2_BACSU	DNA-directed RNA polymerase subunit beta' OS=Bacillus subtilis (strain 168) OX=224308 GN=rpoC PE=3 SV=1
A0A6M3ZAK9	A0A6M3ZAK9_BACSU	ATP-dependent DNA helicase RecG OS=Bacillus subtilis (strain 168) OX=224308 GN=recG PE=3 SV=1
A0A6M3ZAS7	A0A6M3ZAS7_BACSU	Isoleucine--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=ileS PE=3 SV=1
A0A6M3ZAS8	A0A6M3ZAS8_BACSU	DNA polymerase III PolC-type OS=Bacillus subtilis (strain 168) OX=224308 GN=polC PE=3 SV=1
A0A6M3ZKU9	A0A6M3ZKU9_BACSU	Ribonuclease R OS=Bacillus subtilis (strain 168) OX=224308 GN=rnr PE=3 SV=1
A0A6M4JI35	A0A6M4JI35_BACSU	DNA-directed DNA polymerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12350 PE=4 SV=1
A0A6M4JI95	A0A6M4JI95_BACSU	Proline--tRNA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=proS PE=3 SV=1
A0A6M4JP70	A0A6M4JP70_BACSU	DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=helD PE=4 SV=1
A0A6M3ZAX9	A0A6M3ZAX9_BACSU	Chromosome partition protein Smc OS=Bacillus subtilis (strain 168) OX=224308 GN=smc PE=3 SV=1
A0A6M3ZB70	A0A6M3ZB70_BACSU	AMP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09910 PE=4 SV=1
A0A6M3ZC49	A0A6M3ZC49_BACSU	DNA-directed DNA polymerase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10985 PE=4 SV=1
A0A6M3ZD79	A0A6M3ZD79_BACSU	Ribonuclease YeeF family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13010 PE=4 SV=1
A0A6M3ZGR7	A0A6M3ZGR7_BACSU	GTPase Der OS=Bacillus subtilis (strain 168) OX=224308 GN=engA PE=3 SV=1
A0A6M4JJ14	A0A6M4JJ14_BACSU	DNA gyrase subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=gyrA PE=3 SV=1
A0A6M4JJB7	A0A6M4JJB7_BACSU	DNA helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11310 PE=3 SV=1
A0A6M4JLU8	A0A6M4JLU8_BACSU	DNA topoisomerase 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=topA PE=3 SV=1
A0A6M4JMP9	A0A6M4JMP9_BACSU	DNA translocase SftA OS=Bacillus subtilis (strain 168) OX=224308 GN=sftA PE=3 SV=1
A0A6M4JNB0	A0A6M4JNB0_BACSU	Ribonucleoside-diphosphate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10885 PE=3 SV=1
A0A6M4JPK5	A0A6M4JPK5_BACSU	Cell division topological determinant MinJ OS=Bacillus subtilis (strain 168) OX=224308 GN=minJ PE=4 SV=1
A0A6M4JPQ1	A0A6M4JPQ1_BACSU	ATP-dependent helicase ComFA OS=Bacillus subtilis (strain 168) OX=224308 GN=comFA PE=4 SV=1
A0A6M4JPZ1	A0A6M4JPZ1_BACSU	DEAD/DEAH box helicase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20845 PE=4 SV=1
A0A6M4JJK2	A0A6M4JJK2_BACSU	MBL fold metallo-hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15600 PE=4 SV=1
DNA repair		
A0A6M3Z7Y6	A0A6M3Z7Y6_BACSU	Chaperonin GroEL OS=Bacillus subtilis (strain 168) OX=224308 GN=groL PE=3 SV=1
A0A6M3ZEY8	A0A6M3ZEY8_BACSU	Endonuclease MutS2 OS=Bacillus subtilis (strain 168) OX=224308 GN=mutS2 PE=3 SV=1
A0A6M4JE64	A0A6M4JE64_BACSU	Transcription-repair-coupling factor OS=Bacillus subtilis (strain 168) OX=224308 GN=mfd PE=3 SV=1
A0A6M4JN18	A0A6M4JN18_BACSU	Protein RecA OS=Bacillus subtilis (strain 168) OX=224308 GN=recA PE=3 SV=1
A0A6M4JPL4	A0A6M4JPL4_BACSU	UvrABC system protein B OS=Bacillus subtilis (strain 168) OX=224308 GN=uvrB PE=3 SV=1
A0A6M4JET8	A0A6M4JET8_BACSU	Nuclease SbcCD subunit D OS=Bacillus subtilis (strain 168) OX=224308 GN=sbcD PE=3 SV=1
A0A6M4JR91	A0A6M4JR91_BACSU	Recombinase domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03840 PE=4 SV=1
Transcription regulator		
A0A6M3Z7B9	A0A6M3Z7B9_BACSU	MurR/RpiR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01070 PE=4 SV=1
A0A6M3Z813	A0A6M3Z813_BACSU	Transcriptional repressor MtlR OS=Bacillus subtilis (strain 168) OX=224308 GN=mtlR PE=4 SV=1
A0A6M3ZD82	A0A6M3ZD82_BACSU	Transcriptional regulator GmuR OS=Bacillus subtilis (strain 168) OX=224308 GN=gmuR PE=4 SV=1
A0A6M3ZEF9	A0A6M3ZEF9_BACSU	Transcription termination/antitermination protein NusA OS=Bacillus subtilis (strain 168) OX=224308 GN=nusA PE=3 SV=1

A0A6M3ZF32	A0A6M3ZF32_BACSU	Replication initiation membrane attachment protein DnaB OS=Bacillus subtilis (strain 168) OX=224308 GN=dnaB PE=4 SV=1
A0A6M3ZF76	A0A6M3ZF76_BACSU	Fatty acid metabolism transcriptional regulator FadR OS=Bacillus subtilis (strain 168) OX=224308 GN=fadR PE=4 SV=1
A0A6M3ZHF7	A0A6M3ZHF7_BACSU	Transcriptional regulator LicR OS=Bacillus subtilis (strain 168) OX=224308 GN=licR PE=4 SV=1
A0A6M3ZHU0	A0A6M3ZHU0_BACSU	SWIM zinc finger family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20840 PE=4 SV=1
A0A6M4JCC8	A0A6M4JCC8_BACSU	Transcriptional regulator Btr OS=Bacillus subtilis (strain 168) OX=224308 GN=btr PE=4 SV=1
A0A6M4JFI2	A0A6M4JFI2_BACSU	Anti-sigma-I factor RsgI OS=Bacillus subtilis (strain 168) OX=224308 GN=rsgI PE=4 SV=1
A0A6M4JGP4	A0A6M4JGP4_BACSU	AbrB family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05860 PE=4 SV=1
A0A6M4JJQ3	A0A6M4JJQ3_BACSU	Transcriptional regulator LevR OS=Bacillus subtilis (strain 168) OX=224308 GN=levR PE=4 SV=1
A0A6M4JPT2	A0A6M4JPT2_BACSU	LacI family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20650 PE=4 SV=1
A0A6M4JR12	A0A6M4JR12_BACSU	TetR family transcriptional regulator OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22690 PE=4 SV=1
Q03222	RHO_BACSU	Transcription termination factor Rho OS=Bacillus subtilis (strain 168) OX=224308 GN=rho PE=3 SV=3
Transport and chemotaxis and flageller assembly		
Basic transport & secretion system		
A0A6M3ZAN6	A0A6M3ZAN6_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07890 PE=4 SV=1
A0A6M3ZDF8	A0A6M3ZDF8_BACSU	HMP/thiamine ABC transporter permease ThiX OS=Bacillus subtilis (strain 168) OX=224308 GN=thiX PE=4 SV=1
A0A6M3ZGY4	A0A6M3ZGY4_BACSU	Transport permease protein OS=Bacillus subtilis (strain 168) OX=224308 GN=tagG PE=3 SV=1
A0A6M4JE55	A0A6M4JE55_BACSU	ABC transporter ATP-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04570 PE=4 SV=1
A0A6M4JF00	A0A6M4JF00_BACSU	Signal peptidase I OS=Bacillus subtilis (strain 168) OX=224308 GN=lepB PE=3 SV=1
A0A6M4JFL2	A0A6M4JFL2_BACSU	Alanine:cation symporter family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01455 PE=3 SV=1
A0A6M4JK33	A0A6M4JK33_BACSU	PTS fructose transporter subunit IIC OS=Bacillus subtilis (strain 168) OX=224308 GN=levF PE=4 SV=1
A0A6M4JKV9	A0A6M4JKV9_BACSU	Sublancin transporter SunT OS=Bacillus subtilis (strain 168) OX=224308 GN=sunT PE=4 SV=1
A0A6M4JM05	A0A6M4JM05_BACSU	Oligopeptide ABC transporter substrate-binding protein OppA OS=Bacillus subtilis (strain 168) OX=224308 GN=oppA PE=3 SV=1
A0A6M4JN68	A0A6M4JN68_BACSU	Bacitracin ABC transporter permease BceB OS=Bacillus subtilis (strain 168) OX=224308 GN=bceB PE=3 SV=1
P24141	OPPA_BACSU	Oligopeptide-binding protein OppA OS=Bacillus subtilis (strain 168) OX=224308 GN=oppA PE=1 SV=1
A0A6M3Z7C0	A0A6M3Z7C0_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01815 PE=3 SV=1
A0A6M3Z8Y4	A0A6M3Z8Y4_BACSU	HlyC/CorC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05355 PE=3 SV=1
A0A6M3Z946	A0A6M3Z946_BACSU	Sodium:solute symporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05800 PE=3 SV=1
A0A6M3Z9U7	A0A6M3Z9U7_BACSU	Serine/threonine exchanger OS=Bacillus subtilis (strain 168) OX=224308 GN=steT PE=4 SV=1
A0A6M3ZA02	A0A6M3ZA02_BACSU	Magnesium transporter MgtE OS=Bacillus subtilis (strain 168) OX=224308 GN=mgtE PE=3 SV=1
A0A6M3ZBM8	A0A6M3ZBM8_BACSU	MATE family efflux transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09975 PE=4 SV=1
A0A6M3ZCH2	A0A6M3ZCH2_BACSU	Permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01905 PE=3 SV=1
A0A6M3ZEE8	A0A6M3ZEE8_BACSU	M48 family metallopeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05720 PE=3 SV=1
A0A6M3ZF08	A0A6M3ZF08_BACSU	Type II toxin-antitoxin system toxin ribonuclease YobL OS=Bacillus subtilis (strain 168) OX=224308 GN=yobL PE=4 SV=1
A0A6M3ZFZ3	A0A6M3ZFZ3_BACSU	Na+/H+ antiporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19340 PE=3 SV=1
A0A6M3ZG28	A0A6M3ZG28_BACSU	Na+/H+ antiporter subunit A OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18390 PE=3 SV=1
A0A6M3ZG80	A0A6M3ZG80_BACSU	Type VII secretion protein EssC OS=Bacillus subtilis (strain 168) OX=224308 GN=essC PE=4 SV=1
A0A6M3ZGI9	A0A6M3ZGI9_BACSU	SulP family inorganic anion transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19990 PE=3 SV=1
A0A6M3ZI65	A0A6M3ZI65_BACSU	Efflux RND transporter permease subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03825 PE=4 SV=1

A0A6M3ZID9	A0A6M3ZID9_BACSU	Copper transporter YcnJ OS=Bacillus subtilis (strain 168) OX=224308 GN=ycnJ PE=4 SV=1
A0A6M3ZIP3	A0A6M3ZIP3_BACSU	CPBP family intramembrane metalloprotease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_23270 PE=4 SV=1
A0A6M4JG26	A0A6M4JG26_BACSU	Mechanosensitive ion channel protein MscC OS=Bacillus subtilis (strain 168) OX=224308 GN=mscC PE=3 SV=1
A0A6M4JGZ8	A0A6M4JGZ8_BACSU	HAMP domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10110 PE=4 SV=1
A0A6M4JHK6	A0A6M4JHK6_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06820 PE=4 SV=1
A0A6M4JHW6	A0A6M4JHW6_BACSU	Iron ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01035 PE=3 SV=1
A0A6M4JI19	A0A6M4JI19_BACSU	Glycerol-3-phosphate transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=glpT PE=3 SV=1
A0A6M4JJH3	A0A6M4JJH3_BACSU	MFS transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14805 PE=4 SV=1
A0A6M4JLE6	A0A6M4JLE6_BACSU	Diguanylate cyclase DgcK OS=Bacillus subtilis (strain 168) OX=224308 GN=dgcK PE=4 SV=1
A0A6M4JLI3	A0A6M4JLI3_BACSU	HlyC/CorC family transporter OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05340 PE=3 SV=1
A0A6M4JME7	A0A6M4JME7_BACSU	Amino acid permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17705 PE=4 SV=1
A0A6M4JR32	A0A6M4JR32_BACSU	Cyclic-di-AMP phosphodiesterase OS=Bacillus subtilis (strain 168) OX=224308 GN=gdpP PE=3 SV=1
A0A6M4JRR6	A0A6M4JRR6_BACSU	MOP flippase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20470 PE=4 SV=1
O32105	YUIF_BACSU	Putative amino acid transporter YuiF OS=Bacillus subtilis (strain 168) OX=224308 GN=yuiF PE=4 SV=1
P37476	FTSH_BACSU	ATP-dependent zinc metalloprotease FtsH OS=Bacillus subtilis (strain 168) OX=224308 GN=ftsH PE=1 SV=1
A0A6M3Z6T6	A0A6M3Z6T6_BACSU	Iron ABC transporter permease OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01030 PE=3 SV=1
A0A6M3Z9I3	A0A6M3Z9I3_BACSU	Heme-based aerotactic transducer HemAT OS=Bacillus subtilis (strain 168) OX=224308 GN=hemAT PE=4 SV=1
A0A6M3ZAM2	A0A6M3ZAM2_BACSU	PTS transporter subunit EIIA OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07925 PE=4 SV=1
A0A6M4JNY4	A0A6M4JNY4_BACSU	Glycine betaine transporter OpuD OS=Bacillus subtilis (strain 168) OX=224308 GN=opuD PE=3 SV=1
A0A6M3Z8D7	A0A6M3Z8D7_BACSU	PLP-dependent aminotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02975 PE=3 SV=1
A0A6M3Z988	A0A6M3Z988_BACSU	PLP-dependent aminotransferase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06015 PE=3 SV=1
Chemotaxis & flageller movement		
A0A6M3ZJE1	A0A6M3ZJE1_BACSU	Methyl-accepting chemotaxis protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19480 PE=4 SV=1
A0A6M4JLK3	A0A6M4JLK3_BACSU	Methyl-accepting chemotaxis protein TlpB OS=Bacillus subtilis (strain 168) OX=224308 GN=tlpB PE=4 SV=1
Kinase & tranferases		
A0A6M3ZAL1	A0A6M3ZAL1_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinE PE=4 SV=1
A0A6M3ZF44	A0A6M3ZF44_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=phoR PE=4 SV=1
A0A6M3ZFU0	A0A6M3ZFU0_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_18435 PE=4 SV=1
A0A6M4JJL3	A0A6M4JJL3_BACSU	Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=kinC PE=4 SV=1
A0A6M3Z8I8	A0A6M3Z8I8_BACSU	Phosphotransferase enzyme family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03790 PE=4 SV=1
A0A6M3ZJV8		Histidine kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20885 PE=4 SV=1
A0A6M3ZAN5	A0A6M3ZAN5_BACSU	Non-specific serine/threonine protein kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=prkC PE=4 SV=1
A0A6M3Z7F5	A0A6M3Z7F5_BACSU	Aspartokinase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02195 PE=3 SV=1
A0A6M3ZF24	A0A6M3ZF24_BACSU	NAD kinase OS=Bacillus subtilis (strain 168) OX=224308 GN=nadK PE=3 SV=1
A0A6M4JPK3	A0A6M4JPK3_BACSU	Phosphotransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20260 PE=4 SV=1
A0A6M3ZHU2	A0A6M3ZHU2_BACSU	Glycosyltransferase family 8 protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21965 PE=4 SV=1
Metabolic pathway		
C/ N & lipid metabolism		

A0A6M3Z7D1	A0A6M3Z7D1_BACSU	Assimilatory nitrate reductase catalytic subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=nasC PE=4 SV=1
A0A6M3Z8J1	A0A6M3Z8J1_BACSU	Alpha,alpha-phosphotrehalase OS=Bacillus subtilis (strain 168) OX=224308 GN=treC PE=3 SV=1
A0A6M3Z9L3	A0A6M3Z9L3_BACSU	Carbamoyl-phosphate synthase large chain OS=Bacillus subtilis (strain 168) OX=224308 GN=carB PE=3 SV=1
A0A6M3ZB80	A0A6M3ZB80_BACSU	Glycerol-3-phosphate acyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=plsY PE=3 SV=1
A0A6M3ZBL3	A0A6M3ZBL3_BACSU	2-oxoglutarate dehydrogenase E1 component OS=Bacillus subtilis (strain 168) OX=224308 GN=sucA PE=3 SV=1
A0A6M3ZED6	A0A6M3ZED6_BACSU	Levanase OS=Bacillus subtilis (strain 168) OX=224308 GN=sacC PE=3 SV=1
A0A6M3ZEU1	A0A6M3ZEU1_BACSU	Glutamyl-tRNA reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=hemA PE=3 SV=1
A0A6M3ZFN0	A0A6M3ZFN0_BACSU	1,4-alpha-glucan branching enzyme GlgB OS=Bacillus subtilis (strain 168) OX=224308 GN=glgB PE=3 SV=1
A0A6M3ZIU7	A0A6M3ZIU7_BACSU	Maltose phosphorylase OS=Bacillus subtilis (strain 168) OX=224308 GN=mdxK PE=3 SV=1
A0A6M3ZLA7	A0A6M3ZLA7_BACSU	Cardiolipin synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=cls PE=3 SV=1
A0A6M4JDX2	A0A6M4JDX2_BACSU	Glucose-1-phosphate cytidylyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04115 PE=4 SV=1
A0A6M4JES8	A0A6M4JES8_BACSU	Glucose-6-phosphate 3-dehydrogenase NdtC OS=Bacillus subtilis (strain 168) OX=224308 GN=ntdC PE=4 SV=1
A0A6M4JFC1	A0A6M4JFC1_BACSU	Assimilatory nitrate reductase electron transfer subunit NasB OS=Bacillus subtilis (strain 168) OX=224308 GN=nasB PE=4 SV=1
A0A6M4JHH2	A0A6M4JHH2_BACSU	2,3-diketo-5-methylthiopentyl-1-phosphate enolase OS=Bacillus subtilis (strain 168) OX=224308 GN=mtnW PE=3 SV=1
A0A6M4JK59	A0A6M4JK59_BACSU	Long-chain-fatty-acid--CoA ligase LcfA OS=Bacillus subtilis (strain 168) OX=224308 GN=lcfA PE=4 SV=1
A0A6M4JKK3	A0A6M4JKK3_BACSU	Alpha-N-arabinofuranosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_16795 PE=3 SV=1
A0A6M4JN15	A0A6M4JN15_BACSU	3-hydroxyacyl-CoA dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19030 PE=4 SV=1
A0A6M4JNX9	A0A6M4JNX9_BACSU	Acyl--CoA ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_17220 PE=4 SV=1
A0A6M4JPF4	A0A6M4JPF4_BACSU	Oligo-1,6-glucosidase OS=Bacillus subtilis (strain 168) OX=224308 GN=malL PE=3 SV=1
O07012	BGAL2_BACSU	Beta-galactosidase GanA OS=Bacillus subtilis (strain 168) OX=224308 GN=ganA PE=1 SV=2
A0A6M4JEP2	A0A6M4JEP2_BACSU	Fatty-acid peroxygenase OS=Bacillus subtilis (strain 168) OX=224308 GN=cypC PE=4 SV=1
A0A6M4JKS6	A0A6M4JKS6_BACSU	Acyl-CoA dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14365 PE=3 SV=1
A0A6M3ZBX1	A0A6M3ZBX1_BACSU	Acyl-CoA carboxylase subunit beta OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09885 PE=4 SV=1
A0A6M4JQ85	A0A6M4JQ85_BACSU	Nitrate reductase subunit beta OS=Bacillus subtilis (strain 168) OX=224308 GN=narH PE=4 SV=1
Amino acid metabolism		
A0A6M3ZBC2	A0A6M3ZBC2_BACSU	Glutamate synthase large subunit OS=Bacillus subtilis (strain 168) OX=224308 GN=gltB PE=3 SV=1
A0A6M3ZDA0	A0A6M3ZDA0_BACSU	L-threonine dehydratase OS=Bacillus subtilis (strain 168) OX=224308 GN=ilvA PE=3 SV=1
A0A6M3ZF59	A0A6M3ZF59_BACSU	Cysteine synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=cysK PE=3 SV=1
A0A6M3ZGI8	A0A6M3ZGI8_BACSU	Homoserine O-acetyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=metA PE=3 SV=1
A0A6M3ZI13	A0A6M3ZI13_BACSU	Asparagine synthase (Glutamine-hydrolyzing) OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=3 SV=1
A0A6M4JJ65	A0A6M4JJ65_BACSU	Gamma-glutamyl phosphate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=proA PE=3 SV=1
A0A6M4JQT4	A0A6M4JQT4_BACSU	Imidazolonepropionase OS=Bacillus subtilis (strain 168) OX=224308 GN=hutI PE=3 SV=1
A0A6M4JRL8	A0A6M4JRL8_BACSU	Choline dehydrogenase OS=Bacillus subtilis (strain 168) OX=224308 GN=gbsB PE=4 SV=1
O34858	ARLY_BACSU	Argininosuccinate lyase OS=Bacillus subtilis (strain 168) OX=224308 GN=argH PE=3 SV=1
P54420	ASNB_BACSU	Asparagine synthetase [glutamine-hydrolyzing] 1 OS=Bacillus subtilis (strain 168) OX=224308 GN=asnB PE=1 SV=2
A0A6M3Z723	A0A6M3Z723_BACSU	Aminotransferase class V-fold PLP-dependent enzyme OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01585 PE=3 SV=1
A0A6M3ZEL5	A0A6M3ZEL5_BACSU	Serine protease AprX OS=Bacillus subtilis (strain 168) OX=224308 GN=aprX PE=3 SV=1
Nucleotide metabolism		
A0A6M4JG22	A0A6M4JG22_BACSU	Multifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase/5'-nucleotidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04385 PE=3 SV=1

A0A6M4JGM8	A0A6M4JGM8_BACSU	Ribonucleoside-diphosphate reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=nrdE PE=3 SV=1
O31875	NRDEB_BACSU	Ribonucleoside-diphosphate reductase NrdEB subunit alpha OS=Bacillus subtilis (strain 168) OX=224308 GN=nrdEB PE=3 SV=2
A0A6M4JIV5	A0A6M4JIV5_BACSU	NUDIX hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06730 PE=4 SV=1
Central metabolism(TCA/electron transport)		
A0A6M3ZAG6	A0A6M3ZAG6_BACSU	Pyruvate carboxylase OS=Bacillus subtilis (strain 168) OX=224308 GN=pyc PE=4 SV=1
A0A6M3ZBL7	A0A6M3ZBL7_BACSU	Bifunctional cytochrome P450/NADPH--P450 reductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04105 PE=3 SV=1
A0A6M3ZDM0	A0A6M3ZDM0_BACSU	Bifunctional 2-methylcitrate dehydratase/aconitate hydratase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14355 PE=3 SV=1
A0A6M3ZDW2	A0A6M3ZDW2_BACSU	Citrate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=mmgD PE=3 SV=1
A0A6M4JEQ9	A0A6M4JEQ9_BACSU	Citrate synthase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05285 PE=3 SV=1
A0A6M3ZI84	A0A6M3ZI84_BACSU	SDR family oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22765 PE=3 SV=1
A0A6M4JKI9	A0A6M4JKI9_BACSU	Cytochrome c biogenesis protein ResB OS=Bacillus subtilis (strain 168) OX=224308 GN=resB PE=4 SV=1
Cell wall synthesis		
A0A6M3ZG90	A0A6M3ZG90_BACSU	LTA synthase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19310 PE=3 SV=1
A0A6M3ZGJ9	A0A6M3ZGJ9_BACSU	Beta-N-acetylglucosaminidase LytD OS=Bacillus subtilis (strain 168) OX=224308 GN=lytD PE=4 SV=1
A0A6M3ZI35	A0A6M3ZI35_BACSU	Glutamate racemase OS=Bacillus subtilis (strain 168) OX=224308 GN=racE PE=3 SV=1
A0A6M4JBY5	A0A6M4JBY5_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_00075 PE=3 SV=1
A0A6M4JGG3	A0A6M4JGG3_BACSU	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase OS=Bacillus subtilis (strain 168) OX=224308 GN=murE PE=3 SV=1
A0A6M4JJR6	A0A6M4JJR6_BACSU	Serine-type D-Ala-D-Ala carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_08325 PE=3 SV=1
A0A6M3Z9N5	A0A6M3Z9N5_BACSU	UDP-glucosyltransferase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_06745 PE=4 SV=1
A0A6M3ZEE3	A0A6M3ZEE3_BACSU	Transglycosylase SLT domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15330 PE=4 SV=1
A0A6M4JH58	A0A6M4JH58_BACSU	LD-carboxypeptidase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10445 PE=3 SV=1
Uncharacterized protein		
A0A6M3Z8V2	A0A6M3Z8V2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05140 PE=4 SV=1
A0A6M3ZBR5	A0A6M3ZBR5_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10210 PE=4 SV=1
A0A6M3ZC19	A0A6M3ZC19_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11415 PE=4 SV=1
A0A6M3ZC58	A0A6M3ZC58_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10940 PE=4 SV=1
A0A6M4JGA0	A0A6M4JGA0_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_05050 PE=4 SV=1
A0A6M4JJC7	A0A6M4JJC7_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11365 PE=4 SV=1
A0A6M4JPL9	A0A6M4JPL9_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20085 PE=4 SV=1
A0A6M4JQN2	A0A6M4JQN2_BACSU	Uncharacterized protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22330 PE=4 SV=1
O34708	YFLA_BACSU	Uncharacterized transporter YflA OS=Bacillus subtilis (strain 168) OX=224308 GN=yflA PE=3 SV=1
Stress resistance/response proteins		
A0A6M3ZDQ8	A0A6M3ZDQ8_BACSU	PBP1A family penicillin-binding protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13425 PE=4 SV=1
A0A6M3ZC91	A0A6M3ZC91_BACSU	Superoxide dismutase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10535 PE=3 SV=1
A0A6M3ZH49	A0A6M3ZH49_BACSU	Erythromycin resistance protein OS=Bacillus subtilis (strain 168) OX=224308 GN=erm(B) PE=3 SV=1
A0A6M3ZKH0	A0A6M3ZKH0_BACSU	Chaperone protein HtpG OS=Bacillus subtilis (strain 168) OX=224308 GN=htpG PE=3 SV=1
A0A6M4JL07	A0A6M4JL07_BACSU	Glyoxalase/bleomycin resistance/dioxygenase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15210 PE=4 SV=1

A0A6M4JQY7	A0A6M4JQY7_BACSU	General stress protein 30 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_22785 PE=4 SV=1
A0A6M4JP82	A0A6M4JP82_BACSU	FUSC family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19405 PE=4 SV=1
A0A6M3Z8S4	A0A6M3Z8S4_BACSU	Thioredoxin-dependent thiol peroxidase OS=Bacillus subtilis (strain 168) OX=224308 GN=bcp PE=4 SV=1
A0A6M4JM08	A0A6M4JM08_BACSU	Arsenate reductase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_19015 PE=3 SV=1

Sporulation specific proteins

A0A6M3Z716	A0A6M3Z716_BACSU	Sporulation killing factor biosynthesis protein SkfC OS=Bacillus subtilis (strain 168) OX=224308 GN=skfC PE=4 SV=1
A0A6M3ZAJ4	A0A6M3ZAJ4_BACSU	Spore germination protein GerKB OS=Bacillus subtilis (strain 168) OX=224308 GN=gerKB PE=3 SV=1
A0A6M3ZB79	A0A6M3ZB79_BACSU	DNA translocase SpoIIIE OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIIE PE=3 SV=1
A0A6M3ZDY2	A0A6M3ZDY2_BACSU	Stage III sporulation protein AE OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIIAE PE=4 SV=1
A0A6M3ZEK5	A0A6M3ZEK5_BACSU	Stage II sporulation protein SpoIIB OS=Bacillus subtilis (strain 168) OX=224308 GN=spoIIB PE=4 SV=1
A0A6M3ZF09	A0A6M3ZF09_BACSU	Spore coat assembly protein SafA OS=Bacillus subtilis (strain 168) OX=224308 GN=safA PE=4 SV=1
A0A6M3ZGU4	A0A6M3ZGU4_BACSU	SpoIID/LytB domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20485 PE=4 SV=1
A0A6M3ZJ55	A0A6M3ZJ55_BACSU	Sporulation protein YqfD OS=Bacillus subtilis (strain 168) OX=224308 GN=yqfD PE=4 SV=1
A0A6M4JEX2	A0A6M4JEX2_BACSU	Spore germination protein GerKA OS=Bacillus subtilis (strain 168) OX=224308 GN=gerKA PE=3 SV=1
P12254	RPSK_BACSU	RNA polymerase sigma-K factor OS=Bacillus subtilis (strain 168) OX=224308 GN=sigK PE=1 SV=2
A0A6M3Z8L3	A0A6M3Z8L3_BACSU	YfhO family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04765 PE=4 SV=1

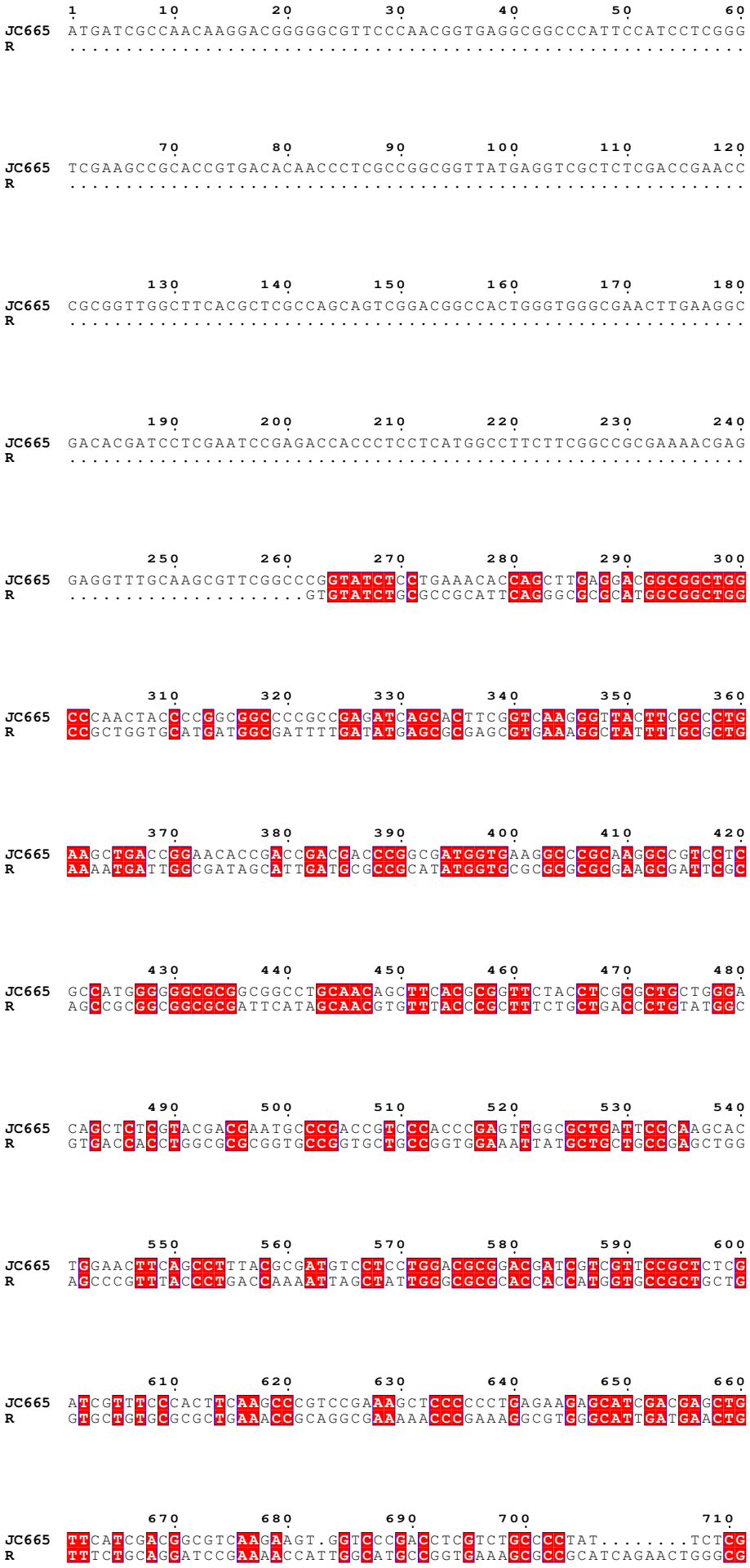
Others

A0A6M4JH86	A0A6M4JH86_BACSU	Non-ribosomal plipastatin synthetase PpsE OS=Bacillus subtilis (strain 168) OX=224308 GN=ppsE PE=3 SV=1
A0A6M3Z6W3	A0A6M3Z6W3_BACSU	DUF4885 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01270 PE=4 SV=1
A0A6M3Z7C8	A0A6M3Z7C8_BACSU	DUF4901 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01660 PE=4 SV=1
A0A6M3Z820	A0A6M3Z820_BACSU	Band_7_1 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03560 PE=4 SV=1
A0A6M3Z989	A0A6M3Z989_BACSU	UPF0374 protein HIR78_04800 OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_04800 PE=3 SV=1
A0A6M3ZBH5	A0A6M3ZBH5_BACSU	KAP NTPase domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_10335 PE=4 SV=1
A0A6M3ZDR1	A0A6M3ZDR1_BACSU	EAL domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07770 PE=4 SV=1
A0A6M3ZI56	A0A6M3ZI56_BACSU	DUF2334 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02455 PE=4 SV=1
A0A6M3ZK18	A0A6M3ZK18_BACSU	4Fe-4S dicluster domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21325 PE=4 SV=1
A0A6M3ZMB6	A0A6M3ZMB6_BACSU	Insulinase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_21445 PE=4 SV=1
A0A6M4JDL6	A0A6M4JDL6_BACSU	DUF58 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03635 PE=4 SV=1
A0A6M4JDN3	A0A6M4JDN3_BACSU	DUF4129 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_03640 PE=4 SV=1
A0A6M4JF49	A0A6M4JF49_BACSU	DUF4901 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_01655 PE=4 SV=1
A0A6M4JKV0	A0A6M4JKV0_BACSU	Prophage_tail domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_12895 PE=4 SV=1
A0A6M4JL77	A0A6M4JL77_BACSU	DUF1405 domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_13530 PE=4 SV=1
A0A6M4JML3	A0A6M4JML3_BACSU	Phage portal protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_15415 PE=3 SV=1
A0A6M4JMV4	A0A6M4JMV4_BACSU	Herpes_ori_bp domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_11330 PE=4 SV=1
A0A6M4JPN1	A0A6M4JPN1_BACSU	YitT family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20305 PE=4 SV=1
A0A6M4JPY1	A0A6M4JPY1_BACSU	VanZ family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20980 PE=4 SV=1
A0A6M4JM03	A0A6M4JM03_BACSU	Insulinase family protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_09100 PE=3 SV=1

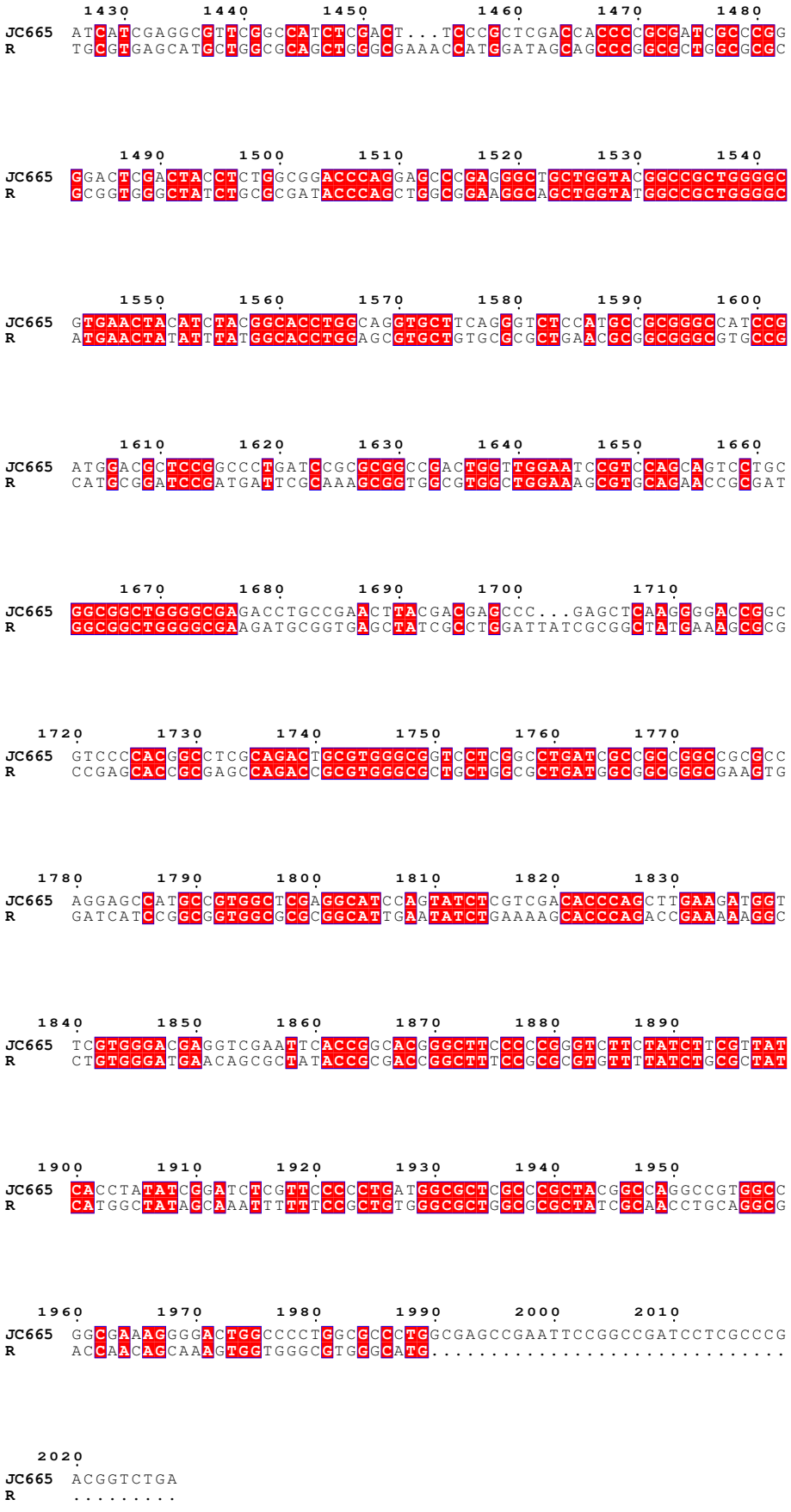
A0A6M4JFE2	A0A6M4JFE2_BACSU	Surfactin non-ribosomal peptide synthetase SrfAC OS=Bacillus subtilis (strain 168) OX=224308 GN=srfAC PE=3 SV=1
A0A6M3Z9W6	A0A6M3Z9W6_BACSU	Altronate oxidoreductase OS=Bacillus subtilis (strain 168) OX=224308 GN=uxaB PE=3 SV=1
A0A6M4JF07	A0A6M4JF07_BACSU	EIICB-Mtl OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_02290 PE=4 SV=1
A0A6M3ZDY7	A0A6M3ZDY7_BACSU	Alpha/beta hydrolase OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_14195 PE=4 SV=1
A0A6M3ZH02	A0A6M3ZH02_BACSU	LTD domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_20185 PE=4 SV=1
A0A6M4JJC3	A0A6M4JJC3_BACSU	AAA domain-containing protein OS=Bacillus subtilis (strain 168) OX=224308 GN=HIR78_07550 PE=3 SV=1

Table S1 D) Protein profiles of spores of *Bacillus subtilis* TB10 produced under H_2O_2 treated condition

Fig. S1: Multiple sequence alignment of squalene hopene cyclase (shc) gene of Paludisphaera rhizosphaerae JC665T with its homolog from Rhodopseudomonas palustris TIE-1. Highly conserved nucleotides are highlighted with red background color.




720 730 740 750 760 770
JC665 TGGGGCAGGTTCTTTCTCGCCGTCGACCGTGGTCTGAAGTGGCCGACCGCCTGCTGCCG
R CTGTTTAAACTGTTTGGCAGCATTGATGCGGTGCTGCGCGTGATTGAACCGGTGATGCCG
780 790 800 810 820 830
JC665 ACCGCCGTTCGTCGTCGCGGCGTCCAGGCCGCTCACCGGTGGATGGTCGAGCACTTCGAG
R AAAGGCATTCGCAAACGCGCGATTGATAAAGCGCTGGCGTTTATTGAAGAACGCCTGAAC
840 850 860 870 880 890
JC665 GACTCGGACGGCCTGGGCGCGATCTATCCGCCGATGGTCTACACGGATCCTCGCTCTGCGT
R GGCGAAGATGGCATGGGCCGCGATTTTCCGCCGATGGCGAACGCGGTGATGATGTATGAA
900 910 920 930 940 950
JC665 TGCCTGGGCTATGAGGACGACTCCGCCAGCGTCGTCTGGGCCTGGAGGCAGCTCGAAGAT
R GCGCTGGGCTATCCGGAAGATTATCGCCGCGCGCGAGCCAGCGCCGCGGCATTGATCTG
960 970 980 990 1000 1010
JC665 CTGAAGATCGACGAGGGCGATACGACTCGCTTCCAACCTTGCGTCTCTCCCGTGTGGGAC
R CTGCTGGTGGATCGCGGCGATGAAGCGTATTGCCAGCCGTGCGTGAGCCCGGTGTGGGAT
1020 1030 1040 1050 1060 1070
JC665 ACGGCGATCACGGCCATCGCTCTCTCCGACGCCGACGAACCCTCCGACGAGGCGGCCCTT
R ACGCGCGTGGCGAGCCATGCGGTGCTGGAAGCGGATGGCCATGAAGGCGCGAAAAGCGTG
1080 1090 1100 1110 1120
JC665 GAGAAGGCGGGCGACTGGC...TTCTCGACAAGGAACTCCGTCGCGGCGGCGACTGGCAG
R CGCCCGGCGCTGGATTGGCTGCTGCCGCGCCAGGTGCTGGATGTGAAAGGCGATTGGGCG
1130 1140 1150 1160 1170 1180
JC665 GTGCGTCGGCCGGGGGTCGAACCGAGCGGCTGGCACTTCGAGTACGCCAACGGGTTCTAT
R GTCAAAGCGCCCAACGTGCGCCCGGGCGGCTGGGCGTTTCAGTATAACAACGCGCATTAT
1190 1200 1210 1220 1230 1240
JC665 CCGGACATCGACGACACGGCCATGGTCCTGATGGCACTGCTCCGGTCGCCTGCATC...C.
R CCGGATCTGGATGATACCGCGGTGGTGGTGATGGCGCTGGATCGCGCGCAAAGATCAG
1250 1260 1270 1280 1290 1300
JC665 GCCGAACCGCGATACGTCGAGTCGATCAAGCGGGGGACCGACTGGCTGCTCGCGATGCAG
R CGGAACCCGGCGTATGATGCGGCGATTGCGCGCGCGCGGAATGGATTGAAGGCATGCAG
1310 1320 1330 1340 1350 1360
JC665 AACGCGGACGGCGGCTGGGCGGCCTTCGATGTGGACATCGACAACGAGGTCCTCACCCAG
R AGCGATGATGGCGGCTGGGGCGCGTTTGATATTAACACACGAATATTATCTGAACAAC
1370 1380 1390 1400 1410 1420
JC665 GTCCCGTTCGCCGACCACAACGCGATGCTCGACCCGAGCTGCGCCGATATCACGGCCCGA
R ATTCCGTTTAGCGATCATGGCGCGATGCTGGATCCGCCGACCGAAGATGTGACCGCGCGC


1430 1440 1450 1460 1470 1480
JC665 ATCATCGAGGCGTTCGGCCATCTCGACT...TCCCGCTCGACCACCCCGCGGATCGCCCGG
R TGCGTGAGCATGCTGGCGCAGCTGGGCGAAACCATGGATAGCAGCCCGGCGCTGGCGCGC
1490 1500 1510 1520 1530 1540
JC665 GGACTCGACTACCTCTGGCGGACCCAGGAGCCCGAGGGCTGCTGGTACGGCCGCTGGGGC
R GCGGTGGGCTATCTGCGCGATACCCAGCTGGCGGAAGGCAGCTGGTATGGCCGCTGGGGC
1550 1560 1570 1580 1590 1600
JC665 GTGAACTACATCTACGGCACCTGGCAGGTGCTTCAGGGTCTCCATGCCGCGGGGCCATCCG
R ATGAACTATATTTATGGCACCTGGAGCGTGCTGTGCGCGCTGAACGCGGCGGGGCGTGCCG
1610 1620 1630 1640 1650 1660
JC665 ATGGACGCTCCGGCCCTGATCCGCGCGGCCGACTGGTTGGAATCCGTCCAGCAGTCCTGC
R CATGCGGATCCCATGATTCGCAAAGCGGTGGCGTGGCTGGAAAAGCGTGCAGAACCGCGAT
1670 1680 1690 1700 1710
JC665 GGCGGCTGGGGCGAGACCTGCCGAACTTACGACGAGCCC...GAGCTCAAGGGGACCGGC
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1720 1730 1740 1750 1760 1770
JC665 GTCCCCACGGCCTCGCAGACTGCGTGGGCGGTCCTCGGCCTGATCGCCGCCGGCCGCGCC
R CCGAGCACCGCGAGCCAGACCGCGTGGGCGCTGCTGGCGCTGATGGCGGCGGGCGAAGTG
1780 1790 1800 1810 1820 1830
JC665 AGGAGCCATGCCGTGGCTCGAGGCATCCAGTATCTCGTCGACACCCAGCTTGAAGATGGT
R GATCATCCGGCGGTGGCGCCGGCATTGAATATCTGAAAAGCACCCAGACCGAAAAAGGC
1840 1850 1860 1870 1880 1890
JC665 TCGTGGGACGAGGTCGAATTCACCGGCACGGGCTTCCCCGGGTCTTCTATCTTCGTTAT
R CTGTGGGATGAACAGCGCTATACCGCGACCGGCTTTCCGCGCGTGTTTATCTGCGCTAT
1900 1910 1920 1930 1940 1950
JC665 CACCTATATCGGATCTCGTTCCCCCTGATGGCGCTCGCCCGCTACGGCCAGGCCGTGGCC
R CATGGCTATAGCAAATTTTTCCGCTGTGGCGCTGGCGCGCTATCGCAACCTGCAGGCG
1960 1970 1980 1990 2000 2010
JC665 GGCGAAAGGGGACTGGCCCCTGGCGCCCTGGCGAGCCGAATTCCGGCCGATCCTCGCCCG
R ACCAACAACAAAGTGGTGGGCGTGGGCATG.....
2020
JC665 ACGGTCTGA
R .....

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Publications

PUBLICATIONS

1. **Smita N**, Indu B, Anusha R, Sasikala Ch, Ramana Ch V. (2023) In silico analysis of sporulene biosynthesis pathway genes in the members of the class *Bacilli. Archives of Microbiology*. 205:233.

2. Anusha R, Jagadeeshwari U, Deepshikha G, **Smita N**, Ipsita S, Sasikala Ch, Ramana Ch V. (2022) *Neoroseomonas marina sp. nov.,* Isolated from a Beach Sand. *Current Microbiology*. 79:233.

3. Lhingjakim K L, **Smita N**, Gaurav K, Jagadeeshwari U, Shabbir A, Sasikala Ch, Ramana Ch V. (2022) *Paludisphaera rhizosphaereae* sp. nov., a new member of the family Isosphaeraceae, isolated from the rhizosphere soil of *Erianthus ravennae*. *Antonie van Leeuwenhoek*. 115:1073-1084.

4. Anusha R, Jagadeshwari U, Deepshikha G, **Smita N**, Sasikala Ch, Ramana Ch V. (2021) Phylotaxogenomics for the reappraisal of the genus *Roseomonas* with the creation of six new genera. *Frontiers in Microbiology*. 677842.

5. Anusha R, **Smita N**, Shabbir A, Jagadeeshwari U, Keertana T, Sasikala Ch, Ramana Ch V. (2021) *Mesobacillus aurantiibacter* sp. nov., isolated from an orange pond near a solar saltern. *Archives of Microbiology*. 203:1499–1507.

6. Dhanesh Kumar, **Smita N**, Gaurav K, Suresh G, Sasikala Ch, Ramana Ch V (2020) *Arenibacter lacus* sp. nov., isolated from Chilika lagoon. *Current Microbiology*. 77:4152-4259.

7. Anusha R, **Smita N**, Suresh G, Shabbir A, Sasikala Ch, Ramana Ch V (2020) *Paracoccus aeridis* sp. nov., an indole producing bacterium isolated from the rhizosphere of an orchid, *Aerides maculosa*. *International Journal of Systematic and Evolutionary Microbiology*. 70:1720-1728.

8. Indu B, Kumar G, **Smita N**, Shabbir A, Sasikala Ch. and Ramana Ch V. (2020). *Chryseobacterium candidae* sp. nov., isolated from a yeast (*Candida tropicalis*). *International Journal of Systematic and Evolutionary Microbiology*, 70:90-93.

9. Anusha R, Indu B, **Smita N**, Deepshikha G, Gaurav K, Dhanesh K, Suresh G, Sasikala Ch and Ramana Ch.V. (2019). Emerging concepts in bacterial taxonomy. In Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications - Volume 1. Microbial Diversity in Normal and Extreme Environments (Eds. T. Satyanarayana, S.K. Das and B.N. Johri), Springer Nature Singapore (Invited introductory chapter).

10. **Smita N**, Sasikala Ch and Ramana Ch. V. (2023). New insights into peroxide toxicity: Sporulenes aid in the resistance of endospores of *Bacillus subtilis* to hydrogen peroxide. *Journal of Applied Microbiology* (Under revision).

CONFERENCES AND SEMINAR

1. Presented a poster at the "National Conference on Frontiers in Plant Biology" (Feb. 2020), University of Hyderabad, Hyderabad.
2. Presented poster at the "59th Annual Conference of Association of Microbiologists of India" (Dec. 2018), University of Hyderabad, Hyderabad.
3. Presented an oral presentation at 13th Plant Science colloquium "Sporulenes of a few bacilli and their role in *Bacillus subtilis* under stress conditions" (Oct. 2021), University of Hyderabad, Hyderabad.

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ORIGINAL PAPER

In silico analysis of sporulene biosynthesis pathway genes in the members of the class *Bacilli*

N. Smita[1] · R. Anusha[1] · B. Indu[1] · Ch. Sasikala[2] · Ch. V. Ramana[1]

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Abstract

Sporulene, a pentacyclic triterpenoid, was discovered in *Bacillus subtilis* and is associated with bacterial endospores. However, the study was not further extended, leaving a trail of questions. One such question is what diversity of sporulenes exists among spore-forming members? Considering the sporulene biosynthesis pathway as a fundamental tool to survey the distribution of this molecule, a genome mining study was conducted. Mining for genes encoding putative proteins of sporulene biosynthesis pathway among the class *Bacilli* members revealed the presence of *hepS*, *hepT*, *ytpB,* and *sqhC* genes in the members of the family *Bacillaceae*, *Caryophanaceae*, *Paenibacillaceae,* and *Sporolactobacillaceae*. However, these genes were completely absent in the members of *Staphylococcaceae*, *Lactobacillaceae*, *Aerococcaceae*, *Carnobacteriaceae,* and *Leuconostocaceae*. Unlike other probable pathway related proteins, a conserved amino acid domain of putative terpenoid cyclase (YtpB) appeared deep-rooted among the genus *Bacillus* members. In-depth analysis showed the constant gene arrangement of *hepS, hepT, ytpB,* and *sqhC* genes in these members, there by demonstrating the conserved nature of sporulene biosynthesis pathway in the members of the genus *Bacillus.* Our study suggests confinement of the sporulene biosynthesis pathway to spore-forming members of the class *Bacilli,* majorly to the genus *Bacillus*.

Keywords Sporulene · *Bacillus* · *sqhC* · *Bacillaceae* · Synteny

Introduction

A five-carbon compound, isoprene serves as the monomeric unit for the synthesis of polymeric isoprenoid or terpenoid molecules (Dominiczak et al. 2014). Isoprenoids are hydrocarbons that contribute majorly to the secondary metabolites (Tian et al. 2019) produced in plants and bacteria (O'connor 2016). Likewise, sterols, an important part of the membrane lipids in bacteria, are synthesized from isoprenoid precursors. Such molecules synthesized from isoprenoid precursors include tetracyclic sterols like cholesterol, polycyclic triterpenoids like hopanoids (Dufourc 2008; Hannich et al. 2011) and sporulenes, which are synthesized from 30 to 35 carbon acyclic isoprenoid precursors like squalene and curcumene (Bosak et al. 2008). Though recently discovered, hopanoids are relatively well-studied triterpenoids that differ from sporulenes in having a pentacyclic ring and are known to give membrane stability and integrity (Bosak et al. 2008; Kontnik et al. 2008). In comparison, sporulenes are less studied, and their actual functions in relation to bacterial endospores still need to be unraveled.

Due to the diverse niche harbored by microorganisms, they evolve continuously to adapt to the given challenges. As a result, they either develop new sets of metabolic pathways which are in accordance with the surrounding or omit the redundant pathways (Wang et al. 2019; Copley et al. 2000). Sporulene biosynthesis is one such metabolic cascade that was observed in *Bacillus subtilis* (Bosak et al. 2008; Sato et al.2011). In the proposed pathway of sporulene biosynthesis in *Bacillus subtilis* by Takigawa and coworkers (Takigawa et al. 2010), two subunits of

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✉ Ch. Sasikala
cvr449@gmail.com

✉ Ch. V. Ramana
chvrsl@uohyd.ernet.in

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500046, India

[2] Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology, J.N.T. University Hyderabad, Kukatpally, Hyderabad 500085, India

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ORIGINAL PAPER

Paludisphaera rhizosphaereae sp. nov., a new member of the family *Isosphaeraceae,* isolated from the rhizosphere soil of *Erianthus ravennae*

Khongsai L. Lhingjakim · Nandardhane Smita · Gaurav Kumar · Uppada Jagadeeshwari · Shabbir Ahamad · Chintalapati Sasikala · Chintalapati Venkata Ramana

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Abstract Two axenic cultures of *Planctomycetota* were isolated from distinct geographical locations of India. Strain JC665^T was isolated from a rhizosphere soil of Loktak lake, Manipur, whereas strain JC747 was isolated from a soil sediment at Pallikkara village, Kerala, India. The two closely related strains shared the highest 16S rRNA gene sequence identity (94.6%) with *Paludisphaera borealis* PX4^T, while the 16S rRNA gene sequence identity between both strains was 100%. Both strains grow aerobically, stain Gram negative, colonies are light pink-coloured, cells are non-motile, spherical to oval-shaped and tolerate NaCl up to 2% (w/v). While strain JC665^T grows well up to pH 9.0, strain JC747 grows only up to pH 8.0. The respiratory quinone in both strains is MK-6. $C_{16:0}$, $C_{18:1}\omega9c$ and $C_{18:0}$ are the major fatty acids. Phosphatidylcholine, two unidentified glycolipids, seven unidentified lipids and two unidentified phospholipids made up the polar lipid composition of both strains. Both strains have genome sizes of about 8.0 Mb and a DNA G+C content of 66.4 mol%. Both strains contain genes coding for enzymes putatively involved in the production of lycopene-related carotenoids. The phylogenetic position together with the results of the analysis of morphological, physiological and genomic features support the classification of strain JC665^T as a new species of the genus *Paludisphaera*, for which we propose the name *Paludisphaera rhizosphaerae*

Khongsai L. Lhingjakim, Nandardhane Smita, Gaurav Kumar have contributed equally and are considered as equal first authors

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the strains JC665T and JC747 are LR746340 and OU374731, respectively. The GenBank/EMBL/DDBJ accessions for the whole genome shotgun sequence for strains JC665T and JC747 are JAALCR000000000 and JAHPZK000000000, respectively

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K. L. Lhingjakim · N. Smita · G. Kumar · S. Ahamad · C. V. Ramana (✉)
Department of Plant Sciences, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500046, India
e-mail: cvr449@gmail.com

Present Address:
G. Kumar
Department of Microbiology, School of Sciences, ITM (SLS) Baroda University, Halol, Vadodara, Gujarat 391510, India

U. Jagadeeshwari · C. Sasikala (✉)
Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology, JNT University Hyderabad, Kukatpally, Hyderabad 500085, India
e-mail: sasi449@yahoo.ie; sasikala.ch@gmail.com

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Neoroseomonas marina sp. nov., Isolated from a Beach Sand

Anusha Rai[1] · Jagadeeshwari Uppada[2] · Deepshikha Gupta[1] · Smita Nandardhane[1] · Ipsita Sahu[1] · Sasikala Chintalapati[2] · Venkata Ramana Chintalapati[1]

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Abstract

A pink-pigmented bacterium (strain JC162^T = KCTC 32190^T) was isolated from a beach sand sample. Cells were Gram-stain-negative, coccoid, non-motile, and strictly aerobic. EzBioCloud BLAST search of 16S rRNA gene sequence showed that strain KCTC 32190^T had the highest sequence identity to the members of the genus *Neoroseomonas* and was closely related to *N. oryzicola* YC6724^T (99.8%), *N. sediminicola* FW-3^T (98.5%), *N. soli* 5N26^T (98.2%), and other members of the genus *Neoroseomonas* (< 97.9%) in the family *Acetobacteriaceae* within the class of *Alphaproteobacteria*. Chemo-organoheterotrophy was the only growth mode and growth was possible on a wide range of organic substrates. Strain KCTC 32190^T was positive for catalase and oxidase. Fatty acid composition of strain KCTC 32190^T includes (in decreasing %) $C_{18:1}\omega7c$, *cyclo*-$C_{19:0}\omega8c$, $C_{18:0}$2-OH, $C_{16:0}$, $C_{18:0}$3-OH, $C_{16:1}\omega7c$/$C_{16:1}\omega6c$, $C_{16:0}$2-OH and $C_{16:1}\omega5c$. Polar lipids comprised of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified amino lipid, and three unidentified lipids. The genomic DNA G+C content of the strain KCTC 32190^T was 70.9 mol%. Strain KCTC 32190^T has a low ANI value of < 92.7% and genome reassociation (based on *digital* DNA-DNA hybridization) value of < 48.8% with the nearest type strains. The genome relatedness is supported by other polyphasic taxonomic data to propose strain KCTC 32190^T as a new species in the genus *Neoroseomonas* with the name *Neoroseomonas marina* sp. nov. The type strain is strain JC162^T (KCTC 32190^T = CGMCC1.12364^T).

Abbreviations

NCBI	National Center for Biotechnology Information
ANI	Average Nucleotide Identity
*d*DDH	Digital DNA-DNA Hybridization
BLAST	Basic Local Alignment Search Tool
MUSCLE	Multiple Sequence Comparison by Log-Expectation
KCTC	Korean Collection for Type Cultures
CGMCC	China General Microbiological Culture Collection Centre

Introduction

Marine habitats are composed of a wide spectrum of environments, each supporting a complex assemblage of potentially unique macroorganisms and microorganisms. Certain habitats like open ocean beaches and intertidal areas are of prime importance as it forms the interface between the terrestrial/freshwater and marine systems [1]. They are subjected to various tidal and fluvial processes; therefore, it continuously shapes their geochemistry, mineralogy, and biological processes [2]. The molecular studies of bacterial diversity have shown that they occupy an important ecological niche in the marine environment [3, 4]. Therefore, these marine habitats are currently being explored mainly for bacteria for various biotechnological applications [5, 6]. While working with the sand sample from a beach, a pink-pigmented bacterium was isolated and 16S rRNA gene sequence analysis indicated its affiliation to the genus *Neoroseomonas*. The members of the genus *Roseomonas* were first described by Rihs et al. in 1993 [7] under the genus *Roseomonas*. Genus *Roseomonas* was highly heterogeneous because of which the taxa was re-evaluated and reclassified into six novel genera [8, 9] and has been validated with their

✉ Sasikala Chintalapati
sasikala.ch@gmail.com; sasi449@yahoo.ie

✉ Venkata Ramana Chintalapati
cvr449@gmail.com

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500 046, India

[2] Bacterial Discovery Laboratory, Centre for Environment, IST, JNT University Hyderabad, Kukatpally, Hyderabad 500 085, India

frontiers in Microbiology

ORIGINAL RESEARCH
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Phylotaxogenomics for the Reappraisal of the Genus *Roseomonas* With the Creation of Six New Genera

Anusha Rai[1†], Uppada Jagadeeshwari[2†], Gupta Deepshikha[1], Nandardhane Smita[1], Chintalapati Sasikala[2] and Chintalapati Venkata Ramana[1*]*

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India, [2] Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology (IST), Jawaharlal Nehru Technological (JNT) University Hyderabad, Hyderabad, India

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***Correspondence:**
Chintalapati Sasikala
sasi449@yahoo.ie;
sasikala.ch@gmail.com
Chintalapati Venkata Ramana
cvr449@gmail.com

† These authors have contributed equally to this work and share first authorship

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The genus *Roseomonas* is a significant group of bacteria which is invariably of great clinical and ecological importance. Previous studies have shown that the genus *Roseomonas* is polyphyletic in nature. Our present study focused on generating a lucid understanding of the phylogenetic framework for the re-evaluation and reclassification of the genus *Roseomonas.* Phylogenetic studies based on the 16S rRNA gene and 92 concatenated genes suggested that the genus is heterogeneous, forming seven major groups. Existing *Roseomonas* species were subjected to an array of genomic, phenotypic, and chemotaxonomic analyses in order to resolve the heterogeneity. Genomic similarity indices (*d*DDH and ANI) indicated that the members were well-defined at the species level. The Percentage of Conserved Proteins (POCP) and the average Amino Acid Identity (AAI) values between the groups of the genus *Roseomonas* and other interspersing members of the family *Acetobacteraceae* were below 65 and 70%, respectively. The pan-genome evaluation depicted that the pan-genome was an open type and the members shared 958 core genes. This claim of reclassification was equally supported by the phenotypic and chemotaxonomic differences between the groups. Thus, in this study, we propose to re-evaluate and reclassify the genus *Roseomonas* and propose six novel genera as *Pararoseomonas* gen. nov., *Falsiroseomonas* gen. nov., *Paeniroseomonas* gen. nov., *Plastoroseomonas* gen. nov., *Neoroseomonas* gen. nov., and *Pseudoroseomonas* gen. nov.

Keywords: phylotaxogenomics, average amino acid Identity (AAI), percentage of conserved proteins (POCP), reclassification, *Roseomonas*

Abbreviations: NCBI, National Center for Biotechnology Information; ANI, Average Nucleotide Identity; AAI, Average Amino acid Identity; POCP, Percentage of Conserved Proteins; *d*DDH, digital DNA-DNA Hybridization; BLAST, Basic Local Alignment Search Tool; MUSCLE, MUltiple Sequence Comparison by Log-Expectation; KCTC, Korean Collection for Type Cultures; CGMCC, China General Microbiological Culture Collection Centre; ATCC, American Type Culture Collection; CIP, Institute Pasteur Collection; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; JCM, Japan Collection of Microorganisms-RIKEN BioResource Center; KACC, Korean Agricultural Culture Collection; NBRC, NITE Biological Resource Center; LMG, Laboratorium voor Microbiologie; MCCC, Marine Culture Collection of China; GDMCC, Guangdong Microbial Culture Collection Center; CECT Colección Española de Cultivos Tipo; KEMB, Korea Environmental Microorganisms Bank; CCUG, Culture Collection University of Göteborg; BCC, BIOTEC Culture Collection; UBCG, Up-to-date Bacterial Core Gene set.

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ORIGINAL PAPER

Mesobacillus aurantius sp. nov., isolated from an orange-colored pond near a solar saltern

Anusha Rai[1] · N. Smita[1] · A. Shabbir[1] · U. Jagadeeshwari[2] · T. Keertana[1] · Ch. Sasikala[2] · Ch. V. Ramana[1]

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Abstract

An endospore producing, strict aerobic, Gram-stain-positive, orange-colored colony forming bacterium designated as strain JC1013^T was isolated from an orange pond near a solar saltern of Tamil Nadu, India. Phylogenetic analysis of the 16S rRNA gene sequences indicated that strain was affiliated to the family *Bacillaceae* of the phylum *Firmicutes*. Strain showed highest 16S rRNA gene sequence identity of 98.7% with *Mesobacillus selenatarsenatis* SF-1 ^{T} and below 98.3% with other members of the genus *Mesobacillus*. Strain JC1013^T produced carotenoid pigments and indole compounds. Major cellular fatty acids of strain JC1013^T were iso-$C_{15:0,}$ anteiso-$C_{15:0}$, $C_{16:0}$ 3-OH, iso-$C_{17:0}\omega10c$ and summed feature 4 (iso-$C_{17:1}$ I/ anteisoB). Polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, two unidentified aminolipids and four unidentified phospholipids. Strain JC1013^T constituted *m*-diaminopimelic acid as diagnostic cell wall amino acids. MK-7 is the predominant menaquinone of strain JC1013^T. The genome size of strain JC1013^T was 4.6 Mbp and its G + C content was 42.7 mol%. For the affirmation of strain's taxonomic status, a detailed phylogenomic study was done. Based on the phylogenetic analyses, low ANI (84.6%), AAI (88.5%) values, in-silico DDH (< 29%) value, morphological, physiological and chemo-taxonomical characteristics, strain JC1013^T was clearly distinguished from the nearest phylogenetic neighbor, *Mesobacillus selenatarsenatis* SF-1^T to conclude that it is a new species of the genus *Mesobacillus*. We propose the name as *Mesobacillus aurantius* with type strain JC1013^T (= NBRC 114146^T = KACC 21451 ^{T}).

Keywords *Mesobacillus* · Sp. nov. · Endospore · Salt tolerant

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The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of strain JC1013^T is LS998022**.** The GenBank/EMBL/DDBJ accession for the whole genome shotgun sequence is JAAVUM000000000.

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✉ Ch. Sasikala
sasikala.ch@gmail.com

✉ Ch. V. Ramana
cvr449@gmail.com

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500 046, India

[2] Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology, J. N. T. University H, Kukatpally, Hyderabad 500 085, India

Abbreviations

NCBI	National centre for biotechnology information
GCM	The global catalogue of microorganisms
ANI	Average nucleotide identity
AAI	Average amino identity
*d*DDH	Digital DNA–DNA hybridization
BLAST	Basic local alignment search tool
MUSCLE	Multiple sequence comparison by log-expectation
TLC	Thin-layer chromatography
HPLC	High-pressure liquid chromatography
NBRC	Biological resource center
NITE	KACC, Korean agricultural culture collection
CMC	Carboxymethyl cellulose

Introduction

Hyper-saline environments like salt pan lakes are hubs for unique ecological layouts spanning diverse growth conditions (pH, alkalinity, salinity and temperature) and

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Arenibacter lacus sp. nov., Isolated from Chilika Lagoon, India

Dhanesh Kumar[1] · Nandardhane Smita[1] · Gaurav Kumar[1] · Gandham Suresh[1] · Uppada Jagadeeshwari[2] · Chintalapati Sasikala[2] · Chintalapati Venkata Ramana[1]

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Abstract
A shiny dark yellow pigmented, Gram-stain-negative, rod-shaped, non-motile bacterium, designated as strain JC631^T, was isolated from the sediment ecosystem of Chilika lagoon, India. Strain JC631^T tolerates up to pH 10 and NaCl up to 3% (w/v). MK6 is the only respiratory quinone. Predominant (> 10%) cellular fatty acids are anteiso-$C_{11:0}$, anteiso-$C_{13:0}$, and anteiso-$C_{15:0}$. Major polar lipids are phosphatidylethanolamine, phosphatidylcholine, a few unidentified phospholipids, and a few unidentified aminolipids. 16S rRNA gene sequencing showed that strain JC631^T shared the highest sequence identity (98.7%) with *Arenibacter latericius* KMM 426^T followed by *Arenibacter certesii* CCUG 48006^T (98.6%) and other members of the genus *Arenibacter* (< 97%). The genome size of strain JC631^T is 4.16 Mb with a GC content of 40.8 mol%. Strain JC631^T has ANI scores of 78.3% and 78.1%; *d*DDH values of 22.2% and 21.8%, respectively, with the type strains of *A. latericius* and *A. certesii*. The genomic distinction is well supported by chemotaxonomic characteristics and differential physiological properties. This supports strain JC631^T to be described as a new species of the genus *Arenibacter,* named as *Arenibacter lacus* sp. nov. The type strain is JC631^T (= KCTC 72002^T = NBRC 114071^T).

Introduction

The genus *Arenibacter* was first described by Ivanova et al. [1] to accommodate a rod-shaped bacterium, *Arenibacter latericius* within the family *Flavobacteriaceae*, which was further amended by Nedashkovskaya et al. [2]. Currently, the genus *Arenibacter* has 10 species with validly published names (https://lpsn.dsmz.de/genus/arenibacter). All species of the genus *Arenibacter* except *A. antarcticus*, *A. catalasegens*, and *A. aquaticus* were reported to have the capacity of degrading polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene and naphthalene, indicating the industrial importance of the genus *Arenibacter* [3]. Members of the genus *Arenibacter* are exclusively marine and are isolated from different habitats such as sediment, sea weed, sea urchin, and tidal flat. During our study on the cultivable bacterial diversity of Chilika lagoon, we have isolated an obligately aerobic bacterium which has highest (94–99%) 16S rRNA gene sequence identity with the members of the genus *Arenibacter*. Polyphasic characterization including phylogenomic analysis was carried out to classify the new isolate.

Materials and Methods

Home, Habitat and Isolation

Strain JC631^T was isolated from a sediment sample collected from Chilika lagoon at Satapada, Odisa, India (GPS of the sampling site was 19° 40′ 12.0″ N 85° 25′ 48.0″ E), depth of 10 feet from the water surface; sample pH was 8.0 and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of the strains JC631^T is LR215975. The GenBank/EMBL/DDBJ accession number for the whole-genome shotgun sequence is VVIN00000000.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00284-020-02205-x) contains supplementary material, which is available to authorized users.

✉ Chintalapati Sasikala
sasikala.ch@gmail.com

✉ Chintalapati Venkata Ramana
cvr449@gmail.com; chvrsl@uohyd.ernet.in

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500046, India

[2] Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology, J. N. T. University Hyderabad, Kukatpally, Hyderabad 500085, India

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TAXONOMIC DESCRIPTION
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MICROBIOLOGY SOCIETY

Paracoccus aeridis sp. nov., an indole-producing bacterium isolated from the rhizosphere of an orchid, *Aerides maculosa*

Anusha Rai[1], Smita N[1], Suresh G[1], Shabbir A[1], Deepshikha G[1], Sasikala Ch[2,*] and Ramana Ch.V[1,*]

Abstract

A Gram-stain-negative, non-motile, coccoid-shaped, catalase- and oxidase-positive, non-denitrifying, neutrophilic bacterium designated as strain JC501^T was isolated from an epiphytic rhizosphere of an orchid, *Aerides maculosa*, growing in the Western Ghats of India. Phylogenetic analyses based on the 16S rRNA gene sequence indicated that strain JC501^T belonged to the genus *Paracoccus* and had the highest levels of sequence identity with *Paracoccus marinus* KKL-A5^T (98.9 %), *Paracoccus contaminans* WPAn02^T (97.3%) and other members of the genus *Paracoccus* (<97.3 %). Strain JC501^T produced indole-3 acetic acid and other indole derivatives from tryptophan. The dominant respiratory quinone was Q-10 and the major fatty acid was $C_{18:1}\omega7c/C_{18:1}\omega6c$, with significant quantities of $C_{18:1}\omega9c$, $C_{17:0}$ and $C_{16:0}$. The polar lipids of strain JC501^T comprised phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, an unidentified glycolipid, two unidentified aminolipids, two unidentified lipids and four unidentified phospholipids. The genome of strain JC501^T was 3.3 Mbp with G+C content of 69.4 mol%. For the resolution of the phylogenetic congruence of the novel strain, the phylogeny was also reconstructed with the sequences of eight housekeeping genes. Based on the results of phylogenetic analyses, low (<85.9%) average nucleotide identity, digital DNA–DNA hybridization (<29.8%), chemotaxonomic analysis and physiological properties, strain JC501^T could not be classified into any of the recognized species of the genus *Paracoccus*. Strain JC501^T represents a novel species, for which the name *Paracoccus aeridis* sp. nov. is proposed. The type strain is JC501^T (=LMG 30532^T=NBRC 113644^T).

Bacteria residing in the rhizosphere of the plants play a vital role in the holistic development of the plant system [1–3]. Epiphytic orchid-associated bacteria have functional and ecological roles in the development of their host plant [4]. Epiphytes do not interact directly with the soil or its microbiota and thus constitute a unique system of ecology. Therefore, epiphytes have their own distinctive structural system for their sustenance, wherein they take up the nutrients and moisture from the atmosphere on the surface of the host plant aided by the microbial association [5, 6]. While investigating this unique diversity and its subsequent role in the development of the orchids, we have isolated strain JC501^T from the rhizosphere of an epiphytic orchid (*Aerides maculosa*). This strain belongs to the genus *Paracoccus* based on 16S rRNA gene sequence analysis. The genus *Paracoccus* was first described by Davis and his co-workers in 1969 [7] and belongs to the family '*Rhodobacteraceae*' of the class *Alphaproteobacteria* in the phylum *Proteobacteria*. There are more than 50 species of *Paracoccus* with validly published names (www.bacterio.net). Members have been isolated from environmental samples such as soil [8, 9], sediment [10, 11], water [12, 13], sludges [14, 15], foodstuffs [16], clinical specimens [17] and insects [18]. *Paracoccus halotolerans* [19], *Paracoccus salipaludis* [20], *Paracoccus fontiphilus* [13], *Paracoccus alimentarius* [16], *Paracoccus endophyticus* [21], *Paracoccus haematequi* [22] and *Paracoccus nototheniae* [23] are the valid names published during the year 2018–2019, while *Paracoccus jeotgali* [24] and *Paracoccus indicus* [25] are effective publications. Members of this genus are Gram-stain-negative, mostly non-motile and chemoorganotrophs [26]. Their major fatty acid is $C_{18:1}\omega7c$ and they are metabolically versatile [27]. The members of the genus *Paracoccus* have a genome size ranging from 2.9 to 5.6

Author affiliations: [1]Department of Plant Sciences, School of Life Sciences, University of Hyderabad P.O. Central University, Hyderabad 500046, India; [2]Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology, J. N. T. University Hyderabad, Kukatpally, Hyderabad 500085, India.
***Correspondence:** Sasikala Ch, sasi449@yahoo.ie; Ramana Ch.V, cvr449@gmail.com
Keywords: *Paracoccus*; epiphytic orchid; Proteobacteria; indole.
Abbreviations: AIC, Akaike information criterion; ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; IAA, indole-3-acetic acid; IAM, indole-3-acetamide; LCB, local collinear block; ML, maximum-likelihood; MLSA, multilocus sequence analysis; NA, nutrient agar.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JC501^T is LT799401. The Whole Genome Shotgun project is SELD00000000. The genome sequence of *P. marinus* NBRC 100637^T is VJYZ00000000.
Five supplementary tables and ten supplementary figures are available with the online version of this article.

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MICROBIOLOGY SOCIETY

Chryseobacterium candidae sp. nov., isolated from a yeast (*Candida tropicalis*)

Indu B,[1] Kumar G.,[1] Smita N.,[1] Shabbir A.,[2] Sasikala Ch.[1,*] and Ramana Ch. V[1]

Abstract

A Gram-stain-negative, rod shaped, non-motile, aerobic bacterium (strain JC507^T) was isolated from a yeast (*Candida tropicalis* JY101). Strain JC507^T was oxidase- and catalase-positive. Complete 16S rRNA gene sequence comparison data indicated that strain JC507^T was a member of the genus *Chryseobacterium* and was closely related to *Chryseobacterium indologenes* NBRC 14944^T (98.7 %), followed by *Chryseobacterium arthrosphaerae* CC-VM-7^T (98.6 %), *Chryseobacterium gleum* ATCC 35910^T (98.5 %) and less than 98.5 % to other species of the genus *Chryseobacterium.*The genomic DNA G+C content of strain JC507^T was 36.0 mol%. Strain JC507^T had phosphatidylethanolamine, four unidentified amino lipids and four unidentified lipids. MK-6 was the only respiratory quinone. The major fatty acids (>10 %) were anteiso-$C_{11:0}$, iso-$C_{15:0}$ and iso-$C_{17:0}$3OH. The average nucleotide identity and *in silico* DNA–DNA hybridization values between strain JC507^T and *C. indologenes* NBRC 14944^T, *C. arthrosphaerae* CC-VM-7^T and *C. gleum* ATCC 35910^T were 80.2, 83.0 and 87.0 % and 24, 26.7 and 32.7 %, respectively. The results of phenotypic, phylogenetic and chemotaxonomic analyses support the inclusion of strain JC507^T as a representative of a new species of the genus *Chryseobacterium,* for which the name *Chryseobacterium candidae* sp. nov. is proposed. The type strain is JC507^T (=KCTC 52928^T=MCC 4072^T=NBRC 113872^T).

The family *Flavobacteriaceae* comprises the genus *Chryseobacterium* which was first established by Vandamme *et al.* [1]. At the time of writing, more than 100 species of bacteria (www.bacterio.net/chryseobacterium.html) have been placed in the genus *Chryseobacterium* and were isolated either from clinical samples [2], fish [3], fresh water [4], sewage and plants [5], soils [6, 7], meat [8] or waste water [9]. New species with valid names added during 2018–2019 included *Chryseobacterium salipaludis* [10] *Chryseobacterium aurantiacum* [11], *Chryseobacterium phosphatilyticum* [12], *Chryseobacterium glaciei* [13] and *Chryseobacterium populi* [14]. The typical characteristics of the genus *Chryseobacterium* include the presence of an aerobic type of metabolism, branched-chain fatty acids, iso-$C_{15:0}$ and iso-$C_{17:0}$3-OH as the major fatty acids, phosphatidylethanolamine as a major polar lipid, menaquinone-6 (MK-6) as the characteristic respiratory quinone, and production of flexirubin-type pigments [15–17]. Some of the members of this genus are associated as endophytes [12, 17] and during our study on yeast diversity, we have isolated an endobacterium (strain JC507^T) of a yeast (*Candida tropicalis*) which was characterized by a genomic and polyphasic taxonomic approach.

A yeast (strain JY101) was isolated from a soil sample collected from Hyderabad, India (22° 98′ N 71° 47′ E). The soil sample was serially diluted and plated onto a nutrient agar plate containing chloramphenicol (100 µg ml^{-1}). Several white-coloured colonies were observed which were further purified and sequenced using yeast-specific primers used for amplification of the ITS regions and the D1/D2 domain of the 26S rRNA gene. The Internal transcribed spacer (ITS) region was amplified with ITS1 (5′-GTCGTAACAAGGTTTCCGTAGGTG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (31). The D1/D2 domain of the 26S rRNA gene was amplified using NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers. NCBI/CBS BLAST analysis of strain JY101 for D1/D2 (599 bp; NCBI accession numberLT999794) and ITS (340 bp; NCBI accession number LT795043) showed 100 % sequence similarity to the yeast *Candida tropicalis*. It was interesting to observe moving objects inside the vacuoles of *C. tropicalis* JY101 (Fig. S1, available in the online version of this article). There are a few reports [18, 19] of yeast hosting bacteria such as *Helicobacter pylori*. However, these reports were

Author affiliations: [1]Department of Plant Sciences, School of Life Sciences, University of Hyderabad, P.O. Central University, Hyderabad 500046, India; [2]Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology, J. N. T. University Hyderabad, Kukatpally, Hyderabad 500085, India.
***Correspondence:** Sasikala Ch., cvr449@gmail.com *or* sasi449@yahoo.ie
Keyword: *Chryseobacterium* .
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strainJC507^T is LT838865. The GenBank/EMBL/DDBJ accession for the whole genome shotgun sequence is SDLV00000000.
One supplementary table and three supplementary figures are available with the online version of this article.

Emerging Concepts in Bacterial Taxonomy

1

Anusha Rai, Indu, N. Smita, G. Deepshikha, K. Gaurav, K. Dhanesh, G. Suresh, Ch. Sasikala, and Ch. V. Ramana

Abstract

Bacterial taxonomy has progressed over the years by virtue of the brisk and competent scientific developments. Ground-breaking molecular techniques have added an edge in the phylogenetic studies, resulting in the quality description of the taxa under studies. New avenues are rapidly developing whose validation has always been embraced and included, which will assist in resolution. It began with the simple application of objective procedures for classification, and now we have arrived at the genome-based taxonomy. This pedantic step has led to the meticulous examination and served to reconcile certain conflicts of the status of the taxa. This field is dynamic and is exploring more options like proteomics and metabolomics in gaining more insights into the lineal heritage. Even though there has been a significant change and addition, there is an ever-growing need for a comprehensive study, which would thread all the attributes together into one functional unit of classification. In this review, we examine the paradigm shift from traditional taxonomy to integrated taxonomy useful in the characterisation of bacteria which in addition aids in the identity of biotechnological targets.

Keywords

Bacterial taxonomy · Polyphasic · Phylogenomics · Integrated taxonomy · Average nucleotide sequence index (ANI)

A. Rai · Indu · N. Smita · G. Deepshikha · K. Gaurav · K. Dhanesh · G. Suresh · C. V. Ramana (✉)
Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana, India

C. Sasikala (✉)
Centre for Environment, Institute of Science & Technology, JNT University Hyderabad, Hyderabad, Telangana, India

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Hopanoids and sporulenes of a few bacteria and insights into sporulenes of Bacillus subtlis

by Smita Nandardhane

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