Groundnut-plant growth promoting rhizobacteria interactions: Changes in root exudates, root proteome and bacterial cell wall proteome

Thesis submitted for the degree of
DOCTOR OF PHILOSOPHY

By
Ankati Sravani
(Regd. No: 14LPPH05)

Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500 046
India

April 2018

University of Hyderabad

(A Central University established in 1974 by an act of parliament)

HYDERABAD – 500 046, INDIA

DECLARATION

This is to declare that the work embodied in this thesis entitled **"Groundnut-plant growth promoting rhizobacteria interactions: Changes in root exudates, root proteome and bacterial cell wall proteome"** has been carried out by me under the guidance and supervision of Prof. Appa Rao Podile, Department of Plant Sciences, School of Life Sciences. The work presented in this thesis is a bonafide research work and has not been submitted for any degree or diploma in any other University or Institute. A report on plagiarism statistics from the University Librarian is enclosed.

Ankati Sravani

Prof. Appa Rao Podile
(Research Supervisor)

Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad – 500046, Telangana, India

CERTIFICATE

This is to certify that this thesis entitled **"Groundnut-plant growth promoting rhizobacteria interactions: Changes in root exudates, root proteome and bacterial cell wall proteome"** is a record of Bonafide work done by **Mrs. Ankati Sravani**, a research scholar for Ph.D. programme under the Department of Plant Sciences, School of Life Sciences, University of Hyderabad under my guidance and supervision. This thesis is free from plagiarism and has not been submitted in part or in full to this or any other University or institution for the award of any degree or diploma.

Parts of the work have been:
A. Published in the following publications:

B. Presented in the following conferences:

1. Sravani Ankati, T. Swaroopa Rani and Appa Rao Podile (2017) *Pseudomonas aeruginosa* induced antifungal molecules in groundnut root and root exudates provide protection against stem rot pathogen *Sclerotium rolfsii*" in 5th Asian PGPR international conference, Indonesia.
2. Sravani Ankati and Appa Rao Podile (2015) Characterization of *Arachis hypogaea* L. root exudates in presence of plant growth promoting rhizobacteria and their role in bacterial cell wall proteome changes in 56th Association of Microbiologists of India, New Delhi.

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

Sl.No.	Course Code	Name	Credits	Pass/Fail
1.	PL 801	Research Methodolgy	4	Pass
2.	PL 802	Research Ethics & Management	2	Pass
3.	PL 801	Lab Work	4	Pass
4.	PL 801	Biostatistics	2	Pass

Supervisor **Head** **Dean**

Contents

Abbreviations

°C	: degree centigrade/degree Celsius
µg	: microgram
µM	: micromolar
•OH	: hydroxyl radicals
2-DE	: 2-dimensional electrophoresis
AFM	: antifungl metabolites
APX	: ascorbate peroxidase
ATP	: adenosine tri phosphate
BSA	: bovine serum albumin
CAS	: Chromo azurol S
CFU	: colony forming units
CHAPS	: 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-opanesulfonate
Cm	: centimeter
CMC	: carboxy methyl cellulose
CW	: cell wall
DAS	: days after sowing
DNA	: deoxy ribonucleic acid
dNTPs	: deoxynucleotides
DTT	: dithiothreitol
EDTA	: ethylene diamine tetra acetic acid
$FeCl_3$	: Ferric chloride
g	: gram
GC	: gas chromatography
GST	: glutathione S-transferase
h	: hour(s)
HCl	: hydrochloric acid
$HgCl_2$	: Mercuric chloride
H2O2	: hydrogen peroxide
HEPES	: 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid
HSP	: heat shock protein
IEF	: isoelectric focusing

kDa	: kilodalton
L	: litre
LB	: Luria Bertini
LC	: liquid chromatography
LDH	: Lactate dehydrogenase
M	: molar
MALDI-TOF	: matrix-assisted laser desorption/ionization-time of light
mg	: milligram
MgCl2	: magnesium chloride
min	: minute(s)
mL	: milliliter
mM	: millimolar
MM	: minimal media
MOA	: methoxyamine hydrochloride
MS	**:** mass spectrometry
MSTFA	: N-methyl-N-(trimethylsilyl)-trifluoroacetamide
MWt	: molecular weight
NaCl	: Sodium chloride
NIST	: National Institute of Standard and Technology
O.D.	: optical density
PAGE	: polyacrylamide gel electrophoresis
PCA	: principal component analysis
PCR	: polymerase chain reaction
PDA	: potato dextrose agar
PGPR	: plant growth promoting rhizobacteria
pI	: isoelectric point
PIPES	: piperazine-N,N′-bis(2-ethanesulfonic acid)
PLS-DA	: partial least squares-discriminant analysis
PMSF	: phenyl-methyl sulphonyl fluoride
PR proteins	: pathogenesis-related proteins
PVP	: polyvinylpyrrolidone
REs	: Root exudates
RI	: Retention index

RNA	: ribonucleic acid
SA	: salicylic acid
SDS	: sodium dodecyl sulphate
TCA	: trichloroacetic acid
TEMED	: tetramethylethylenediamine
Tris	: tris-(Hydroxymethyl) aminoethane
V	: volume
W	: weight

List of Tables

List of Figures

1. Introduction

1.1. Groundnut phytomorphology

Groundnut is an annual, herbaceous cash crop with edible-oil-rich, and nutritious seeds. It grows up to 1 to 1.6 feet tall with opposite, pinnate leaves and seeds below the ground. Groundnut is scientifically known as *Arachis hypogaea* L. and taxonomically classified into the family Fabaceae. It is commonly known as peanut, earthnut, goober peas, pygmy nut, and monkey nut. Groundnuts mostly germinate by 5-10 days after sowing based on the environmental temperature and moisture content. Normal pH and temperature conditions required for obtaining good yield are 5.9-7 and 23 °C, respectively. Well-drained, sandy loam soils with warm, moist conditions are highly favorable for good germination and fast seedling development. Flowering starts after 20-60 days of seed germination and they are in yellowish orange color with reddish veins. Groundnut is a self-pollinating plant with unique pod development. The fertilized ovaries will develop into long thread-like structures called peg. The peg grows horizontally into the soil and develops into pods with 2 to 4 seeds normally. A complete crop requires up to 95-150 days based on the climatic conditions and groundnut variety (Spanish, Runner, Virginia, and Valencia).

1.2. Economic importance and statistics

Groundnut is an economically important plant as every part of it is useful for various purposes. Seeds of it are consumed as a food source with high amount of proteins (20-50%), carbohydrates (10-20%), fatty acids (40-50%) and used for extracting vegetable oil (Dutta et al., 2011). The oil is rich in antioxidants (like oleic acid and resveratrol) and used for cooking purposes. The shells and stems are used in cellulose production for paper synthesis, plastic manufacturing, in cosmetics, fuel generation, and as an animal feed.

China is the largest producer (42.4%) of groundnut followed by India (14.5%), Nigeria (7.8%), United States (4.4%), Burma (3.7%), Indonesia (3.1%), Argentina (2.6%), and Sudan (2.2%). Despite high area (up to 30% of the world's groundnut area) under its cultivation, India stands second in terms of its productivity owing to inappropriate fertilizer management (Mathivanan et al., 2014). As per Directorate of Economics and Statistics (Crop division, Government of India), Gujarat is the highest producer of groundnut in India followed by Rajasthan, Tamil Nadu, Andhra Pradesh, Karnataka etc. (Fig. 1.1). In India, it is grown in two-crop cycles (Karif

and rabi) and harvested twice in a year (in March and October). A survey report on groundnut crop in 2017 by Indian oilseeds and produce export promotion council shows an export of 7, 26,535.91 million tonnes, worth of 5,456.72 crores in the financial year 2016-17.

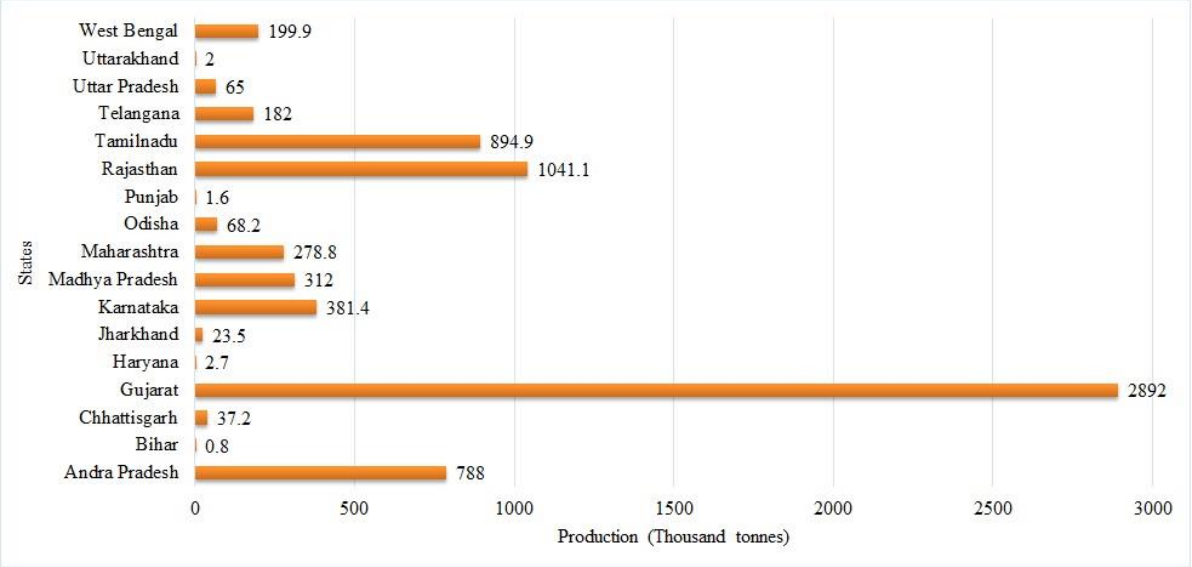

Fig.1.1: Groundnut production in various states of India in 2015-2016 (source web page: visualize.data.gov.in)

1.3. Factors effecting groundnut agronomy

Groundnut is usually grown in poorly fertile, nutrient-deficient, tropical and semi-arid tropical regions of the world (Krishi and Medinipur, 2014). This affects the crop yield, despite high area used for farming. According to Directorate of Economics and Statistics, for the past two years, there is a decrease of 221.2 thousand tonnes in groundnut production (from 7401.7 thousand tonnes 2014-15 to 7180.5 thousand tonnes 2015-16 in India). Several environmental, economical, abiotic and biotic factors influence the crop yield. The factors like temperature, pH, rain, drought, and soil salinity are the major abiotic threats. Whereas, biotic factors like pathogenic microbes, pests, and weeds contribute to poor yield and low quality of pods. A study carried out in Sudan, to evaluate the damage caused by various pests and diseases attacking groundnut showed a 57.3% yield loss without controlling pests and diseases. By controlling foliar insects, diseases, and pests, yield loss was restricted to 37%, 32% and 27% respectively (Tanzubil and Yahaya, 2017). More than 55 microbial pathogens like bacteria, fungi, viruses, nematodes, and mycoplasma affecting groundnut yield were reported. Among these, fungal pathogens cause highest economic yield loss (Podile and Kishore, 2002). *Aspergillus niger*, *Puccinia arachidis*, *Cercospora arachidicola*, *Sclerotium rolfsii*, and

Cercosporidium personatum are the few serious, notable fungal pathogens causing crown/collar rot, rust, stem rot, early and late leaf spot in groundnut. Along with insects, pests, and pathogenic microbes, weeds also affect crop yield by competing with crop plant for nutrients. Olayinka and Etejere (2015) reported high groundnut growth parameters like relative growth rate, dry matter, and crop yield in weed managed regions than weeded regions. All these studies show the direct impact of biotic factors on groundnut yield.

1.3.1. Practices to overcome yield loss caused by environmental factors

The challenges due to nutrient deficiency, damage caused by pests and pathogens have serious limitations in enhancing the productivity of the groundnut. Use of fertilizers to improve nutrients seem to be a necessary agricultural practice for a long time. The fertilizers increase the crop yield, which is the major requisite in the scenario of an inverse relation between available agricultural land and increasing population. Fertilizers are either nutrient based non-organic fertilizers or organic fertilizers. The non-organic fertilizers contain nitrogen (N), potassium (K), phosphate (P), and ammonium salts. Whereas, organic fertilizers include vermicompost, farmyard manure, poultry manure, and sewage sludge. India uses around 1,214 Kg/ha of N+P+K fertilizer for increasing crop yields. Whereas, the lowest and highest users are Turkey (1,004 Kg/ha) and Netherlands (6,655 Kg/ha), respectively (Savci, 2012). The combined use of organic and non-organic fertilizers is also an attractive practice, to boost the positive effects of fertilizers than their individual application. For example, vermicompost and N+P+K based fertilizers co-application provided the highest yield in many crops like maize, okra, onions and tomato (Sial et al., 2008; Sharma et al., 2009; Chanda et al., 2011). Overall, the use of fertilizers can combat the yield loss caused by soil nutrient deficiency.

1.3.2. 'Pros and cons' of fertilizers usage

The increase of crop yield by application of organic and non-organic fertilizers has its own advantages and disadvantages. The non-organic fertilizers are simple nutrients, in readily available form for the uptake of plants. Even though they are economically costly, a small amount of it is sufficient to improve the crop growth. The excessive or regular application of these fertilizers leads to adverse effects on plant growth, soil structure, soil microflora, animals and humans health. In a broad point of view, continuous use of these fertilizers causes the eutrophication of water bodies, groundwater contamination, and air pollution. Eventually, this affects biological processes of the life (Bhardwaj et al., 2014). The excess amount of nutrients

from the chemical fertilizers accumulate in the soil and cause many adverse effects. The accumulated nitrogen softens the plant tissues and affects the colonization of many microbes including beneficial organisms. High phosphate concentration leads to soil acidification, more organic material decomposition, and loss of soil structure. Finally, the over use of chemical fertilizers and changes in the traditional crop growing practices lead to deterioration of physicochemical and biological vigour of the cultivable soil (Dadarwal, 1997).

Organic fertilizers cannot be used as a direct nutrient source by the plants. They increase solubilization and exchange capacity of nutrients in the soil by increasing biological activity of surrounding microorganisms. This makes the nutrients available to plants in a steady state for long time growth. Along with improving plant growth, organic fertilizers combat against many soil associated problems like acidity, salinity, alkalinity, toxic heavy metals, and pesticides. However, the negative effects of organic fertilizers include slow plant growth, highly variable growth rate, accumulation of salts and heavy metals (Chen, 2006). The concerns about the residual toxicity of fertilizers (either organic or non-organic) led agriculturists to find a cost-effective alternative. To promote sustainable agriculture, a simple solution to these complex environmental threats and technical problems need to be evolved. A viable alternative is the use of soil microbes with plant growth stimulating ability without disturbing the natural habitat of the environment.

1.4. Plant-microbe interactions

The interactions between plant and microbes can be either beneficial, neutral or harmful (Fig 1.2). The outcome is dependent on the delicate balance among soil and plant type. Beneficial microbes include nitrogen fixers (NF), phosphate solubilizing bacteria (PSB), vesicular-arbuscular mycorrhiza (VAM), and plant growth promoting rhizobacteria (PGPR). Mostly, these microbes are in a mutual relationship with plants for carbon source and simultaneously improve plant growth by various mechanisms. Few microbes in soil have no relation to the plant processes. Neither they get to benefit from the plant nor they provide benefit to the plant; such organisms could be classified under the neutral category of microbes. The harmful microbes invade plants for their own benefit to get nutrients or to propagate. They cause severe damage to plant developmental processes even sometimes leading to the plant death.

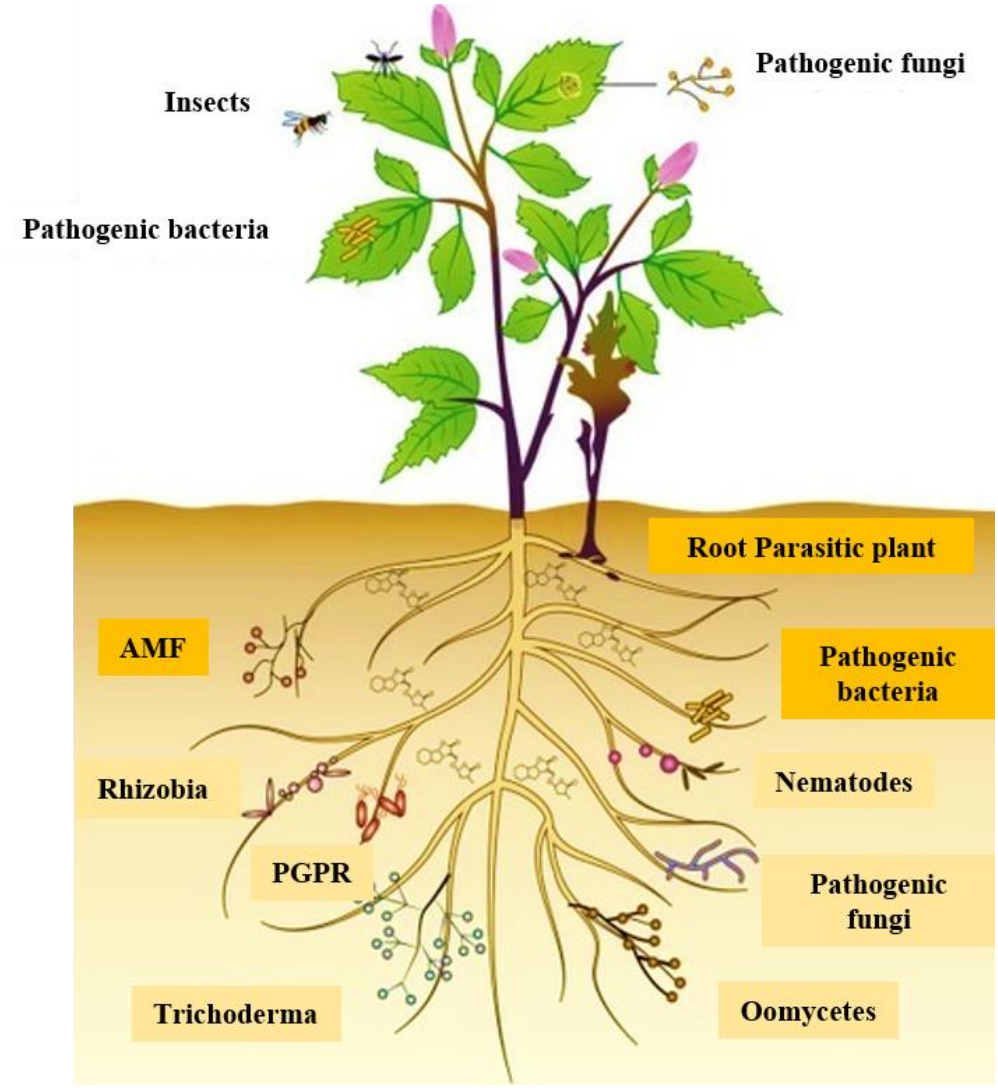

Fig.1.2: Plant-microbe interactions in the below ground region (Lopez-Raez et al., 2017).

1.4.1. Nitrogen Fixers

Nitrogen-fixing bacteria are either in symbiotic or non-symbiotic association with the host plants. Rhizobia are well-known symbiotic NF of legumes and they fix approximately 50-300 Kg N/ha (Mahdi et al., 2010). They form root nodules by chemo-attracting to plant secreted compounds and maintain a symbiotic relationship. Free-living *Azotobacter* and *Azospirillum* also fix nitrogen by an associative symbiotic relationship in the soil. They can fix up to 20-40 Kg N/ha (Mahdi et al., 2010) and a specific host plant is not required for their association. Maize, sorghum, sugarcane, wheat, and pearl millet are mostly recommended by this type of NF (Rajaee et al., 2007; Gholami et al., 2009). The NF increase soil fertility, seed germination, plant growth and produce antibiotics against pathogens. Another group of NF is blue-green algae/cyanobacteria which can fix up to 20-30 kg N/ha in rice fields. They are typically found

in the rice fields, hence named as paddy organisms (Nayak et al., 2004). *Azolla*, a free-floating nitrogen-fixing fern is in symbiotic association with *Anabaena*, alleviates P, S, K, Zn, Mb and Fe (nutrients) deficiency to the host plant (Yatazawa et al., 1980). This *Azolla- Anabaena* complex is also used as a nitrogen fertilizer along with cyanobacteria to increase rice yield (Watanabe et al., 1977).

1.4.2. Phosphate solubilizing bacteria

Mostly phosphate in soils is available in bound form (di/tricalcium phosphate, hydroxyapatite, and rock phosphate) which cannot be absorbed by the plants. PSB can solubilize the bound phosphate and make it available to plants without using phosphate fertilizers. As a first step, PSB secrete organic and inorganic acids that decrease the soil pH. Later, the acid phosphatases released by PSB mineralize the bound phosphate for plants' uptake (Mahdi et al., 2010; Sharma et al., 2011). *Aereobacter, Achromobacter, Agrobacterium, Bacillus, Burkholderia, Erwinia, Flavobacterium, Micrococcus, Pseudomonas,* and *Rhizobium* are the few PSB. For example, *Pseudomonas fluorescens* and *Bacillus megaterium* with phosphate-solubilizing ability increased the plant growth, and P and N levels in *Cicer arietinum* and *Phaseolus vulgaris* (Sharma 2007; Collavino et al., 2010). PSB like *Pantoea* sp., *Enterobacter* sp., *Burkholderia* sp., *and Sphingomonas* sp. can also phytostabilize heavy metals (like lead and aluminum) along with phosphate solubilization in rice seedlings (Park et al., 2011; Panhwar et al., 2014). This indicates the advantage of using PSB in different situations like plant growth and phytostabilization.

1.4.3. Vesicular-arbuscular mycorrhiza

The Glomeromycota phylum fungi penetrate into the vascular plant roots and form vesicles and arbuscules. They capture nutrients (P, N, and S) from the soil and provide to plants for their growth. This is a symbiotic relationship between fungi and plant root system was earlier referred as mycorrhizae. The arbuscular mycorrhizal fungi (AMF) form a sheath like protective cover around roots to protect plants from different environmental conditions like drought, salinity, other pathogenic fungal attacks. They increase the root elongation rate and improve the capturing ability of less available or inaccessible mineral nutrients (zinc, copper, calcium, and molybdenum) from the soil. *Artemisia annua* inoculated with AMF significantly increased plant growth, essential oil, and artemisinin content (Kumar et al., 2017).

A consortium of beneficial microbes was applied to test its effect on crop yield (Zaidi et al., 2017). The consortium may exhibit positive or negative effects on plants, depending on the nature of interaction between the members of the consortium. Mostly combined use of beneficial microbes resulted in increased plant growth, yield and enhanced biocontrol potential towards pathogens than their individual application (Jain et al., 2015a; Jain et al., 2015b; Sarma et al., 2015). Alagawadi and Gaur (1994) first used the dual inoculum of *Azospirillum brasilense* (NF) and *Pseudomonas striata* or *Bacillus polymyxa* (PSB) on sorghum. This application increased grain yield by improving N and P absorption by the plants. In *Rhizobium* and PSB dual application, yield increased by 20% in comparison to their individual use in wheat (Afzal and Bano, 2008). Dual inoculation of PSB and *Glomus fasciculatum (arbuscual* mycorrhizal AM fungus - AMF) also increased alfalfa plants growth (Piccini and Azcon, 1987). *Rhizobium leguminosarum* and AMF, dual inoculation on faba bean, provided tolerance to alkaline conditions along with improved plant growth (Abd-Alla et al., 2004). This consortium of beneficial microbes was presently used in the restoration of the degraded landscape. For example, the mass multiplication of beneficial AMF, NF, and rhizobacteria in 30 legume species restored the soil fertility (Ghosh and Dutta, 2016). *Arachis hypogaea,* out of all the tested legumes, showed the highest colonization by this tri-partite symbiotic relationship along with increased plant yield and elevated drought stress. These NF or PSB or AMF associations provide benefit to plants by increasing the nutrient availability and alleviate some environmental stress conditions.

1.5. Plant growth promoting rhizobacteria (PGPR)

Bacteria that provide benefit to plant can be symbiotic or free-living in the soil. They are abundant near the roots. The fraction of soil influenced by roots is called as rhizosphere (Parray et al., 2016). The term rhizosphere was first coined by a German plant physiologist and agronomist Lorenz Hiltner in 1904. In Greek the word ‘rhizo’ means root. The rhizosphere is divided into three zones: ectorhizosphere- soil near the root, rhizoplane- surface of the root and endorhizosphere- inside the root tissue, including cortical layers and endodermis (Badri and Vivanco, 2009; Oburger and Schmidt, 2016). The rhizosphere is colonized by a diverse group of microorganisms than the surrounding bulk soil. Approximately 10^{10}-10^{12} microflora will be present in a gram of rhizosphere soil, which is 1000-2000 times higher than the bulk soil microbial population. Some of the rhizobacteria in the soil promote plant growth, yield, and control diseases. Such free-living beneficial bacteria are termed as plant growth promoting

rhizobacteria (PGPR) (Podile and Kishore, 2006). About 2-5% of the total rhizosphere bacteria constitutes PGPR (Antoun, 2006). Strains of the genera characterized as PGPR are *Acetobacter, Acinetobacter, Aeromonas, Alcaligenes, Azoarcus, Azospirillum, Azotobacter, Arthobacter, Bacillus, Beijerinckia, Burkholderia, Clostridium, Derxia, Enterobacter, Exiguobacterium, Gluconacetobacter, Herbaspirillum, Klebsiella, Methylobacterium, Ochrobactrum, Pantoea, Paenibacillus, Pseudomonas, Rhodococcus, Serratia, Stenotrophomonas,* and *Zoogloea* (Podile and Kishore, 2006; Chauhan et al., 2015; Jha and Saraf, 2015).

PGPR are classified on the basis of functional and physiological aspects. Based on function, Somers et al., (2004) classified PGPR as biofertilizers, phytostimulators, rhizoremediators, and biopesticides. This was less accepted as the functions of PGPR are overlapping with each other. Whereas, Gray and Smith (2005) classified PGPR into two simple groups based on their colonization ability. The intracellular PGPR (iPGPR): for bacteria colonizing inside the root (also known as endophytes) and extracellular PGPR (ePGPR): for bacteria colonizing in the rhizosphere, rhizoplane or intercellular spaces of the root. Rhizobial interaction with legumes is a simple example of iPGPR. They form nodules in the root and fix the atmospheric N and promote plant growth. The gram-negative, rod-shaped bacterial population is dominant in iPGPR than gram-positive, cocci, rods or pleomorphic bacteria. The ePGPR are not able to form nodules, but they colonize roots and influence plant nutrient uptake, growth, and yield by an array of direct and indirect mechanisms (Fig 1.3). Some PGPR directly regulate the plant processes by impersonating synthesis of plant hormones, increasing soil minerals availability, as a way to enhance growth directly (Persello-Cartieaux et al., 2003; Taurian et al., 2010). Whereas, other groups of PGPR contribute indirectly by providing biocontrol against pathogens. They compete with pathogenic microorganisms for nutrients and niche (Dutta and Podile, 2010), produce lytic enzymes and antimicrobials (Kavino et al., 2010; George et al., 2015) and induce systemic resistance (Tjamos et al., 2005) to kill the pathogens.

1.5.1. Direct plant growth promoting mechanisms

1.5.1.1. Facilitating nutrient acquisition

PGPR facilitate the uptake of mineral nutrients like nitrogen, phosphate, iron, and zinc from the soil by converting the nutrients to soluble form. Nitrogen is a vital mineral for many living organisms including plants. It is required for the synthesis of building blocks like nucleotides,

DNA, RNA, amino acids, and proteins etc. In the atmosphere, nitrogen is available in diatomic form (N≡N) with a strong triple bond. This form of nitrogen is inert and won't be able to react with any other chemicals as well as non-absorbable by plants or animals. Nitrogen-fixing PGPR convert atmospheric nitrogen into ammonia and contribute to the N requirement of the plants. All nitrogen-fixing PGPR possess metalloenzymes known as nitrogenase, coded by *nif* genes. They include structural genes, iron protein activating genes, iron-molybdenum cofactor biosynthesis genes and regulatory genes necessary for the nitrogen fixation. Nitrogen-fixing *Pseudomonas* strain K1 increased grain yield and shoot biomass of two basmati rice varieties in comparison to non-nitrogen fixing *Zoogloea*, *Azospirillum brasilense*, and *Azospirillum lipoferum* (Mirza et al., 2006). A similar result was observed by Kuan et al., (2016) in maize with nitrogen-fixing *Klebsiella* sp., *Bacillus pumilus*, and *Acinetobacter* sp. inoculation.

After nitrogen, phosphorus is the second most essential macronutrient required for the growth of plants. Mostly phosphate exists in insoluble form, even in phosphate-rich soils. PGPR or PSB secrete organic acids (gluconic, glycolic, malonic, oxalic, and succinic acid) and phosphatases to disturb soil phosphate dynamics. They convert insoluble phosphates into soluble mono or di basic ($H_2PO_4^-$and HPO_4^{2-}) ions, referred as mineral phosphate solubilization (Mallick, 2016). This leads to an increase in phosphate availability in rhizosphere and plants phosphate utilization. *Bacillus*, *Enterobacter*, *Erwinia* and *Pseudomonas* spp. are the most potent PSBs (Gyaneshwar et al., 2002). As an example, a gluconic acid producing endophytic *Pseudomonas* sp. improved growth and yield of *Pisum sativum* L. in phosphate-limiting conditions (Oteino et al., 2015).

Like phosphate, zinc also exists as insoluble complexes in soil and cannot be utilized by the plants. PGPR fulfil the plant's zinc requirement by solubilizing zinc complexes and releasing zinc into the rhizosphere. Zinc solubilization is achieved by production of organic acids, inorganic acids (sulphuric acid, nitric acid, and carbonic acid), chelating ligands, proton extrusion, and/or with the oxidoreductive systems present on the cell membranes of PGPR (Wakatsuki, 1995; Saravanan et al., 2004; Goteti et al., 2013).

1.5.1.2. Phytohormones production

Phytohormones are plant growth regulators that influence plant development; produced by plants, algae, and few prokaryotic microorganisms. There are five important phytohormones, i.e. auxins, gibberellic acids, cytokinins, abscisic acid, and ethylene. PGPR secreted phytohormones influence the root zone and the plant developmental process. For instance,

indole-3-acetic acid (IAA) is an auxin that controls many important plant physiological processes, that include cell elongation, cell division, tissue differentiation, lateral root formation, and response to light and gravity (Parray et al., 2016). PGPR with IAA producing capability will have a capability to regulate these developmental processes by adding IAA to the plant's auxin pool (Vessey, 2003). Plant's potential to absorb nutrients and water depends on the root surface area. The increase in root surface by IAA improves absorption capacity from large volume of soil and their utilization for plant growth (Volkmar and Bremer, 1998).

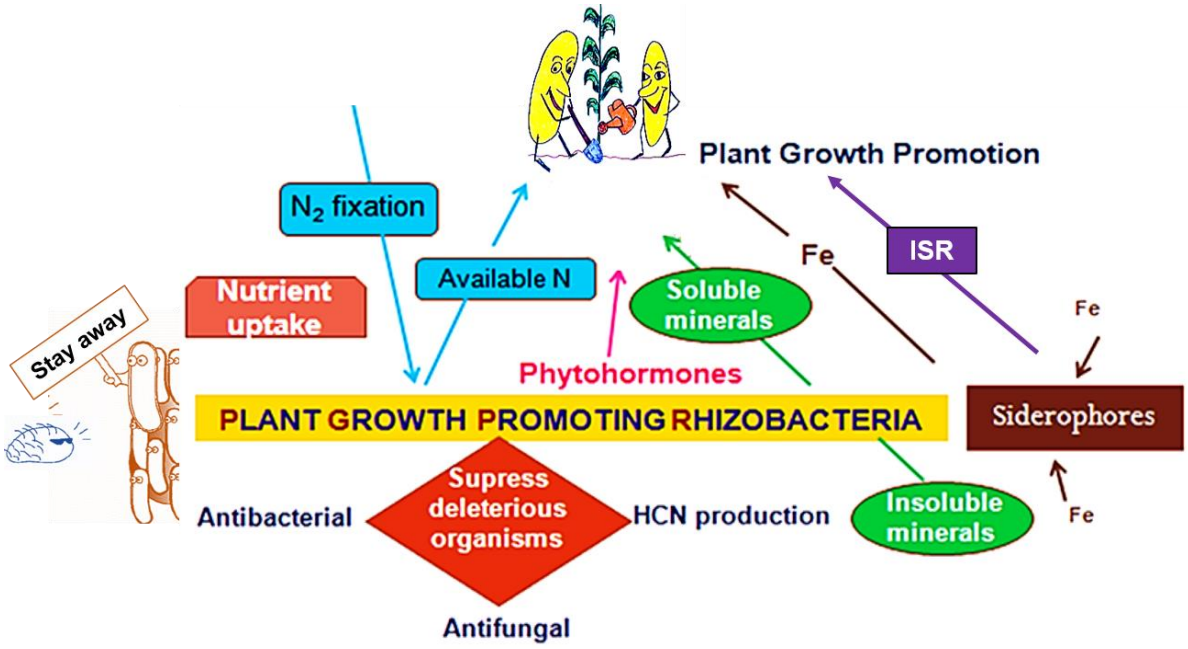

Fig. 1.3: Overview of plant growth promotion by PGPR: Direct and indirect mechanisms in improving the plant growth. They increase nitrogen (N), phosphate, zinc, and iron (Fe) availability and release phytohormones for improving plant growth. Antimicrobial metabolites, lytic enzymes, and siderophores secreted by PGPR prevent pathogens attack by ISR.

Gibberellic acids (GA) are tetracyclic diterpene compounds that promote seed germination, sex expression, stem elongation, flowering, and senescence in plants. They are produced by higher plants, bacteria, and fungi. All the GAs available in the rhizosphere are not biologically active. PGPR with potential to deconjugate gibberellin-glucosyl bonds can generate active dihydroxylated GAs (like GA1, GA3, and GA4) in the root zone. This active form of GAs promotes plant growth. For example, *Bacillus cereus*, *B. macrolides*, and *B. pumilus* significantly increased red pepper growth by producing biologically active GAs (Joo et al., 2004). A new PGPR, *Leifsonia soli* SE134 also influenced the growth and yield of cucumber, rice, tomato, and radish by producing active GAs (Kang et al., 2014). The third group of phytohormones, cytokinins are involved in cell division of root and shoot formation.

Arabidopsis thaliana mutants lacking cytokinin signalling genes (*CRE1, AHK2* and *AHK3*, and *RPN12*) showed impaired plant growth in presence of plant growth promoting *Bacillus megaterium*. This shows the complementary role of cytokinin and PGPR in plant growth promotion (Ortíz-castro et al., 2008). Other two phytohormones abscisic acid (ABA) and ethylene are stress-tolerant hormones. ABA influences seed dormancy and bud growth, whereas ethylene affects the cell shape and growth. *A. thaliana* elevated salt stress in presence of *Azospirillum brasilense* and showed a two-fold increase of plant ABA content (Cohen et al., 2008). This explains the importance of ABA in stress tolerance along with growth promotion. Plant internal ethylene levels are triggered by many biotic and abiotic factors. The increased ethylene levels inhibit plant growth by hindering DNA synthesis and cell division processes. PGPR able to synthesize 1-aminocyclopropane-1-carboxylate (ACC) deaminase can counteract the negative effects of ethylene *via* decreasing its levels. The ACC deaminase cleaves ACC, the precursor of ethylene into ammonia and α-ketobutyrate. *Pseudomonas putida* inoculated *Papaver somniferum* plants resisted against the negative effects of downy mildew (caused by *Peronospora* sp.) by decreasing ACC levels, increasing IAA and significantly improved the plant growth. Rkh1- Rkh4 PGPR, isolated from weed rhizosphere showed significant growth promotion of soybean by secreting IAA, GB, and ABA to elevated salt stress (Naz et al., 2009), indicating the interlink of phytohormones secreted by PGPR in regulating growth and stress alleviation in plants.

1.5.1.3. Siderophores production

Iron is an important micronutrient and serves as a cofactor for many redox maintaining enzymes of cell. Iron is available in insoluble ferric hydroxide form in soils. This limits the iron availability even in iron-rich regions for proper plant growth. Siderophores are low molecular weight, small, iron chelating compounds released by the bacteria (including PGPR), fungi and plants. The siderophores have a high affinity to Fe^{3+} and form Fe^{3+}-siderophore complexes that are absorbed by the plants. Plants adapted mechanisms to absorb Fe^{3+} bound to the bacterial siderophores (Masalha et al., 2000) by ligand exchange process. PGPR with siderophore producing ability can chelate iron in the soil and make it available to plant, and limit its availability to pathogens that cannot produce affinity siderophores. This generates a competition between PGPR and pathogen for iron utilization. Further, they suppress pathogens by inducing defense mechanisms of the plant. Pyoverdine, a yellow-green pigment produced by many fluorescent Pseudomonads functions as siderophore, and suppresses the pathogens

(Becker and Cook, 1988). Few siderophores can even chelate heavy metals and radionuclides (such as Al, Cu, Cd, In, Ga, Pb, U, and Np) and alleviate the stress imposed on plants (Neubauer et al., 2000).

1.5.2. Indirect plant growth promotion mechanisms

PGPR effectively compete with pathogens for nutrients or niche by releasing lytic enzymes/ antimicrobial compounds and by inducing systemic resistance (Podile and Kishore, 2006) in plants.

1.5.2.1. Antibiosis by PGPR

A biological association of two or more microorganisms, in which one is detrimental to another by its antagonistic behavior is called as antibiosis. PGPR secrete a wide variety of antibiotics to suppress phytopathogens. These antibiotics can be antibacterial or antifungal and inhibit pathogens at even very low concentrations. Some reported antibiotics produced by Pseudomonads are aerugine, amphisin, azomycin, butyrolactones, cepaciamide A, ecomycins, hydrogen cyanide, 2,4-diacetylphloroglucinol, phenazine, oomycin A, pyoluteorin, tensin, tropolone, pyrrolnitrin, viscosinamide, cyclic lipopeptides, rhamnolipids, kanosamine, zwittermycin-A, pseudomonic acid, antitumor antibiotics, cepafungins, and karalicin. They are reported for antimicrobial, antioxidant, antitumor, antiviral, antihelminthic, cytotoxic, phytotoxic, and plant growth promotion (Goswami et al., 2016; Parray et al., 2016). Antibiotics produced by *Streptomyces*, *Bacillus*, and *Stenotrophomonas* include oligomycin A, kanosamine, zwittermicin A and xanthobaccin (Parray et al., 2016). The volatile hydrogen cyanide (HCN), among them, inhibits cytochrome C oxidase, an important electron transport chain enzyme and reduces the energy supply to cell. This eventually leads to death of pathogen. As the HCN is not a specific inhibitor of pathogens, it may inhibit PGPR or plant energy mechanisms as well. The phytotoxic effects of HCN in reducing plant growth also reported in crops (Devi et al., 2007; Kumar et al., 2015). Rijavec and Lapanje (2016) proposed the phosphate regulating ability of HCN, over the biocontrol activity against pathogens, which needs further evaluation.

1.5.2.2. Induced systemic resistance

PGPR or antimicrobial compounds released by them, trigger a mild innate immune response after colonizing plants, this is referred as priming. First, microbe-associated molecular patterns

of PGPR are recognized by pattern recognition receptors of plant cells. Later, the plant develops an induced systemic resistance (ISR) by producing phytoalexins, expressing PR proteins, activating mitogen-activated protein kinase, and altering cellular calcium (Ca^2) levels. This prepares the plant to fight against subsequent pathogens attack. ISR in plants is attained by activating the signalling pathways regulated by jasmonic acid or salicylic acid or ethylene. In *Pseudomonas* spp. 'O' antigenic side chain of lipopolysaccharides, 2, 4-diacetylphloroglucinol, volatiles (like, acetoin and 2, 3-butanediol), siderophores (like pseudobactin and pseudomanine), flagella, cyclic lipopeptides, and homoserine lactones are few determinants of ISR in plants (Ryu et al., 2004; Gupta et al., 2015; Goswami et al., 2016). Diseases and damage caused by fungi, bacteria, viruses, nematodes and insects can be reduced by the PGPR application through activation of ISR (Ramamoorthy et al., 2001). Inoculation of *Bacillus velezensis*, *B. mojavensis*, *B. safensis*, *B. subtilis*, and *B. altitudinis* individually or in mixtures, reduced the *Heterodera glycines* (nematode) population and increased yield of soybean by activating the ISR (Xiang et al., 2017). *B. pumilus* SE34, *Pseudomonas fluorescens* 89B61, and *P. putida* inoculation in tomato plants reduced the late blight (fungal) disease and *Spodoptera litura* (insect) infestation by exerting ISR (Yan et al., 2002; Bano and Muqarab, 2017). From this, ISR can be considered as a crucial defense mechanism in plants against most of the pathogens like nematodes, insects, and fungi.

1.5.2.3. Cell wall degrading enzymes

Several PGPR show hyperparasitic activity on fungal pathogens by producing fungal cell wall degrading enzymes. As the fungal cell walls have a considerable amount of chitin and β-glucans as structural components, chitinases, and β-glucanases are considered as major mycolytic enzymes active against the number of phytopathogenic fungi (García-Cristobal et al., 2015; Kim et al., 2015). Degrading fungal cell walls by these enzymes inhibits the fungal growth and propagation. Chitinase producing PGPR include *Bacillus* spp., *Serratia marcescens*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa*, and *P. fluorescens.* Few glucanase producers are *Paenibacillus, B. cepacia* and *Streptomyces* (Goswami et al., 2016). This is the major mechanism adapted by most of the PGPR to overcome fungal attacks on plants.

1.6. Biofertilizers

A substance with soil microorganisms after applying to soil or seed, colonizes the plant internal region or rhizosphere with a potential to improve plant growth by increasing nutrient availability is called as biofertilizer (Vessey, 2003). The use of microorganisms to optimize crop productivity is an ancient practice since Theophrastus of 372-287 BC. He is the first person to suggest the use of mixing of different soils as a means of 'remedying defects and adding heart to the soil'. This mixing of soils promotes the beneficial microflora interchange and provides positive benefits to the plants (Tisdale and Nelson, 1975).

Commercialization of PGPR depends on safety, stability, shelf life, and consistency of the prepared formulation. Demand for a product in the market, academic-industrial tie-up, and funding (Gupta, et al., 2015). A biofertilizer is formulated by mixing beneficial microbes (mostly PGPR) with a suitable carrier material. Carrier material should have high water retention capacity, chemically and physically uniform, nontoxic, biodegradable, non-polluting, supports bacterial growth and survival. It should be able to protect cells from environmental stress conditions and keep them viable. A good carrier delivers the specified number of viable cells at accurate physiological conditions. Biofertilizers can be prepared in different dispersal forms (Fig 1.4). The solid powders or granules are prepared with soil, clay, peat, vermiculite, or perlite as carrier material. Slurries and liquids are made by using chemicals as carriers (cellulose, starch, carboxymethyl cellulose etc.) to improve stickiness, surfactant, stabilization and dispersal ability of the formulation. Different compounds other than the carrier that are added to formulations to support microbe's viability are additives. These include protectants (like carboxymethyl cellulose, cellulose, starch, trehalose, glycerol, and hydroxyl ectoine), carbon source (sucrose) and elicitors (chitin and chitosan). Gum arabica and polyvinylpyrrolidone (PVP) have dual functions as adhesives and desiccation protectants by holding sufficient amount of water for cells viability in the formulation.

Each formulation has its own advantages and disadvantages (Arora et al., 2010). Solid biofertilizers are prepared by evaporating water from the formulation. The viability of cells in the solid formulation will be in an inverse relationship with the moisture content of the environment. Humidity in the atmosphere affects the dried microbes viability in such formulations. These are less miscible in soil and prone to contaminations. Whereas, liquid formulations are less prone to contaminations and more miscible in the soil after application. The shelf life of a formulation is one of the major constraints in the commercialization of the

biofertilizer. It depends on the carrier material used, production technology, storage place and transport mechanism employed. In liquid formulations, shelf life is as high as up to 2 years; whereas, in solid formulations it is approximately 6 months. Presently 2×10^9 CFU/mL inoculum concentration is prepared in liquid formulations. This concentration suites for lower application and higher application of the inoculum based on the requirement (Bashan et al., 2014). Each step plays a significant role in producing an efficient biofertilizer.

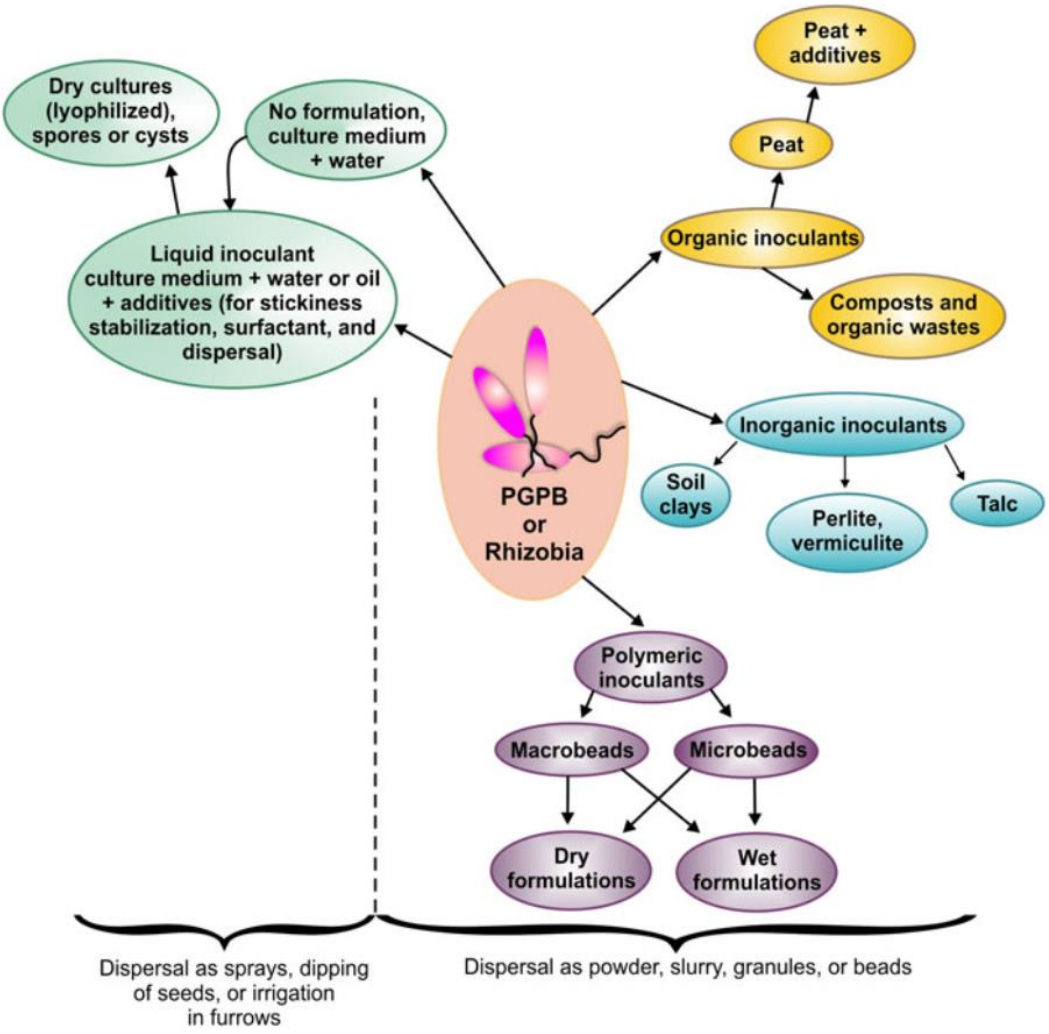

Fig. 1.4: Formulation strategies with different carrier materials and their mode of application in field.

Biofertilizers can be inoculated as seed or soil inoculations in fields (Fig 1.4). This depends on the inoculum requirement and environmental factors. The soil inoculation method introduces high inoculum into the soil than seed inoculation method. In seed inoculation method a small amount of formulation can sufficiently coat many seeds. Inoculum attachment will be dependent on seed size. Big seeds have more surface area, can accompany more inoculum than

the small seeds. In case of small seeds, soil inoculation is more advantageous to get more beneficial bacteria population. Finally, a potent biofertilizer contains a specified number of beneficial microbe population and exhibit significant plant growth promotion after application.

1.6.1. Drawbacks of PGPR biofertilizers

Currently, a number of PGPR, with potential to enhance crop yield, are being commercialized. Various PGPR have been formulated individually or in a consortium for plant growth and defense against pathogens. Groundnut yield was significantly increased in combined application of *Rhizobium*, *Pseudomonas*, and *Bacillus* (Mathivanan et al., 2014). Defense against anthracnose, angular leaf spot, and wilt causing pathogens in cucumber plants was reported by Raupach and Kloepper (1998) by application of *B. subtilis*, *B. pumilus*, and *Curtobacterium flaccumfaciens*.

The main drawback in using PGPR as biofertilizers is their inconsistency and irreproducibility in their performance under field conditions. The variation in PGPR biofertilizers performance is due to environmental factors that affect their stability and growth. Eventually, this hinders their potential growth promotion on plants. About 90% of the applied biofertilizer is noted to be lost into the air while applying and not used by the plants (Vejan et al., 2016). The remaining population might not be optimum for colonization. Ultimately this leads to a rapid decline in the population (Arora et al., 2010). This might be one of the reasons for negative results in the field conditions vs. positive outcomes in the laboratory observations. Other factors affecting may be the poor expertise of farmers for inoculum application, handling, storage, and large field area for inoculation (Bashan et al., 2014).

To maximize interactions of nursery seedlings and PGPR, it is essential to determine, how they exert their positive effects on plants. Understanding the interactions at molecular and physiological levels (Vessey, 2003) with the focus on genes, proteins, and metabolites (Parray et al., 2016) can fill the gaps. New concepts like rhizo-engineering for pointing the exotic biomolecules responsible for the plant-microbe interactions (Gupta et al., 2015) and nanotechnology for the production of PGPR based nano-fertilizer for an efficient application (Vejan et al., 2016) can be potent alternatives. Gregorio et al., (2017) generated nanofibers immobilized with PGPR; *Pantoea agglomerans* and *Burkholderia caribensis*. The coating of soybean seeds with these fibers showed increased plant growth. The processes involved in the

colonization of PGPR to the root system and chemical signalling involved in plant-PGPR interactions need special attention, to increase the usage of PGPR in agriculture.

1.6.2. Chitosan-based biofertilizer

Chitosan is a biologically active linear polymer. It is made of D-glucosamine and *N*-acetyl-D-glucosamine units linked by β-(1→4)-glycosidic bond. Chitin, the major cellular component of fungal cell walls, insects, crustaceans acts as a precursor for generating chitosan. Chitosan is a deacetylated form of chitin. It has many applications in pharmaceuticals, biopesticides, and plant growth enhancement. Chitosan induces the synthesis of callose, lignin, defense response and phytoalexins in plants. Use of chitosan in biofertilizers to alleviate fungal diseases in crop plants is in trend. For example, crustaceous chitosan and *Cunninghamella elegans* chitosan alleviated *Fusarium oxysporum* infection in cowpea plants by inducing catalase, reactive oxygen species, and peroxidases (Berger., 2016). The pinewood nematode, *Bursaphelenchus xylophilus* causes severe wood damage in pine plants. Application of chitosan in soil reduced the nematode population and damage caused by them (Silva et al., 2014). A combined application of diazotrophic bacteria (with N, P, K, P accumulation ability) and crustaceous chitosan improved cowpea nodules formation, shoot biomass and yield by increasing nutrients availability (Berger et al., 2013). Along with agricultural applications, chitosan also had many pharmacological uses like drugs, siRNA, DNA, and proteins delivery in humans. Ippolito et al. (2017) studied crustacean chitosan role in inhibition of potato pathogens *Phytophthora infestans* and *Fusarium solani*. These findings indicate the role of chitosan as an elicitor and growth promoter in biofertilizers. Chitosan amendment can add an additional benefit in preparing an efficient biofertilizer.

1.7. Root exudates

Plants communicate with its underground environment (soil microbes and other plants roots) through the secretion of various high and low molecular weight organic compounds called root exudates (REs). The ability to release variety of chemical compounds into the rhizosphere through REs is one of the remarkable metabolic features of the plants. REs play a major role in root-root, root-microbe communications and regulate different mechanisms below ground. They regulate soil microbial community in the neighbourhood, fight with herbivores, boost beneficial interactions, modify soil texture, and inhibit the competing plants' growth (Nardi, et al., 2000). Mechanical functions attributed to REs include maintenance of root-soil

communication, root tip lubrication, protection against desiccation, soil micro-aggregates stabilization, specific ions adsorption and storage (Bais et al., 2004). Traditionally REs are grouped into low molecular weight and high molecular weight compounds. The high molecular weight (HMW) REs comprises mucilage (polygalacturonic acid and polysaccharides) and proteins. Whereas, low molecular weight (LMW) compounds are amino acids, phenolics, organic acids, fatty acids, sugars, alcohols and secondary metabolites. (Fig 1.5). Over 1,00,000 diverse LMW compounds are believed to exist and cover the majority of REs secreted by the plants (Bais et al., 2004).

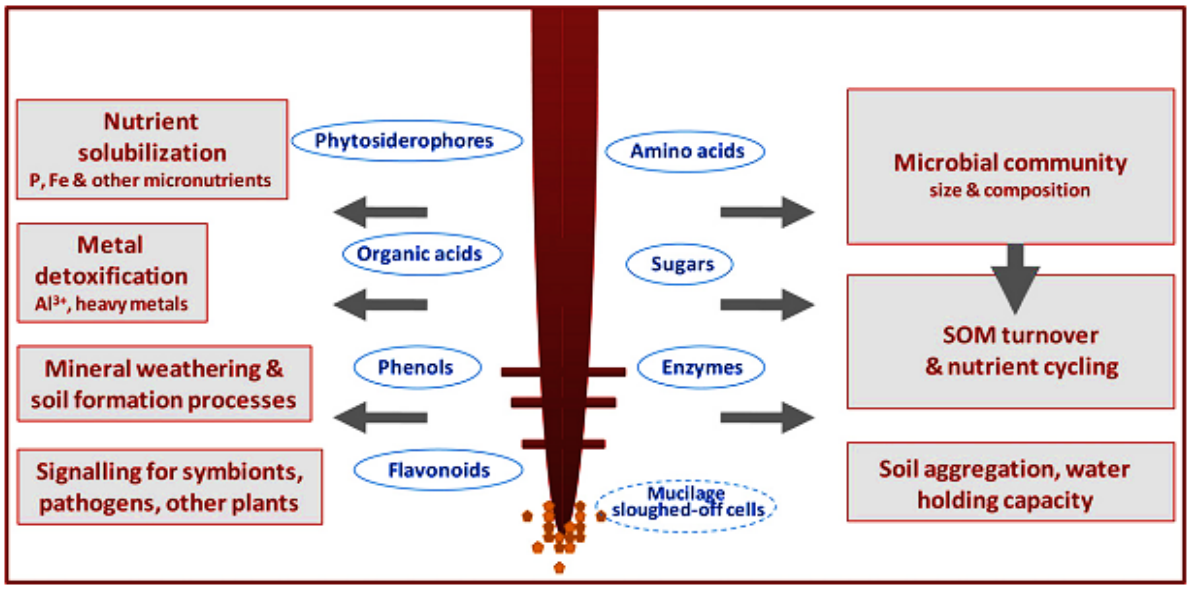

Fig. 1.5: Schematic representation of root exudate compounds driven rhizosphere processes (Oburger et al., 2013).

1.7.1. Mechanism of root exudation

Root exudation is the net efflux of LMW compounds of REs that are cycled across the root cell plasma membrane. REs composition varies with the growth media used. It indicates the role of specific nutrition used for the plant growth in root exudation process (Badri and Vivanco, 2009). Garcia et al., (2001) reported a positive correlation between the actively growing root system and root exudation. More REs are secreted by actively growing roots than young or old roots. Factors like light, temperature, pH, moisture, elicitors (jasmonic acid, salicylic acid, nitric oxide), mechanical impedance, minerals and metal content in growth media also affects the root exudation pattern (Pramanik et al., 2000; Vranova et al., 2013; Das et al., 2017).

Root exudation is mediated by three different pathways: passive diffusion, active transport by ion channels, and vesicular transport (Neumann and Romheld, 2000; Bertin et al., 2003). Small, uncharged, polar molecules are released by passive diffusion across the lipid membrane. This depends on cytosolic pH and the membrane permeability limit. Amino acids, sugars, and carboxylates are transported with the help of proteins, depending on the electrochemical gradient (mM range of root cell to µM range in soil) between root cytosol and external soil (Badri and Vivanco, 2009). Cell mitochondria and cytosol generated organic acids are build up in vacuoles or apoplast with the help of specific carrier proteins (Lopez-Bucio, et al., 2000). Even though the organic acids pool of vacuoles is important for the storage, the high concentration gradient developed between the soil solution and cytosolic pool releases them out. This drives the passive efflux of organic acids, as the l M concentrations of them in the soil are 1000 fold lower than in the cytosol (Fig 1.6). In some conditions, they are even secreted by H^+-ATPase active transport mechanism (Vranova et al., 2013).

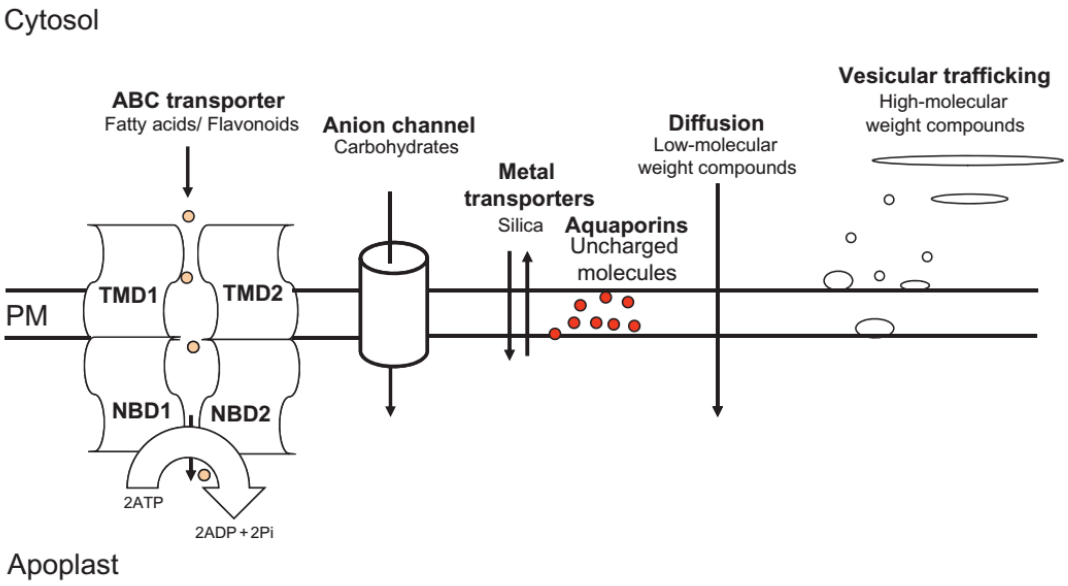

Fig 1.6: Mechanism of root exudation across the root cell membrane. PM, Plasma membrane; NBD, nucleotide binding domain; TMD, transmembrane domain (Badri and Vivanco, 2009)

Roots also secrete defense-related phytochemicals through ABC transporters. REs profile of *Arabidopsis* showed differences when ABC transporters and P-type ATPase transporters are inhibited, shows the role of these transporters in root exudation (Loyola-Vargas et al., 2007). Another line of molecules involved in defense is secreted from ‘front line' of detached border cells. They constitute 98% of the total carbon source of the REs (Fig 1.7F). These living border

cells release various organic compound which can fight against pathogenic fungi and bacteria (Bais et al., 2004). Proteins and mucilage like HMW compounds are secreted by root cap cells though Golgi mediated vesicular transport (Vranova et al., 2013).

1.7.2. Root exudates collection and analysis

Despite technical difficulties inherent with the REs collection, significant research in the field made it easy to choose an appropriate collection method for diverse REs studies. Numerous parameters play a significant role in REs collection. First, the plant growing medium used. Soil, nutrient agar, vermiculite, soil rite and nutrient solution are mostly used for the growth of plants. Second, collection solution (either deionized water or nutrient solution) with buffering salts ($CaCl_2$, KCl, $CaSO_4$) or antimicrobial components (cycloheximide) used. Third, sterile or non-sterile conditions provided while collection process. Plant growing containers for REs collection are distinguished into three groups by Oburger and Schmidt (2016). They include pot or compartment system, vertical or horizontal rhizoboxes, and rhizotrons. The use of pot system gives a simple differentiation between rhizosphere and soil samples. Whereas, rhizoboxes provide the picture of rhizosphere samples and mostly used for biogeochemical studied. The rhizotrons are more advanced of all the three methods listed with a potential to study a single root along its axis. It is used in 2D image analysis techniques.

Different sampling methods are in practice in collection of REs, include soil/rhizobox growth-hydroponics sampling (Sun et al., 2016; Mwita et al., 2016) referred to be a ‘quick and dirty approach’, hydroponic growth-sampling (Badri et al., 2013) and soil growth-rhizobox sampling (Neumann et al., 2014). Each sampling method has its own advantages interlinked with disadvantages (Table 1.1). As plants, natural habitat is soil, use of soil to grow plants seems to be more relevant and often used by researchers to collect REs. Use of soil grown plants over hydroponics will give a better picture of REs to field conditions. Hydroponic growth- sampling method is an easy and controllable way to collect REs in all the three sampling methods. But, relevance to field conditions is an unanswered question. Soil growth-rhizobox sampling is more advantageous over the above two methods with a non-destructive collection of REs. This sampling system requires a complex setup and the recovery of REs by this method needs to be tested (Oburger and Schmidt, 2016).

Different metabolites were detected in REs of various plants (Table 1.1). The analysis of REs in plant-microbe interactions, plant-plant interactions, and phytoremediation by targeted

approach (specified metabolite/group of metabolites) is common practice for a long time (Fig 1.5). For instance, organic acids like quinic acid, lactic acid, maleic acids and a flavonoid 7,4-dihydroxyflavone of *Pinus radiata* and *Medicago sativa* REs showed significant role in altering soil microbial community (Shi et al., 2011; Szoboszlay et al., 2016). Li et al. (2016) established the role of maize REs in inducing flavonoid synthesis in faba bean; it further increases nodulation and stimulation of nitrogen fixation. This shows the importance of REs in plant-plant communication. Phytoremediation of lead and pyrene by *Sedum alfredii* REs components like alanine, proline, oxalic acid, malic acid, glucose, and serine was reported (Lu et al., 2017; Luo et al., 2017). The non-targeted approach in REs analysis over targeted approach will help to identify the variations in interactions without any bias. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are the two potent tools in non-targeted analysis of REs. Overall, the soil-grown hydroponic sampling is more appreciated even though this method has limitations (slight damage to root during excavation). It is more suitable to field samples in evaluating plant-microbe interactions by whole community metabolomics (Oburger and Schmidt, 2016), as well as the assessment of comparative metabolome data by untargeted approaches using GC-MS or LC-MS (Dam and Bouwmeester, 2016).

1.7.3. Root exudates in plant-microbe interactions: specific to PGPR

Plants secrete about 50% of its total photosynthetic products through REs (Dam and Bouwmeester, 2016) as a positive or negative signal to contiguous microbes (Badri and Vivanco, 2009). REs serve as signalling molecules in highly specific chemical communication between roots and soil microflora leading to a biologically significant plant-microbe interaction. The positive signals help to establish a beneficial association with rhizobia, mycorrhizae, and PGPR. In contrast, negative signals prevent pathogenic microbes, parasitic plants, and herbivores association with plants (Fig 1.7E).

In legume-rhizobia interaction, flavonoids (2-phenyl-1,4-benzopyrone derivatives) present in the plant REs induce rhizobia nod genes responsible for the symbiotic association. The activated *nod* genes, synthesise and secrete lipo-chitooligosaccharides (LCOs), referred as nodulation (Nod) factors. The LCOs are recognized and perceived by the plant with the help of root epidermal receptor kinases. This triggers the nodule formation by rhizobia for nitrogen fixation in legumes (Fig 1.7A). An overlapping LCO-nod signalling mechanism is also seen in plant and AMF mutual association in the rhizosphere. In this symbiosis also plant releases some

Table 1.1: Different sampling methods to detect metabolites in root exudates: advantages and limitations

Method	Advantages	Limitations	Plants species	metabolites detected	References
Solid (soil /MS agar/sand) growth media-hydroponic sampling	a simple collection, Applicability to field	Slight root damage	*Beta vulgaris*	Citramalic and Salicylic acid	Khorassani et al. (2011)
			Eperua falcata	Chalcone and Flavone	Michalet et al. (2013)
			Arabidopsis thaliana	Sugars, Sugar alcohols, Phenolics and Amino acids	Chaparro et al. (2013)
			Spirodela polyrrhiza	Oleamide and Erucamide	Sun et al. (2016)
Hydroponic growth-sampling	Controlled, easy manipulation of conditions	Artificial growth conditions, poor comparability to the soil system	*Arabidopsis thaliana*	Amino acids, Fatty acids Nucleosides, Dipeptides, Phytohormones, and Phenylpropanoids	Strehmel et al. (2014)
			Arabidopsis thaliana	Oligolignol and Coumarin	Ziegler et al. (2015)
			Avena sativa, *Brachiaria decumbens*, *Medicago sativa*, and *Brassica juncea*	Flavanone, Flavone, Isoflavone, 7-Hydroxyflavanone, 7-hydroxyflavone, and 6-hydroxyflavone	Pino et al. (2016)
			Cucumis sativus	Tryptophan and Raffinose	Liu et al. (2017)
Soil growth - rhizobox sampling	Non-destructive, repeatable sampling	Semi-natural growth conditions; complicated set-up	*Lactuca sativa* L. cv. Tizian	Sugars, Sugar alcohols, Organic acids, Benzoic and Lauric acids	Neumann et al. (2014)
			Medicago sativa	7,4-dihydroxyflavone and Naringenin	Szoboszlay et al. (2016)

uncharacterized molecules (Nadal and Paszkowski, 2013) that signal mycorrhizal fungi to produce Myc-LCOs (Maillet et al., 2011). These are similar to LCOs of legume-rhizobia symbiosis. As mycorrhizal associations are considered as primitive (according to fossil records, at least from 400 million years) in comparison to legume-rhizobia symbiosis, rhizobia might co-opt AMF signalling mechanism in evolution. But, the intense research in legume-rhizobia symbiosis might predispose the first detection of this mechanism in them, rather than in plant-AMF association (Venturi and Keel, 2016). In plant-AMF association the unknown plant signalling molecules were identified as strigolactone and cutin in the REs (Fig 1.7B) (Haldar and Sengupta, 2015; Dam and Bouwmeester, 2016). *Medicago truncatula* mutants impaired in cutin monomers production were unable to show AMF symbiosis, explains the role of cutin in interaction (Liu et al., 2011).

Root parasitic plants (*Striga* spp., *Alectra* spp., *Orobanche* spp., and *Phelipanche* spp.) over deemed this strigolactone signaling of plants for attracting AMF. They developed mechanisms to utilize host plant strigolactones and show negative effects on plants by competing with beneficial AMF and host plant nutrients (Dam and Bouwmeester, 2016). Other negative signals in the REs include antimicrobial compounds which act against pathogens. The cowpea seedlings, chitinases like protein in the REs inhibited *Fusarium oxysporum* growth (Nobrega et al., 2005). The coumaric acid of rice REs displayed inhibition of *F. oxysporum* wilt in watermelon when grown in intercropping (Hao et al., 2010). Li et al., (2013b) identified increased exudation of antifungal compounds (against *F. oxysporum* and *F. solani*) like *p*-coumaric acid, *p*-hydroxybenzoic acid, and benzoic acid by resistance peanut cultivars. These findings show the role of REs in inhibiting pathogens by releasing antimicrobial compounds.

REs are perceived as the first line of chemical communication between roots and PGPR in the rhizosphere and may serve as a source of nutrition (Hirsch et al., 2003; Bais et al., 2006). The quality and quantity of REs varied among plant species, age of the plant (at different stages of plant growth), biotic and abiotic factors (Badri and Vivanco, 2009). Distinct REs could specifically modulate the rhizosphere microbiome and shape their functional diversity. The rhizosphere microbial communities of many plants like *Arabidopsis*, maize, *Medicago*, sugar beet, pea, wheat etc. are modulated by plant developmental specific root exudation (Badri et al., 2013; Chaparro et al., 2013; Haldar and Sengupta, 2015). Organic acids or flavonoids or sugars in the REs play a specific role as a carbon source (Jones, 1998) and molecular signals in plant-microbe interactions (Fig 1.7D).

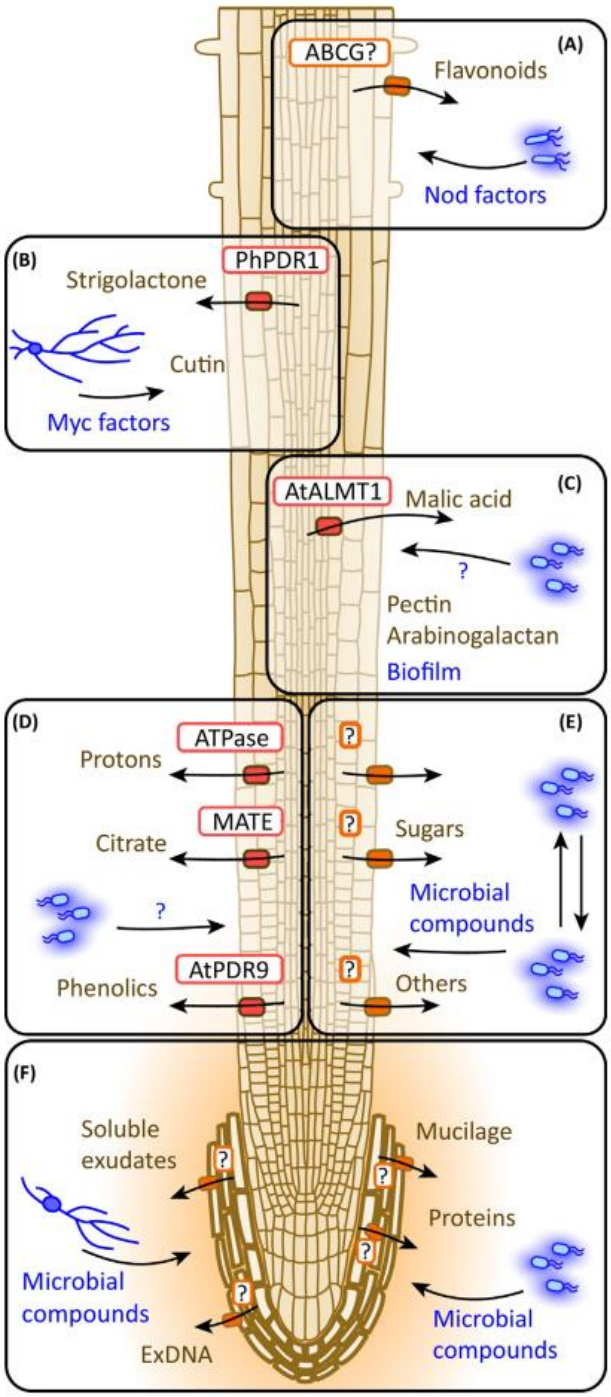

Fig. 1.7: Metabolites interchange in plant-microbe interactions in the rhizosphere. (A) Legume-Rhizobia symbiosis. Flavonoids of plant REs are sensed by rhizobia, produces Nod factors (LCOs) and initiates nodulation for nitrogen fixation. (B) Plant-AMF association. strigolactones or cutin of REs are sensed by *Glomeromycota*, intern releases Myc factors (Myc-LCOs) for colonization and establishment (C) Chemotaxis of beneficial microbes towards REs and biofilm formation. In *Arabidopsis*, malic acid in the REs chemo-attracts beneficial bacteria and allows their biofilm formation by using root pectin and arabinogalactan (D) REs transport for microbial utilization and communication (E) Microbe-plant and microbe-microbe signalling (F) Border cells in plant-microbe interactions. exDNA-extracellular DNA, ABCG-type transporter, PDR-Pleiotropic drug-resistant transporter, MATE- Multidrug and toxic compound extrusion, ALMT1-Aluminium-Activated Malate Transporter 1 (Sasse et al., 2017).

Changes in the diversity and quantity of the REs compounds alters upon colonization by beneficial or pathogenic microbes. For instance, more of tryptophan was detected in cucumber REs after treatment with *Bacillus,* promoting further colonization. On the other hand, decreased raffinose exudation reduced the colonization by pathogenic *Fusarium* in a tripartite association (Liu et al., 2017a). REs even drive the PGPR adherence, colonization and biofilm formation in the rhizosphere besides chemoattraction (Fig 1.7C). For example, *Bacillus* spp. and *Paenibacillus* spp. showed improved colonization, chemotaxis, and biofilm formation in presence of organic acids of banana, tomato and watermelon REs (Ling et al., 2011; Tan et al., 2013; Yuan et al., 2015). Malic acid exudation increased in *Arabidopsis* REs in presence of pathogenic *Pseudomonas syringae* pv. *tomato* to recruit (by chemo- attracting and biofilm formation) beneficial *Bacillus subtilis* (Rudrappa et al., 2008a). Simultaneously, the plant pectin and arabinogalactan stimulated biofilm formation of this *B. subtilis* for establishing on the root surface (Beauregard et al., 2013). Colonization of PGPR on plant roots triggers the release of metabolites involved in the phytoremediation along with antimicrobial compounds. For example, flavone, flavanone, and isoflavone detected in *Pseudomonas* spp. and *Stenotrophomonas* spp. colonized *Avena sativa*, *Brachiaria decumbens*, *Medicago sativa*, and *Brassica juncea* plant REs degraded polychlorinated biphenyls (Pino et al., 2016). These studies indicate the importance of plant REs in orchestrating the rhizosphere processes for establishing PGPR. However, the key chemical compounds of REs responsible for plant-PGPR interaction are yet to be deciphered in comparison to rhizobia and AMF interactions.

1.8. Omics approach to study plant-PGPR interactions

Omics is a study to identify and quantify metabolites (metabolomics) or proteins (proteomics) or genes (transcriptomics) involved in a process at a given time. Recent advances in analytical techniques (particularly GC-MS, LC-MS, 2D electrophoresis, and microarray) have opened the doors for unraveling many biological processes. In understanding plant-PGPR interactions, REs were focused by many researchers for more than a decade. Choosing a specific metabolite or group of metabolites (like organic acids/ sugars/ flavonoids/phenols/amino acids) present in the REs was reported by targeted metabolomics approach with the help of HPLC analysis. This narrow downs the REs profiling in the detection of signalling molecules in plant-PGPR interactions. Untargeted analysis of REs with the help of GC-MS or LC-MS analysis initially provides a ‘fingerprint' of metabolites differentiated in the interaction. Statistical analysis of the fingerprint by multivariate analysis using principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), visualization techniques (heat map, dendrogram

clustering, and Venn diagram) will provide significant metabolites need to be pointed. The significant metabolites can be tested for their role in the interaction by different bioassays. This approach was experimentally proved in *Arabidopsis* and maize REs profiling, as well as in detecting lead phytoremediators of *Sedum alfredii* REs (Luo et al., 2017; Pétriacq et al., 2017).

Exploring the REs metabolome in plant-PGPR interactions is not sufficient to get a complete picture of this beneficial interaction. Other biological parameters need to be focused include proteins and genes involved in the interaction from both the partners. Transcriptome analysis of two *Bradyrhizobium* spp. in presence of soybean REs showed variations in genes involved in the nodulation like chemotaxis, a two-component system, ABC transporters, and IAA metabolism (Liu et al., 2017b). Both, plant and PGPR, simultaneously regulate their cellular proteins to adjust with the beneficial interaction and to synthesise rhizosphere signalling compounds. Differential expression of root carbohydrate, nitrogen metabolism, defense, stress and antioxidant-related proteins was reported in cucumber- *Pseudomonas putida* UW4 and *Arabidopsis*- *P. polymyxa* E681 beneficial interaction (Li et al., 2013a; Kwon et al., 2016). Root proteins that are directly or indirectly associated with growth promotion have changed significantly in canola, upon *P. fluorescence* treatment under salt stress (Banaei-asl et al., 2015). Dubrovskaya et al. (2017) reported the polycyclic aromatic hydrocarbons degradation ability of alfalfa and sorghum REs peroxidases. *Bacillus mycoides* endosphere (EC18) and rhizosphere (SB8) colonizing isolates transcriptomics in presence of potato REs showed differential gene expression patterns. In endosphere (EC18), membrane proteins and amino acid biosynthesis genes were upregulated. Whereas, in the rhizosphere (SB8), carbohydrate transport genes were upregulated, suggesting the role of REs in specific induction of biological pathways according to their colonization habitat (Yi et al., 2017). A correlative study of REs metabolome data and genome sequences detected variations in between 19 *Arabidopsis thaliana* accessions in their natural habitat. Three biosynthetic pathways differed in them are indolic glucosinolate hydrolysis, hydroxycinnamic acid amine and a flavonoid triglycoside (Monchgesang et al., 2016). These findings show the importance of proteins as one of the major stations, needed to be focused in plant-PGPR interactions.

1.9. Objectives of the study

PGPR have enormous potential as biofertilizers for sustainable agriculture. At the same time, the results in the field experiments are sometimes inconsistent. If we understand the mechanism of interaction, it would be possible to find ways to overcome the inconsistency after

understanding plant-PGPR interactions at molecular level. Plant REs are identified to be involved in various plant-microbe interactions. Still, the specific metabolites of the plant that attracting PGPR and facilitate establishment on the roots, along with proteome and genome level changes are not elucidated. Use of 'Omics' approach to identify the key metabolites, proteins, and genes of plant-PGPR interaction would be a holistic approach to comprehend these interactions in depth. Against this background, by choosing groundnut as an experimental plant, we framed few objectives to understand groundnut-PGPR interactions.

1. Isolation and characterization of groundnut PGPR and preparation of liquid formulation
2. Identification of specific metabolites in the root exudates in groundnut- PGPR interaction
3. Detection of variations in groundnut root proteome and PGPR cell wall proteome in groundnut- PGPR interaction
4. *Pseudomonas* sp. (RP2) interaction specific groundnut root exudates and root proteins in bacterial attachment and defense against *Sclerotium rolfsii*

2.1. Isolation of groundnut associated bacteria

2.1.1. Sample collection

Groundnut plants were randomly picked from 14 different fields of two states, Telangana and Andhra Pradesh, India. Plants were uprooted in triplicates from each field and transferred to a sterile cover. Non-rhizospheric and rhizospheric soil samples also collected from the fields and stored at 4 °C till the bacteria isolated.

2.1.2. Cultivable bacteria isolation

One gram of rhizospheric soil closely attached to the roots and non-rhizospheric soil samples were suspended in 10 mL of sterile double distilled water (DDW). The rhizoplane and phylloplane bacteria were isolated by washing 1 g of the root or leaf surfaces with sterile water, followed by suspending in 10 mL of DDW. All the samples were vortex, serially diluted till 10^{-8} dilution, spread plated on Luria Bertani (HiMedia Laboratories, India) agar and incubated for 24 h at 37 °C. Morphologically distinct bacterial colonies were selected and pure cultures were prepared by quadrant streaking method. Pure cultures were maintained at -80 °C as glycerol stocks for further use.

2.2.1. Media composition

2.2.1.1. Nutrient agar

Peptone- 5 g, beef extract- 3 g, NaCl- 5 g, and agar- 15 g were dissolved in 900 mL of DDW, pH was set to 6.8, and volume was adjusted to 1.0 L.

2.2.1.2. National Botanical Research Institute Phosphate (NBRIP) medium

Glucose-10 g, $Ca_3(PO_4)_2$- 5 g, $(NH_4)_2SO4$- 0.1 g, KCl- 0.2 g, $MgSO_4$- 0.25 g, $MgCl_2$- 0.5 g, and agar-15 g were dissolved in 900 mL of DDW, pH was set to 7.0, and volume was adjusted to 1.0 L.

2.2.1.3. Zinc agar

Glucose-10 g, $(NH_4)_2SO_4$-1 g, KCl- 0.2 g, $MgSO_4$- 0.2 g, K_2HPO_4- 0.1 g, ZnO- 5 g and agar-15 g were dissolved in 900 mL of DDW, pH was set to 7.0, and volume was adjusted to 1.0 L.

2.2.1.4. Chitin agar

Colloidal chitin-2.5 g, Na_2HPO_4-0.065 g, KH_2PO_4-1.5 g, NaCl-0.25 g, NH_4Cl-0.5 g, $MgSO_4$-0.12 g, $CaCl_2$-0.005 g and agar-15 g were dissolved in 900 mL of DDW, pH was set to 7.0, and volume was adjusted to 1.0 L.

2.3. Screening for plant growth promoting traits

2.3.2. Phosphate solubilization

The NBRIP media was used to investigate the phosphate solubilization ability of the test isolates. Briefly, 0.5% of $Ca_3 (PO_4)_2$ emended plates were spot inoculated with overnight grown active cultures and incubated for 72 h at 30 °C. A clear zone around the spotted inoculum was noted as positive for phosphate solubilization (Nautiyal, 1999).

2.3.2. Zinc solubilization

To check the zinc solubilization capacity of the bacterial isolates, 0.5 % of zinc oxide amended minimal medium (MM) agar plates were prepared. Overnight grown active cultures were spotted on these plates and incubated for 72 h at 37 °C. Bacterial isolates, able to utilize zinc, make a clear zone around the inoculum by utilizing the zinc oxide, are positive for zinc solubilization (Saravanan et al., 2004).

2.3.3. Chitinase(s) production

The 0.25 g of colloidal chitin, the substrate of chitinases, was added in 100 mL of MM having 1.5 g of agar (Das et al., 2010). Chitin agar was sterilized in autoclave poured in petri plates. The 12 h grown active bacterial isolates were spot inoculated on the petri plates and kept for 96 h at 37 °C. A clear zone around the test colony formed by the breakdown and/or utilization of the colloidal chitin was considered as positive for chitinase(s) production.

2.3.4. Siderophore(s) production

As described by Neilands (1987), Chrome Azurol S (CAS) agar was used to detect the siderophores producing ability of the test bacterial isolates. CAS plates were prepared using solution-1 containing 2 mM $FeCl_3$ in 10 mM HCl (1 mL), solution-2 having 6.05 mg of CAS dissolved in 5 mL of DDW, and solution-3 with 7.3 mg HDTMA (cTAB) dissolved in 4 mL of DDW. All the 3 solutions were mixed one by one slowly by stirring, till solution attains a

dark blue color. Another solution including 75 mL of DDW, 10 mL of 10X MM salts, 3.02 g PIPES, and 1.5 g of agar. The pH was maintained at 6.8 with NaOH solution. Both the mixtures were autoclaved and cooled to 50 °C. To this solution, 3 mL of filter-sterilized l0% casamino acids solution was added under sterile conditions. Finally, the CAS dye solution was added to the above mixture along the conical flask walls, slowly agitated, and plates were poured. The plates were inoculated with 12 h grown active test cultures and incubated for 72 h at 37 °C. Orange halo on blue agar CAS plate, around the test bacterial colony, indicated the chelation of iron by siderophore(s).

2.3.5. Indole-3-acetic acid production

Bacterial isolates were tested for IAA production using a microtiter plate method (Sarwar and Kremer, 1995). Bacteria were grown in 10 mL of LB broth at 37 °C for 12 h. The 150 µL of bacteria grown culture filtrate was dispensed into 96-well plate and 100 µL of Salkowski reagent was added. The reagent was prepared by mixing 50 mL of 35 % of $HClO_4$ and 1 mL of 0.5 M $FeCl_3$ solutions. Samples kept at 25 ± 2°C for 30 min and allowed to react. The color intensity was measured using a microtiter plate reader at OD_{530}.

2.3.6. ACC deaminase assay

ACC deaminase assay was performed with a slight modification procedure of Li et al. (2011). The overnight grown bacterial isolates were inoculated in MM (Glucose- 5 g, Na_2HPO_4- 6 g, KH_2PO_4- 4 g, NaCl-0.25 g, $MgSO_4$- 0.2 g, and $CaCl_2$- 0.005 g) containing 3 mM of ACC, the substrate of ACC deaminase. Cultures were incubated for 48 h at 37 °C. Un-inoculated MM containing ACC was used as control. The culture supernatant was collected and 500 µL of it was mixed with ninhydrin reagent (1 mL). Reagent was prepared from 0.5 g of ninhydrin and 15 mg of ascorbic acid dissolved in 60 mL of the ethylene glycol. The reagent and supernatant were mixed properly, boiled for 30 min in a water bath, and allowed to cool to 25 ± 2°C. If purple colour developed, it was considered as positive for ACC deaminase activity that was measured at OD_{570}.

2.3.7. Hydrogen cyanide production

The production of HCN was evaluated by growing bacterial isolates in 0.44 g of glycine (precursor of HCN) supplemented in 100 mL of nutrient agar medium as described earlier (quote a recent paper from our lab that quoted this method). Briefly, the agar plates were

streak-inoculated with test bacterial isolates. A Whatman No.1 filter paper dipped in 2 % Na_2CO_3 and 0.5 % picric acid sterile solution was air dried in laminar flow. Then the reagent paper was overlaid on the lid of streak-plate and kept at 37 °C for 72 h. The conversion yellow filter paper in to orange or red or brown indicated the HCN production; range from lower to higher, accordingly.

2.3.8. Nitrogen fixation ability

Nitrogen fixation ability of the bacterial isolates was tested by inoculating in nitrogen deficient Jensen's broth (HiMedia Laboratories, India). The inoculated samples were grown at 37 °C for 24 h and a 200 µL of culture aliquot was collected after 24 h of growth in broth. The sample was spread onto Jensen's plates and grown for 3-4 days. The growth of bacterial colonies on nitrogen-deficient medium indicated the possible nitrogen-fixing ability of the test isolate (Chandrababu and Raj, 2009).

2.3.9. Biofilm formation

Biofilm formation was assessed by microtiter dish biofilm formation assay (O'Toole, 2011). Overnight grown bacterial cultures were 100-times diluted and 100 µL of it was added in a 96-well plate in triplicates. The plate was kept undisturbed for 24 h at 37 °C. Culture was dumped out by turning the plate upside down and shaking out the bacterial culture. The plate was washed twice by submerging it in a tub of water. Plate was dried at 30 °C for 30 min to remove all the water content. To each well 125 µL of 0.1 % aqueous crystal violet solution was added and incubated for 15 min at 30 °C. The plate was rinsed thrice with water to eliminate excess die attached, as described above, and dried overnight. Retention of crystal violet on the walls of the microtiter plate was considered positive for biofilm formation.

2.3.10. Antagonistic activity against phytopathogens

The bacterial isolates ability to inhibit five pathogenic fungi *Sclerotium rolfsii*, *Fusarium oxysporum*, *Curvularia lunata*, *Rhizoctonia solani*, and *Alternaria alternata* was assessed by dual culture inoculation method. Briefly, 1 cm fungal plug was positioned at centre of the potato dextrose agar (HiMedia Laboratories, India) followed by test bacteria was inoculated as a spot, at an equidistance of 3 cm from the fungal plug (Vaikuntapu et al., 2014). The fungal mycelium growth inhibition was observed by incubating plates at 28 °C for 72 h. The antibacterial ability of test isolates was screened by pour plate method, against phytopathogens

Xanthomonas citri, *X. oryzae*, and *Bacillus licheniformis*. Potato semi-synthetic agar plates were mixed with 1 mL (OD_{600} of 0.6) of respective pathogenic bacterial cultures and pour plated. The test cultures were spot inoculated on the dried plate and kept for 72 h at 28 °C. The zone of growth inhibition of bacteria around test bacterial isolate indicated antibacterial activity.

2.4. Identification of bacteria

Genomic DNA was isolated from overnight grown bacterial isolates in LB broth at 37 °C. Briefly, cultures were pelleted at 5000 × g for 5 min by centrifugation. The pellet was dissolved in 200 µL of TE buffer containing 10 mM of Tris-HCl and 1 mM EDTA (pH 8). To this 50 ng of RNase and 400 µL of 1 % SDS, 0.5 M NaCl, and 1 % Sarkosyl solution were added. Samples were incubated for 10 min after proper mixing at 37 °C. Equal volume of PCI (Phenol: Chloroform: Isoamyl alcohol; 25:24:1) was added to above sample and mixed. Aqueous phase was obtained by centrifuging at 10,000 × g, 37 °C for 5 min. To this, 0.1 volume of 3 M sodium acetate of pH 5.2 and 0.6 volume of isopropanol were added, mixed gently and centrifuged as previous step. Resulting pellet was washed twice with 1 mL of 70 % ethanol, air-dried, and eluted with 100 µL of sterile nuclease-free water. The purity and concentration of the genomic DNA was tested using Nanodrop spectrophotometer (Thermo Scientific, UK).

The 16S rRNA gene amplification was performed from 100 ng of isolated genomic DNA for bacterial identification. Thermocycler (Eppendorf Mastercycler Gradient, Germany) was used to amplify the 16S rRNA gene sequence using 27F (5'-GTTTGATCCTGGCTCAG-3') forward and 1494R (5'-CTACGGYTACCTTGTTACGAC-3') reverse primers. The PCR mixture was prepared by mixing 0.1 mM of forward and reverse primers individually, 10 mM of each dNTPs (Fermentas, USA), PCR buffer with 1.5 mM $MgCl_2$ (Sigma Aldrich, USA), and 2 U of Taq DNA polymerase (Sigma Aldrich, USA). The PCR was carried out for 34 cycles including a pre-denaturation step for 4 min at 95 °C, denaturation at 94 °C for 1 min, annealing at 54 °C for 1.5 min, polymerization at 72 °C for 2 min, and a post PCR for 10 min at 72 °C. The PCR products were loaded on 1.5 % agarose gel, the amplicon was extracted and sequenced at 1st Base, Malaysia. The resulting sequence of nucleotides was uploaded in BLASTn (nucleotide-nucleotide basic local alignment search tool provided by National Centre for Biotechnology Information (NCBI) database for similar bacterial sequences. The identity of bacteria was assigned on the basis of the degree of similarity. The sequence of identified bacteria was deposited in NCBI, GenBank and accession numbers were obtained.

2.5. Plant growth promotion studies

2.5.1. Plant material and bacterial strains

Mature seeds of groundnut (ICGV-91114 variety) were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India). Three isolated groundnut associated bacteria and six PGPR available in our lab (Table 2.1) were used for preliminary growth promotion studies in MS media. The *Pseudomonas* sp. (RP2) and *B. sonorensis* (RS4) with potential groundnut growth promotion was used in molecular studies of groundnut-PGPR interactions.

Table 2.1: Bacterial isolates used in comparative plant growth promotion studies

S.No	Abbreviation	Isolate
1	R20	Groundnut rhizosphere *Pseudomonas aeruginosa*
2	RP2	Groundnut rhizoplane *Pseudomonas aeruginosa*
3	RP28	Groundnut rhizoplane *Pseudomonas aeruginosa*
4	BBli	PGPR strain from Banaras Hindu University *Bacillus licheniformis*
5	BPse	PGPR strain from Banaras Hindu University *Pseudomonas fluorescence*
6	CBli	Commercial PGPR strain *Bacillus licheniformis*
7	CBls	Commercial PGPR strain *Bacillus subtilis*
8	RS4	Tomato rhizosphere *Bacillus sonorensis*
9	TBrac	Tomato rhizosphere *Brahybacterium conglomeratum*

2.5.2. Seed bacterization and *in vitro* plant growth parameters

Seed surface sterilization was performed by removing seed coat and washing seeds for 3 min in 0.1 % aqueous mercuric chloride ($HgCl_2$) solution (Sharma and Anjaiah, 2000). Then, seeds were thoroughly washed 5-6 times with sterile DDW to remove the sterilant. Bacterial isolates chose for seed bacterization were grown for overnight at 37 °C in 50 mL of LB broth. Cells were harvested by centrifugation at 6,000 x g for 10 min and the pellet was suspended in 1 % sterile carboxymethylcellulose (CMC) to get a density of $1x10^5$ CFU/mL. Sterilized seeds were incubated in prepared test bacterial suspension at 28 ± 2 °C for 2 h. A slight agitation was maintained for the proper bacterial coating on the seed surface. Seeds were dried in the laminar hood and placed in half strength MS-agar containing bottles. MS media was prepared by mixing 2.2 g of MS media powder (Duchefa Biochemie, Netherlands), 15 g sucrose, and 9 g agar in 1 L of DDW. All the components mixed properly, sterilized using the autoclave, and poured in sterile tissue culture bottles for solidification. Seeds were germinated in dark at 25 ± 2 °C and grown until 15 days with 16 h of light and 8 h dark photoperiod. Sterile CMC treated

seeds without bacteria served as control (referred as non-bacterized). Growth parameters like shoot height, root length, the fresh and dry weight of the plants were recorded at 15 days after sowing (DAS). The dry weight was measured by keeping plants in an oven for 24 h at 80 °C.

2.5.3. Root colonization

Seed bacterization of groundnut seeds was done as described above with *Pseudomonas* sp. (RP2) and *B. sonorensis* (RS4). Dried seeds coated with bacteria were suspended in sterile phosphate buffer and vortex vigorously. A 100 µL aliquot of the prepared suspension was used to measure the seed binding capacity of respective bacterial isolate by spread plating on LB agar. The number of bacteria attached to seed surface was counted by incubating the plates at 37 °C for 12 h. Sterile CMC treated seeds served as control. The air-dried seeds were germinated in half strength MS medium at 25 ± 2 °C with 16 h of light and 8 h dark photoperiod. Bacterial colonization on plant root surface was measured at 5, 10, and 15 DAS. Roots were carefully uprooted from MS media and suspended in sterile phosphate buffer. The suspension was vortex and 100 µL aliquot was used to calculate the number of colonies attached to root surface as described for seed binding capacity measurement. Sterile CMC treated non-bacterized plant roots served as control.

2.5.4. Plant growth parameters in greenhouse

Bacterial strains *Pseudomonas* sp. (RP2) and *B. sonorensis* (RS4) were seed bacterized, sowed in sterile soil: sand mixture (2:1) containing pots (2 seeds/pot) in the greenhouse. Pots were maintained at 25 ± 2 °C by watering daily. Growth parameters like shoot and root length, fresh and dry weight were recorded from 12, and 24 DAS.

2.5.4.1. Estimation of chlorophyll content

Leaf chlorophyll (Chl) content was quantified for non-bacterized and bacterized plants at 12 and 24 DAS. Chlorophyll was extracted from leaves, estimated, and total Chl, Chl-a and Chl-b content were calculated according to Sankar et al. (2013) from the absorbance values in mg/mL. Briefly, 400 mg of leaf material was ground in liquid N_2, chlorophyll was extracted with 20 mL of 80 % acetone. Absorbance was read at OD_{645}, OD_{663}, and OD_{480} in a U.V spectrophotometer against 80 % acetone blank.

2.5.4.2. Root parameters

Plants uprooted for chlorophyll content estimation were used for observing root morphological parameters. The soil remnants attached to the roots were washed under running tap water. Roots were spread properly in a loading tray containing water with least overlapping and scanned using Typhon scanner. Images were analyzed using WinRHIZO Pro V 5.0a software at the division of Crop Physiology (ICRISAT, Hyderabad). Root morphological parameters like root length, surface area, average diameter, and volume of bacterized plants were recorded against non-bacterized plants.

2.6. Characterization of groundnut root exudates

2.6.1. Root exudates collection

REs were collected from bacterized (RP2, RS4) and non-bacterized control plants grown in sterile soil, sand (2:1) mixture at 12 and 24 DAS. Ten plants in each treatment were carefully uprooted, washed and immersed for 48 h in flasks containing 100 mL of sterile DDW with similar pH as that of soil: sand mixture (Yuan et al., 2015; Sun et al., 2016; Mwita et al., 2017). REs were collected in three biological triplicates, filter sterilized (0.22 µM, Millipore, USA), normalized to 100 mL/g of root fresh weight and lyophilized (ScanVac, Germany) for further use (Slavov et al., 2004).

2.6.2. GC-MS analysis of root exudates

Lyophilized REs powder was dissolved in 80 % methanol and incubated for 2 h on ice. This will allow the precipitation of high molecular weight compounds like proteins, polysaccharides, and mucilage of the REs. After incubation, samples were centrifuged at 12,000 × g for 10 min; 4 °C, supernatant was collected and concentrated using lyophilizer (Nagahashi and Douds, 2000). The samples were stored at -80 °C till analysed. The 20 mg of dried supernatant was resuspended in 1 mL of 70 % methanol having 50 µL of heptadecanoic acid (0.2 mg/mL of methanol). The C17 fatty acid, heptadecanoic acid served as an internal standard. Briefly, 1 mg of the REs methanol extract was derivatized with 20 µL of MOA (20 mg/mL pyridine) at 30 °C for 90 min. Then, 20 µL of MSTFA was added and incubated at 70 °C for 30 min. The derivatized samples were analysed through GC-MS (Agilent 7890 series) by employing the below parameters: EI-voltage 70 eV; column DB-1HT /IntegraGuard (Restek GmbH, Germany), 29.3 m x 0.25 mm i.e., 0.10 µm film thickness column (Agilent, USA);

source temperature of 230 °C; injection temperature: 240 °C, One µL was injected by splitless injection, carrier gas helium at a constant flow of 1.5 mL/min; Program temperature used: 70 °C (5 min), 70-300 °C, 300 °C (4 min), mass range of m/z 35 to 1000. Data acquisition and evaluation was done with LECO-GCMS.

The raw SMP files were generated and processed using ChromaTOF software 4.44.0.0 chromatography version (LECO Corporation, USA). Signal to noise ratio was set at ≥ 1, further, alignment and ion-wise mass signal extraction were performed. The mass spectra extracted in the above step was opened in NIST MS search v 2.0 software for identifying the compounds using NIST Library. The compound hit with >700 similarity value and least RI deviation (±30) were used to identify the putative metabolites. Unknown metabolites were eliminated and only annotated metabolites were considered. During identification, the metabolites with different trimethylsilyl derivatives were summed together as a single entity. Variations between metabolites of non-bacterized and bacterized plant REs were studied by applying multivariate analysis using MetaboAnalyst 3.0 web tool (Xia and Wishart, 2016). An unsupervised PCA, cluster analysis, and a supervised PLS-DA were performed. To detect significant metabolites in groundnut-PGPR interaction, one-way ANOVA analysis was performed.

2.7. Influence of groundnut REs on physiological functions of PGPR

2.7.1. Bacterial growth

The actively growing RP2 and RS4 isolates were inoculated (to attain an initial OD_{600} of 0.01) in 20 mL of MM containing groundnut REs. MM was amended with filter-sterilized non-bacterized plant REs of 12 and 24 DAS to obtain a final concentration of 1% in the media (from 5% REs collected. i.e. 5 g of fresh roots used to collect REs in 100 mL DDW). Bacterial growth was recorded using spectrophotometer at OD_{600} for every 3 h. The OD_{600} values of bacteria grown in presence of REs were plotted against bacteria grown in alone MM, to check the bacterial utilization of the REs.

2.7.2. Induction of biofilm formation

The ability of groundnut REs to induce biofilm formation of RP2 and RS4 was tested in 10 mL of MM with 1% of REs in sterile test tubes. The respective active bacterial culture was inoculated in media to obtain an initial OD_{600} of 0.1. The inoculated tubes were kept

undisturbed for 3 days at 37 °C. Un-inoculated tubes served as blank, whereas bacteria grown in MM without REs served as control. Biofilm formation was assessed according to Yuan et al. (2015) with slight modifications. Briefly, glass tubes with culture were dipped in a tray containing water twice and dried. Staining was done with 10 mL of 0.1 % aqueous crystal violet solution for 30 min at 25±2 °C. The staining solution was decanted and washed thrice to remove excess stain and dissolved in 10 mL of 4:1 (v:v) ethanol and acetic acid. Absorbance was recorded at OD_{550}.

2.7.3. Chemotaxis of PGPR

A capillary assay was performed with slight modification to Yuan et al. (2015) with the help of multichannel pipette. The 12 and 24 DAS groundnut plant REs were concentrated ten times in a lyophilizer. One hundred µL of the filter-sterilized two times concentrated 12 and 24 days REs were pipetted along with DDW, which served as control. The set up was placed by just touching the tips in 96-well plate containing 200 µL of actively grown (RS4 and RP2 isolates at an OD_{600} of 0.8 in chemotaxis buffer (20 µM EDTA and 100 mM potassium phosphate buffer with pH 7.0). After 30 min of incubation under laminar flow at 25 ± 2 °C, the REs with chemo-attracted bacteria by capillary action were collected from the pipette tips. The number of bacteria moved by chemotaxis was counted by serial dilution and spread plate counts.

2.8. Groundnut root proteome in PGPR interaction

2.8.1. Isolation of root proteins

Groundnut root proteins were isolated from RP2, RS4-bacterized and non-bacterized plant roots at 12 and 24 DAS. Briefly, 1.0 g of the frozen root tissue was ground to fine powder with liquid nitrogen, followed by re-suspended in the extraction buffer (0.5 M Tris–HCl of pH 7.5, 0.7 M sucrose, 0.1 M KCl, 50 mM EDTA, 2 % β mercaptoethanol, and 1 mM PMSF) in a ratio of 1:4 (w/v). The samples were mixed for 10 min at 4 °C and centrifuged at 12,000 × g for 20 min, at 4 °C. An equal volume of phenol saturated with Tris–HCl (pH 7.5) was added to the supernatant, mixed for 30 min at 4 °C and centrifuged at 5,000 × g for 30 min at 4 °C. The upper phenolic phase was collected. The same step was repeated. The proteins in the phenolic phase were precipitated with four volumes of 0.1 M ammonium acetate in methanol at -20 °C overnight and centrifuged at 10,000 × g at 4 °C for 30 min. The precipitate was washed thrice with ice-cold methanol and twice with ice-cold acetone and air dried for few minutes. The pellet was solubilised in rehydration solution (8 M urea, 2 M thiourea, 4 % CHAPS, 30 mM

DTT). Amido black method was used to check the concentration of isolated proteins using bovine serum albumin as standard (Saravanan and Rose, 2004). Root protein of 300 µg was rehydrated on immobilized gradient (IPG) strips for 12 h with rehydration solution containing 1% 4-7 pH IPG buffer and 0.004% bromophenol blue. The final volume of the sample was made up to 320 µL. The IPG strip with 11 cm length, 4-7 linear gradient pH (Amersham, GE Healthcare) were rehydrated at 25 ± 2 °C in an Ettan IPGphor II (GE Healthcare). Whereas isoelectric focusing (IEF) was done using the following program: 300 V for 1 h, 600 V for 1 h, 1000 V for 1 h, 2000 V for 1 h, gradually increasing to 4000 V-12000 Vh for 5 h and then a step voltage up to 4000 V-2000 Vh. The equilibration of rehydrated strips was performed initially with 2% DTT, followed by 2.5% iodoacetamide, each for 15 min. Proteins were separated on 12% SDS-PAGE and 2D gels were analysed with Image Platinum 6.01 software (Rani and Podile, 2014). Proteins were identified by MALDI-TOF and mass spectral data searching in SwissProt and NCBInr databases using MASCOT program employing Biotools software (Bruker Daltonics, Bremen, Germany). For each root sample, gels were run in biological triplicates. Protein identity was considered if at least two peptides were matched and the significant threshold level ($P < 0.05$) of the MASCOT probability was observed.

2.8.2. Validation of proteomics data through qRT-PCR

RNA was isolated by Qiagen RNeasy plant mini kit from the root samples used for the proteomics study. Using 2 µg of the isolated RNA and PrimeScript™ 1st strand cDNA synthesis kit, first strand of cDNA was synthesized. The cDNA prepared was diluted 7 times and 2 µL was used as a template to quantify the specific gene levels by RT-PCR. Gene-specific primers for the selected genes (chitinase and ascorbate peroxidase) were designed using primer 3.0 software (Table 2.2). The reaction conditions include an initial denaturation at 94 °C for 3 min followed by 30 cycles of amplification (denaturation at 94 °C, 30 sec; annealing at 50 °C, 30 sec; extension at 72 °C, 30 sec) with a final extension step at 72 °C for 10 min. Triplicate reactions were performed and the RNA transcripts fold change was calculated by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) using alcohol dehydrogenase 3 as an internal reference gene.

Table 2.2: List of primers used in the RT-PCR

Gene I.D	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
ADH 3	GCTTCAAGAGCAGGTCACAAGT	GAGACATCCTCCTTCGTGCATA
Chi II	AGAGCATTTCCCGCCTTT	ACCTGTTGTCTCGTGTGATG
APX	TGGCATGATGCTGGTAC	ACGAGTCCAGCATTGGCT

2.9. *Bacillus sonorensis* (RS4) cell wall proteome in presence of groundnut REs

2.9.1. Isolation of cell wall proteins (CWP)

Isolation of bacterial CWP was performed as described by Dutta et al. (2013). Briefly, bacterial culture was harvested at 6,000 × g for 10 min; 4 °C by centrifugation and incubated for 5 min on ice. Ten mL of the chilled phosphate buffer was used to wash the pellet twice and resuspended in 1.15 mL of ice-cold mutanolysin mix. Mutanolysin mix was prepared freshly before use and placed on ice (1 mL of tris EDTA-Sucrose (TES) buffer, 50 µL of 5,000 U/mL mutanolysin prepared in 0.1 M K_2HPO_4 of pH 6.2, 100 µL of 100 mg/mL lysozyme prepared in TES). The bacterial pellet, dissolved in mutanolysin mix, was incubated at 37 °C for 2 h with shaking. Subsequently, the mixture was centrifuged at 8,000 × g for 20 min; 4 °C. The supernatant containing solubilized cell wall-associated proteins fraction was collected and dialyzed against DDW with TE buffer. Dialyzed proteins were precipitated using acetone at 1:3 ratio and spun down at 10,000 × g for 20 min at 4 °C. The pellet was washed thrice with ice-cold acetone, air dried, till all the acetone evaporated, by keeping on ice. The resulting protein was re-suspended in potassium phosphate buffer. The 300 µg of CWP protein was resolved on 2D-gel electrophoresis, gels were analysed, and proteins identification was described in root proteome.

2.9.2. Lactate dehydrogenase (LDH) assay

The purity of the isolated CWP fraction was tested by LDH assay, a cytosolic marker enzyme. Whole cell proteins (WCP) were isolated by sonication of bacterial pellet in potassium phosphate buffer. The supernatant containing WCP fraction was obtained by centrifuging the suspension at 10,000 × g for 15 min at 4 °C. This WCP fraction was used as a positive control for LDH activity. LDH assay was performed by adding 50 µg of the respective isolated protein into 0.77 mM sodium pyruvate, 100 mM NADH prepared in 50 mM potassium phosphate buffer. The decrease in absorbance at OD_{340} until 6 min was noted for every minute and used for calculating LDH activity (Bergmeyer and Bernt, 1963).

2.10. Challenge inoculation of groundnut plants with *Sclerotium rolfsii*

S. rolfsii, a stem rot causing fungal pathogen of groundnut, was obtained from ICRISAT, Hyderabad. The fungal strain was grown on sterile sorghum seeds for 2 weeks to maintain the pathogenicity. The sclerotia were collected and germinated on PDA at 28 °C for 4 days. Two

discs each with 0.8 cm actively growing mycelium were inoculated on the stem nodal region of 12 days old RS4-bacterized, RP2-bacterized, and non-bacterized seedlings. Seedlings were covered with transparent covers having pores for 3 days to maintain the humidity and allow proper fungal growth. Later, covers were removed and the number of wilted seedlings was noted every day for 20 days. The experiment was repeated thrice each time with 12 replicates.

2.11. Chitosan-amended liquid biofertilizer of *Bacillus sonorensis* (RS4)

2.11.1. Preparation of liquid formulation

Actively growing RS4 cells were inoculated in 20 mL of nutrient broth and grown at 37 °C. One mL of culture was harvested, every 12 h, till 48 h and checked for sporulation by RS4. Spores were observed using Schaeffer–Fulton stain. The 100 µL of culture was spread on a glass slide, followed by heat fixed, steamed in a water bath for 5 min. The surface of glass slide was covered with filter paper by adding 0.5% malachite green continuously. The dye was rinsed to counter fix with 2.5% of safranine solution for 2 min. The endospores fixed with malachite green and vegetative cells with pink color of safranine were observed under light microscope.

Different combinations of CMC, glycerol, polyvinylpyrrolidone (PVP), chitin, chitosan, tween, and skimmed milk were prepared, sterilized using the autoclave, and cooled (Table 2.3). RS4 was grown in 500 mL of nutrient broth at 37 °C until reaches sporulation stage. Cells were centrifuged at 7,000 × g for 15 min. The resulting pellet was washed twice with sterile saline (0.8 % NaCl) solution to remove media components. Approximately 1×10^8 cells containing pellet was suspended in 5 mL of sterile water for preparing a homogenous suspension of bacterial cells. To avoid bacterial aggregation. The 5 mL of bacterial suspension was mixed in sterile formulation mixtures prepared by manually agitating. This will allow the formation of a uniform homogenous liquid formulation.

2.11.2. Shelf-life of the formulation

The bottles inoculated with formulation mixtures were stored at 25 ± 2 °C and shelf-life was recorded, every month, for 24 months. The viability of RS4 cells in different formulation mixtures was assessed by suspending 1 mL of the mixture in 99 mL of saline and incubated at 37 °C, 120 rpm for 1 h. One mL of the above suspension was serially diluted and incubated at 72 °C for 20 min for spore germination. The resulting sample was pour-plated by amending in

nutrient broth with 2% agar. The number of CFU in 1 mL of formulation mixture was calculated to evaluate the shelf life.

Table 2.3: Details of different components used in liquid formulation

Component (100 mL)	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8
CMC (g)	2	2	2	2	2	2	2	2
PVP (g)	1	4	6	6	6	1	6	6
Glycerol (ml)	5	5	5	5	5	5	5	5
Chitin (g)	-	-	-	-	-	-	0.5	0.5
Chitosan (g)	-	-	-	-	0.1	0.25	-	-
Tween (ml)	-	-	-	-	-	-	-	2
Skimmed milk (g)	-	-	-	0.1	-	-	-	-

M: Mixture

3.1. Introduction

Groundnut is one of the major oilseed crops of India, having a high yield loss due to environmental factors, mainly by the fungal pathogens attack. The productivity of a wide range of agricultural crops, including groundnut, under decreasing land resources and shrinking biological potential of the soil need to be improved. To achieve this requirement, extensive use of chemical fertilizers, pesticides, and insecticides has been in practice, which pollutes the environment and leads to adverse effects on human health. On the other hand, use of genetically modified disease-resistant crop varieties for alleviating the pathogens was also in the run through for impressive food production (Sharma and Anjaiah, 2000). However, the usage of these genetically modified resistant varieties has limitations like low social acceptance and the longtime requirement for their generation. Therefore, search for useful soil microorganisms surrounding the plant roots became a hot spot for sustainable agriculture.

Plant Growth Promoting Rhizobacteria (PGPR) gained an increased attention for their multiple benefits to plants. Currently, several PGPR are available for different crops as fungicide, insecticide, heavy metal solubilizing bacteria, salinity tolerant, and plant growth promoters. In groundnut too, many bacteria isolated from rhizosphere, phylloplane, and chitin contaminated areas showed their potential in improving groundnut growth upon seed treatments (Kishore et al., 2005a; Kishore et al., 2005c; Das et al., 2010). Moreover, application of these PGPR in fields effectively condensed the effects of several phytopathogens in groundnut (Manjula and Podile, 2005). The best of PGPR must be rhizo-competent with other surrounding microbes (Parray et al., 2016), while colonization of root surface is an equally important factor for the success of PGPR (Podile et al., 2014). Root colonization is one of the major limiting factors for the success of PGPR in the field conditions (Podile and Kishore, 2006). Generally in screening a PGPR, either of PGPR attributes i.e. growth promotion or biocontrol of isolated rhizobacteria were considered as a practice. But, finding a PGPR with both the parameters, with multiple direct and indirect growth promotion traits, can be an additional benefit to maximize their effects.

Despite availability of several PGPR, there could always be a possibility of having a potent PGPR depending on the source and region of isolation. Screening rhizobacteria inhabiting in the plant phytosphere (such as rhizosphere, rhizoplane, and phylloplane) for growth promotion and biocontrol, besides comparative testing with other reported PGPR, could be an advantageous method to identify the best PGPR for any crop. Understanding the effect of

PGPR colonization and root topology for improved growth can be an efficient way to pick a more potent PGPR from the rhizobacteria pool. Hence, in this study, we focused on the following objectives:

1) Isolation, screening, and identification of groundnut habitat bacteria for plant growth promoting traits.
2) Characterization of the identified PGPR in root colonization and altering plant morphological parameters contributing growth.

3.2. Results

3.2.1. Isolation of groundnut habitat bacteria

A total of 126 groundnut-associated bacteria were isolated and maintained as pure cultures. Among these, 32 were from rhizosphere, 38 from rhizoplane, 33 from non-rhizosphere, and 23 from phylloplane (Table 3.1). Out of the 14 villages sampled, Venkatapuram showed a number of morphologically diverse groundnut associated bacteria. Whereas, minimum number of isolates could be obtained in Yellandoddi village of Mahabubnagar district.

Table 3.1: Number of morphologically distinct bacteria isolated from groundnut habitat and sampling regions

S.No	Village	District	R	RP	L	NR	No. of morphologically distinct bacteria
1	Kuchinerla	Mahabubnagar	1	3	1	2	7
2	Sulthanapur	Mahabubnagar	2	1	3	2	8
3	Sompur	Mahabubnagar	1	2	2	3	8
4	Yellandoddi	Mahabubnagar	2	1	1	1	5
5	Sidhanapalli	Mahabubnagar	1	1	1	3	6
6	Chintalakunta	Mahabubnagar	2	5	3	1	11
7	Bunyadpur	Mahabubnagar	4	4	2	4	14
8	Sugoor	Mahabubnagar	1	1	3	2	7
9	Venkatapuram	Mahabubnagar	4	5	1	5	15
10	Venkatapur	Mahabubnagar	3	3	1	3	10
11	Sheripalli	Mahabubnagar	2	3	3	1	9
12	Doudarpalli	Mahabubnagar	5	4	1	2	12
13	Kurnool-1	Kurnool	3	1	1	1	6
14	Kurnool-2	Kurnool	1	4	0	3	8
Total isolates			32	38	23	33	126

R, Rhizosphere; RP, Rhizoplane; L, Phylloplane; NR, Non-rhizosphere

3.2.2. Screening plant growth promoting (PGP) traits

About 10, 92, 55, 2, and 26% of the total 126 isolates were positive for phosphate solubilization, production of IAA, siderophores, chitinases, and HCN, respectively (Fig 3.1). Among the 126 bacterial isolates, 14 isolates, positive for multiple PGP traits, were further tested for their ability to fix nitrogen, zinc solubilization, phytases production, ACC deaminase activity, biofilm formation, and antagonism against soil-borne phytopathogens. Seven of the 14 isolates were able to fix nitrogen, while 6 were able to solubilize zinc and produce phytases. Most of the 14 isolates were positive for ACC deaminase activity and biofilm formation except L22, NR8 isolates. However, the extent of activity varied with the trait and the isolate (Table 3.2). Of the 14 isolates, six isolates were not antagonistic to test phytopathogenic fungi or bacteria. In the remaining 8 isolates R20, RP2, and RP28 inhibited most of the test phytopathogens *viz*., *Sclerotium rolfsii, Fusarium oxysporum, Curvularia lunata, Rhizoctonia solani, and Alternaria alternata, Xanthomonas citri, Xanthomonas oryzae, and Bacillus licheniformis*.

3.2.3. Identification of bacteria

Among the 14 isolates, three isolates *viz*., R20, RP2, and RP28 with multiple plant growth promoting traits were selected for 16S rRNA gene sequencing to identify the bacteria. The 16S rRNA gene amplified PCR products of three isolates were run on 1.5% agarose gel (Fig 3.2). Gel extraction of the amplified product was performed using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Germany) and sequenced. The 16S rDNA gene sequence of all the three bacteria showed more than 99% similarity with taxonomical class γ-proteobacteria, genus *Pseudomonas*. The 16S rDNA gene sequences were deposited in NCBI Gene bank database (accession numbers KJ631606, KJ631607, KJ631608, respectively). These isolates were tentatively identified as *Pseudomonas aeruginosa*.

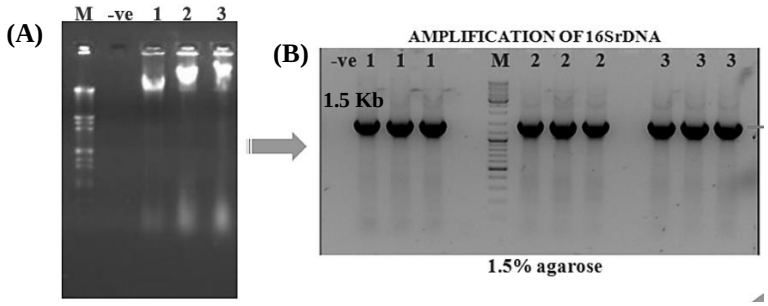

Fig 3.2: Identification of groundnut associated bacteria. (A) Genomic DNA of R20, RP2, and RP28 isolates on 0.8% agarose gel (B) Amplified 16S rRNA gene product on 1% agarose gel.

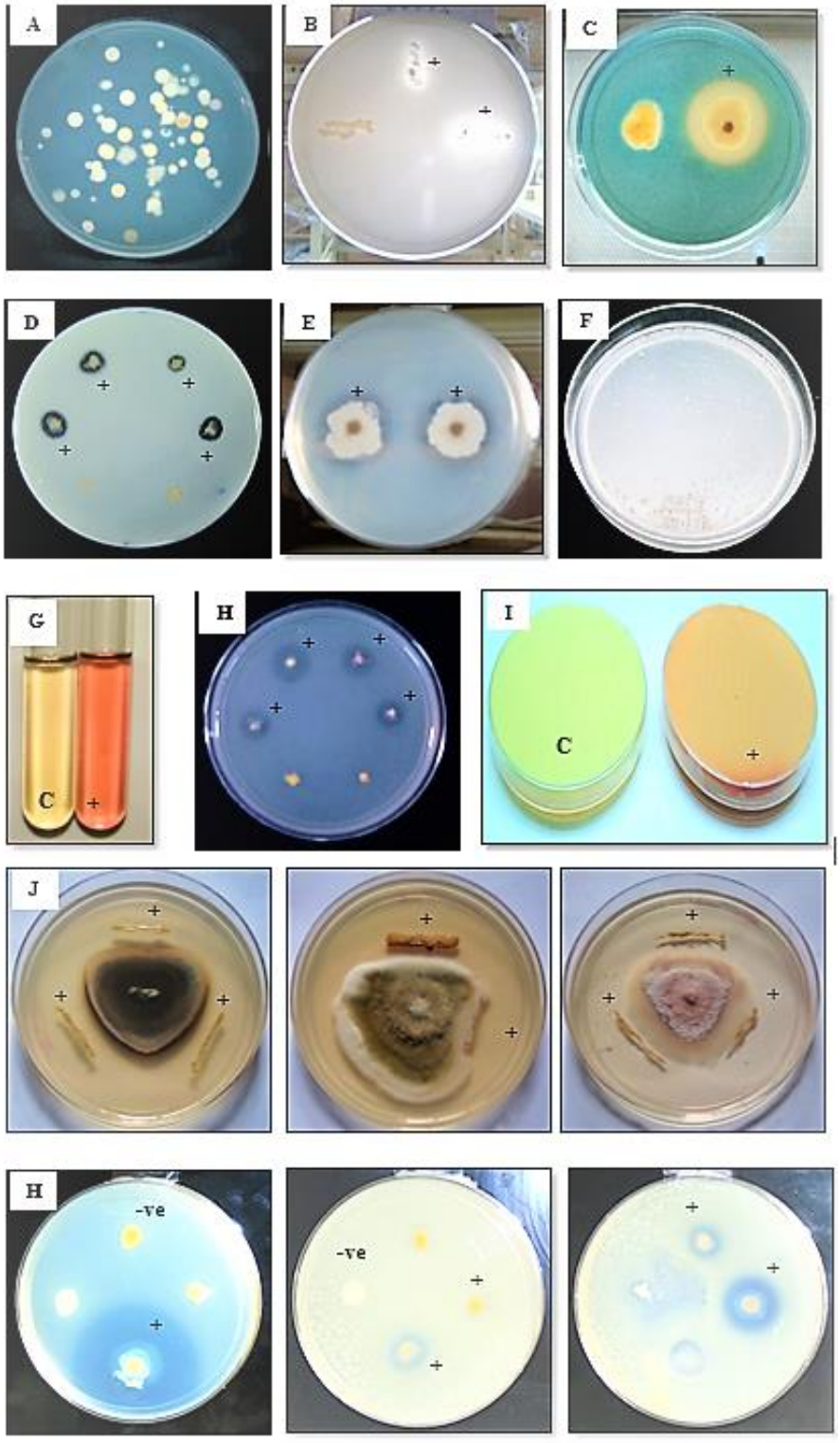

Fig 3.1: Representative pictures of plant growth promoting traits screened with groundnut associated bacteria (A) Isolation plate with diverse morphological bacteria (B) Phosphate solubilization on NBRIP agar plates (C) Siderophores production on CAS agar plates (D) Zinc solubilization (E) Colloidal chitin degradation on chitin agar (F) Bacteria growth on nitrogen deficient media (G) IAA production detection by Salkowski reagent (H) Phytase production (I) HCN production (J) Antifungal activity against *C. lunata*, *A. alternata*, and *F. oxysporum* from left to right of the row (K) Antibacterial activity against *B. licheniformis*, *X. citri*, and *X. oryzae* from left to right of the row.

Table 3.2: Screening of groundnut-associated bacteria for their plant growth promoting characteristics

Isolate code ǂ	Solubilization		Production of								Antagonism against							
											Fungi					Bacteria		
	Phosphate	Zinc	Chitinase	Phytase	Siderophore	IAA	ACC deaminase	HCN	N	Biofilm	*Aa*	*Fo*	*Cl*	*Rs*	*Sr*	*Xc*	*Xo*	*Bl*
R13	-	-	-	-	++	+	++	-	+	+	-	-	-	-	-	-	-	-
R16	-	-	-	-	++	+	+	+	+	+++	-	-	-	+	-	-	-	-
R20	-	+++	-	+++	+++	+	+++	+++	-	++	+	+	+	+	+	+	+	-
RP2	++	+++	-	+++	+++	++	+++	+++	-	+++	+	+	+	+	+	+	+	-
RP15	+	++	-	+++	-	+	+++	+	+	+	-	-	-	-	-	+	+	-
RP28	-	+++	-	+++	+++	+	+++	+++	-	++	+	+	+	+	+	+	+	+
RP34	-	-	-	-	++	++	+++	+	+	+	-	-	-	-	-	-	-	-
RP37	-	++	-	++	++	++	+++	+	-	+++	-	-	-	-	-	+	-	-
L10	+	+	-	++	++	+++	+++	-	-	+	-	-	-	+	-	+	-	-
L22	-	-	-	-	+	++	-	++	+	+	-	-	-	-	-	-	-	-
L23	-	-	-	-	+++	+++	+++	+	+	+	-	-	-	-	-	-	-	-
NR8	-	-	-	-	++	++	-	++	-	-	-	-	-	-	-	-	-	-
NR14	-	-	+++	-	++	+++	++	-	-	+++	+	-	+	-	-	+	-	+
NR18	-	-	-	-	++	++	+++	-	+	++	-	-	-	-	-	-	-	-

'+', positive; '-', negative result for the test. For phosphate (P), zinc (Zn) solubilization, chitinases, phytase and siderophores production: '+' represents zone of clearance < 0.2 mm; '++', zone of clearance 0.2-0.4 mm; '+++', > 0.4 mm. For IAA production: '+', absorbance < 0.1; '++', absorbance between 0.1-0.3; '+++', absorbance > 0.3. For biofilm and ACC deaminase '+', absorbance < 0.05; '++', absorbance between 0.05-0.1; '+++', absorbance > 0.1. For HCN production: '+', slight orange colour; '++', red colour; '+++', dark brown colour. For nitrogen (N) fixation or growth on nitrogen-deficient medium: '+', CFU/ml > 10^5 after 48 h of growth at 37 °C. ǂ R, Rhizosphere; RP, Rhizoplane; L, Phylloplane; NR, non-rhizosphere; *Aa, A. alternata; Fo, F. oxysporum; Cl , C. lunata; Rs, R. solani; Sr, S. rolfsii; Xc, X. citri; Xo, X. oryzae; Bl, B. licheniformis*

3.2.4. Plant growth promotion studies

3.2.4.1. *In vitro* growth parameters

The three groundnut bacterial isolates R20, RP2, RP28, and 6 available PGPR from our lab were used for seed bacterization. Bacterized seeds were sown in half strength MS media. After 15 DAS, RP2, RS4, BBli, and TBrac bacterized groundnut plants had significant effect on dry weight compared to non-bacterized plants (Fig 3.3). Shoot height increased significantly with RP2, R20, and RS4 bacterization. Whereas, increase in the root length was observed in RP2, RS4, and BPse bacterized plants. Remaining isolates did not enhance the plant growth significantly. Of the nine tested bacteria, RP2 and RS4 enhanced all the examined growth parameters, upon seed bacterization, compared with other isolates and the non-bacterized plants. Therefore, RP2 and RS4 were selected for further studies.

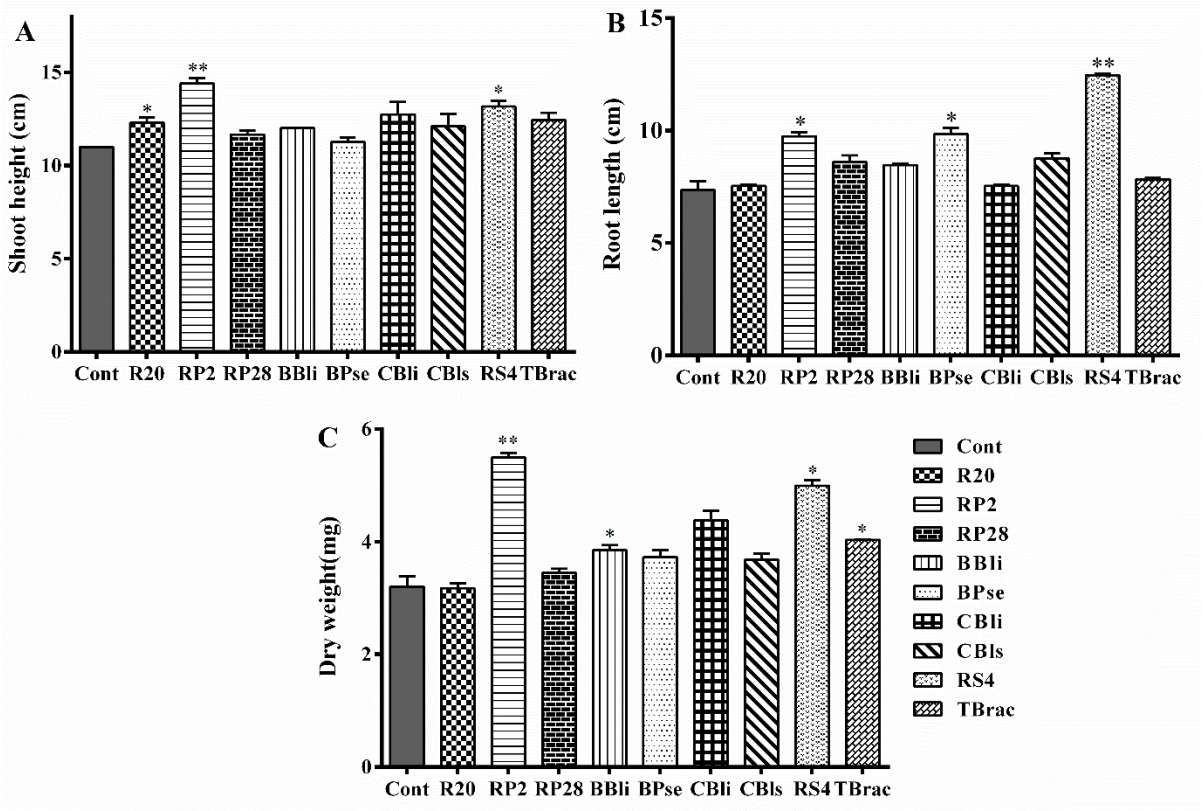

Fig 3.3: Effect of seed bacterization on groundnut growth parameters in MS media. (A) Shoot height (B) Root length (C) Dry weight. Seeds bacterized with test bacterial isolates (approximately1x10^5 CFU/seed) were grown *in vitro* in MS medium. After 15 days of growth, shoot height and root length were measured in centimeters, while dry weight of the entire plant was measured in milligrams. Data represent the mean of the three independent experiments. Error bars indicate standard deviation (n=3). The data was subjected to one-way ANOVA followed by Dunnett's multiple comparisons test. *$P \leq 0.05$, ** $P \leq 0.01$.

3.2.4.2. Growth parameters in greenhouse

The growth of bacterized plants increased significantly at 12 and 24 DAS in comparison to non-bacterized plants. Both the isolates, RP2 and RS4 equally improved the plant growth. The RS4-bacterized plants benefited by 33% and 30% increase in shoot height at 12 and 24 DAS respectively. While, RP2-bacterized plants, increased shoot height by 29% and 22%, respectively (Fig. 3.4A) after 12 and 24 DAS. RP2 bacterization improved root length and fresh weight slightly more than RS4 bacterization on groundnut. RP2-bacterized plants root length and fresh weight increased by 22% and 53% at 24 DAS, in case of RS4-bacterized plants it was up to 18% and 45% in comparison to non-bacterized plants (Fig. 3.4B, C). The dry weight of both the bacterized plants increased by 58% in comparison to non-bacterized at 24 DAS. Whereas, at 12 DAS it was 49% and 54% enhancement in RS4 and RP2 bacterization, respectively (Fig. 3.4D).

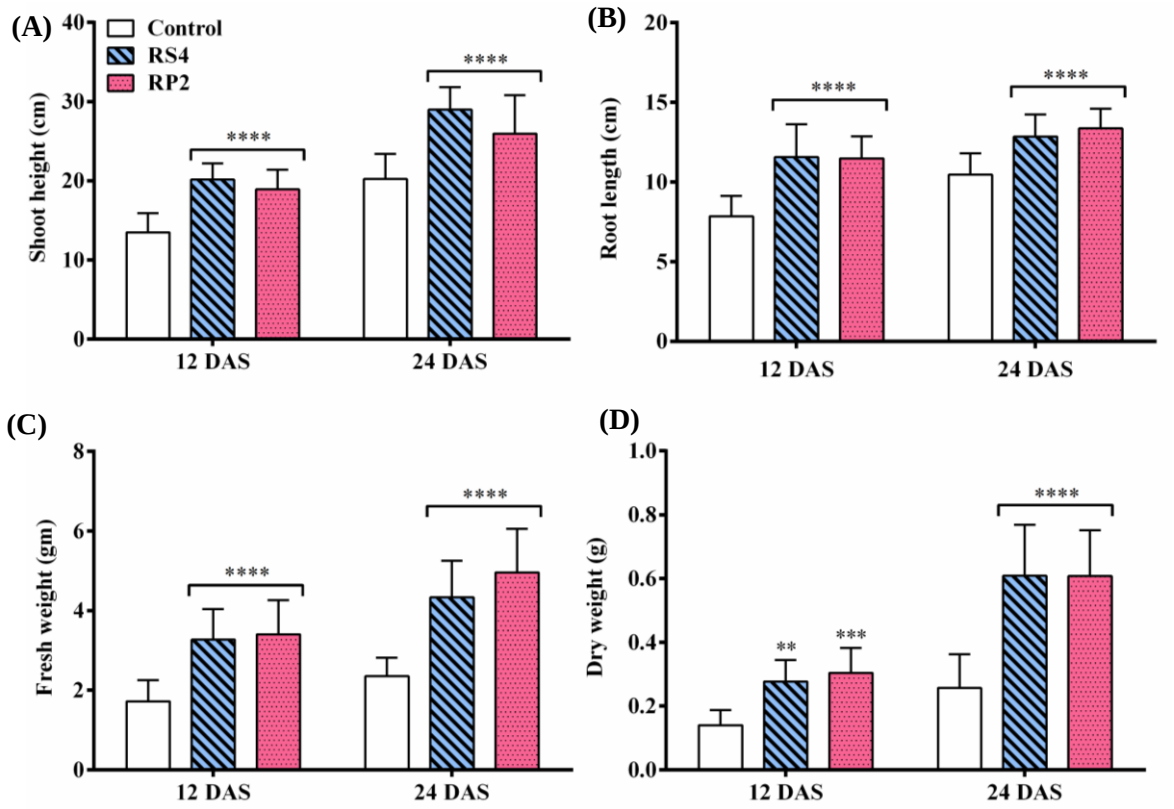

Fig 3.4: Effect of seed bacterization with *Pseudomonas* sp. (RP2) and *B. sonorensis* (RS4) on the growth of groundnut. (A) Shoot height (B) Root length (C) Fresh weight (D) Dry weight. Seeds bacterized with approximately1x10^7 CFU/seed were grown *in vitro* in sterile soil. After 12 and 24 days of growth, shoot height and root length were measured in centimeters, while dry weight of entire plant was measured in milligrams. Data represent the mean of the three independent experiments each time with 5 replicates. The vertical line indicates standard deviation. Statistical analysis was performed using two-way ANOVA ($p<0.05$, $n=15$) followed by Bonferroni's multiple comparison post hoc test. ** $P\leq0.01$, ***$P\leq0.001$, **** $P\leq0.0001$.

The chlorophyll content of groundnut leaves was high in RP2-bacterized plants at both 12 and 24 DAS, but not in RS4 bacterized plants (Fig 3.5). The total Chl and Chl-a content increased by 14% and 17% at both 12 and 24 DAS, while, Chl b has not varied by RP2 bacterization as well.

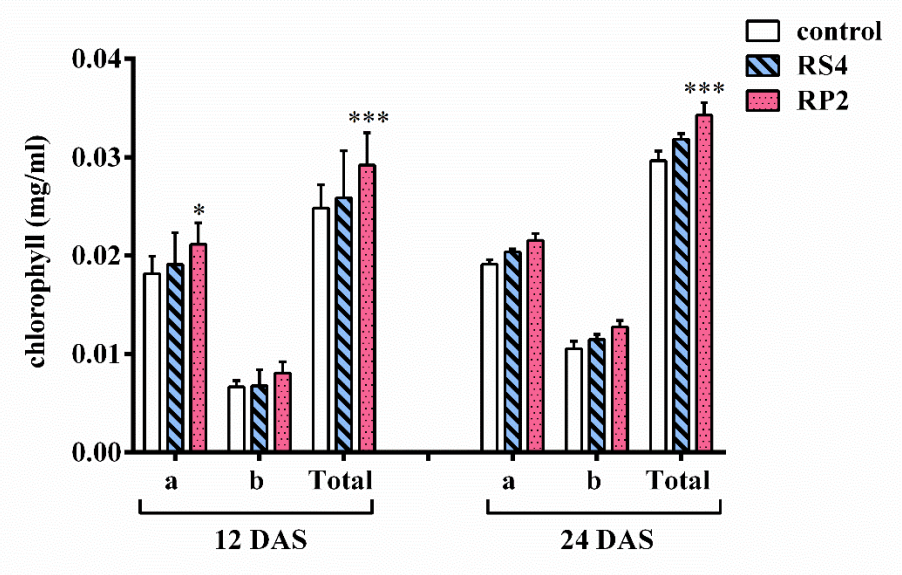

Fig 3.5: Chlorophyll content of PGPR treated groundnut plants after 12 and 24DAS. Data represent the mean of the three independent experiments. Error bars indicate standard deviation (n=6). The data was subjected to two-way ANOVA followed by Dunnett's multiple comparisons test. *$P \leq 0.05$, ** $P \leq 0.01$.

The root morphological characters analyzed using a root analyzer displayed significant morphological variations in bacterized plant roots. In RP2-bacterized plants whole root length increased by 50% in 12 DAS and 65% in 24 DAS, whereas RS4-bacterized plants increase was 55% and 57%, respectively (Fig. 3.6A). Similarly, both the volume and surface area also increased in bacterized roots (Fig. 3.6B, C). In RP2 bacterization, the surface area of roots increased by 49% and 58%, while, volume increased by 49% and 56% at 12 and 24 DAS respectively. Volume increase by RS4 was similar to RP2 bacterization. RS4-bacterized roots surface area increased by 59% and 53% at 12 and 24 DAS plants, respectively. The average root diameter was constant and unchanged in both the treatments (Fig. 3.6D).

3.2.5. Root colonization

Colonization on groundnut root surface by RP2 and RS4 showed a linear correlation with the plant development. Though, the colonization efficiency varied with the isolate. The number of CFU of RP2 and RS4 increased from 5 days (8.8×10^3 CFU of RP2 and 9.75×10^2 CFU of RS4) to 15 days (1.95×10^6 CFU of RP2 and 1.35×10^4 CFU of RP2) of the plant development (Fig.

3.7A). The examination of 15 DAS plant root samples under SEM clearly showed the colonization of bacteria on the root surface (Fig. 3.7B, C, D). Even though, both the RP2 and RS4 isolates colonized and exhibited growth promotion on groundnut, RP2 seed binding and simultaneous colonization on groundnut roots was more than the RS4.

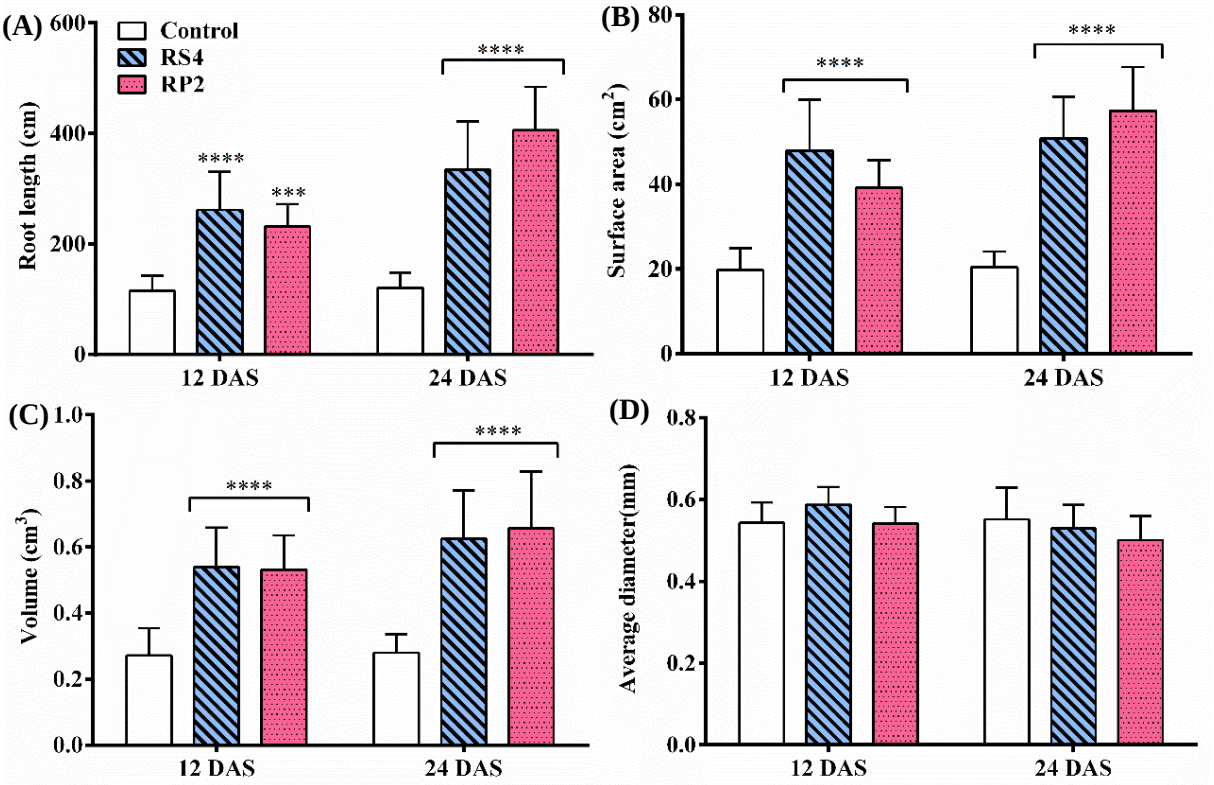

Fig 3.6: Effect of seed bacterization with *Pseudomonas* sp. (RP2) and *Bacillus sonorensis* (RS4) on groundnut roots. RP2 and RS4-bacterized and non-bacterized groundnut roots were harvested at 12 and 24 days after sowing (DAS) from the greenhouse. Roots were analyzed using WinRHIZO root analyzer for topological parameters: (A) Root length (B) Average diameter (C) Surface area (D) Root volume. Error bars indicate standard deviation (n=9) and significance analysis between RP2-bacterized and non-bacterized samples was performed by two-way ANOVA followed by Bonferroni's multiple comparisons test. ***$P \leq 0.001$, **** $P \leq 0.0001$.

3.2.6. Effect of bacterization on groundnut resistance to *Sclerotium rolfsii*

To determine whether RP2 and RS4 bacterization induce defense response against phytopathogens, we tested the defense response of groundnut by *S. rolfsii* infection. The RP2-bacterized seedlings survived better against *S. rolfsii* with 55% decrease in wilting in comparison to non-bacterized seedlings at 20th day after fungal inoculation (Fig. 3.8). In case of RS4-bacterized plants, only 17% decrease in wilting was recorded. Hence, the RP2-bacterized groundnut seedlings had a lower incidence of stem rot disease than the RS4-bacterized and non-bacterized seedlings, showing biocontrol ability of RP2 over RS4 isolate.

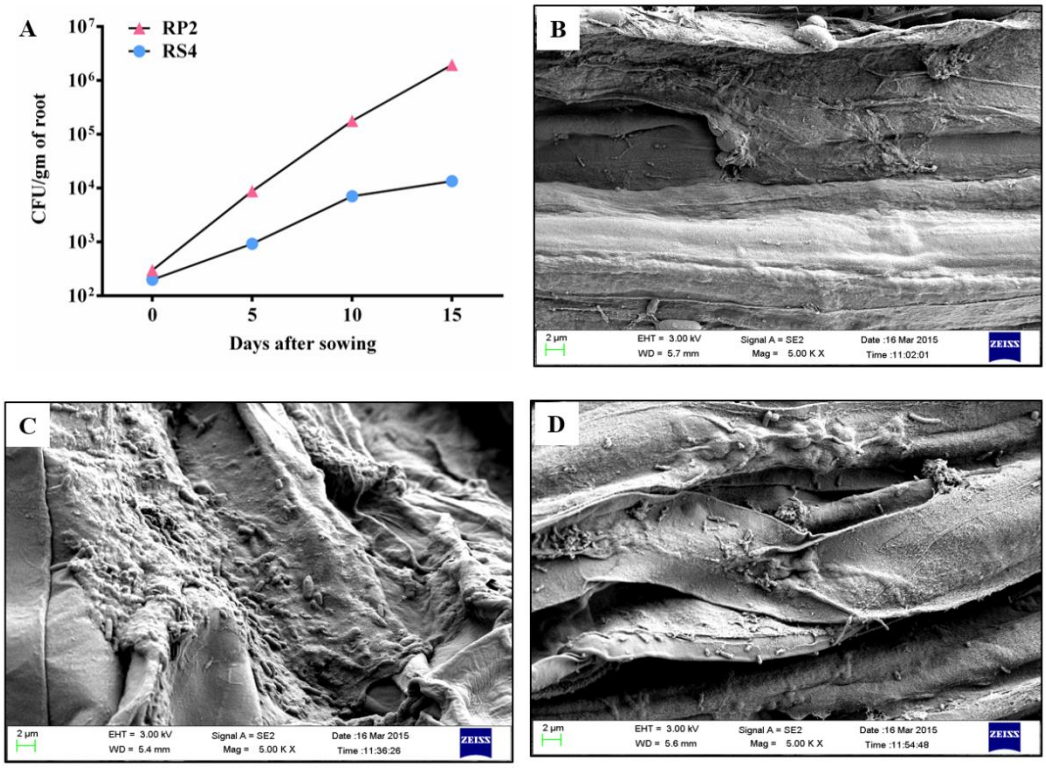

Fig. 3.7: Colonization of *Pseudomonas* sp. (RP2) and *Bacillus sonorensis* (RS4) on groundnut roots. (A) Number of CFU colonized on a gram of root surface. Error bars indicate standard deviation (n=3). (B) Scanning electron microscopic image of the non-bacterized root (C) RS4-bacterized root (D) RP2-bacterized root. Roots were uprooted at 15 DAS and fixed with glutaraldehyde and osmium tetraoxide fixatives. Images were taken at 5000X magnification.

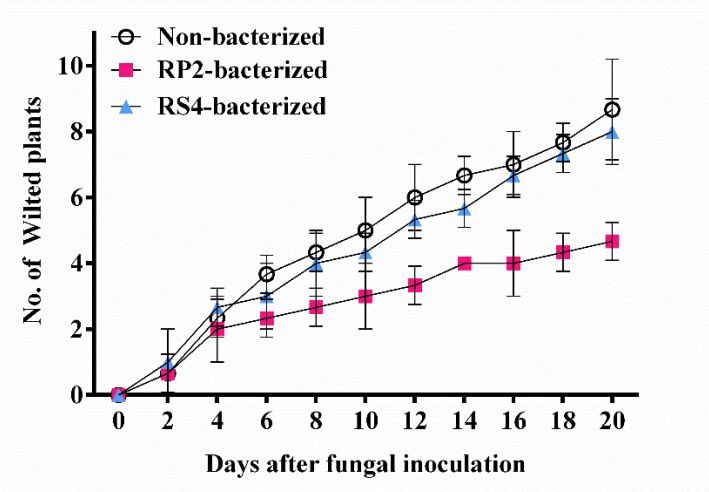

Fig. 3.8: Suppression of stem rot of *Sclerotium rolfsii* by *Pseudomonas* sp. (RP2) bacterization in groundnut. Non-bacterized and RP2-bacterized 15 days' old seedlings of groundnut were inoculated with *S. rolfsii***.** The number of wilted plants was recorded till 20 days. Data was obtained from three independent experiments with each time 12 replicates. Error bars indicate standard deviation (n=3).

3.2.7. Shelf-life of RS4 in formulations

3.2.7.1. Initial screening RS4 viability in different mixtures

Eight combinations of CMC, PVP, glycerol, chitin, chitosan, tween, and skimmed milk were prepared and inoculated with RS4. The number CFU per mL of formulation mixtures (M) were calculated every month till 6 months to initially screen the mixture allowing stability of RS4 cells. After the mixing of RS4 with prepared mixtures, the number of CFU were calculated and considered as 0 time point. Data were analyzed statistically to know the significant increase or decrease in the number of RS4 cells in the mixtures over 6 months of storage at 25±2 °C. The M-1, M-4, M-7, and M-8 showed significant change in the number of RS4 cells in the formulation mixtures (Fig. 3.9). The M-1 having CMC, low amount of PVP, and glycerol as components, promoted the increase of RS4 cells, while M-2 and M-3 with higher proportion of PVP than in M-1 showed no significant change in RS4 cells count.

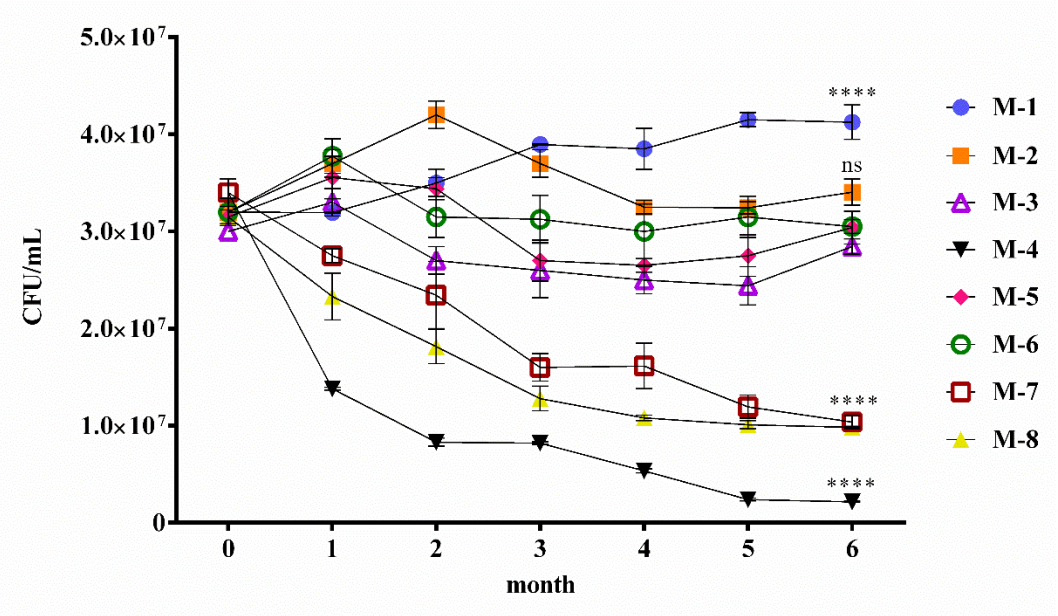

Fig. 3.9: Shelf-life of RS4 in different formulation mixtures. The RS4 was mixed with different components at sporulation stage and stored. Cells viable in the mixtures were counted up to six months, at one month interval, by pour-plate method. Bars indicate the standard deviation. Statistical analysis was performed using one-way ANOVA ($p<0.05$, n=3) followed by Tukey's multiple comparison post hoc test. 'ns' if non-significant and **** $P \leq 0.0001$.

M-4 with skimmed milk an additional component over M-3 drastically reduced the number of RS4 cells. The M-7 and M-8 with the basic proportion of M-3, also decreased the RS4 cells count considerably by 6 months of storage, where chitin is commonly added to both the mixtures and tween to M-8 alone. The M-5 and M-6 with chitosan displayed no significant variation in RS4 cells count (Fig.3.9). Chitosan amendment was made as an addition to M-3

components in two different concentrations. Thus, chitosan has efficiently stabilized the RS4 and the shelf-life of chitosan-amended RS4 formulation mixtures were constant. However, M-2 and M-3 with basic components also exhibited stable shelf-life, deviations were more in them than the M-5 and M-6. The M-5 and M-6 were selected for further long term shelf-life evaluation.

3.2.7.2. RS4 shelf-life screening in chitosan amended mixtures

The shelf-life of RS4 was evaluated for 24 months in M-5 and M-6 with two months' time interval. Mix-6 with 0.25 % of chitosan showed stable and viable RS4 cell count till 24 months of storage. There is no significant change in the number RS4 cells between initial 0 time point and 24 months after keeping the mixture. Whereas M-5 with 0.1% of chitosan, the number of CFU per mL of the mixture declined significantly after 8 months of storage, which is consistent till 24 months (Fig.3.10). The clear variation between RS4 count was noted after 12 months of formulation prepared between Mix 5 and 6. Therefore, higher concentration of chitosan helped in RS4 viability till long time. Both these mixtures were applied on crop plants for growth promotion studies.

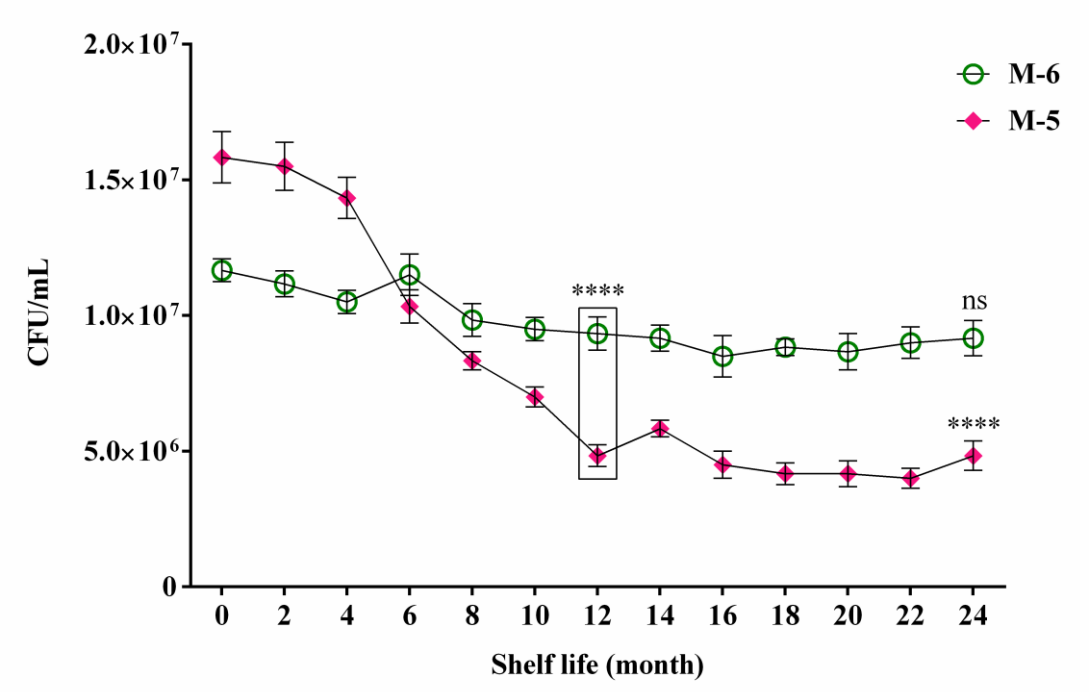

Fig. 3.10: Chitosan- amended RS4 liquid formulation shelf-life. Vertical bars indicate the standard deviation. Statistical analysis was performed using one-way ANOVA ($p<0.05$, n=6) followed by Tukey's multiple comparison post hoc test. 'ns' if non-significant and **** $P \leq 0.0001$. Square box shows the significant variation between Mix-5 and 6 after 12 months.

3.3. Discussion

An array of candidate bacteria were screened and evaluated in soil-less or soil media to unveil their ability to act as PGPR (Dey et al., 2004; Vaikuntapu et al., 2014; Majeed et al., 2015). In this study, a total of 126 morphologically distinct bacteria were isolated from groundnut habitat including rhizosphere, rhizoplane, phylloplane, and bulk soil. The rhizosphere and rhizoplane regions together constitute the highest number of culturable bacteria than the phylloplane or bulk soil. This is a common observation in many bacterial isolations, as these regions are highly influenced by the plant secreted compounds referred to as root exudates (Badri and Vivanco, 2009). This makes them comparatively an attractive sink rich in nutrients and plant released photosynthetic compounds for microbes (Dam and Bouwmeester, 2016).

3.3.1. Plant growth promotion characters of bacterial isolates

All the bacterial isolates were tested for phosphate solubilization, production of IAA, siderophores, chitinases, and HCN. Out of 126 bacterial isolates 14 (namely R13, R16, R20, RP2, RP15, RP28, RP34, RP37, L10, L22, L23, NR8, NR14, and NR18) were positive for most of the growth-promoting traits. The selected isolates were further tested for zinc solubilization, nitrogen fixation, and production of phytases, biofilm, ACC deaminase, and antibiosis against pathogens. All the 14 isolates produced IAA. Variations in the production of IAA levels between the isolates bacteria was observed in this study, relate with the earlier observations in tomato and maize (Vaikuntapu et al., 2014; Zahid et al., 2015). Application of IAA producing PGPR facilitated many crop plants like groundnut, wheat, maize, and ginger growth (Kishore et al., 2005b; Majeed et al., 2015; Zahid et al., 2015). This shows the importance of PGPR produced IAA in promoting the plant growth. Siderophores production by PGPR will not only suppress soil-borne plant pathogens but also provide the iron to plants for growth promotion (Sharma and Johri, 2003). For example, siderophores producing *Alcaligenes faecalis* was reported to increase the groundnut seed germination percentage, shoot, and root length (Sayyed et al., 2010). At the same time, inhibition of broad range of fungal pathogens like *Mucor* sp., *A. niger*, *F. solani*, *F. oxysporum*, *Rhizopus oryzae*, *R. solani*, *S. rolfsii*, and *Sclerospora graminicola* by siderophores producing *P. fluorescence* of groundnut, chilli, cotton, and soybean was reported in iron-deficient King's medium (Yeole and Dube, 2000). In the same way, *P. fluorescence* isolated from groundnut rhizosphere provided resistance to *Macrophomina phaseolina* causing charcoal rot of groundnut by secreting siderophores, HCN,

and IAA (Shweta et al., 2008). All the 14 bacterial isolates, except RP15, produced siderophores in CAS medium to sequester the ferric ions and can act as antagonists.

3.3.2. Nitrogen fixation and phosphate solubilization by selected isolates

Nitrogen and phosphorus are the two vital plant nutrients that are available in the bound form in the soil. Fixation of nitrogen and solubilization of bound phosphates by PGPR would enhance their availability to plants, contributing to growth promotion. About half of the 14 test isolates viz., R13, R16, RP15, RP34, L22, L23, and NR18 were able to grow on nitrogen-deficient Jenson's medium, shows their ability to fix nitrogen by this basic test, though acetylene reduction assay is more appropriate. Phosphate solubilization was displayed by only three isolates i.e. RP2, RP15, and L10. Strains of *Pseudomonas, Klebsiella*, and *Ochrobactrum* were reported to enhance groundnut growth promotion by improved phosphorous and nitrogen uptake of plants along with IAA production and ACC deaminase activities. They even alleviated salt stress caused by NaCl up to 8% (Sharma et al., 2016). Zinc solubilization and phytases production were commonly detected in isolates R20, RP2, RP15, RP28, RP37, and L10. Phytates are the organic forms of the phosphorus in soil along with bound inorganic forms (tri or dicalcium phosphates). Phytases mineralize the organic phosphates of soil for plant phosphorus uptake. PGPR like *Advenella*, *Tetrathiobacter* spp. PB-03, and *Bacillus* spp. PB-13 with phyase producing ability, improved the phosphate solubilization and biomass of Indian mustard and maize seedlings (Idriss et al., 2002; Kumar et al., 2013; Singh et al., 2014).

3.3.3. ACC deaminase activity of selected isolates

Assessment of ACC deaminase activity of isolated rhizobacteria has been one of the common practices to find the best PGPR (Siddikee et al., 2010; Barnawal et al., 2017). ACC deaminase cleaves ACC, the precursor of ethylene and makes it unavailable for ethylene synthesis. Here, all the 14 groundnut isolates showed ACC deaminase activity, except two isolates L22 and NR8. Despite soil salinity, groundnut yield enhancement was noted in presence of *P. fluorescens* strain TDK1 with ACC deaminase activity (Saravanakumar and Samiyappan, 2007). The cumulative assessment of this finding with Sharma et al., (2016), discussed above, supported the importance of ACC deaminase activity in providing tolerance against salt stress in groundnut plants.

3.3.4. Indirect effect of seed bacterization on groundnut growth

Indirect growth promotion of PGPR by controlling pathogens was assessed by their ability to produce HCN, chitinases, and antibiosis. In the present study, HCN production was observed in most of the test isolates except R13, L10, NR14, and NR18. The HCN produced by PGPR plays a crucial role in biological control of soil pathogens by inhibiting enzymes involved in the energy metabolism of pathogens. However, the inhibition was not specific and can even affect the plant growth (Alstrom and Burns, 1989; Rudrappa et al., 2008b).

Certain strains of *Pseudomonas* spp. have been involved in the suppression of soil fungal pathogens by producing chitinases (Dowling and Gara, 1994). The NR18, out of all the test isolates was able to produce chitinases, that could be involved in biocontrol against fungi by degrading chitin in their cell wall (Podile and Prakash, 1996). Antimicrobial metabolites secreted by PGPR hinder and inhibit the invading pathogens growth on plants (Goswami et al., 2016; Parray et al., 2016). Though specific antimicrobial compounds were not detected in this study, inhibition of pathogens growth might be due to secreted compounds of the test isolates. Here, the groundnut isolate RP28 displayed antagonism against all the tested phytopathogens i.e. *A. alternata*, *F. oxysporum*, *C. lunata*, *R. solani*, *S. rolfsii*, *X. citri*, *X. oryzae*, and *B. licheniformis.* The R20 and RP28 isolates inhibited the growth of all the pathogens except *B. licheniformis*. In contrast, RP15 and RP37 could able to inhibit only *B. licheniformis*. Three more isolates, R16, L10, and NR14 displayed antibiosis against two or three test pathogens. The inhibition of test phytopathogens by these isolates might be with their potential to produce siderophores, HCN, chitinase, and/or by secreted antimicrobial compounds.

R20, RP2, and RP28 isolates were positive for most of the growth-promoting traits screened with the exception of nitrogen fixation and chitinase production. These were identified by 16s rDNA sequence analysis as *P. aeruginosa*, one of the most abundant group of rhizobacterial population in numerous plants rhizosphere. This supports the earlier identification of *Pseudomonas* spp. as PGPR in rhizobacteria isolation from other crop plants including the groundnut (Kishore et al., 2005c; Kachhap, 2015; Zahid et al., 2015; Sherathia et al., 2016). The ability of a PGPR to improve plant growth and resistance towards phytopathogens are the main factors considered to use bacterial inocula as PGPR. *Pseudomonas* spp. are mostly capable of producing bioactive metabolites such as siderophores, antimicrobial compounds, and HCN as the primary mechanism of biocontrol. Here, the three groundnut isolates R20,

RP2, and RP28, positive for the above traits showed inhibition of most of the test pathogenic fungi and bacteria (Table 1.2) show their usefulness as biocontrol agents.

3.3.5. Benefits of plant growth with selected isolates

In a comparative growth promotion study on groundnut, out of the nine PGPR tested maximum growth promotion was attained by *Pseudomonas* sp. RP2 of groundnut and *B. sonorensis* RS4 of tomato rhizosphere (Fig 3.3). These two isolates significantly increased groundnut plant biomass *in vitro* and greenhouse. This observation was in agreement with the earlier reports of *Pseudomonas* spp. and *Bacillus* spp. in increasing the growth of groundnut, maize, ginger, peppermint as potent PGPR (Kishore et al., 2005b,c,d; Cappellari et al., 2015; Dinesh et al., 2015; Zahid et al., 2015; Sherathia et al., 2016). Similarly, in groundnut *B. firmis* GRS123, *B. megaterium* GPS55, *P. aeruginosa* GPS21, *S. marcescens* GPS-5, and *B. licheniformis* were reported as best PGPR (Dey et al., 2004; Kishore et al., 2005c; Tripura et al., 2007; Goswami et al., 2014). Inoculation of virulent *S. rolfsii*, a stem rot causing fungus, on 2 weeks old non-bacterized, RP2 and RS4-bacterized groundnut seedlings showed reduced wilting in RP2-bacterized seedlings (Fig 3.8). It can be attributed to the RP2 ability in producing high levels of HCN and siderophores, which can inhibit fungal growth and activate ISR (Gupta et al., 2015). Sherathia et al., (2016) also reported the inhibition of groundnut soil-borne fungal pathogens, i.e. *A. niger*, *A. flavus*, and *S. rolfsii* by three Pseudomonads *P. putida*, *P. fluorescens* and *P. aeruginosa*. Inclusive, RP2 promoted the growth and reduced the severity of infection caused by dry stem rot pathogen *S. rolfsii* in groundnut. The RS4-bacterized seedlings were unable to resist the *S. rolfsii* wilting, in *vitro* antagonism towards *F. solani*, *F. moniliforme*, and *M. phaseolina*, although it can able to produce chitinases (Vaikuntapu et al., 2014). As the expression of bacterial chitinases depends on the chitin availability (Hamid et al., 2013), which was lacking due to inoculation of *S. rolfsii* on shoot nodal regions rather in the soil, where RS4 was colonized. Moreover, the RS4 was deficient in HCN production and showed low levels of siderophores production on CAS plates (< 0.2 mm of the zone of clearance). The low levels of defense compounds production, non-expression of chitinases, might be the reason for no antibiosis of RS4 against fungal pathogens and wilting caused by *S. rolfsii*.

3.3.6. Root colonization and biofilm formation

The ability of PGPR to promote plant growth is dependent on their colonization ability and biofilm formation. The RP2 and RS4-bacterized roots observed under SEM, revealed the

bacterial attachment to the roots in the biofilm, strongly suggesting their ability to colonize groundnut roots (Fig 3.7). Root morphological characters like root length, surface area, and volume are the indicators for potential uptake of water and nutrients by the plants to support growth have significantly increased in RP2 and RS4-bacterized plants. Additionally, plant growth promotion by these isolates was evident from the enhanced shoot height and dry weight. *Pseudomonas* sp. (RP2) and *B. sonorensis* (RS4) with significant plant growth promotion and root colonization could be potent groundnut PGPR. The chitosan based liquid formulation of RS4 displayed a constant number of cell viability for long time of storage up to 24 months. This can be used as an environmental friendly biofertilizer to improve groundnut yield as an alternative to the chemical fertilizers. Furthermore, selecting RP2 and RS4 as PGPR in understanding groundnut- PGPR interactions at the molecular level could be a right choice, as these are the most reported PGPR members of many crops.

4.1. Introduction

Root exudates (REs) are perceived to be highly variable chemical cues of plant communication network to interact with the rhizosphere microbes. They act as the first line of interactive prompts between roots and PGPR and also serve as nutrition source (Hirsch et al., 2003; Bais et al., 2006). The chemical composition of REs differs among plant species, by the plant age, biotic and abiotic factors (Badri and Vivanco, 2009). Such as, rice REs have p-coumaric acid, which was not detected in watermelon REs, contributed to alleviating watermelon *Fusarium* wilt by intercropping with rice (Hao et al., 2010). In another study, analysis of soybean REs revealed changes in flavonoids detected in presence of biotic, *Chryseobacterium balustinum* and abiotic, NaCl. Flavonoids like quercetin and naringenin were not detected in *C. balustinum* colonized soybean REs, whereas, in presence of NaCl, daidzein and naringenin were not detected. These REs further lack of inducing the *nodA* gene of *Sinorhizobium fredii* and its nodulation ability on soybean roots (Dardanelli et al., 2010).

The diversity and quantity of REs compounds vary upon colonization by beneficial or pathogenic microbes. For example, more of tryptophan was detected in cucumber REs after treatment with *Bacillus*, promoting further colonization. On the other hand, decreased raffinose exudation reduced the colonization by pathogenic *Fusarium* in a tripartite association (Liu et al., 2017a). Similarly, in tomato REs, citric acid and succinic acid levels were directly proportional to the plant interaction with biocontrol *P. fluorescence* and pathogenic *Fusarium* respectively (Kamilova et al., 2006). These reports suggested the differences in REs profiles by the associated microbe and established interaction. However, the plant can specifically modulate the metabolite level in the REs to recruit beneficial PGPR over pathogen. *A. thaliana* released malic acid through REs to chemoattract the beneficial *B. subtilis* FB17 than *P. syringae* pv tomato (Rudrappa et al., 2008a). At the same time, plant can even send deleterious signals in inhibiting the growth of self-plant, parasitic plants, and pathogenic microbes through the REs. E.g. cinnamic acid of cucumber REs reduced the leaf transpiration and net assimilate rate of cucumber seedlings (Yu et al., 2003). The rosmarinic acid increased in REs of *P. aeruginosa* infected sweet basil roots to defend against the pathogen (Walker et al., 2004). Thus, plants specifically secrete metabolites through REs to alleviate stress, allow beneficial microbe, and inhibit pathogenic microbe association.

REs drive chemotaxis, colonization, and biofilm formation of PGPR in the rhizosphere. Such as *Bacillus* spp. and *Paenibacillus* spp. showed improved colonization, chemotaxis, and biofilm formation in presence of banana, tomato, and watermelon organic acids present in the REs (Ling et al., 2011; Tan et al., 2013; Yuan et al., 2015). The 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one is a benzoxazinoid detected in the maize REs, chemoattracted PGPR *P. putida* KT2440 and its further root colonization in soil studies (Neal et al., 2012). PGPR chemotaxis and colonization governed by plant REs was evident from these results, however, REs can similarly impact the plant growth promoting fungus root colonization for beneficial effects. Such as the beneficial *Trichoderma harzianum* T-E5 showed improved colonization in presence of cucumber REs compounds oxalic acid and malic acid (Zhang et al., 2014a). Walker et al., (2011) demonstrated the first evidence for PGPR effects on plant secondary metabolite levels. Two cultivars of maize significantly showed variation in benzoxazinoid levels by *Azospirillum* inoculation in a cultivar-dependent manner. This shows the impact of PGPR on plant metabolite alterations. However, the key chemical compounds present in the REs, responsible for plant- PGPR interaction are not clearly known. Against background, we tried to address the following questions to evaluate the groundnut REs changes in presence of PGPR. The potent groundnut PGPR used include, a groundnut self-isolate RP2 and a tomato rhizosphere isolate RS4.

1) Can groundnut REs specifically influence the PGPR physiological characteristics?
2) Are there any profile variations in groundnut REs by PGPR?
3) What type of PGPR interaction-specific metabolites will be present in the REs?

4.2. Results

4.2.1. Groundnut REs impact on PGPR physiological parameters

4.2.1.1. PGPR growth in presence of root exudates

Groundnut REs promoted the growth of two PGPR, RS4 and RP2. In both, the isolates growth was detected to be more in 24d-REs- amended minimal media (MM) followed by 12d-REs and control (MM without REs). The growth rate of RS4 was fast in comparison to RP2 isolate. The stationary phase of RS4 growth curve was observed at 21 h after incubation in control sample, while in presence of REs it reached by 42 h (Fig. 4.1A). In case of the RP2 growth curve, stationary phase was detected after 39 h incubation in control sample and 42 h in REs (Fig. 4.1B). RP2 isolate showed a slow growth than RS4 in presence of REs. The RS4 and RP2

control samples had maximum growth at 42 h and 48 h, with an OD_{600} of 0.4 and 0.25, respectively. By 42 h, RS4 isolate reached approximately an OD_{600} of 0.5 in presence 12d-REs and 0.6 in 24d-REs. A slight decline was observed in the RS4 growth curve, which was not detected in RP2. On the other hand, RP2 isolate reached maximum growth of 0.55 in 24d-REs at 42 h. Thus, REs had a role in PGPR growth by providing the required nutrients. At the same time, difference in the growth pattern of the two PGPR was observed in presence of groundnut REs. Therefore, REs might specifically impact the growth of PGPR with their chemical makeup of metabolites.

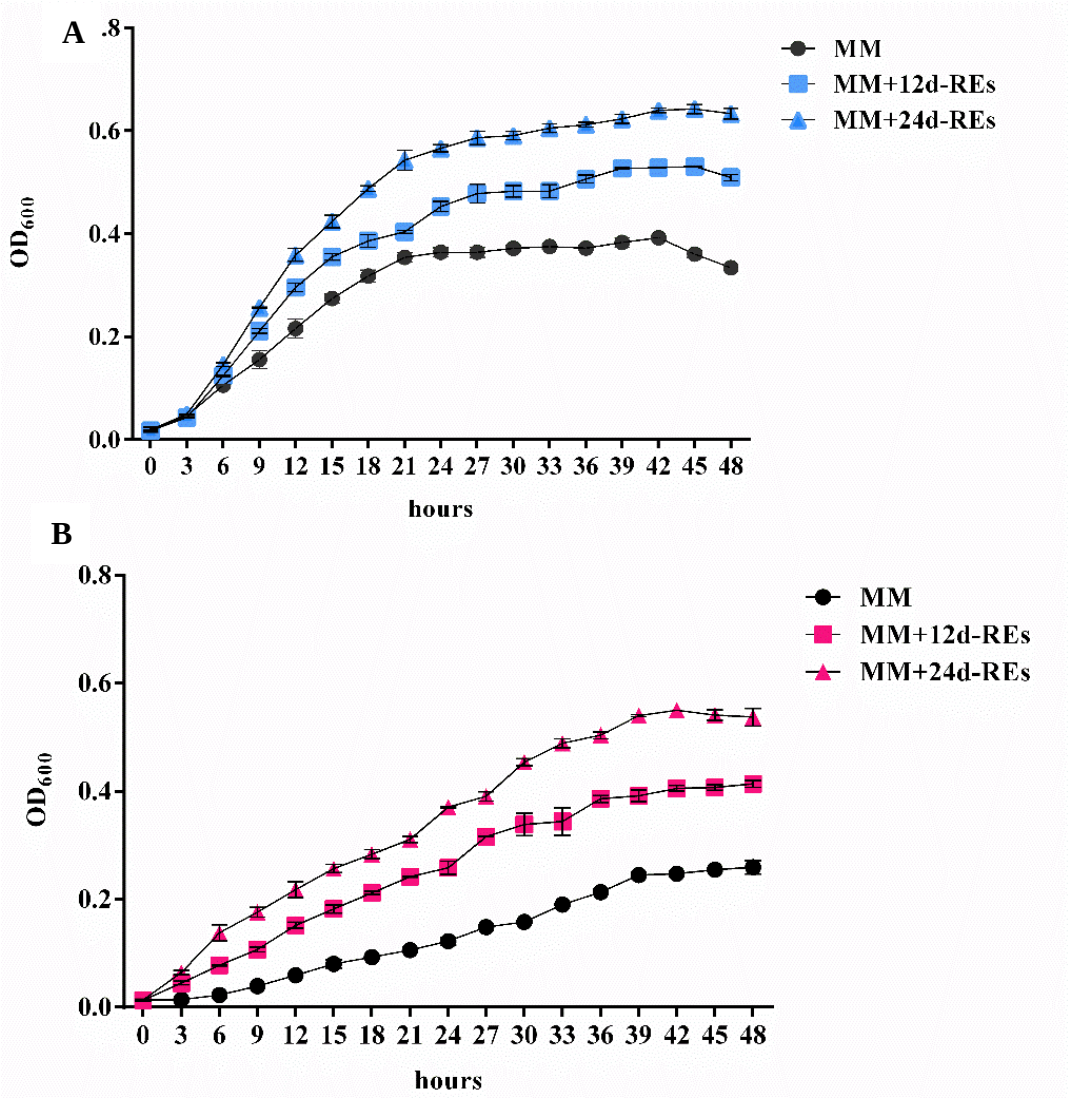

Fig. 4.1: Growth curve of RS4 and RP2 shown groundnut root exudates. (A) RS4 growth in REs (B) RP2 growth in REs. Minimal media amended with 12d and 24d-REs were inoculated with respective active PGPR and incubated at 37 °C. Growth was recorded for every 3 h at OD_{600} and data was plotted. Vertical bars indicate the standard deviation of the means from three replicates.

4.2.1.2. Induction of PGPR biofilm by root exudates

Biofilm formation of PGPR isolates was significantly induced in presence of groundnut REs. In both RS4 and RP2 isolates, biofilm formation was higher in REs- amended samples in comparison with their respective controls. Among these isolates, biofilm production was more in all samples of RS4 over RP2. However, the variation between control and REs samples was less in RS4 when compared to RP2 (Fig. 4.2). There was no significant difference between the RS4 biofilm formed between 12d and 24d-REs amendment, in contrast, RP2 biofilm formed was significant between 12d and 24d-REs. Thus, the biofilm formation of PGPR was modulated by groundnut REs composition depending on the plant age in RP2.

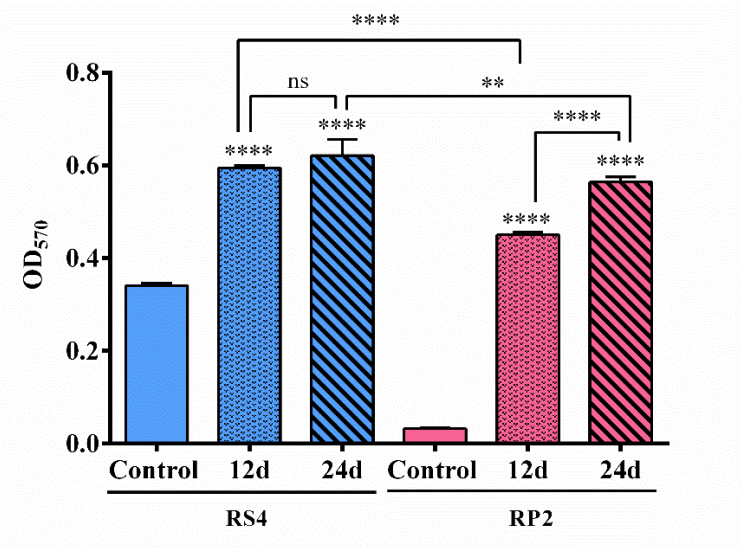

Fig. 4.2: Biofilm formation of RS4 and RP2 in presence of groundnut root exudates. Biofilm was estimated by crystal violet staining and value of OD_{570}. Bars indicate the standard deviation of the means from three replicates. One-way ANOVA followed by Sidak's multiple comparisons test was performed. 'ns' if non-significant, ** $P \leq 0.01$, **** $P \leq 0.0001$.

4.2.1.3. Chemotaxis of PGPR towards root exudates

A capillary chemotaxis assay was performed to quantitatively measure the chemotactic response of PGPR isolates (RS4 and RP2) towards the groundnut REs. To check, whether chemotaxis of PGPR is specific to plant REs, a groundnut non-PGPR (III NR1) reported by Vaikuntapu et al., (2014) and a non-plant associated bacteria (*E. coli*) were also tested for their chemotactic movement towards REs. The number of bacterial CFU/mL in the REs, after capillary movement were counted with respect to water control. A significant increase in the PGPR capillary movement by chemotaxis was noted towards the 12d and 24d-REs. Whereas, capillary movement of III NR1 and *E. coli* was constant irrespective of presence of REs. They

showed a similar number of CFU/mL in presence of water and REs. This might be due to the lack of their specific chemoattracting compounds in the groundnut REs. RP2 isolate showed the highest chemotaxis towards the groundnut REs regardless of plant age in comparison to RS4 isolate (Fig. 4.3). To attract PGPR, no significant difference was detected in RS4 movement with 12d and 24d-REs. However, RP2 chemotaxis was triggered maximum by 24d-REs. These results indicate the variation in REs profiles between 12d and 24d-REs, which specifically attracting the respective PGPR. Overall, the REs played a significant role in PGPR physiological processes and demonstrated the importance of REs in plant-PGPR interaction.

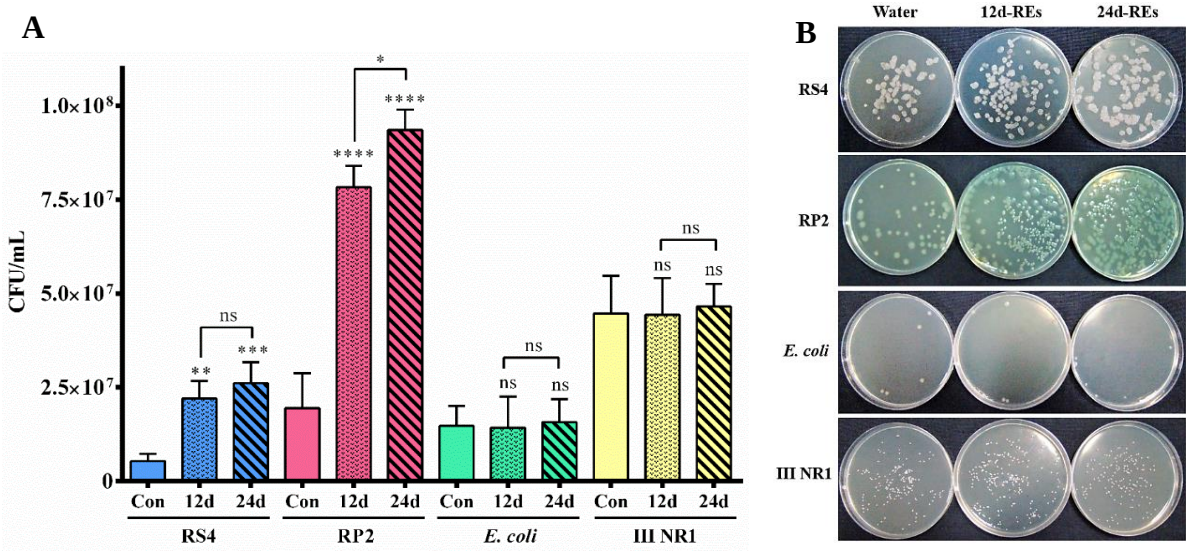

Fig. 4.3: Chemotaxis of PGPR and non-PGPR towards groundnut root exudates. (A) Capillary chemotaxis assay (B) Representative image of bacterial colonies on spread plate. The vertical bars in histogram indicate standard deviation. Statistical analysis was performed using one-way ANOVA ($p<0.05$, $n=6$) followed by Tukey's multiple comparison post hoc test. 'ns' if non-significant, * $P\leq0.05$, ** $P\leq0.01$, *** $P\leq0.001$, **** $P\leq0.0001$

4.2.2. GC-MS/MS profiling of groundnut root exudates

To identify the effect of PGPR bacterization on groundnut REs profiles, GC-MS/MS was performed with non-bacterized and PGPR-bacterized 12 and 24d-REs. A total of 75 organic compounds were detected by GC-MS/MS. The putatively identified metabolites were analyzed for variations in relative content using multivariate analysis methods. The variation in groundnut REs profiles by RS4 and RP2 bacterization, plant development i.e., between 12 and 24 days was clear from both PCA and PLS-DA approaches used. Here, the 12d-REs were sorted separately from the 24d-REs in both non-bacterized and RS4 or RP2-bacterized samples. This was clearly detectable from the first principal component (PC1) of PCA which resolved

plant developmental specific variation (50.7%) in samples. The second principal component (PC2) resolved the bacterization specific metabolites along with developmental specific metabolites with 10.1% of the variation. The PC2 showed the shift in RP2-bacterized plant REs profiles from their respective controls at 12d and 24d-REs. However, the variation became less in 24d-REs than 12d-REs. In contrast, RS4-bacterized plant REs varied more in 24d-REs, while 12d-REs very less deviated from their non-bacterized plant REs (Fig. 4.4A).

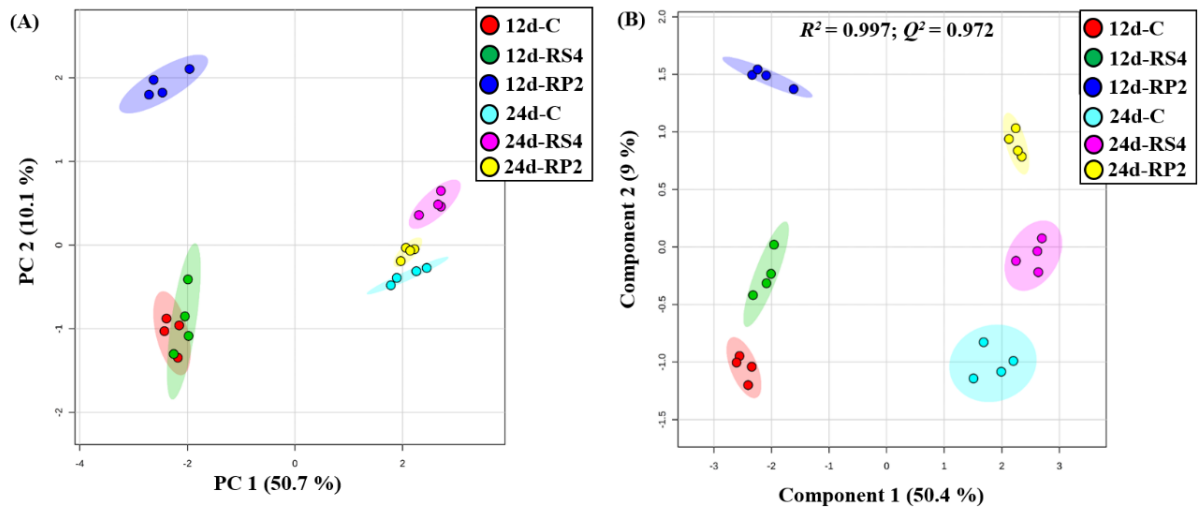

Fig. 4.4: Global differences in metabolite profiles of root exudates from non-bacterized and PGPR bacterized groundnut. (A) Unsupervised principal component analysis (PCA) with variance in percentage within parenthesis (B) Supervised partial least squares discriminant analysis (PLS-DA). R^2 and Q^2 values indicate the correlation and predictability respectively. Each symbol represents biological replicate.

As the PCA is an unsupervised multivariate analysis, a supervised PLS-DA method was also used to check the variations in the REs profiles. PLS-DA method displayed prominent segregation of all the six samples apart with each other. The separation between bacterized and non-bacterized plant REs, variation within RS4 and RP2-bacterized plant REs was clearly evident in PLS-DA (Fig. 4.4B). Variation in component 1 of PLS-DA was noted to be 50.4% whereas component 2 was 9%. These values were almost similar as detected in PC1 and PC2 variations in PCA analysis. PLS-DA cross-validation results showed good correlation (R^2 = 0.997) and predictability (Q^2 = 0.972) of the data. Further, dendrogram generated by using hierarchical cluster analysis with Pearson correlation showed separation between non-bacterized and bacterized REs. The RP2-bacterized 12d and 24d-REs clustered separate and relatively closely with each other than their respective non-bacterized control plants over RS4-bacterized REs (Fig. 4.5). Whereas, RS4-bacterized 12d-REs were initially clustered with the control sample, which was clearly separated in 24d-REs. This was similar to observations from

PCA and PLS-DA analysis, where RP2 or RS4 treatment shifted both 12d and 24d-REs metabolites profiles away from their controls. The complete analysis of samples showed a clear separation of RS4 or RP2-bacterized and non-bacterized samples along with plant developmental age. This indicates the clear change of metabolite profiles in groundnut REs upon bacterization with PGPR depend on the PGPR isolate.

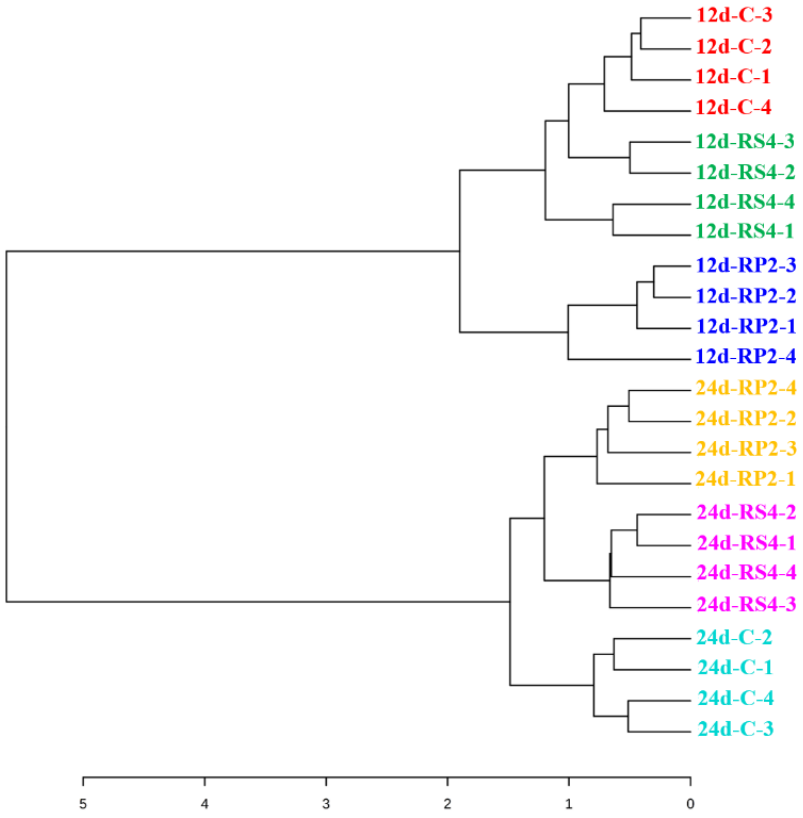

Fig. 4.5: Dendrogram representing the cluster analysis (Pearson's correlation) of non-bacterized and PGPR bacterized groundnut root exudates. Below scale shows the distance between samples by complete linkage method.

The 75 metabolites were categorized into six groups based on chemical structure. In total, 4 sugar, 7 fatty acids, 9 amino acids, 15 hydrocarbons, 28 organic acids (except fatty acids), and 12 compounds grouped as others. Their composition and changes are listed (Table 4.1). The number of metabolites detected was 34 in 12d-C, 33 in 12d-RS4, 37 in 12d-RP2, 47 in 24d-C, 43 in 24d-RS4, and 47 in 24d-RP2 plant REs samples. Only thirteen metabolites (Fig. 4.6) were common in all the six samples (12d-C, 12d-RS4, 12d-RP2, 24d-C, 24d-RS4, and 24d-RP2), with variation in their abundance. Thirty-one metabolites were identified in at least three samples, of these 12 were common to 12d-REs and 18 were common to 24d-REs of both bacterized and non-bacterized plant samples, while one was detected specifically in RS4 and

RP2-bacterized 12d and 24d-REs. There are 17 metabolites which were detected in any of the two samples. No metabolite was identified commonly in both 12d-C and 24d-C sample, however, eight common metabolites were found in non-bacterized and RS4-bacterized REs (3 in 12d-REs and 5 in 24d-REs), while only two were common in non-bacterized and RP2-bacterized REs (3-aminoisobutyric acid in 12d-REs and D-glucopyranoside in 24d-REs). One metabolite-pentanoic acid was in common between 12d-RS4 and 12d-RP2 REs. Five metabolites were present in both 24d-RP2 and 24d-RS4, L-serine was in 12d-RP2 and 24d-RS4 samples. Eight metabolites were detected only in one sample (among them 4 metabolites in 24d-C, 2 in 12d-RS4, and 2 in 12d-RP2 samples). However, the abundance of particular metabolite varied among the different treatments. The exudation of unique metabolites was more in RS4-bacterized REs samples.

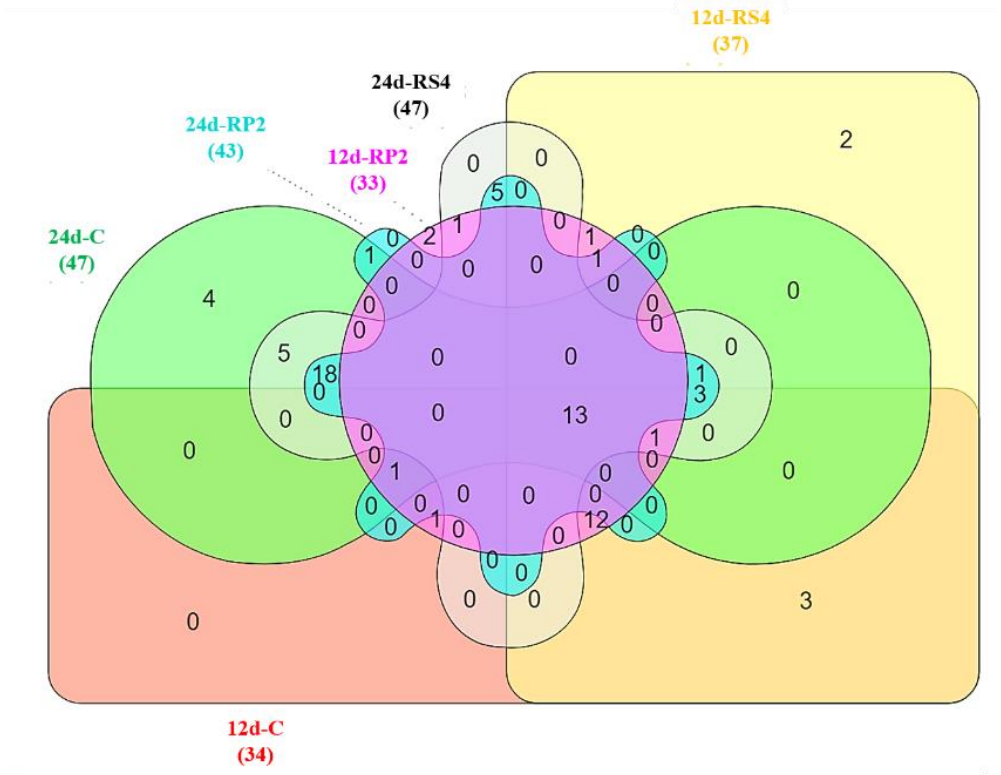

Fig. 4.6: Venn diagram of PGPR bacterized and non-bacterized groundnut root exudates with the number of metabolites detected in different groups.

Table 4.1: Metabolic profiling of root exudates by GC-MS in PGPR bacterized and non-bacterized groundnut. R.T, retention time; Values in are mean of four biological replicates in µM/ mg of REs sample used in GC-MS/MS.

S.No	Sample	R.T (min)	Exact mass	12 DAS			24 DAS		
				Control	RS4	RP2	Control	RS4	RP2
	Amino acids								
1	Glycine (1TMS)	5.63	147.07	0	0	0	38.75	100.13	141.74
2	L-Valine	8.28	189.11	54.74	6.38	191.01	0	0	0
3	3-Aminoisobutyric acid	7.09	103.06	1.99	0	2.98	0	0	0
4	N-Methyl-L-prolinol	9.07	115.09	0	0	0	0	18.90	19.02
5	L-Alanine	9.55	233.12	17.32	7.29	15.04	0	0	0
6	L-Leucine (1TMS)	11.55	203.13	28.12	1.40	40.58	0	0	0
7	N-Formylglycine	12.46	103.02	0.62	0.09	2.96	0	0	0
8	L-Serine	15.59	321.16	0	0	20.20	0	6.31	0
9	L-Threonine (2TMS)	15.87	263.13	0	0	4.15	0	0	0
10	L-Proline	18.82	259.14	0	0	0	3.74	28.44	45.58
	Organic acids								
11	Propanoic acid	7.22	322.14	114.42	3.30	174.29	408.39	576.73	298.25
12	Ethanedioic acid (2TMS)	8.79	234.07	97.67	35.31	50.85	39.55	81.28	49.78
13	2-Furancarboxylic acid (1TMS)	8.87	184.05	6.64	3.63	5.78	0	0	0
14	3-Hydroxybutyric acid (2TMS)	9.16	248.12	111.35	52.22	0	26.38	5.83	44.91
15	Propanedioic acid (2TMS)	10.54	248.09	19.14	11.14	0	5.30	11.44	13.30
16	Butanoic acid	11.24	336.16	16.86	5.03	29.30	0.65	0.06	0
17	(2-Ethoxyethoxy)acetic acid (1TMS)	12.41	220.11	0	0	0	0.41	0	0
18	3-Pyridinecarboxylic acid (1TMS)	13.22	195.07	4.00	13.62	2.32	0.92	0.85	2.18
19	(E)-2-Butenedioic acid (2TMS)	14.96	260.09	1.50	0.49	0	0	0	0
20	Methylmaleic acid (2TMS)	15.26	274.10	0	0.18	0	0	0	0
21	Butenedioic acid (2TMS)	16.35	260.09	0	0	0	0.58	1.37	0.42
22	Hydroxypyruvic acid (2TMS)	17.39	248.09	0	0	0	0.03	11.24	0
23	3,4-Dihydroxybutanoic acid (3TMS)	17.67	336.16	3.75	1.71	0.88	0	0	0

24	Benzoic acid	17.74	194.07	1.91	9.59	20.55	1.781	10.75	12.51
25	Pentanoic acid	21.13	333.19	0	0.37	54.19	0	0	0
26	L-Tartaric acid (4TMS)	21.27	438.17	0	0	0	1.00	3.82	2.52
27	Pentanedioic acid	22.39	276.12	0	0.68	0	6.11	7.78	4.62
28	Butanedioic acid	22.61	350.14	7.23	4.32	3.27	1.89	6.83	2.09
29	3,4-Dimethylbenzoic acid (1TMS)	23.19	222.10	0	0	0	0.47	0	0
30	2-Hexenoic acid, 5-(1-ethoxyethoxy) (1TMS)	26.86	302.19	0	0	0	0.90	0	0
31	1,2,3-Propanetricarboxylic acid (3TMS)	27.14	480.18	12.99	5.03	2.26	5.22	11.23	6.80
32	1,2-Benzenedicarboxylic acid (2TMS)	27.52	310.10	0	0	0	0.29	0.44	0.40
33	Cinnamic acid	28.8	308.12	2.77	1.08	0.35	0	0	0
34	Hydrocinnamic acid (2TMS)	29.26	310.14	0	0	0	0.74	2.18	0
35	1H-Indole-5-carboxylic acid (2TMS)	30.58	305.12	0	1.59	0	0	0	0
	Fatty acids								
36	Dodecanoic acid (1TMS)	21.48	272.21	0	0	0	1.26	2.22	2.76
37	Tetradecanoic acid (1TMS)	26.5	300.24	0	0	0	10.00	8.88	6.25
38	Pentadecanoic acid (1TMS)	28.41	314.26	0	0	0	0.64	1.32	0.67
39	9,12-Octadecadienoic acid (1TMS)	32.32	352.27	0	0	0	1.64	3.29	0
40	Octadecanoic acid (1TMS)	33.21	356.31	0	0	0	302.62	227.41	215.81
41	trans-9-Octadecenoic acid (1TMS)	34.26	354.29	0	0	0	1.41	2.94	0.92
42	Hexadecanoic acid	38.56	474.35	0	0	0	88.08	245.77	91.57
43	2-Monostearin (1TMS)	41.11	502.38	0	0	0	1.05	1.11	0.26
	Hydrocarbons								
44	Butane	6.13	322.18	2.49	0.81	1.75	0.61	1.99	2.39
45	Trisiloxane (2TMS)	10.69	384.14	0	0	0	0.06	0.24	0.26
46	Pentasiloxane, dodecamethyl-	10.96	384.14	0.52	0.45	0	2.17	0.43	0.74
47	2,10-disilaundecane, 2,2,10,10-tetramethyl-	11.43	250.14	0	0	0	1.70	4.97	1.54
48	Hexadecane	21.12	226.26	5.52	4.72	0	0	0	0
49	Tridecane	22.1	198.23	4.73	3.42	1.69	0	0	0
50	Tetradecane	22.39	198.23	0	0	0	0.02	0.43	2.29
51	Undecane	26.41	296.10	4.67	5.56	10.80	0.07	0.45	0.15

52	1,8-cis-Undecadien-5-yne (2TMS)	27.27	324.19	0	0	0	0.17	0.67	0
53	Heptadecane	31.28	268.31	1.14	1.50	1.55	0	0	0
54	Octadecane, 2-methyl-	32.57	268.31	0	0	0	0	1.09	2.92
55	Dodecane, 2,6,11-trimethyl-	32.75	212.25	0	0	0	0.11	5.75	1.12
56	Nonadecane, 2-methyl-	33.93	282.32	2.82	2.90	3.71	0	0	0
57	Eicosane, 2-methyl-	36.2	296.34	0.57	0	0.91	1.22	0	2.79
58	Heptacosane	44.81	380.43	3.14	0.35	2.58	2.08	1.17	0.56
	Sugars								
59	2,3,4-Trihydroxybutyric acid (4TMS)	25.9	424.19	0	0	0	2.08	3.21	1.18
60	Ribonic acid (5TMS)	32.79	526.24	0.82	0.47	3.36	0	0	0
61	D-Talofuranose (5TMS)	35.03	540.26	0	0	0	0	0.30	0.56
62	D-Glucopyranoside (4TMS)	35.19	496.25	0	0	0	0	2.04	2.33
63	D-Galacturonic acid (5TMS)	36.46	554.24	0	0	0	0.20	0	0.14
	Others								
64	N,N-Diethyl carbamate (1TMS)	6.98	189.11	8.55	2.04	19.32	3.58	5.97	10.40
65	2-Pyrrolidinone	8.13	99.06	0	7.75	1.03	0	0	6.59
66	Tris-borate (1TMS)	8.4	278.13	758.69	733.02	374.62	155.14	42.93	38.84
67	2-methylpyrrolidine	10.75	129.11	0	0	0	0	0.10	0.17
68	Glyoxylic oxime acid (2TMS)	11.2	233.09	0	0	12.02	0	0	0
69	Urea (2TMS)	12.13	204.11	0.67	2.75	47.69	1.98	14.75	19.41
70	Glycerol (3TMS)	13	308.16	1.28	7.70	0.59	5.41	6.27	6.45
71	2-(2-Butoxyethoxy)ethoxy (1TMS)	18.54	234.16	0	0	0	3.97	3.519	4.07
72	2-Ethylacetoacetate (2TMS)	20.38	274.14	6.24	4.60	7.19	0	0	0
73	2-(2-butoxyethoxy)acetate (1TMS)	20.74	248.14	0	0	0	1.48	0	0
74	1,2-Benzenediol, O-(4-methoxybenzoyl)-	36.25	244.07	0.17	0.87	0	0	0	0
75	5-Methyluridine (3TMS)	36.48	474.20	0	0	0	0.19	1.70	0

4.2.3. PGPR interaction specific root exudates metabolites

4.2.3.1. Group of metabolites influenced by PGPR in root exudates

REs metabolites detected in GC-MS/MS were divided into six groups. The cumulative concentration of exuded compounds in the respective group was calculated and their log_2 values were plotted. This helped to distinguish the potential group of metabolites influenced by PGPR bacterization in groundnut REs. Analysis of data was done in three steps. First, to detect the group of REs that varied significantly by PGPR bacterization, in which fatty acids, hydrocarbons, and other group have not varied by PGPR bacterization in 12d-REs. Amino acids, organic acids, and sugars cumulative concentration levels were influenced by PGPR bacterization. These groups significantly increased in RP2-bacterized 12d-REs, while they decreased in RS4-bacterized 12d-REs (Fig. 4.7). Conversely, the amino acids, hydrocarbons, and sugars cumulative levels significantly increased by PGPR bacterization in 24d-REs, whereas organic acids and fatty acids were unchanged, other group metabolites decreased considerably. Therefore, amino acids, sugars, hydrocarbons, and organic acids out of the six groups were influenced by PGPR bacterization, except organic acids in RS4-bacterized REs. However, organic acids and hydrocarbons were specific to 12d and 24d-REs, respectively.

As a second step, groups that varied considerably in the RS4 and RP2-bacterized plant REs were explored. In them, amino acids, sugars, and organic acids showed the strain-specific deviations in root exudation by groundnut. However, initial two groups were significant in 12d-REs and last group in both 12d plus 24d-REs. So these groups might be influenced PGPR strain specific, within RS4 and RP2 isolates in groundnut REs. In the third step, particular metabolite groups responsible for plant development were detected. Above mentioned three groups also varied developmentally between 12d and 24d-REs of non-bacterized and RS4-bacterized plants, but not changed in RP2-bacterized REs (Fig. 4.7). Hydrocarbons have not changed in either RP2 or RS4-bacterized 12 and 24d-REs. This indicates amino acids, sugars, and organic acid as specific groups changed by RP2 bacterization in groundnut REs rather than due to development. There was no specific group detected to be influenced by RS4-bacterization. Hydrocarbons could be a common group of metabolites influenced by PGPR bacterization on groundnut, as they were neither developmentally significant nor within RS4 and RP2-bacterized REs, but they were significant by PGPR bacterization. Fatty acids and other group metabolites cumulative concentration was significant, during the course of the development in all the samples.

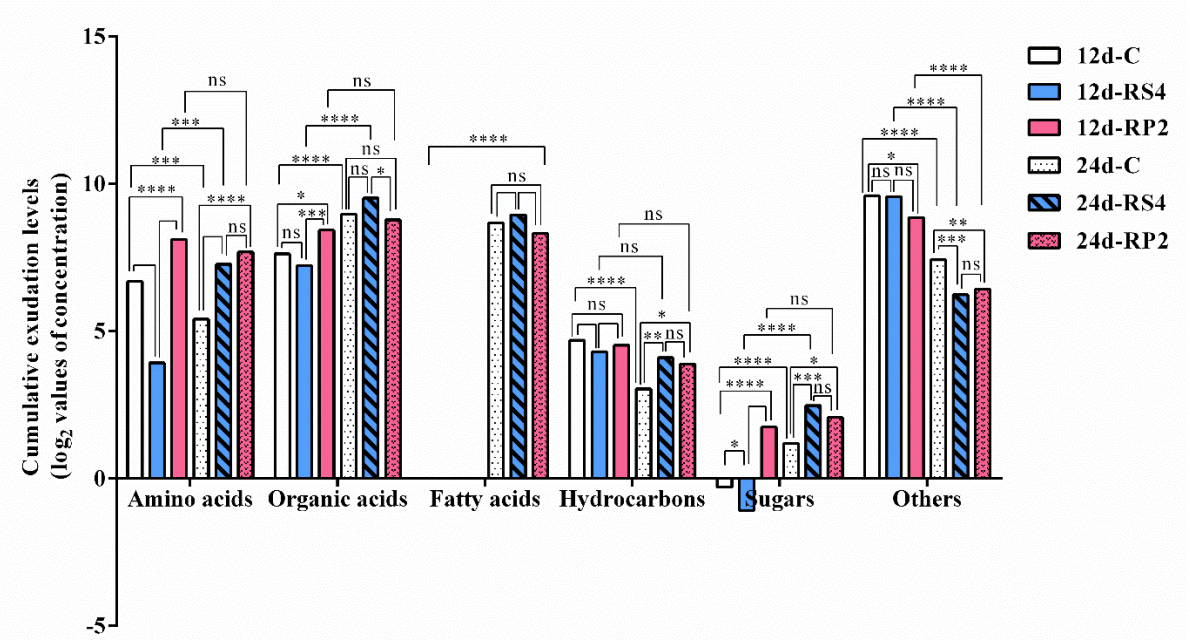

Fig 4.7: Groundnut root exudates composition in presence of PGPR. The putatively identified root exudates compounds were grouped into chemical classes. The log_2 values of cumulative exudation levels after normalization were plotted. Statistical analysis was performed using two-way ANOVA ($p<0.05$, n=6) followed by Tukey's multiple comparison post hoc test. 'ns' if non-significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

4.2.3.2. Root exudates specific metabolites varied by PGPR

There was a significant change in various metabolites level in all the samples on plant development basis between 12d and 24d-REs. But, as the interest of study was to find the PGPR interaction specific metabolites, a one-way ANOVA was carried out individually between 12d and 24d-REs metabolites detected in GC-MS/MS. This will pinpoint the interaction specific metabolites significant between bacterized and non-bacterized plant REs. About 18 metabolites, 8 in 12d-REs, 7 in 24d-REs, and 3 commonly in both, changed significantly due to bacterization with PGPR. Most of these metabolites fall under groups of organic acids, amino acids, and sugars.

The metabolites glyoxylic oxime acid and L-threonine are specifically detected in RP2-bacterized 12d-REs, while D-glucopyranoside, pentanoic acid, L-serine, and 2-pyrrolidinone detected in either 12d or 24d-REs of both RS4 and RP2-bacterized plant REs. Nine metabolites (3 in either of RS4 and RP2, 6 in both) increased in bacterized REs over non-bacterized REs. In them, L-valine was more in 12d-RP2 and less in 12d-RS4, while 5-methyluridine and 9, 12-octadecadienoic acid were more in 24d-RS4 (Fig. 4.8). Four metabolites *viz.*, benzoic acid, L-proline, propanedioic acid, and urea were more in RP2-bacterized REs over RS4. In contrast,

the pentanedioic acid (in 12d and 24d-REs) and L-tartaric acid (in 24d-REs) were detected more in RS4-bacterized REs than RP2. Inclusive, these six metabolites concentrations were more in bacterized plant REs over the non-bacterized. Apart from them, three more metabolites decreased due to RS4 and RP2 bacterization, i.e. 2-hexenoic acid, 3,4-dimethylbenzoic acid, and 2-butenedioic acid. The first two were detected only in 24d-C and last one in 12d-C and 12d-RS4.

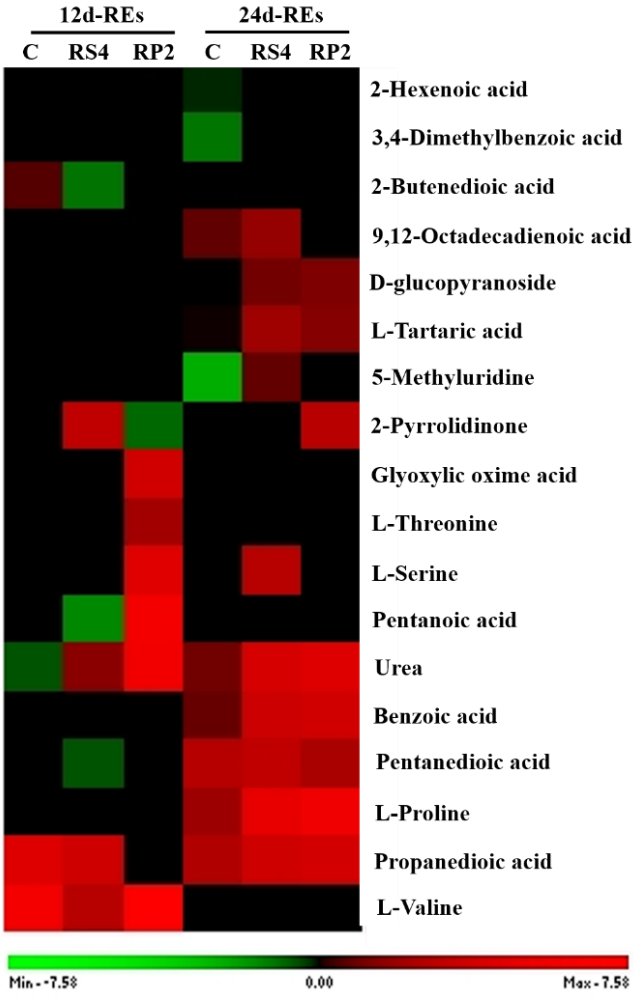

Fig 4.8: Heatmap of interaction specific significant metabolites of groundnut root exudates. The average concentration values of metabolites were transformed into $\log_2$ values and the heat map was generated using PERMUTMATRIX graphical interface software. Each row represents the concentration of the labeled metabolite in color, according to the scale at the bottom of the figure.

The glyoxylic oxime acid and L-threonine were specific to RP2 bacterization, but no specific metabolite by RS4 bacterization was detected (Fig. 4.8). Metabolites like serine, D-glucopyranoside, pentanoic acid, and 2-pyrrolidinone specific to PGPR bacterization. Proline, valine, urea, benzoic acid, propanedioic acid, tartaric acid, and pentanedioic acid increased by

PGPR bacterization, while 5-methyluridine and 9, 12-octadecadienoic acid were specifically increased only in RS4-bacterized REs. Therefore, PGPR bacterization significantly influenced the plant REs with respect to individual metabolites and concentrations. Thus the groundnut REs profiles were varied by the PGPR bacterization, within the PGPR isolates, and plant age.

4.3. Discussion

4.3.1. Root exudates and chemotaxis of PGPR

REs govern distinct functions in the rhizosphere along with providing a nutrient source to microbes. The specific group of metabolites secreted by the plants through REs could play a specific role in the rhizosphere. For instance, amino acids, purines, vitamins, and sugars provide microbial growth, while organic acids and phenols help in chemoattracting microbes along with chelating the metals (Dakora and Phillips, 2002). In this study groundnut REs analyzed by GC-MS/MS possess above all groups, displayed an enhanced growth, biofilm formation, and chemotaxis of PGPR isolates RS4 and RP2. Earlier reports showed the role of metabolites of REs in controlling microbial physiological characteristics. Such as, an increased chemotaxis and biofilm induction of *B. amyloliquefaciens* NJN-6 was observed in presence of organic acids of banana REs (Yuan et al., 2015). The palmitic acid detected in the *Festuca arundinacea* L. REs promoted the *Klebsiella* sp. D5A chemotaxis and growth (Liu et al., 2015). Likewise, in groundnut REs increased levels of palmitic acid (hexadecanoic acid) was detected in PGPR bacterized REs over the non-bacterized plants. However, within RS4 and RP2 isolates the higher levels were reported in 24d-RS4. At the same time, palmitic acid was observed only in 24d-REs control samples, that correlates with the increased chemotaxis of PGPR towards 24d-REs than 12d-REs. Organic acids released by the plants into REs were not only chemotactic signals to promote beneficial bacteria, but they can even provide tolerance to metals. For example, a Cu accumulator castor plant REs have high concentrations of tartaric acid and citric acid, the low concentrations of oxalic acid and cysteine contributed to the Cu tolerance by the plant (Huang et al., 2016). Same way the levels of tartaric acid and oxalic acid were modulated in PGPR-bacterized groundnut REs. This shows the importance of these organic acids even in PGPR interaction along with Cu tolerance.

4.3.2. Sugars and amino acids in root exudates

The chemoattraction of the particular microbe towards REs is an initial step put forward by the plant for their efficient root colonization (Bais et al., 2004). Sugars and amino acids of plant

REs can also specifically chemoattract bacteria. For example, *B. subtilis* was chemoattracted towards numerous tested sugars including glucopyranoside specifically detected in PGPR bacterized groundnut REs (Ordal et al., 1979). The *P. fluorescens* Pf0-1 chemoattraction was highly influenced by the amino acids present in the tomato REs, where the amino acids sensory chemotaxis proteins mutant strain was unable to compete with the wild strain and colonize the tomato roots (Oku et al., 2012). Threonine is one of the major amino acid determinant required for the pilus formation in many bacteria (Podile et al., 2014). The increased levels of threonine specifically RP2-REs indicates their involvement in pilus formation which might contribute to higher chemotaxis of RP2 towards groundnut REs. In another report cucumber PGPR, *Acinetobacter calcoaceticus* ameliorated the plant growth by increasing plant threonine and proline levels (Kang et al., 2012).

The chemoattraction and enhanced root colonization of PGRP by plant REs are mediated in depth by modulating the PGPR genome. For instance, *Arabidopsis* ABC transporter mutant significantly reduced the levels of many organic acids and amino acids secretion in REs. Further the lack or reduced levels of these secondary metabolites in mutant *Arabidopsis* REs unable to induce *B. cereus* AR156 chitinase and siderophores producing genes (Zhou et al., 2016). In these metabolites serine, proline, threonine, oxalic acid, fumaric acid, succinic acid, and hydroxybutyric acid were also detected in the current study of groundnut REs. All these metabolites increased in either of PGPR bacterized 12d and 24d-REs groundnut REs. The gene level modifications in the PGPR by the influence of plant REs was well reported. E.g. The *P. aeruginosa*, *B. amyloliquefaciens* FZB42, and *B. subtilis* OKB105 grown in sugar beet, maize, and rice REs, respectively, altered the genes involved in chemotaxis, type III secretory system, antimicrobial synthesis, transport, sporulation, and metabolism (Mark et al., 2005; Fan et al., 2012; Xie et al., 2015). Thus, it explains the importance of REs metabolites in altering the PGPR gene profiles, which ultimately helps in plant growth and defense.

4.3.3. Phenolic acids in root exudates

The REs facilitated specific rhizosphere microbial selection. But, there is no specific information available on the PGPR- triggered variations in the plant REs through the plant development. All the available information on REs mediated PGPR process were tested with a specific group of REs. Here, we followed an unbiased approach and able to detect five major groups of metabolites include organic acids, amino acids, fatty acids, hydrocarbons, and sugars. Most of these groups were detected in REs of other plants, but the detection of hydrocarbons

in plant REs was not much explored. The variation in sugars, amino acids, and phenolic acids of groundnut REs was earlier reported between susceptible and mid-resistant cultivars. They found the higher amount of sugars and total amino acids in susceptible cultivar, while benzoic acid, coumaric acid, and total phenolic acids in resistant cultivar. These variations were contributed to tolerance against soil-borne *Fusarium* spp. (Li et al., 2013b). Similarly, we found increased levels of few antimicrobial compounds like benzoic acid and hydroxycinnamic acid in PGPR bacterized REs, which might have a role in plant defense. By the help of multivariate analysis, a clear variation in the plant REs profiles in the course of plant development as well as by PGPR bacterization was detected. However, the level of variance differed by the PGPR isolate applied. Similarly, metabolites present in the REs of *Arabidopsis* were significantly varied as the plant development ensues. The amount of sugars decreased as the plants grow, while the amino acids and phenols were increased in REs, correlated with their synthesizing and transportation-related gene expression profiles (Chaparro et al., 2013). In contrast, the groundnut REs showed increased sugars cumulative levels between 12d and 24d-REs, but amino acids were increased inconsistent with this report. The variation in REs within RP2 (groundnut PGPR) and RS4 (tomato rhizosphere PGPR) was also evident from multivariate analysis (PCA, PLS-DA, and cluster analysis) performed. This was correlated with the observed results of higher chemotaxis of RP2 towards groundnut REs over RS4, even significance between 12d and 24d-REs.

4.3.4. Key metabolites of root exudates in groundnut- PGPR interaction

The group of groundnut REs significantly varied by PGPR bacterization includes amino acids, organic acids, and sugars. This correlates with the significant metabolites detected by the ANOVA belong to these groups (D-glucopyranoside, pentanoic acid, 2-pyrrolidinone, proline, valine, benzoic acid, propanedioic acid, tartaric acid, pentanedioic acid, and 5-methyluridine). However, few metabolites grouped as others were also evidently modulated in REs by PGPR. Moreover, the groundnut REs provided increased chemotaxis of PGPR isolates over non-PGPR isolates tested. The significant metabolites detected in these groups might be responsible for the observed enhanced PGPR physiological parameters. Here, serine was detected to be more in PGPR bacterized samples. Serine was to play a reported role in soil bacterial community composition (Ziegler et al., 2013). Likewise, proline levels also increased in PGPR bacterized groundnut REs, reported for osmoprotectant and spore germinant functions (Moir, 1990; Faiza et al., 2011). The increased proline levels were detected in plant growth promoting fungus

Trichoderma virens inoculated *Arabidopsis* REs in presence of salt stress, to alleviate the osmotic stress (Contreras-Cornejo et al., 2014).

Overall in this study, we could detect the variations in groundnut REs composition in presence of PGPR. Most of the groups detected in groundnut REs were earlier reported in other plant REs, however, the individual metabolites in the groups varied. Here, we detected a very less of explored group of plant REs i.e. hydrocarbons in REs. At the same time, the variation occurred in the REs by PGPR bacterization were clearly noted with the help of multivariate analysis methods. This shows the influence of PGPR bacterization on plant REs composition and quantity. The organic acids, amino acids, and sugars which earlier reported in many REs mechanisms include plant-plant interactions, plant-microbe interactions, and phytoremediation etc. were shown to be highly influenced groups by RP2-bacterization. Hydrocarbons whose levels were non-significant through the plant development were observed to be RS4 interaction specific REs group. Evaluating few significant REs metabolites specifically detected by PGPR bacterization is useful to know their role in groundnut-PGPR interaction. Further, understanding the impact of these metabolites on PGPR physiological processes and gene profiles will help to know the PGPR strain specific modifications.

5.1. Introduction

Proteins are the major functional outputs, resulted by the environmental trigger on genes expression. A cumulative proteome profile of an organism can help in developing an image corresponding to its state. It has a wide range of applications in agricultural, biomedical, and food microbiology (Chandrasekhar et al., 2014). Several proteins involved in plant-microbe interaction were elucidated by the application of proteomics. De-la-Pena et al., (2008) reported increased secretion of plant and bacterial origin proteins into REs in *Medicago sativa-Sinorhizobium meliloti* symbiotic interaction, which was not induced in *M. sativa-Pseudomonas syringae* interaction. In contrast, in *Arabidopsis thaliana- P. syringae* pathogenic interaction, defense-related proteins were highly triggered. However, these proteins were not detected in *A. thaliana- S. meliloti* incompatible interaction, indicating the difference in proteins expression and requirement of particular proteins in establishing a specific root-microbe association.

Plant proteomic data could be a snapshot of bacterial effects on plant physiology (Feussner and Polle, 2015). Roots are the major plant regions of PGPR colonization and they are expected to be highly influenced by their interaction. For example, proteins involved in the carbohydrate, amino acid metabolism, antioxidant, defense, stress response, photosynthesis, and plant hormones were differentially expressed in *A. thaliana* root in presence of growth promoting *P. polymyxa* E681 (Kwon et al., 2016). *Bacillus* spp. and *Aeromonas* spp. showed priming effect in rice seedlings against abiotic salt stress and biotic- *Xanthomonas campestris* infection. Both the PGPR protected the rice seedlings against salt and pathogen attack, induced proteins involved in the oxidative stress, ROS generation, and pathogenesis-related (PR) proteins activities. However, the enhanced activity varied with respect to stress and PGPR (García-Cristobal et al., 2015). The wild-type and ethylene-insensitive tomato lines inoculated with *Enterobacter* sp. C7, promoted plant growth irrespective of the plant genotype, while *B. megaterium* specifically promoted wild-type plants growth. Further, root proteomes were highly affected based on the bacterial strain and plant genotype (Ibort et al., 2018).

As PGPR induce the root proteome modifications, the specific region of the plant can also influence the expression pattern of PGPR proteins and colonization ability. In a comparative proteomic analysis between root colonizing and planktonic *B. amyloliquefaciens* SQR9 displayed variations in expression of proteins involved in bacterial motility, chemotaxis,

biofilm formation, biocontrol, transport, and plant polysaccharides degradation. The highly expressed ResE protein was characterized to have a role in colonization and biofilm formation (Qiu et al., 2014). Similarly, *Methylobacterium extorquens* a phylloplane colonizer of *A. thaliana* displayed the differential expression of many proteins, including PhyR having a role in epiphytic colonization and regulation of oxidative stress-responsive genes (Gourion et al., 2006).

Even though these results explain the proteome changes in plant or PGPR, the mechanisms are frequently strain or plant-specific (Long et al., 2008) and less known (Puhler et al., 2004). The changes in proteins expression in PGPR or its plant partner were reported detailed, but a proteomic study as both plant and microbe is lacking in the plant- PGPR interaction. Therefore, an attempt was made to address the following objectives with the help of traditional two-dimensional electrophoresis (2DE), followed matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) identification.

1) What type of protein changes occur in RS4-bacterized groundnut roots?
2) Can groundnut REs alter the proteome of RS4 cell wall?

5.2. Results

5.2.1. Purity of RS4 cell wall proteins

The isolated cell wall proteins (CWP) and whole cell proteins (WCP) were loaded on SDS-PAGE for checking their integrity. Both protein fractions of RS4 varied clearly in the SDS-PAGE analysis (Fig 5.1A). For the purity of CWP, lactate dehydrogenase (LDH) assay was performed. The 50 µg of CWP fraction added to substrate sodium pyruvate reaction displayed a constant OD_{340}, concurrent LDH activity from initial to end of the reaction. This shows the absence of cytoplasmic marker protein LDH in isolated CWP fractions, confirming the purity of CWP. While, in case of WCP fraction, once the protein was added to the reaction, OD_{340} was continuously decreased and by the last time point at 6 min, the enzyme activity was reduced to twice the initial activity. It confirms that CWP fraction was free from the cytosolic proteins (Figure 5.1B).

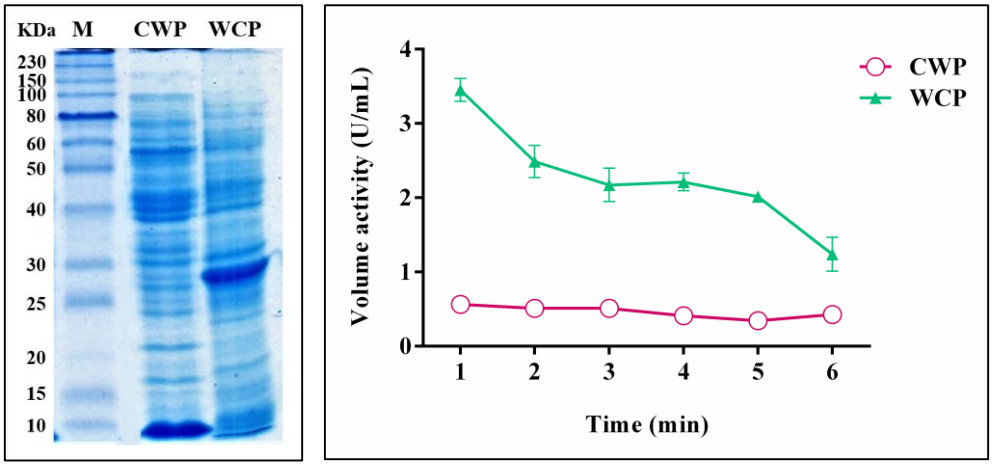

Fig 5.1: Assesment of isolated cell wall protein fractions for cytosolic enzyme activity. Isolated cell wall proteins visualization and purity (A) Visualization of RS4 cell wall proteins (CWP) and whole cell proteins (WCP) on 12% SDS-PAGE. (B) Lactate dehydrogenase activity of CWP and WCP fractions of RS4. Data represent the mean of three independent experiments. The vertical bars indicate standard error. M: molecular weight marker.

5.2.2. Cell wall proteins varied in presence of groundnut REs

The cell wall proteome of RS4 was varied in presence of groundnut REs. The proteins detected with a ratio of ≥1.5 in control *vs.* REs amended RS4 cell wall proteome were considered as up-regulated and ≤0.5 as down-regulated. A total of 24 proteins expression (22 up-regulated and 2 down-regulated) was detected to significantly differ in presence of groundnut REs. In presence of 12d-REs, outer membrane porin protein- NmpC and α-glutamate decarboxylase were specifically up-regulated, whereas none were varied in presence of 24d-REs in the RS4 cell wall. In the 22 proteins commonly differentiated in presence of both 12d and 24d-REs, elongation factor Tu and multispecies: alkyl hydroperoxide reductase subunit C expression was slightly down-regulated, while the remaining 20 proteins were up-regulated. Proteins like 2,3,4,5-tetrahydropyridine-2,6 dicarboxylate N-succinyl transferase, polyketide synthase-PksJ, multidrug transporter- AcrB, and β-N-acetylglucosaminidase expression was noted to be constantly increased in presence of both 12 and 24d-REs. Two glutamate decarboxylase alpha proteins, spore coat protein O, formate acetyltransferase 1, an uncharacterized ABC transporter permease- YknZ, multispecies: aspartate kinase, PTS sugar transporter subunit IIA, Met-tRNAi formyl transferase proteins expression was up-regulated more in presence of 12d-REs than 24d-REs. Whereas, chain A, X-ray crystal structure of protein Yxim_bacsu, alkyl hydroperoxide reductase subunit C, oxalate decarboxylase- oxdD, glutamate 5-kinase 2, three uncharacterized proteins (YqaM YlqG, and YphB), and a leukotoxin were expressed more in presence of 24 than 12d-REs.

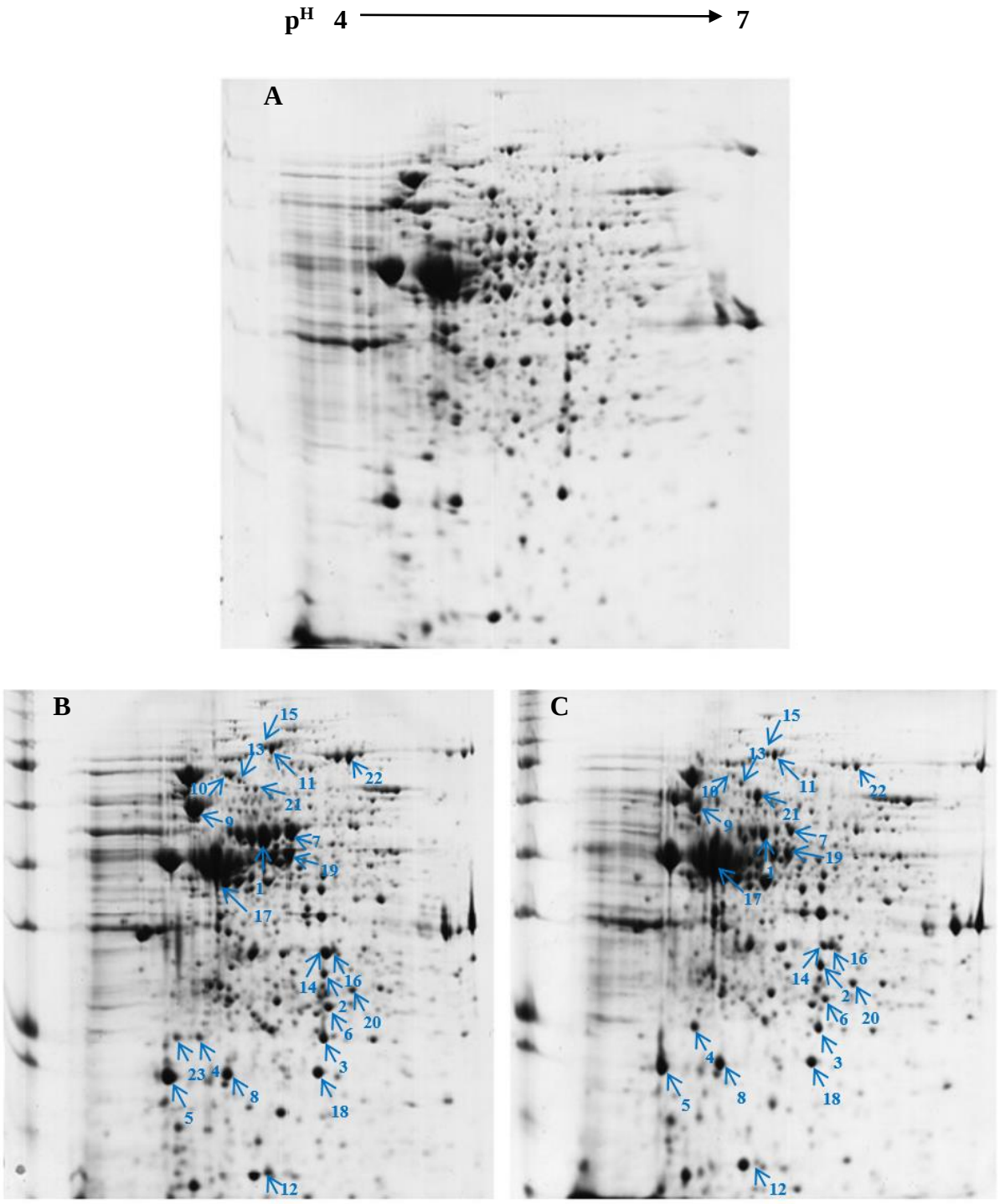

Fig. 5.2: Representative 2DE gels of RS4 cell wall proteome in presence of groundnut root exudates. (A) Proteome without REs, control (B) Proteome with 12d-REs (C) Proteome with 24d-REs. Arrows indicate the differentially regulated proteins ($p<0.05$) and selected for MS/MS analysis.

5.2.3. Groundnut root proteins differed by RS4

Root proteome of groundnut varied upon RS4 bacterization. The proteins detected with a ratio of ≥1.5 in non-bacterized *vs.* RS4-bacterized root proteome were considered as up-regulated

and ≤0.5 as down-regulated. In total 22 root proteins were differentially expressed in RS4-bacterized 12 and 24 DAS plant roots on 2D gels (Fig. 5.2) and identified using MALDI-TOF MS/MS. Protein- Ycf2, Allergen- Ara h 1 expression was down and up-regulated at 12 and 24 DAS RS4-bacterized plant roots respectively.

Of the two proteins differentially expressed at 12 DAS, a hypothetical protein was down-regulated, while pentatricopeptide repeat-containing protein was up-regulated. Out of the 19 proteins varied at 24 DAS, three proteins wound-induced protein (WIN2), SEC14 cytosolic factor family protein, and thaumatin-like protein were down-regulated whereas 16 proteins namely Magnesium-chelatase subunit- ChlH, β-1,3-glucanase, sucrose synthase 4, two heat shock 70 KDa proteins, Hsp70-Hsp90 organizing protein (HOP), chitinase, Ran3 GTP binding protein, pleiotropic drug resistance protein 1, alpha-methyl-mannoside-specific lectin, phospho-2-dehydro-3-deoxyheptonate aldolase, thioredoxin, β-fructofuranosidase, nuclear export mediator factor- NEMF, a hypothetical protein SELMODRAFT, and BnaC03g33460D were up-regulated by RS4-bacterization.

5.3. Discussion

The application of PGPR into fields had its own concerns about environmental factors and other inhabiting soil microflora (Bashan et al., 2014). To gain a complete understanding of PGPR in improving crop yield, in-depth understanding of mechanisms underlying plant- PGPR interaction was necessary. Proteomics is a potent tool to understand many plant-microbe interactions at a functional level (Feussner and Polle, 2015). Though most of the proteomic studies in PGPR were reported in the whole cell (Paul and Nair, 2008; Qiu et al., 2014), the bacterial cell wall is the highly influenced region by the plant REs signals (Dutta et al., 2013). Therefore, an attempt was made to unravel the proteomic changes in groundnut and *B. sonorensis* (RS4) in their beneficial interaction. The RS4 was well elucidated as a PGPR in crop plants like tomato (Vaikuntapu et al., 2014) and chilly (Thilagar et al., 2016). Significant changes in the RS4 cell wall and groundnut root proteome were detected with a vital role in plant- PGPR interactions, growth, and defense. Similarly, in *B. cereus, B. amyloliquefaciens* FZB42, and *B. amyloliquefaciens* SQR9, these proteins or respective genes were differentially expressed in presence of tobacco, groundnut, and maize REs (Dutta et al., 2013; Fan et al., 2012; Zhang et al., 2015).

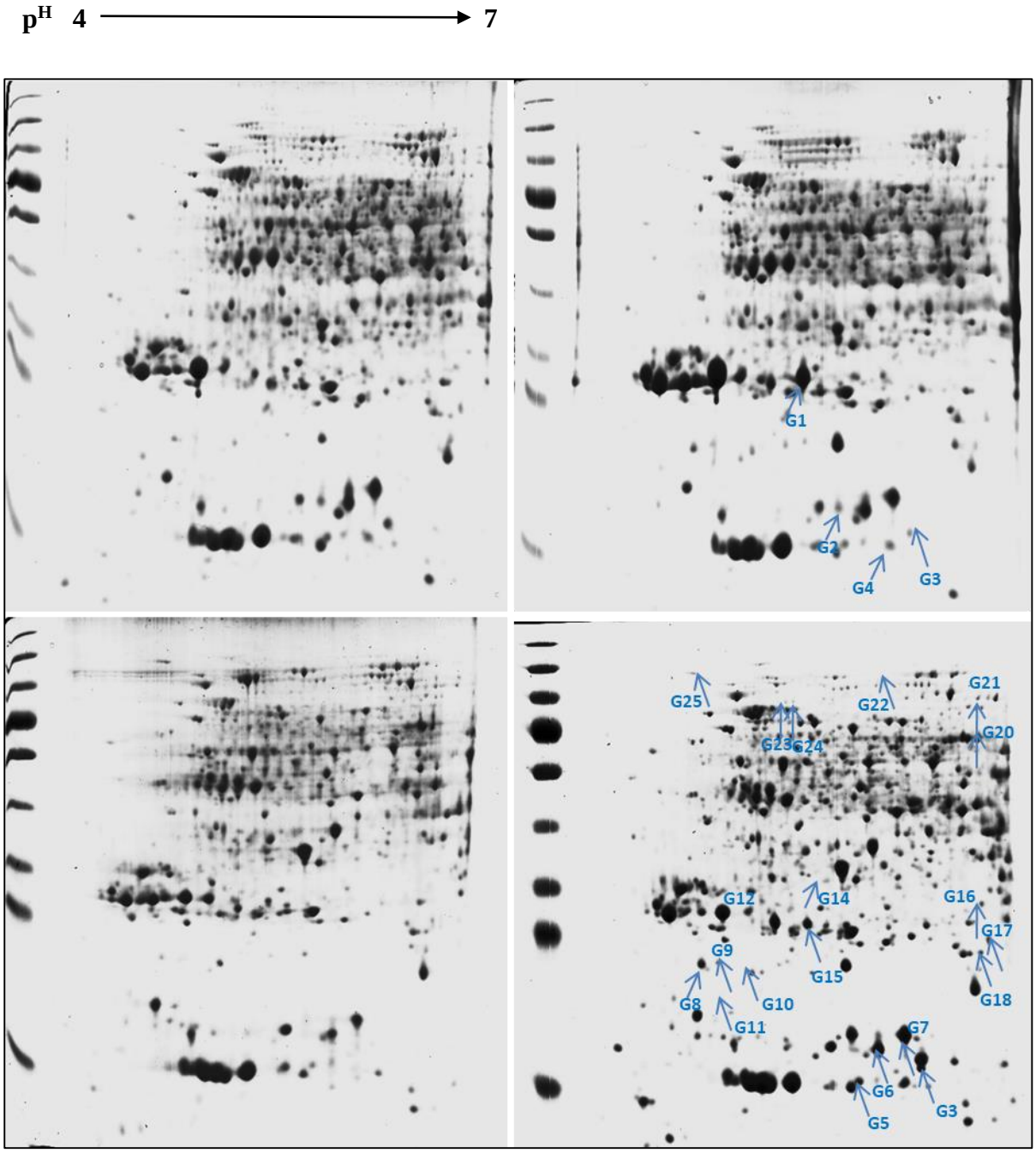

Fig. 5.3: Representative 2DE gels of root proteome in RS4 bacterized and non-bacterized groundnut. Top and bottom panels represent the 2DE maps of 12 and 24 days after sowing plant samples. Left and right panels represent non-bacterized and RS4-bacterized root proteome maps respectively. Arrows indicate the differentially regulated proteins ($p<0.05$) and selected for MS/MS analysis.

Table 5.1. Proteins differentially expressed in cell wall fraction of RS4 in 12 and 24d-REs; Spot number as given in Fig. 5.2; Fold change represents the average of relative fold change of 12 and 24d-REs amended cell wall proteins compared with the control. SM, secondary metabolites

Spot No.	Putative protein name (coding gene)	Fold change in REs		Biological function	Gene identity	Organism
		12d	24d			
B1	Glutamate decarboxylase alpha (GadA)	6.73	3.39	Acid stress resistance	DCEA_ECOLI	*E. coli*
B2	2,3,4,5-tetrahydropyridine-2,6 dicarboxylate N-succinyl transferase (dapD)	5.21	5.80	Amino acid biosynthesis	DAPD_ECOBW	*E. coli*
B3	Spore coat protein O (CotO)	7.44	4.60	Spore morphogenesis	COTO_BACSU	*B. subtilis*
B4	Elongation factor Tu (Tuf)	0.36	0.41	Protein synthesis	gi/270341199	*B. subtilis*
B5	Multispecies: alkyl hydroperoxide reductase subunit C (AhpC)	0.61	0.50	Reactive species resistance	gi/489420424	*B. subtilis*
B6	Chain A, X-ray crystal structure of protein Yxim_bacsu	1.57	2.50	Hypothetical protein	gi/122921265	*B. subtilis*
B7	Glutamate decarboxylase alpha (GadA)	13.59	3.88	Acid stress resistance	DCEA_ECOLI	*E. coli*
B8	Alkyl hydroperoxide reductase subunit C (AhpC)	3.89	4.98	Reactive species resistance	AHPC_ECOLI	*E. coli*
B9	Formate acetyltransferase 1 (Pfl)	6.57	2.75	Carbohydrate metabolism	PFLB_ECOLI	*E. coli*
B10	Uncharacterized ABC transporter permease YknZ	3.67	1.73	Transporter	YKNZ_BACSU	*B. subtilis*
B11	Polyketide synthase (PksJ)	3.34	3.92	SM biosynthesis	PKSJ_BACSU	*B. subtilis*
B12	Multispecies: aspartate kinase (CheY)	3.20	1.26	Amino acid biosynthesis	gi/504071537	*B. subtilis*
B13	PTS sugar transporter subunit IIA	3.16	2.69	Transporter	gi/489327114	*B. subtilis*
B14	Met-tRNAi formyltransferase	3.15	1.54	Protein synthesis	gi/1772500	*B. subtilis*
B15	Uncharacterized protein YqaM	2.36	3.89	-	YQAM_BACSU	*B. subtilis*
B16	Uncharacterized protein YlqG	2.28	4.75	-	YLQG_BACSU	*B. subtilis*
B17	Multidrug transporter AcrB (AcrB)	1.53	1.35	Transporter	gi/647261937	*B. subtilis*
B18	Oxalate decarboxylase (oxdD)	1.43	2.11	Glyoxylate metabolism	gi/751881743	*B. subtilis*
B19	Glutamate 5-kinase 2 (proJ)	1.25	3.61	Pro biosynthesis	PROB2_RHILO	*Rhizobium loti*
B20	Leukotoxin (LtxA)	0.97	1.70	SM biosynthesis	gi|407960125	*Synechocystis sp.*

B21	Uncharacterized protein YphB	0.78	3.01	-	YPHB_BACSU	*B. subtilis*
B22	β-N-acetyl glucosaminidase (NagZ)	5.06	5.12	Carbohydrate metabolism	gi|740679031	*B. subtilis*
B23	Putative outer membrane porin (NmpC)	3.11	-	Transporter	NMPC_ECOLI	*E. coli*
B24	Glutamate decarboxylase alpha (GDA)	3.86	-	Acid stress resistance	DCEA_ECOLI	*E. coli*

Table 5.2. Differentially expressed groundnut root proteins by RS4 bacterization; Spot number as given in Fig. 5.3; Fold change represents the average of relative fold change of RS4-bacterized root proteins compared with the non-bacterized.

Spot No.	Putative protein name	Fold change	Function	Localization	Gene identity	Organism
	Proteins specifically regulated at 12 DAS					
G1	Pentatricopeptide repeat-containing protein (PPR)	11.30	Nucleic acids transport	Mitochondria, Plastids	gi|460394808	*Solanum tuberosum*
G2	Hypothetical protein	0.24	-	-	gi|293335003	*Zea mays*
	Proteins specifically regulated at 24 DAS					
G5	Thioredoxin (TRX)	2.36	Defense response	Cytosol	gi|115187464	*Arachis hypogaea*
G6	β-fructofuranosidase (INV)	1.67	Carbohydrates metabolism	Vacuole, cytosol, cell wall	INV5_ORYSJ	*Oryza sativa* sub sp. *japonica*
G7	Nuclear export mediator factor (NEMF)-like	1.61	Nucleic acids transport	Nucleus	gi|571518396	*Glycine max*
G8	Thaumatin-like protein	0.45	Defense response	Cytosol, secreted	gi|441482380	*Actinidia chinensis*
G9	α-methyl-mannoside-specific lectin	2.68	Symbiosis	Cytosol, secreted	LECC1_ARAHY	*Arachis hypogaea*
G10	Phospho-2-dehydro-3-deoxyheptonate aldolase or DAHP synthase	4.97	Amino acids biosynthesis	Plastids	AROF_SOLTU	*Solanum tub*
G11	Wound-induced protein WIN2	0.11	Defense response	-	gi|356573113	*Glycine max*
G12	Pleiotropic drug resistance protein 1 (PDR)	2.45	Transporter	Multi-pass membrane protein	gi|587891564	*Morus notabilis*
G14	SEC14 cytosolic factor family protein	0.19	Lipids transport	Transmembrane	gi|590603864	*Theobroma*
G15	Magnesium-chelatase subunit ChlH	1.70	Growth	Plastid envelope	CHLH_ORYSI	*Oryza sativa*
G16	Chitinase	15.83	Defense response	Vacuolar, extracellular	gi|1237027	*Arachis hypogaea*

G17	Ran3 GTP binding protein	3.50		Nucleic acids and proteins transport	Nucleus, cytoplasm	gi|157467219	*Iberis amara*
G18	BnaC03g33460D	13.09		-	-	gi|674941598	*Brassica napus*
G20	Hypothetical protein SELMODRAFT_428785	1.77		-	-	gi|302817336	*Selaginella moellendorffii*
G21	Hsp70-Hsp90 organizing protein (Hop)	1.96		Co-chaperones and binds to Hsp90	Nucleus	HSOP_WHEAT	*Triticum aestivum*
G22	Sucrose synthase 4 (SUS4)	1.99		Carbohydrates metabolism	Cytosol, plasma membrane, vacuole	gi|672132636	*Phoenix dactylifera*
G23	Check, Heat shock 70 kDa protein (Hsp70)	2.23		Defense response	Cytosol, nuclear matrix	HSP70_SOYBN	*Glycine max*
G24	Heat shock 70 kDa protein (Hsp70)	2.11		Defense response	Cytosol, nuclear matrix	HSP7C_ARATH	*Arabidopsis thaliana*
G25	β-1,3-glucanase	4.11		Defense response	Vacuolar, secreted	gi|82949442	*Sesbania rostrata*
	Proteins specifically regulated both at 12 and 24 DAS						
G3	Allergen Ara h 1	**12 DAS**	**24 DAS**	Allergin	Seed storage	ALL11_ARAHY	*Arachis hypogaea*
		0.18	2.81				

5.3.1. RS4 cell wall proteins influenced by groundnut REs

5.3.1.1. Amino acids and signal transduction related proteins

Aspartate kinase is a crucial enzyme involved in the biosynthesis of methionine, lysine, and threonine by phosphorylating the aspartate (Vold et al., 1975). RS4 showed increased expression of aspartate kinase in presence of 12 and 24d-REs of groundnut. Other proteins involved in lysine and methionine biosynthesis, 2,3,4,5-tetrahydropyridine-2,6 dicarboxylate N-succinyl transferase and met-tRNAi formyl transferase were also up-regulated in 12 and 24d-REs. Aspartate kinase (CheY) is part of two-component signaling pathway responsible for many cellular functions of bacteria like cell division, chemotaxis, stress response, antibiotic resistance, and metabolites utilization (Falke et al., 1997). A transmembrane chemoreceptor histidine kinase (CheA) transmits the signal to receiver aspartate kinase (CheY) by phosphorylating, which binds to FliM protein of flagellar motor base and controls the motor rotation (Falke and Hazelbauer, 2001). The CheY mutants displayed failed phosphorylation, followed by bacterial tumbling (Falke et al., 1997). This shows the role of aspartate kinase in bacterial chemotaxis by effectively regulating the flagellar motion.

Glutamate 5-kinase 2 is a proline synthesizing enzyme (Belitsky et al., 2001) which has a functional role in spore germination (Moir, 1990). Proline is well-known osmoprotectant, while it can even act as a thermo-protectant and inducer of antioxidants. For example, endogenously synthesized and exogenously provided proline served as osmo and thermo-protectant in *Bacillus* sp. and *Lactococcus* sp., respectively (Holtmann and Bremer 2004; Faiza et al., 2011). *B. subtilis* showed the up-regulation of proline biosynthesis ProHJ transcripts in salt and heat stress (Hahne et al., 2010), which has a functional role in outgrowing the spores (Nagler et al., 2016). Proline can even exhibit oxidative stress response in *E. coli* by up-regulating the hydroperoxidase (KatG) (Zhang et al., 2015a). In this study, the increased expression of this enzyme in RS4 in presence of groundnut 12 and 24d-REs, could help in alleviating stress conditions and sporulation.

5.3.1.2. Antioxidant related proteins

Antioxidant enzymes provide tolerance to damage caused by reactive oxygen or nitrogen or sulfur species generated in the respiration or by environmental stress (García-Cristobal et al., 2015). Alkyl hydroperoxide reductase (Ahp) is a thiol-specific peroxidase protein reported for

detoxification of these reactive species in the cell (Chen et al., 1998; Storz and Imlayt 1999). It has two components, a major alkyl hydroperoxide reductase subunit C (AhpC) with peroxidase role, the second flavoprotein disulfide reductase (AhpF) regenerates the oxidized AhpC (Zuo et al., 2014). *B. subtilis*, *E. coli, and B. aquimaris* showed tolerance to multiple abiotic stress conditions like heat, salt, carbofuron, cadmium, copper, UV-B, and t-butyl hydroperoxide by overexpressing AhpC (Antelmann et al., 1996; Mishra et al., 2009; Natalia et al., 2016). AhpC has additional physiological functions in colonization and maintaining cell morphology. For instance, *mutating in Ahp*C gene *in A. brasilense* Sp245 altered the *cell morphology,* cell-to-cell aggregation ability, motility, and flocculation (Wasim et al., 2009). Similarly, loss of colonization was reported in animal pathogens, *Helicobacter* sp. and *Staphylococcus* sp. by mutating *Ahp*C gene (Olczak et al., 2003; Cosgrove et al., 2007; Charoenlap et al., 2012). In contrast, competitive co-inoculation of wild-type and *Ahp*C mutant *A. brasilense* Sp245 on wheat roots implied no competitive advantage of wild-type over mutant in root colonization. However, these mutants lost the motility (Wasim et al., 2009). Here, in presence of 12 and 24d-REs AhpC expression was up-regulated in RS4 might have major role in cell morphological parameters and stress tolerance over colonization on plants.

5.3.1.3. Carbohydrate metabolism

β-*N*-acetylglucosaminidase and formate acetyltransferase involved in the carbohydrates metabolism were up-regulated in presence of both 12d and 24d-REs. The β-*N*-acetylglucosaminidase encoded by *Nag*Z gene is a cell wall-associated protein. It cleaves the non-reducing ends of N-acetylglucosamine residues present in the cell wall muropeptide and performs cell wall recycling in many gram-positive bacteria including *Bacillus* spp. For example, *B. subtilis* W-23, *B. megaterium, B. cereus*, *Lactobacillus acidophilus* and many other bacteria release up to 50% of their cell wall murine, recycles them for growth (Chaloupka and Kreckova**,** 1971; Mauck et al., 1971; Boothby et al., 1973; Smith et al., 2000). The first evidence for a muropeptide catabolic pathway linked cell wall recovery by NagZ was reported by Litzinger et al., (2010) in *B. subtilis*, indicating the importance of NagZ in bacterial cell wall regeneration and growth promotion.

Formate acetyltransferase commonly known as pyruvate formate lyase (PFL), catalyzes a reversible reaction between pyruvate + CoA and acetyl CoA + formate. The up-regulation of PFL at gene and protein level in *S. aureus,* when grown in biofilm than planktonic, appears to be an important survival strategy in the biofilm (Leibig et al., 2011). A PGPR *P. fluorescens*

B16 displayed antibacterial activity of PFL against root pathogens *Agrobacterium tumefaciens* and *Ralstonia solanacearum* (Jin-Woo et al., 2003). Zelcbuch et al., (2016) showed the PFL-dependent increase of *E. coli* biomass by efficiently utilizing acetate and formate for growth. Together, PFL and NagZ have functional roles in bacterial antibiosis and cell wall regeneration for bacterial growth.

5.3.1.4. Bacterial transporters

Four different transporter proteins were detected to be differentially regulated in RS4 in presence of groundnut 12d and 24d-REs. They are ABC transporter, PTS sugar transporter subunit IIA, multidrug transport AcrB, and a putative outer membrane porin. ABC (ATP binding cassette) transporter permease, YknZ is part of an unusual four-component transporter *YKnWXYZ* operon. This operon provides resistance to endogenously generated antimicrobial peptides including sporulation-delaying protein (SDP). by effluxing them out of the cell using YKnY ATPase and YKnZ permease activity (Butcher and Helmann 2006; Zgurskaya et al., 2015). SDP promotes the cells to irreversibly convert to spores. Even though the exact mechanism for protection against SDP by *YKnWXYZ* operon was unclear (Yamada et al., 2012), in *Bacillus* spp. this might happen by delaying the onset of sporulation in the biofilm (Xu et al., 2016). It was supported by the increased sensitivity to SDP after deleting of *YKnWXYZ* operon in *B. subtilis* cells (Butcher and Helmann, 2006).

AcrB is an efflux transporter of Resistance Nodulation cell Division (RND) superfamily. It is localized in the membrane along with its partner proteins AcrA and TolC (Oswald et al., 2016). It recognizes the various group of toxic substrates like antibiotics, dyes, detergents, organic solutes, antimicrobial agents and expels them out through the H^+ antiport gradient. (Rosenberg et al., 2003; Oswald et al., 2016); Lennen et al., (2013) reported the reduced cell viability and abolished free fatty acids production ability of *E. coli* deletion strains of *acrAB* and *tolC*, indicating the role in fatty acids efflux and cell integrity. Similarly, induction of AcrAB efflux pump in *E. coli* provided more resistance towards lipophilic antibiotics (Rosenberg et al., 2003).

The phosphotransferase system (PTS) is specifically detected in eubacteria for the uptake of multiple sugars from its surrounding environment. PTS system is an active transporter, contains two cytosolic proteins, enzyme I (EI) and histidine protein, which transfers phosphate from phosphoenolpyruvate (PEP) to membrane-bound transporters EIIA, EIIB, and EIIC. These EII

transporters are specific to incoming hexoses or hexitols (Clore and Venditti, 2013) and phosphorylate to prevent their efflux from the cell (Siebold et al., 2001). PTS system proteins have a role in cell growth. For instance, deletion of PTS system $EIIA^{Man}$ involved in the mannose transport decreased the growth of symbiotic nitrogen fixer *Sinorhizobium meliloti* (Pinedo et al., 2008). Similarly, $EIIA^{Ntr}$ mutant cells, lost K^+ efflux transporter inhibiting capacity, simultaneous increased K^+ levels reduced the *E. coli* growth (Powell et al., 1995; Lee et al., 2007).

Porins are distinguished from other transport proteins for their ability to allow passive diffusion of many hydrophilic compounds with different sizes and charges through the membrane. The NmpC is a tolC dependent protein (Morona and Reeves, 1982) with an additional role in cell protection against temperature stress. The high expression of NmpC in heat resistant *E. coli* than sensitive strain allowed them to survive in high heat conditions, indicating the role of NmpC in heat tolerance (Ruan et al., 2011).

5.3.1.5. Acid stress homeostasis

Acidic pH in the external environment makes the bacterial membrane leaky to promote the influx of H^+ and volatile fatty acids (Capitani et al., 2003). This leads to the acidification of the cellular compartment and activation of homeostasis related molecules in the cell. Glutamate decarboxylase (GAD) system is well reported for acid stress tolerance in microorganisms (Feehily and Karatzas, 2013). It is up-regulated in low pH conditions, recruits to the membrane from the cytosol (2012) and acts as a buffering agent (Capitani et al., 2003). Factors like high acid conditions (Feehily and Karatzas, 2013), sodium (Richard and Foster, 2007), hypoxia (Jydegaard-Axelsen et al., 2004) and polyamines (Jung and Kim, 2003) were reported to induce GAD system in *E. coli* and *Listeria monocytogenes*. The identification of GAD thrice in RS4 cell wall proteome with high expression in presence of both 12 and 24d-REs represents its importance in RS4- groundnut interaction.

5.3.1.6. Spore germination and sporulation proteins

Bacillus spp. are distinctly known for their spore-forming ability in the bacterial phylum. These bacterial spores covered with nearly 50 coat proteins in a complex interaction (Kim et al., 2006) to provide a tough barrier to the external environment. Mcpherson et al., (2005) described the morphogenic function of spore coat protein O (CotO) in *B. subtilis*. The *cotO* mutant spores exhibited a 10-fold decrease in lysozyme resistance, indicating the importance of CotO protein

in fully intact coat formation. Apart from cellular acid homeostasis, GAD was reported to play a key role in germination of bacterial spores. In *B. megaterium* the GAD activity increased in germinating spores, while inhibition or mutation in GAD further inhibited the spore germination, suggesting the role of GAD in spore germination (Foerster and Foerster, 1973).

In RS4 cell walls, GAD and CotO protein levels increased in presence of 12d-REs, which got down in 24d-REs. However, the increased expression of spore germinating GAD and spore morphogenic CotO proteins seems to be contradictory, the expression of GAD was very much higher in comparison to CotO. At the same time, GAD levels decreased in 24d-REs as the CotO levels decreased. Therefore, the sporulation of RS4 might be suppressed by GAD expression along with glutamate 5 kinase 2, synthesizing spore germinant proline to keep RS4 in the active state.

5.3.1.7. Antibiosis and secondary metabolism-related proteins

Oxalate decarboxylase (OxdD) catalyzes the conversion of oxalate to fumarate. The plant beneficial *Burkholderia* spp. have a specific oxalotrophic ability over the plant and human pathogenic *Burkholderia* spp. to use oxalate as a carbon source. *B. phytofirmans* endophytic root colonization was impaired by the mutation in the oxalate decarboxylase gene in lupin and maize (Kost et al., 2013). It was even reported for its antifungal property, such as the transgenic lettuce, soybean, and grass pea plants with *Flammulina* sp. OxdD showed tolerance to *S. sclerotiorum* infection (Dias et al., 2006; Kumar et al., 2016). Other proteins, polyketide synthases PksJ producing antibiotic bacillaene, and a protein like leukotoxin involved in pathogenesis (Kachlany et al., 2000) increased in presence of REs. The polyketide synthases PksJ was reported in PGPR genome of *Bacillus* sp. JS (Song et al., 2012) and *P. polymyxa* (Xie et al., 2016).

5.3.2. Groundnut root proteins regulated by RS4-bacterization

5.3.2.1. Carbohydrate metabolism proteins

In plants, sucrose is the prime translocatable sugar molecule catabolized into fructose and UDP-glucose or glucose by two enzymes, sucrose synthase (SUS) and β-fructofuranosidase (referred as invertase- INV). The breakdown of sucrose by these enzymes is considered as one of the major routes of carbon availability for plant cellular metabolism (Bieniawska et al., 2007). The produced glucose participates in the synthesis of cellulose, starch, cell wall, and

incorporates into growing cell components (Coleman et al., 2009; Baroja-Fernandez et al., 2012). A direct relation between expression of these enzymes with respect to plant growth was reported in many plants like tobacco, poplar, and *Arabidopsis* (Sturm and Tang, 1999; Coleman et al., 2006; Coleman et al., 2009). The prime lead by one of these enzymes is still in controversy with respect to the plant development. For instance, mutations in the genes of INV over SUS proved to affect the cellulose synthesis, growth, and root development in *A. thaliana* (Barratt et al., 2009). Moreover, INV were reported in regulating the cell proliferation, differentiation, and plant development by stimulating D-type cyclins. The CycD2 and CycD3 are involved in G1 to S phase transmission of the cell cycle through the cytokinins or sucrose and glucose stimulation (Sturm, 1999; Roitsch and Gonzalez, 2004; Tauzin and Giardina, 2014). However, Baroja-Fernandez et al., (2012) opposed this observation and consistent with the potential contribution of SUS.

Apart from this functions, INV had a regulatory role in various other functions including delaying leaf senescence, salt stress response, plant-herbivore interactions, defense, and symbiotic mycorrhiza or rhizobia interactions (Roitsch and Gonzalez, 2004). These observations indicate the importance of SUS or INV in plant growth and defense. Nevertheless, the combined enhancement of two enzymes might have a similar or additional benefit to the plant. In this study, RS4-bacterized groundnut roots exhibited an increased expression of both SUS and INV at 24 DAS. This is inconsistent with the enhanced plant growth observed in RS4-bacterization (Fig 3.3, 3.4). Similarly, increase in plant growth by stimulating the expression of plant SUS or INV was reported earlier by the application of PGPR. For example, *Burkholderia* Q208 inoculated sugarcane roots exhibited over-expression of sucrose synthases up to 3 folds for growth (Paungfoo-Lonhienne et al., 2016). Soil enriched with *Azospirillum* and *Azotobacter* increased invertase contributed to safflower growth (Nosheen and Bano, 2014).

5.3.2.2. Amino acids synthesis and antioxidant-related proteins

The aromatic amino acids phenylalanine, tyrosine, and tryptophan are synthesized by DHAP synthase, a key regulatory enzyme of Shikimate pathway. These amino acids not only serve as building blocks of protein synthesis, they even act as precursors for generation of secondary metabolites. The flavonoids, lignins, phytoalexins, and alkaloids produced by them have a significant role in the plant-microbe interaction, defense, and cell wall development (Herrmann, 1995). The increased expression and activity of DHAP synthase was observed in

potato cells exposed with a herbicide glyphosate as a part of defense mechanism (Pinto et al., 1988). Further, Entus et al., (2002) reported the ferredoxin/thioredoxin (TRX) redox-mediated control of DAHP synthase levels in the plant cell. Therefore, the increased expression of DAHP synthase and TRX in RS4-bacterized groundnut roots specifies their importance in generating multifunctional amino acids.

TRX plays a crucial role in numerous other plant metabolic processes including synthesis of starch, lipids, in cell-cell communication, and act as a redox agent in oxidative stress response (Santos and Rey 2006; Geigenberger and Fernie 2014; Geigenberger et al., 2017). Sanz-Barrio et al., (2013) reported the increased starch levels in tobacco leaves upon overexpression of TRX, as a way to improve nutritional status as well as biomass. Similarly, the mutation in *Trx*h, a resident of plasma membrane displayed impaired *Arabidopsis* growth and development (Meng et al., 2010). Another side, TRX also interlinked with the Mg-chelatase enzyme of chlorophyll biosynthesis (Luo et al., 2012), mutations in ChlH subunit of Mg-chelatase leads to the retrograde signalling of photosynthetic genes encoded by the nucleus (Ikegami et al., 2007). This indirectly affected the plant photosynthesis and growth. In the current study, Mg-chelatase ChlH levels increased in RS4-bacterized groundnut root. The inclusive expression of TRX, DAHP synthase, and Mg-chelatase ChlH in groundnut roots in presence of RS4 indicates their role in plant growth and enhanced antioxidant system of the plant.

5.3.2.3. Nucleic acid binding proteins

Pentatricopeptide repeat (PPR) protein is mostly a eukaryotic protein with major functions in RNA processing, editing, splicing, translation in mitochondria or chloroplast, and organogenesis (Jiang et al., 2015). On the other hand, these proteins were reported to have a role in plant development (Saha et al., 2007), cytoplasmic male sterility inhibition (Wang et al., 2006), photosynthesis (Meierhoff et al., 2003), various stress tolerance mechanisms (Jiang et al., 2015), and defense against pathogens (Laluk et al., 2011). The highly increased expression of PPR protein in RS4-bacterized 12 DAS plant roots indicates the activated tolerance to different stress conditions and growth. Further, nuclear export mediator factor (NEMF) and Ran3 GTP binding protein also increased in RS4-bacterized plants at 24 DAS over non-bacterized. Ran3 acts as a transporter for non-coding RNA and proteins in nucleocytoplasmic space (Haizel et al., 1997). It played an important role in chromatin condensation, during the cell cycle in tobacco cells (Yano et al., 2006). However, its biological importance in plant-microbe interactions is still unexposed.

5.3.2.4. Plant defense-related proteins

The main cell wall component of higher plants is callose, which is made up of (1, 3)-β-D-glucans, it is synthesized by callose synthase complex, while degraded by hydrolytic enzyme β-1, 3-glucanase. Plant β-1, 3-glucanase falls under pathogenesis-related proteins, PR2, along with chitinases, PR3, and thaumatin-like proteins, PR5. These proteins are induced as a part of ISR in plants to cope up with an upcoming pathogenic attack or abiotic stress. Chitinases and glucanases directly degrade the fungal cell wall and play a vital role in plant defense towards fungal pathogens. For instance, volatiles of *Bacillus* sp. JS developed ISR in tobacco leaves by stimulating glucanases and chitinases genes, further suppressed fungal pathogens *Rhizoctonia solani* and *Phytophthora nicotianae*. Besides defense response glucanases were reported for alleviating temperature stress in red grapes (Romero et al., 2008), draft in wheat (Gregorova et al., 2015), and heavy metal stress in maize (Pirselova et al., 2011).

Increased expression of chitinase in the beneficial interaction of tobacco-*Bacillus* sp. and rice-*Aeromonas* sp. was also known (Garcia-Cristobal et al., 2015; Kim et al., 2015). A cumulative superior activity of glucanases and chitinases was reported in tea plants in interaction with *P. fluorescens* Pf1 to fight against blister blight pathogen *Exobasidium vexans*. Similarly, in this study both chitinase and β-glucanase increased highly in RS4-bacterized plant roots. However, a chitin binding, defense responsive wound-induced protein WIN2 (Chrispeels and Raikhel 1991; Friedrich et al., 1991; Lee 1993) was slightly down-regulated in RS4-bacterized groundnut roots over non-bacterized. Transgenic wheat and tobacco lines with constitutive expression of rice and groundnut TLP genes showed enhanced resistance to fungal pathogens *Fusarium graminearum* and *Rhizoctonia solani,* respectively (Chen et al., 1999; Singh et al., 2013).

5.3.2.5. Plant cell transporters

Pleiotropic drug resistance (PDR) protein, PDR1 is an ATP-binding cassette transporter localized in the plasma membrane. This protein is constitutively expressed and involved in the transport of a diverse group of compounds including antimicrobial metabolites. Such as, *Nicotiana plumbaginifolia* NpPDR1 is involved in the transport of an antifungal diterpene compound sclareol. The expression of NpPDR1 was induced in leaf after infection with pathogenic fungus or bacteria. Similarly, NpPDR1 gene silenced transgenic tobacco plants reduced the resistance towards *Botrytis cinerea* infection (Stukkens et al., 2005). These results

show the importance of PDR1 protein in transporting antimicrobial compounds for fighting against pathogens. Hormones like salicylic acid, methyl jasmonate, or ethylene highly induce the PDR protein levels in many plants. For example, AtPDR12 of Arabidopsis, OsPDR9 of rice, and NpPDR1 of tobacco are induced by methyl jasmonate. Pathogens like *Alternaria brassicicola* or *Sclerotinia sclerotiorum*, and *P. syringae* pv. tomato DC3000 induced *Arabidopsis* PDR1 protein strongly (Crouzet et al., 2006). It shows the pathogen-induced defensive expression of PDR genes along with their regular constitutive expression in plants. In the present study, increased expression of PDR1 in groundnut roots bacterized with RS4 indicates increased defense potential of the cell by RS4 bacterization.

SEC14 cytosolic factor family protein is a transmembrane lipid transfer protein involved in the homeostasis of phosphoinositides in the cell. SEC14 had a reported functional role in root hairs development and elongation. In *Arabidopsis*, SEC14 protein *atsfh1* mutation lead to the improper tip direction and root hair elongation (Denancé et al., 2014; Campos and Schaaf, 2017). An impaired root tip growth and morphology were also observed in *Arabidopsis* and rice with a mutation in SEC14 (Bohme et al., 2004; Huang et al., 2013). Therefore, SEC14 has a vital role in root architecture, but in case of groundnut roots bacterized with RS4 showed a slight decrease in the expression. However, a proper and improved root system was observed in RS4-bacterized groundnut roots in comparison to non-bacterized by the root analyzer.

5.3.2.6. Protein folding

Heat-shock proteins (Hsp) are chaperonins with functions in protein folding. They refold the misfolded proteins in plants to meet the continuous biotic and abiotic responses. Hsp70 is one of the major plants Hsp with increased cytosolic expression deciphered non-host resistance in tobacco and citrus plants (Kanzaki et al., 2003; Rani and Podile 2014). Apart, Hsp70 performs protein biogenesis, folding, prevents aggregates, and degradation (Park and Seo, 2015). Increased expression of the Hsps along with co-chaperon, Hsp70-Hsp90 organizing protein (HOP) in RS4-bacterized plants might be to keep the requirement of increased protein expression for various growth and defense-related proteins generation.

In conclusion, both groundnut and *B. sonorensis* (RS4) influenced each other by altering the expression of the proteins. RS4 cell wall proteins related to chemotaxis, growth, cell wall regeneration, antimicrobial compounds synthesizing, and transporters were enhanced in presence of 12 and 24d-REs of groundnut. Whereas, in groundnut root, carbohydrate

metabolism, amino acids synthesizing, defense responsive, and transport proteins were differentially expressed in RS4-bacterized 12 and 24 DAS plants (Fig 5.4). These observations can be an initial path to find the new underlying networks taking place in groundnut and *B. sonorensis* (RS4) beneficial interaction. For example, in RS4 cell wall proteome, sporulation (spore coat O) and spore germinating (glutamate decarboxylase and glutamate 5 kinase) proteins were detected together with increased expression. Root proteins like DHAP synthase and Mg-chelatase along with their regulating thioredoxin enhanced expression by RS4-bacterization (Fig 5.4). The exact role of these proteins as a set need to be exploited, this will undo the novel pathways detected and helps in elucidating their role in plant- PGPR interactions.

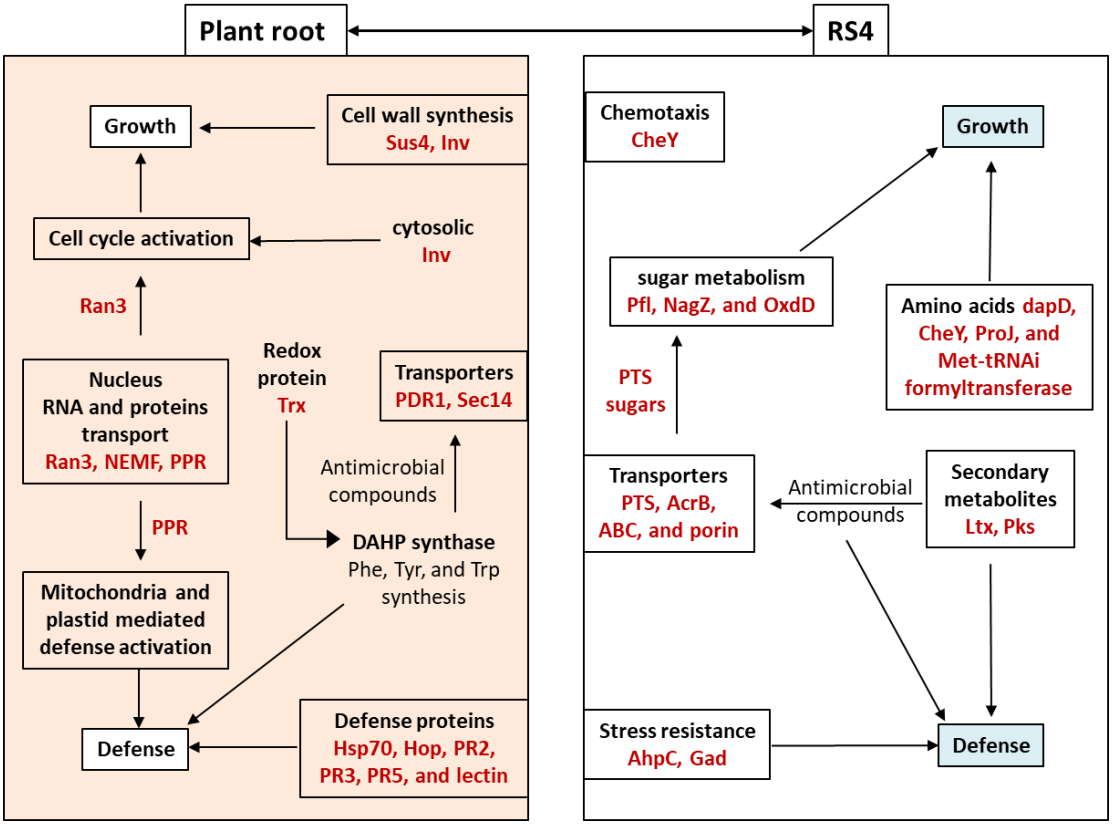

Fig 5.4: Schematic representation of proteins differentially varied in groundnut- *Bacillus sonorensis* (RS4) interaction with the putative functional role. PPR, Pentatricopeptide repeat; PR, Pathogenesis-related; PDR, Pleiotropic drug resistance; Sus, Sucrose synthases; Hsp, Heat shock proteins; Hop, Heat shock proteins organizing protein; NEMF, Nuclear export mediator factor; Ran3, Ras-related nuclear protein; Trx, Thioredoxin; SEC, phosphatidylcholine/phosphatidylinositol transfer protein; DHAP synthase, Phospho-2-dehydro-3-deoxyheptonate aldolase; Gad, Glutamate decarboxylase; Ahp, Alkyl hydroperoxide reductase; ABC transporter, ATP binding cassette transporter; PTS, Phosphotransferase system; AcrB, Multidrug transporters; CheY, aspartate kinase; GK, glutamate kinase; NagZ, β-N-acetylglucosaminidase; Pks, polyketide synthase.

6.1. Introduction

The main route of chemical communication between plants and soil micro flora is through root exudates (REs). Organic compounds like flavonoids, organic acids, and sugars present in the REs play a specific role as carbon source and molecular signals in plant-microbe interactions (Jones, 1998). Changes in the diversity and quantity of compounds in the REs, alter upon colonization of beneficial or pathogenic microbes. More of tryptophan was detected in cucumber REs after treatment with *Bacillus,* promoting further colonization. On the other hand, decreased raffinose exudation reduced the colonization by pathogenic *Fusarium* in a tripartite association (Liu et al., 2017a). *Bacillus* spp. and *Paenibacillus* spp. showed improved colonization, chemotaxis and biofilm formation in presence of organic acids of banana, tomato, and watermelon REs (Ling et al., 2011; Tan et al., 2013; Yuan et al., 2015).

Root proteins are also the major stations of plant-PGPR interactions. Differential expression of root carbohydrate, nitrogen metabolism, defense, stress, and antioxidant-related proteins was reported in cucumber- *P. putida* UW4 and *Arabidopsis*- *Paenibacillus polymyxa* E681 beneficial interaction to overcome hypoxic condition (Kwon et al., 2016; Li et al., 2013). Similarly pepper plants treated with *B. licheniformis* K11 showed tolerance to drought stress by up-regulating root proteins (Lim and Kim, 2013). Root proteins that are directly or indirectly associated with the growth promotion have changed significantly in canola, up on *P. fluorescence* treatment under salt stress (Banaei-asl et al., 2015). Alteration in the root proteins, in the presence of PGPR, to overcome stress conditions in different crops, indicate the need for an in-depth understanding of plant–PGPR interaction.

Profiling metabolites of REs has become necessary to understand various plant chemical networks involved in alleviating the biotic and abiotic factors. However, the specific metabolites in the REs, involved in the plant-PGPR interaction, with respect to groundnut-PGPR interaction are not known. Simultaneously understanding the changes in plant root proteome in presence of PGPR, further evaluating the contribution of such changes in improved performance of the plant growth or disease suppression can fill the gaps.

Therefore, in this chapter we addressed objectives in this direction. A rhizoplane isolate *Pseudomonas* sp. (RP2), identified as a potential PGPR, was selected to assess the changes in REs metabolome and root proteome of groundnut up on bacterization.

1) Is there a difference in REs metabolites profile up on seed bacterization with *Pseudomonas* sp. (RP2)?
2) Will these changes in REs metabolites contribute to *Pseudomonas* sp. (RP2) physiological characters?
3) Which type of groundnut root proteins will be varied in presence of *Pseudomonas* sp. (RP2)?
4) Whether these interaction-specific metabolites and proteins have a role in suppression of *Sclerotium rolfsii*?

6.2. Results

6.2.1. RP2-bacterized groundnut root exudates profile

To identify key metabolites involved in the groundnut-RP2 beneficial interaction, REs were analysed by GC-MS at 12 and 24 DAS from the RP2-bacterized and non-bacterized groundnut plants. Overall changes in 74 putatively identified metabolites of REs was examined through PCA. In multivariate data analysis with unsupervised PCA, each point served as a sample and similar biochemical composition in samples grouped together. Dissimilar samples were grouped apart with each other. In the present study, PCA displayed variation in groundnut REs profiles of non-bacterized plants and RP2-bacterized plants. The first principal component (PC1) resolved plant developmental specific metabolites between 12 and 24 DAS plant REs. The metabolites present in 12 DAS were clearly separate from those in 24 DAS in both non-bacterized and RP2-bacterized plants. The second principal component (PC2) also showed similar differences in REs after 12 and 24 DAS. The PC2 also indicated that RP2 bacterization has shifted metabolite profiles of REs separately from their respective non-bacterized controls. These data, indicated that the differences in REs composition is influenced by RP2 bacterization and plant developmental age (Fig. 6.1A). Cluster analysis with Euclidean correlation showed clear segregation between non-bacterized and RP2-bacterized REs. Although the RP2-bacterized 12d and 24d REs clustered relatively closely than their respective non-bacterized control plants (Fig. 6.1B). This was similar to observations from PCA analysis, where RP2 treatment shifted both 12 and 24 DAS metabolites profiles of REs away from their controls. The differences between REs samples were more prominent in supervised PLS-DA method. This method showed good correlation (R^2 = 0.996) and predictability (Q^2 = 0.586) (Fig. 6.1C). The shift in bacterized samples observed in PC2 of PCA analysis was also evident clearly in PLS-DA. Comprehensive analysis of all samples revealed a clear separation between

RP2-bacterized and non-bacterized samples along with plant developmental age, indicating that there is a clear change of metabolite profile upon RP2 bacterization.

The 74 compounds, identified as above, were classified and categorized into seven groups based on specific structure. In total, 5 alcohols, 4 amino acids, 5 hydrocarbons, 9 sugars, 10 fatty acids, 26 organic acids, and 15 compounds grouped as others were identified and their composition and changes are listed (Table 6.1).

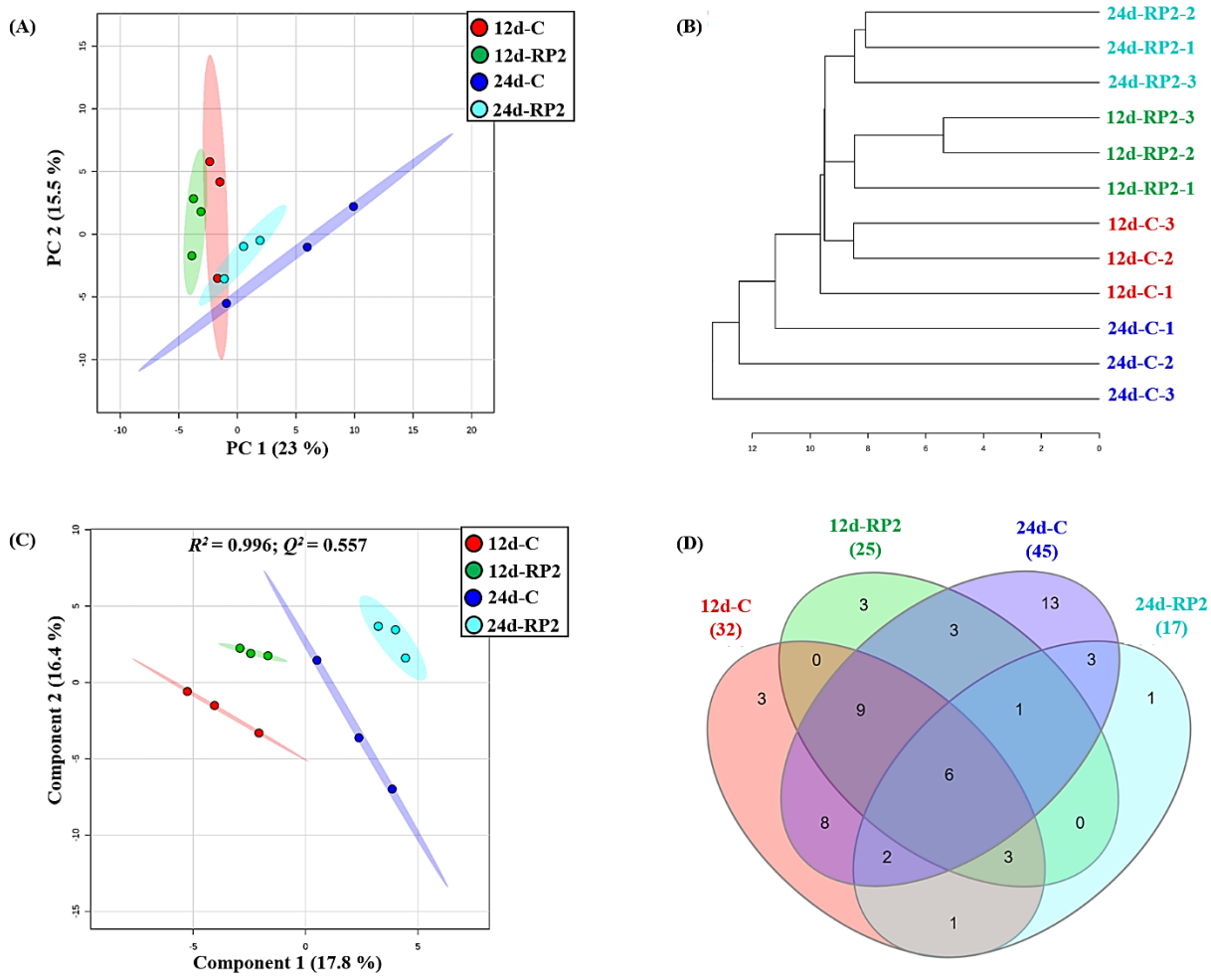

Fig. 6.1: Global differences in metabolite profiles of root exudates from non-bacterized or *Pseudomonas* sp. (RP2) bacterized groundnut. Multivariate, hierarchical clustering analysis and Venn grouping of REs GC-MS data from RP2-bacterized and control plants. (A) Unsupervised principal component analysis (PCA) with percent of variation of each principal component. Each symbol represents biological replicate. The variance of each component (PC1, PC2) is given as percentage within parenthesis (B) Dendrogram of cluster analysis (Euclidean correlation) (C) Supervised partial least squares discriminant analysis (PLS-DA). R^2 and Q^2 values indicate the correlation and predictability respectively (D) Venn diagram of REs with number of metabolites detected in different groups.

Table 6.1: Metabolic profiling of root exudates by GC-MS in *Pseudomonas* sp. (RP2) bacterized and non-bacterized groundnut

S.No	Retention time	Identified compound	12 DAS		24DAS	
			Control	RP2	Control	RP2
	Organic acids					
1	7.58	Propanoic acid	0.77±0.75	0.23±0.19	0.11±0.13	0.00±0.00
2	7.92	2-Furancarboxylic acid (1TMS)	0.00±0.00	0.00±0.00	0.07±0.12	0.00±0.00
3	7.93	3-Furoic acid (1TMS)	0.00±0.00	0.00±0.00	0.09±0.15	0.00±0.00
4	8.60	3-Hydroxybutyric acid (2TMS)	0.00±0.00	0.00±0.00	0.47±0.81	0.00±0.00
5	8.83	2-Ethylhexanoic acid (1TMS)	0.00±0.00	0.00±0.00	0.57±0.40	0.00±0.00
6	9.10	Propanedioic acid (2TMS)	0.00±0.00	0.00±0.00	0.07±0.13	0.00±0.00
7	9.83	3-Pyridinecarboxylic acid (1TMS)	0.02±0.02	1.43±1.24	0.00±0.00	0.01±0.00
8	10.30	Benzoic acid	0.39±0.08	0.90±1.04	0.17±0.15	0.00±0.00
9	10.33	Butanedioic acid	1.68±5.05	11.46±9.92	0.21±0.25	0.00±0.00
10	11.81	2-Picolinic acid	0.00±0.00	0.00±0.00	0.09±0.16	0.00±0.00
11	13.45	Adipic acid	0.00±0.00	0.00±0.00	0.75±1.22	0.00±0.00
12	13.54	Butanoic acid	0.07±0.11	0.00±0.00	0.07±0.06	0.00±0.00
13	14.11	4-methylbenzoate (1TMS)	0.08±0.03	0.11±0.19	0.00±0.00	0.00±0.00
14	15.55	Pentanedioic acid	0.00±0.00	0.00±0.00	0.06±0.11	0.00±0.00
15	16.25	3,4-Dihydroxybutanoic acid (3TMS)	0.00±0.00	0.00±0.00	0.08±0.15	0.00±0.00
16	17.58	Salicylic acid	0.00±0.00	0.00±0.00	0.00±0.00	0.08±0.02
17	20.82	Tartaric acid (2TMS)	0.22±0.19	0.00±0.00	0.24±0.11	0.05±0.00
18	22.40	Octanedioic acid (2TMS)	0.00±0.00	0.00±0.00	0.25±0.13	0.00±0.00
19	24.47	Azelaic acid (2TMS)	0.00±0.00	0.00±0.00	1.56±0.22	0.00±0.00
20	35.59	Myristic acid (2TMS)	1.75±2.37	0.25±0.07	0.00±0.00	0.00±0.00
	Fatty acids					
21	25.28	Tetradecanoic acid (1TMS)	0.05±0.09	0.00±0.00	0.10±0.17	0.00±0.00
22	28.97	Hexadecanoic acid	11.81±2.13	5.45±0.26	5.99±4.31	0.49±0.59
23	30.66	Heptadecanoic acid (1TMS)	0.24±0.22	0.08±0.07	0.24±0.42	0.00±0.00
24	32.12	9,12-Octadecadienoic acid (1TMS)	0.24±0.20	0.00±0.00	0.10±0.10	0.00±0.00

Continued

25	32.29	Oleic acid (1TMS)	0.15±0.25	0.07±0.13	0.32±0.12	0.00±0.00
26	32.62	Lauric acid (2TMS)	0.28±0.48	0.00±0.00	0.00±0.00	0.00±0.00
27	36.15	Eicosanoic acid	0.52±0.48	0.18±0.15	0.18±0.17	0.00±0.00
28	38.17	2-Monopalmitoylglycerol	3.65±5.24	0.00±0.00	0.00±0.00	0.00±0.00
29	40.96	2-Monostearin	2.97±3.54	0.92±0.28	0.00±0.00	0.00±0.00
30	41.32	Octadecanoic acid	15.36±4.83	12.88±1.99	9.19±2.45	0.44±0.28
	Sugars					
31	19.89	2,3,4-Trihydroxybutyric acid (4TMS)	0.00±0.00	0.00±0.00	0.07±0.07	0.00±0.00
32	25.08	Ribonic acid (5TMS)	0.00±0.00	0.16±0.29	0.00±0.00	0.00±0.00
33	29.51	Galactopyranose (5TMS)	0.00±0.00	6.01±10.41	7.26±6.67	0.00±0.00
34	52.03	Maltose	0.00±0.00	0.00±0.00	0.14±0.24	0.00±0.00
	Amino acids					
35	18.18	5-oxo-Proline, (2TMS)	0.17±0.29	1.29±1.65	0.61±0.52	0.01±0.01
36	18.27	2-pyrrolidone carboxylic acid	0.08±0.07	0.00±0.00	0.00±0.00	0.00±0.00
	Hydrocarbons					
37	12.49	Tridecane	0.00±0.00	0.00±0.00	1.06±1.83	0.00±0.00
38	20.46	Hexadecane	0.13±0.13	0.00±0.00	0.03±0.04	0.00±0.00
39	24.75	Octadecane	0.09±0.16	0.00±0.00	0.15±0.25	0.00±0.00
40	40.13	Heptacosane	0.00±0.00	0.07±0.06	0.22±0.26	0.00±0.00
	Alcohols					
41	6.82	Ethanol, 2-methoxy-, carbonate	0.00±0.00	0.00±0.00	8.41±14.57	0.00±0.00
42	12.48	Silanol, trimethyl-, phosphate (3:1)	0.39±0.24	0.41±0.35	0.39±0.68	0.00±0.00
43	23.19	Ribitol (5TMS)	0.82±1.42	1.76±2.98	0.00±0.00	0.00±0.00
44	27.67	Butanol (4TMS)	0.00±0.00	0.12±0.10	0.00±0.00	0.00±0.00
45	31.32	Myo-Inositol (6TMS)	0.09±0.07	0.50±0.58	0.05±0.09	0.00±0.00
	Others					
46	5.04	Tris Borate (1TMS)	0.00±0.00	0.33±0.58	0.33±0.58	0.66±1.15
47	8.49	Azulene	0.00±0.00	0.00±0.00	0.48±0.45	0.00±0.00
48	9.08	2,4-dimethyl benzaldehyde,	0.11±0.19	0.00±0.00	0.00±0.00	0.00±0.00
49	9.98	Urea (3TMS)	0.99±1.15	0.00±0.00	0.08±0.09	0.00±0.00

Continued

50	10.60	2-(2-Butoxyethoxy)ethoxy (1TMS)	0.87±0.78	0.46±0.40	0.00±0.00	0.00±0.00
51	11.05	Nicotinic acid	0.00±0.00	0.00±0.00	0.06±0.09	0.00±0.00
52	16.62	Tris(hydroxymethyl)aminomethane (3TMS)	0.47±0.14	3.75±6.49	1.55±0.96	0.00±0.00
53	17.66	Amine (3TMS)	0.12±0.21	0.00±0.00	0.07±0.11	0.00±0.00
54	18.97	Propachlor	0.00±0.00	0.09±0.08	0.00±0.00	0.00±0.00
55	19.47	Silanamine (4TMS)	0.00±0.00	1.85±2.09	0.21±0.25	0.00±0.00
56	39.79	Silane	0.00±0.00	0.00±0.00	0.06±0.10	0.00±0.00

Values are expressed as mean ± standard deviation. TMS, trimethylsilylation.

The number of metabolites detected was more in non-bacterized plant REs than in RP2-bacterized plant REs. Only six metabolites (Fig. 6.1D) were common in all the four samples (12d-C, 12d-RP2, 24d-C, and 24d-RP2), with variation in their abundance. Fifteen metabolites were identified in at least three samples. Nine metabolites were identified in both 12d-C and 24d-C samples, however, none of the metabolites was in common between 12d-RP2 and 24d-RP2 REs. Three metabolites were present in both 24d-C and 24d-RP2, no metabolite was in common between 12d-C and 12d-RP2 samples. Thirty metabolites were detected only in one sample (among them 5 metabolites in 12d-C, 25 in 24d-C, 4 in 12d-RP2 and 3 in 24d-RP2 samples). However, the abundance of particular compounds varied among the different treatments.

6.2.2. RP2 interaction-specific metabolites

There was a significant change in eight metabolites *viz.*, octadecanoic acid, hexadecanoic acid, tartaric acid, myristic acid, benzoic acid, salicylic acid, azelaic acid, and octanedioic acid. Out of eight metabolites detected, relative levels of benzoic acid and salicylic acid were more at 12d-RP2 and 24d-RP2 REs, respectively (Fig. 6.2). The relative content of hexadecanoic acid and octadecanoic acid (commonly referred as palmitic acid and stearic acid) decreased in RP2-bacterized REs, irrespective of plant age. Two more metabolites, myristic and tartaric acid also decreased in bacterized plants but not detected in 12d-RP2 samples. Azelaic and octanedioic acid were detected only in 24d non-bacterized (24d-C) REs (Fig. 6.2). The data indicated an important role for these compounds in groundnut-RP2 beneficial interaction.

6.2.3. Interaction-specific metabolites in RP2 physiological characters

The potential signals which possibly affect the root colonization of RP2 were identified based on the difference in abundance of the metabolites in RP2-bacterized *vs.* non-bacterized REs (Fig. 6.2). Further, the effect of six metabolites (except azelaic acid and octadecanoic acid) on chemotaxis, bacterial growth, biofilm formation and root colonization was tested by using commercially available pure compounds. All the six compounds supported *in vitro* growth of RP2. Bacterial growth was maximum at 24 h for four metabolites, while palmitic acid and benzoic acid supported maximum growth at 48 h and 72 h, respectively (Fig. 6.3B).

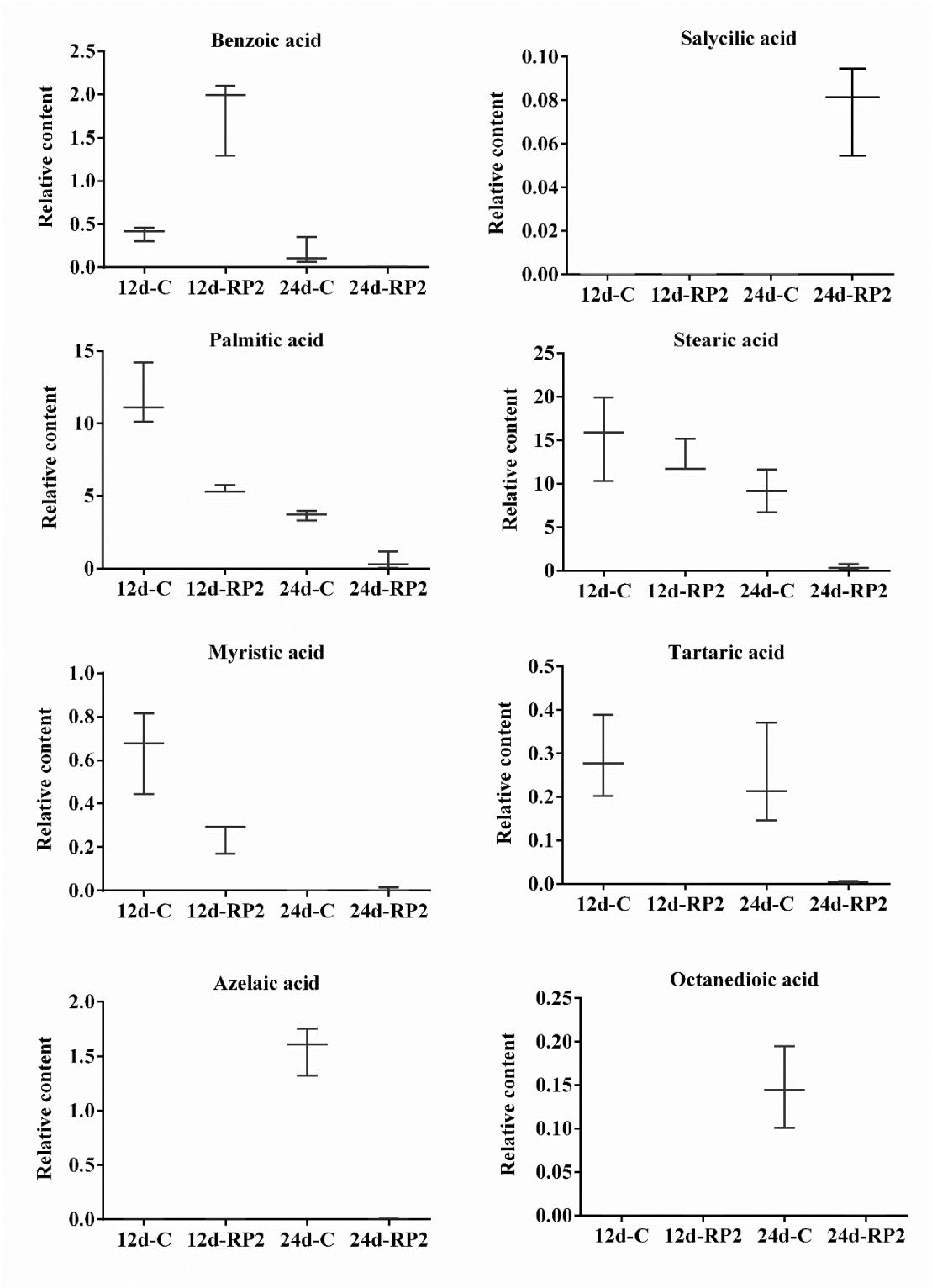

Fig. 6.2: Representation of relative contents of metabolites in root exudates of *Pseudomonas* sp. (RP2) bacterized or non-bacterized groundnut seedlings.

Myristic acid, benzoic acid and salicylic acid enhanced RP2 colonization on groundnut roots (Fig. 6.3D), and enhanced biofilm formation of RP2 (Fig. 6.3C) although they were not chemo-attractants. However, increase in chemotaxis occurred at 10 µM of benzoic acid and was

negligible at a higher dose (Fig. 6.3A). Except for salicylic acid, both myristic acid and benzoic acid supported *in vitro* growth of RP2 (Fig. 6.3B). Although stearic acid and palmitic acid levels in RP2-bacterized REs was less compared with that of non-bacterized REs, both the chemicals have shown a dose-dependent increment in chemotaxis, bacterial growth, biofilm formation and root colonization. Even though tartaric acid supported the *in vitro* growth of RP2, it has not supported chemotaxis, biofilm formation and bacterial recruitment to the groundnut roots (Fig. 6.3).

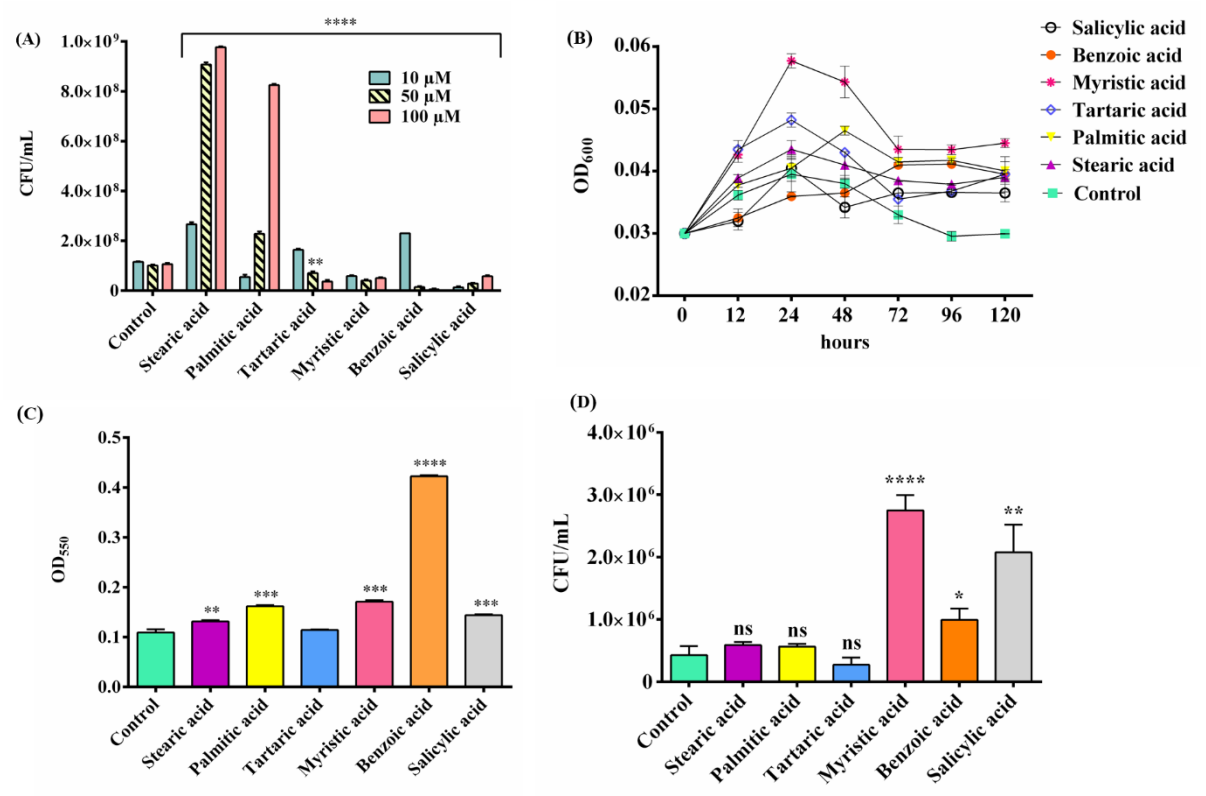

Fig. 6.3: Effect of selected metabolites from groundnut root exudates on *Pseudomonas* sp. (RP2). Selected metabolites in the root exudates of groundnut that showed significant modulation during groundnut-*Pseudomonas* sp. (RP2) interaction were further tested with respective pure chemicals: (A) Chemotaxis of RP2 towards test compounds at different concentrations (10, 50, and 100 µM). Bacterial count was represented as CFU/mL, n=3 (B) Growth of RP2 in presence 100 µM of test compound. Data was recorded at regular intervals from 0 to 120 h with the help of spectrophotometer at OD_{600}, n=3 (C) Effect of test compound on biofilm formation was checked by incubating RP2 in 100 µM concentration for 3 days, n=6 and (D) Root colonization of RP2 in presence of 100 µM of test compound, n=3. Error bars indicate standard deviation. The data was subjected to two-way ANOVA followed by Dunnett's multiple comparisons test for chemotaxis; t-test was applied for biofilm formation and colonization studies 'ns' if $P>0.05$, *$P\leq0.05$, ** $P\leq0.01$,***$P\leq0.001$, **** $P\leq0.0001$.

6.2.4. Root proteins varied by RP2 interaction

Root proteome of groundnut was varied up on RP2 bacterization. From the 2DE analysis 250 proteins were resolved. The proteins detected with a ratio of ≥1.5 in non-bacterized *vs.* RP2-bacterized root proteome were considered as up-regulated and ≤0.5 as down-regulated. A total of 11 proteins, differentially expressed (9 up-regulated and 2 down-regulated) during groundnut-RP2 beneficial interaction (Fig. 6.4), were identified using MALDI-TOF MS/MS.

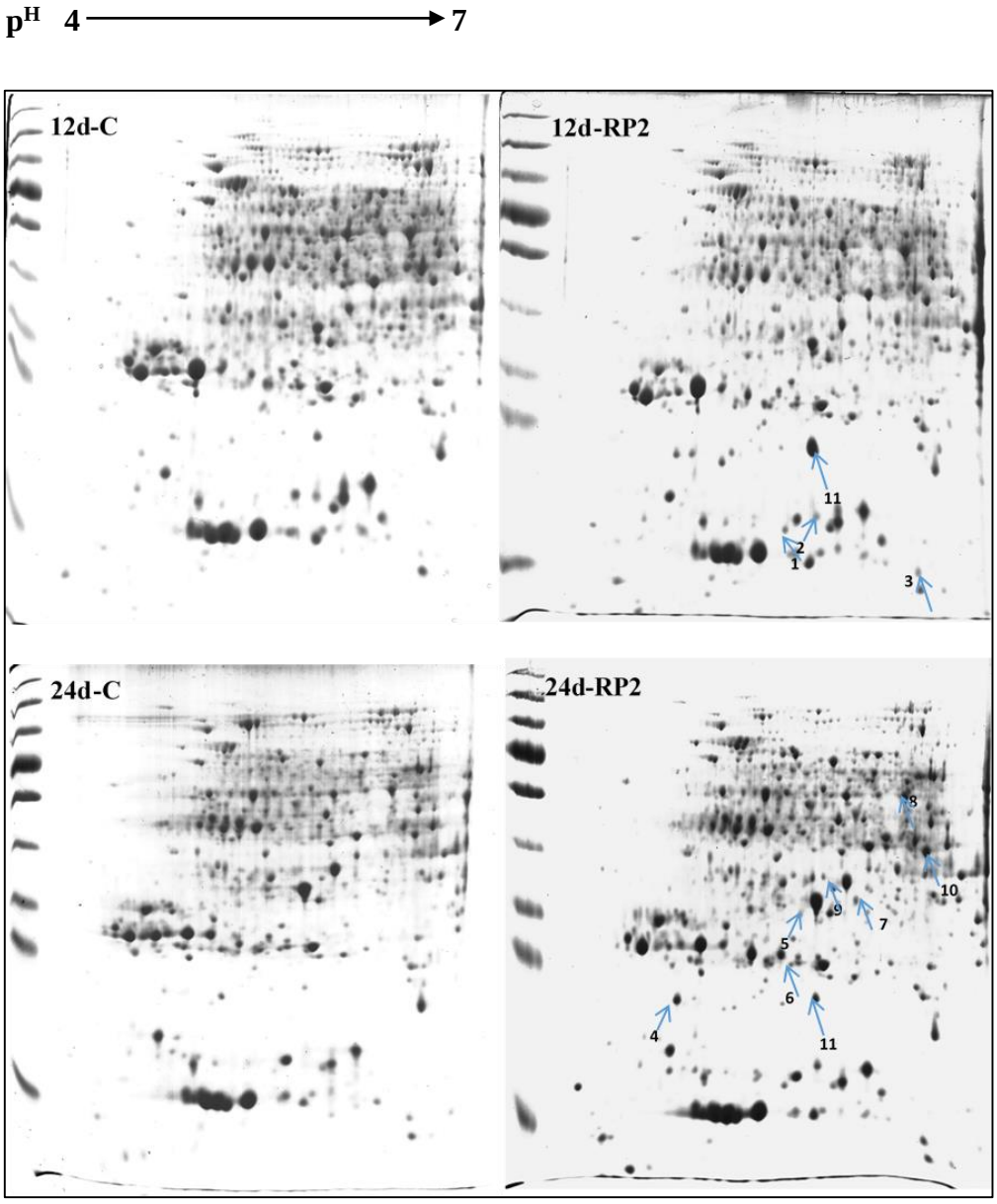

Fig. 6.4: Representative 2DE gels of root proteome in *Pseudomonas* sp. (RP2) bacterized or non-bacterized groundnut. Root proteins of RP2 bacterized and non-bacterized groundnut roots were profiled on 11 cm IPG strips. Protein spots were visualized on 12% SDS-PAGE by Coomassie blue staining. Top and bottom panels represents the 2DE maps of 12 and 24 days

after sowing samples. Left and right panels represent non-bacterized and RP2-bacterized proteome maps, respectively. Arrows indicate differentially regulated proteins.

In 12d-RP2 treated root proteome, hypothetical protein and Class II small heat shock protein Le-HSP17.6 were up-regulated and protein Ycf2 allergen Ara h1 was down-regulated. Whereas, in 24d-RP2, Glutathione S-transferase F9-like (GST), ascorbate peroxidase (APX), enolase, formate dehydrogenase, thaumatin-like protein (TLP), SEC14 cytosolic factor family protein were up-regulated and magnesium-chelatase subunit ChlH was down-regulated. Only chitinase (Chi II) was up-regulated in both 12d-RP2 and 24d-RP2 samples (Table 6.2). The Chi II transcripts increased (Fig. 6.5) upon RP2 bacterization, in agreement with the proteomic data. While APX had a different trend in transcript abundance, recorded by qRT-PCR, compared with 2D gel-based proteome analysis. This may be due to posttranscriptional or post-translational modifications which may have led to the altered steady-state protein amounts.

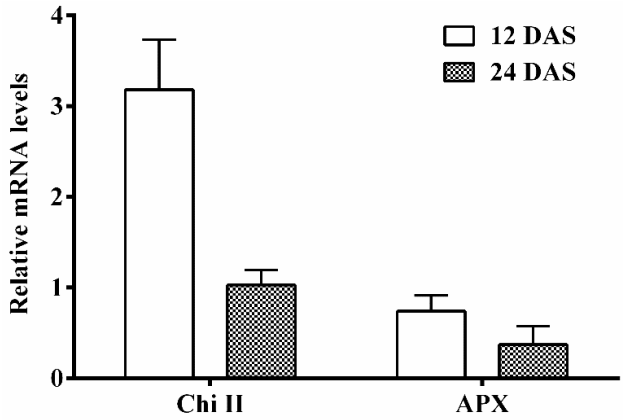

Fig. 6.5: **Real-time quantification of root proteins differentially expressed in bacterized with *Pseudomonas* sp. (RP2) or non-bacterized groundnut.** RNA was isolated from RP2 bacterized and non-bacterized groundnut roots at 12 and 24 days after sowing (DAS). cDNA was prepared and Chi II, APX gene levels were quantified using polymerase chain reaction. Error bars indicate standard deviation (n=3).

6.2.5. Effect of the selected RE-associated chemicals on *S. rolfsii* growth

The decrease of six metabolites exudation might be due to the utilization of these metabolites by RP2 for growth, chemotaxis or biofilm formation. The increased benzoic acid and salicylic acid could play a role in *S. rolfsii* inhibition. Antifungal test was carried out against *S. rolfsii* with benzoic acid, salicylic acid, propanoic acid and furoic acid detected in REs (propanoic acid and furoic acid levels were not statistically significant). The test compounds inhibited *S. rolfsii* growth. Among the test compounds, salicylic acid showed a highest of 89% fungal growth inhibition at 1 mM, followed by propanoic acid, benzoic acid and furoic acid with 37%,

17%, and 9%, respectively (Fig. 6.6). Similar results were observed with 0.1 mM of test compounds except for propionic acid. Ten mM of all the test compounds inhibited fungal growth by 88% suggesting a definite role for these compounds in suppression of groundnut stem rot pathogen growth.

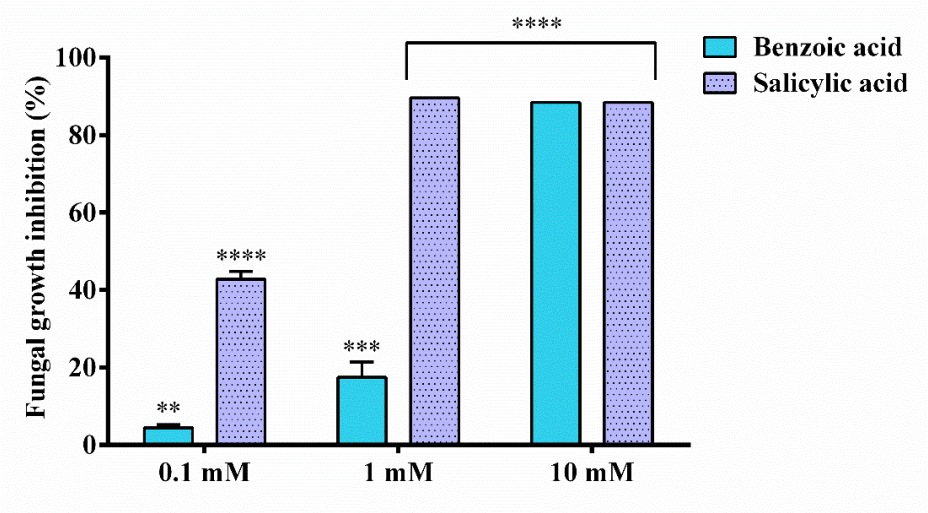

Fig. 6.6: Effect of selected metabolites from groundnut root exudates on the growth of *Sclerotium rolfsii* growth. Growth inhibition of *S. rolfsii* by selected metabolites from the root exudates of groundnut was tested at three different concentrations (0.1, 1, and 10 mM). The percentage of growth inhibition with respect to control, after 3 days of *S. roflsii* growth was plotted. Error bars indicates standard deviation (n=3). Statistical analysis was performed using t-test with P<0.05. ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

6.3. Discussion

6.3.1. Alteration in root exudates profile of RP2-bacterized and non-bacterized plants

Plants secrete different chemical compounds through REs for a variety of purposes. The two major benefits for plants with REs is to chemo-attract beneficial bacteria towards roots to enhance their colonization over other organisms and allow them to establish symbiotic relationship with plants. There are different methods in practice to collect REs like hydroponic growth and sampling (Badri et al., 2013), soil growth-hydroponics sampling (Mwita et al., 2016; Sun et al., 2016), soil growth- rhizobox sampling (Neumann et al., 2014). We have followed soil grown-hydroponic approach to collect REs. Though this method has limitations (slight damage to root during excavation), is more suitable to field samples in evaluating plant-microbe interactions by whole community metabolomics, as well as assessment of comparative metabolome data by targeted or untargeted approaches (Oburger and Schmidt, 2016).

Table 6.2 Proteins differentially expressed in RP2-bacterized groundnut roots

Spot No.	Gene identity	Putative protein name	Fold change		Function
	Proteins specifically regulated at 12 DAS				
1	gi/293335003	Hypothetical protein	1.53		-
2	ycf2_PHAVU	Protein Ycf2, Allergen Ara h 1	0.19		Peanut allergin
3	gi/194466081	Class II small heat shock protein Le-HSP17.6	1.65		Heat shock protein
	Proteins specifically regulated at 24 DAS				
4	gi/441482380	Thaumatin-like protein	3.4		Defense to biotic/abiotic stress, PR protein, hydrolysing β-1,3- glucans
5	gi/590603864	SEC14 cytosolic factor family protein	4.5		Transporter activity, lipids binding and root hair elongation
6	CHLH_ORY SI	Magnesium-chelatase subunit ChlH	0.49		Positive regulator in seed germination, post-germination growth
7	gi/356526968	Glutathione S-transferase F9-like	2.11		Antioxidant, herbicide detoxification, express in stress, pathogen attack and PGPR treatment
8	gi/357463525	Ascorbate peroxidase	2.3		Antioxidant, expresses in PGPR treatment
9	gi/351724891	Enolase	3.34		Carbohydrate metabolism
10	gi/502090619	Formate dehydrogenase	2.4		Catabolism of methanol
	Proteins specifically regulated at both 12 and 24 DAS				
11	gi/1237027	Chitinase	**12 DAS**	**24 DAS**	Anti-fungal, PR protein
			15	2.3	

Organic acids released in to the rhizosphere play major role over other metabolites in supporting bacterial growth and chemotaxis (Jones, 1998). In the present study, six organic acids and two fatty acids showed variation in REs during groundnut-RP2 beneficial interaction. Out of these, benzoic acid and salicylic acid were more in RP2-bacterized root exudates, whereas, tartaric acid was abundant in non-bacterized REs. Role of stearic, palmitic, myristic and tartaric acid in bacterial chemotaxis, biofilm formation and root colonization are not known as compared with malic, fumaric and citric acids (Badri and Vivanco, 2009). For instance, Yuan et al., (2015) reported role of malic acid and fumaric acid of banana REs, in chemotactic response and induced biofilm formation of *B. amyloliquefaciens* NJN-6. Sood (2003) showed the preferential chemotaxis of *P. fluorescens* towards organic acids (citric acid and malic acid) and *Azotobacter chroococcum* towards sugars of tomato REs. Similarly, *P. fluorescens* WCS365 and *B. amyloliquefaciens* NJN-6 move towards tomato and banana REs (Yuan et al., 2015).

In the present study, RP2 showed the highest chemotaxis towards stearic acid and palmitic acid that not promoted maximum bacterial growth but promoted recruiting them on to the root surface (Fig. 6.3). Benzoic acid also showed a similar impact on RP2 except in chemo-attraction. (Liu et al., 2015) reported increase in palmitic acid resulted in chemotaxis and root colonization, while bacterial recruitment to fescue roots declined in presence of benzoic acid and stearic acid. However, in groundnut-RP2 beneficial interaction, RP2 chemotaxis was more towards stearic acid and palmitic acid, while myristic acid, palmitic acid and tartaric acid supported *in vitro* RP2 growth. The recruitment of RP2 increased significantly in myristic acid, salicylic acid, and benzoic acid.

Two different species of Bacilli from cucumber and banana rhizosphere, that showed chemotaxis, improved biofilm in presence of organic acids citric and fumaric acids (Zhang et al., 2014b). *Klebsiella* sp. D5A showed highest chemotaxis towards palmitic acid as in our observation with *Pseudomonas* sp. (RP2) (Fig. 6.3A), followed by benzoic acid and stearic acid detected in REs of *Festuca arundinacea* L. (Liu et al., 2015). High tartaric acid and citric acid levels detected in Cu-tolerant *Ricinus communis* indicated their role in abiotic stress (Huang et al., 2016). Azelaic acid was reported as a plant systemic immunity component, involved in inducing accumulation of salicylic acid during infection (Jung et al., 2009). Thus, the decrease in azelaic acid and increase in salicylic acid in RP2-bacterized REs indicated their role in inducing defense responses in groundnut.

6.3.2. RP2 induced proteome changes in groundnut roots

Alterations in root proteome of groundnut induced by RP2 bacterization was performed through 2D gel electrophoresis. Among the significantly modulated proteins, two defense-related proteins, Chi II and TLP, known to have antifungal activity (Vigers et al., 1992; Sturrock and Ekramoddoullah, 2010) were induced in RP2-bacterized plants. Increased expression of chitinase gene in the beneficial interaction of tobacco-*Bacillus* sp. and rice-*Aeromonas* sp. was also known (García-Cristobal et al., 2015; Kim et al., 2015). Transgenic wheat and tobacco lines with constitutive expression of rice and groundnut TLP genes showed enhanced resistance to fungal pathogens *Fusarium graminearum* and *Rhizoctonia solani,* respectively (Chen et al., 1999; Singh et al., 2013). A mitochondrial protein formate dehydrogenase involved in the oxidation of formate into CO_2 (Herman et al., 2002) was induced up on RP2 bacterization. Accumulation of formate dehydrogenase transcripts in potato leaves by wounding (Hourton-Cabassa et al., 1998) and in *Arabidopsis* up on salicylic acid and methyl jasmonate treatment indicates its role in inducing defense response (Schenk et al., 2000). Similarly, accumulation of Chi II, TLP, and formate dehydrogenase in RP2-bacterized plants (Table 6.1) and salicylic acid (Fig. 6.2) in RP2-bacterized REs, might have a role in inducing resistance against phytopathogens.

Antioxidant-metabolism related proteins GST and APX were up-regulated up on RP2 bacterization. These are multifunctional enzymes that play a key role in detoxification of reactive oxygen species and protect the cell from H_2O_2 induced cell death (Sheehan et al., 2001). Expression of GST increased in *M. truncatula* roots up on *Glomus mossease, P. fluorescens* or *Sinorhizobium meliloti* colonization (Sanchez et al., 2004). Similarly, overexpression of GST occurred in rice-*P. fluorescence* and *Arabidopsis-P. polymyxa* rhizobacterial interactions (Kandasamy et al., 2009; Kwon et al., 2016). Elevated expression of APX was observed in response to abiotic, biotic stress and also in the presence of PGPR like *Bradyrhizobium japonicum* and *Serratia* spp. (Han and Lee, 2005; Mostafa, 2012). Synchronized overexpression of both antioxidant enzymes and defense response proteins in RP2-bacterized plants can be explained to have a role in increasing tolerance of plant towards various environmental stresses.

Enolase is involved in carbohydrate metabolism that may contribute to increased growth and biomass in RP2-bacterized plants. SEC14 plays a role in lipid binding, transporter activity, and homeostasis of phosphoinositides. The SEC14 domain of *A. thaliana,* was involved in proper

root hair elongation and mutations in this gene lead to deformation of root hairs (De Campos and Schaaf, 2017). Exogenous application of trehalose and plant activator benzothiadiazole altered *Arabidopsis* and rice SEC14 transcript levels, respectively (Bae et al., 2005; Chen et al., 2015). Similarly, up-regulation of SEC14 was reported in two inbred lines of Chinese cabbage, in temperature stress (Lee et al., 2013). Therefore, increase of enolase and SEC14 strongly suggested a positive role of these two proteins in plant growth and root development in groundnut-RP2 interaction.

6.3.3. Effect of RP2 inoculation on groundnut in suppression of *S. rolfsii*

In the present study, RP2 inhibited *in vitro* growth of *S. rolfsii* as well as provided resistance against stem rot infection in groundnut. This supports earlier reports of *P. aeruginosa* (GSE 18 and GSE 19 strains) (Kishore et al., 2005c) and *P. fluorescence* that decreased disease incidence against *S. rolfsii* in groundnut (Asadhi et al., 2013). The relative content of benzoic acid and its successive metabolic partner salicylic acid levels increased in RP2-bacterized REs. Benzoic acid was reported as an induced antifungal metabolite in apple fruits up on the attack of *Necfria galligena* (Brown and Swinburne, 1971). Li et al., (2013) reported the increased release of benzoic acid in fungal resistant cultivar REs to that of a susceptible cultivar. The difference in the quantity of benzoic acid was considered to regulate wilt resistance mechanism in the rhizosphere. Salicylic acid is considered as a signalling molecule in plant-microbe interaction. Antifungal action of salicylic acid against *F. oxysporum*, *Eutypa lata* and *Penicillium expansum* by inhibiting spore germination and mycelium growth was reported (Amborabe et al., 2002; Wu et al., 2008; Neto et al., 2015). The increase in benzoic acid and salicylic acid (relative content) in REs and *in vitro* growth inhibition of *S. rolfsii* by benzoic acid and salicylic acid (Fig. 6.2 and 6.6) indicate their role in resistance against *S. rolfsii*.

Our results strongly suggest RP2 as a potential rhizobacterial inoculant for groundnut. Significant changes in groundnut roots were induced by RP2 bacterization. Exudation of metabolites in to the REs was affected by RP2 bacterization along the plant developmental stage. Groundnut exuded stearic acid and palmitic acid into REs that act as chemo-attractants of RP2 along with myristic acid which might support bacterial growth and root colonization. After establishing on groundnut roots, RP2 further increased the exudation of antifungal metabolites like benzoic acid and salicylic acid. Plant defense against *S. rolfsii*, in presence of RP2, might be due to induced expression of defense-related root proteins Chi II, TLP along with exudation of benzoic acid and salicylic acid (Fig. 6.7). Over all the RP2-induced changes

in REs and root proteome have implications in root colonization, suppression of pathogen, promotion of plant growth and activation of defense response against pathogens.

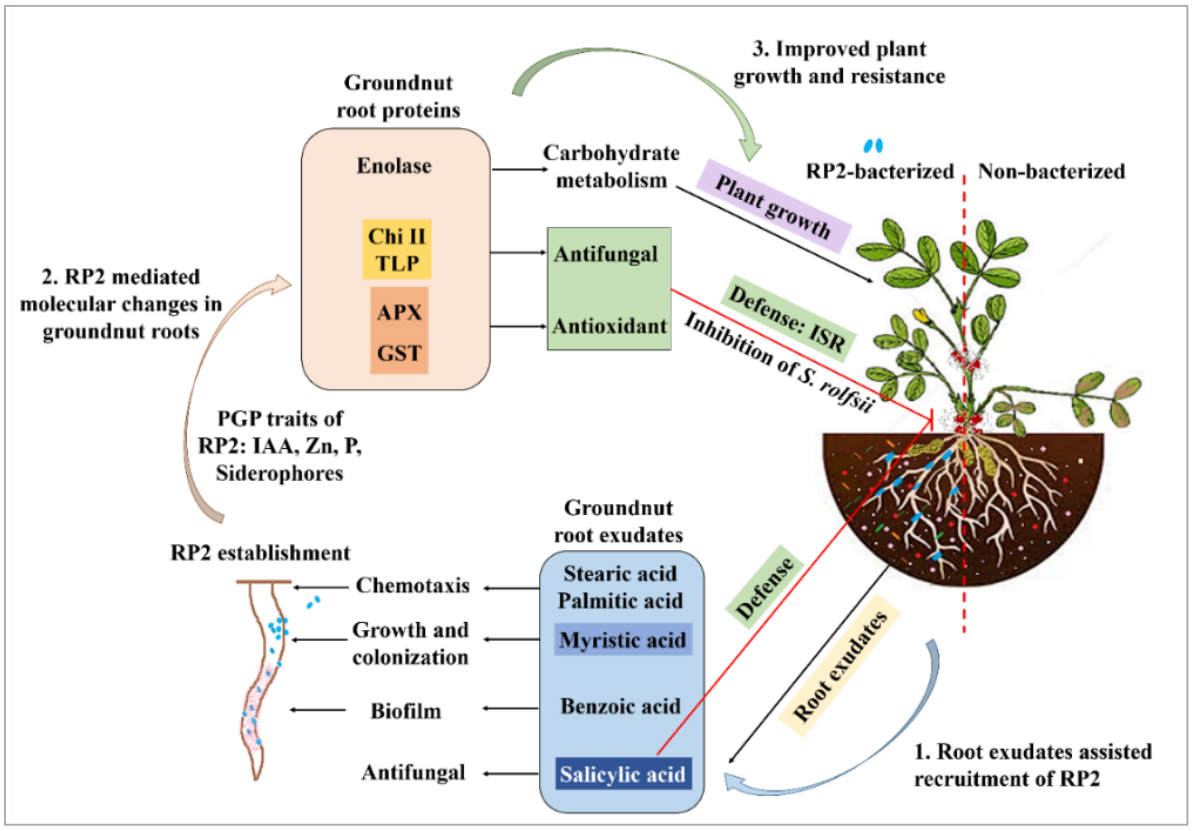

Fig. 6.7: Proposed model on groundnut- ***Pseudomonas*** **sp. (RP2) interaction**

Groundnut and RP2 interaction driven by the plant root exudates and RP2-mediated molecular changes in groundnut root with potential role in plant growth and defense. Root exudate metabolites of groundnut enhanced root colonization of RP2. Stearic acid and palmitic acid from the REs promoted RP2 chemotaxis and growth. Colonization was promoted by myristic acid, while benzoic acid induced biofilm formation on groundnut roots. The colonized RP2 improved plant growth by producing IAA, Zn, P, and siderophores and further induced the release of antifungal benzoic acid and salicylic acid from groundnut root exudates. Parallelly RP2 colonization has also increased the expression of defense-related Chi II and TLP proteins and antioxidant APX and GST proteins in the roots.

7. Summary and conclusions

Tthe use of PGPR is a sustainable agricultural practice to improve the crop yield and boost plant immunity against pathogenic microbes. Irrespective of numerous PGPR characterized for various plants, their application will yield an irreproducible outcomes in the field conditions. This might be due to the poor understanding of plant-PGPR interactions at molecular level. The specific attraction of a rhizobacteria towards a plant or enhanced plant growth promotion by a particular rhizobacteria is like a chemical lock and key between them through the signaling molecules. These interaction specific key signalling molecules are still not elucidated. Therefore, finding the molecular level variations in the plant and PGPR is highly desirable. As each plant has its own microbial web in its habitat, characterizing plant specific potent PGPR is mandatory for further molecular understanding. We identified two efficient groundnut PGPR *Pseudomonas* spp. (RP2) and *Bacillus sonorensis* (RS4), and elucidated their interaction specific metabolites and proteins. In addition, an efficient chitosan based liquid formulation of *B. sonorensis* (RS4) was developed and evaluated.

7.1. Finding potent PGPR and development of liquid formulation

To identify the potent PGPR of groundnut, fourteen different fields were sampled for rhizosphere soil, non-rhizosphere soil, and whole plants. A total of 126 groundnut-associated bacteria were isolated and screened for eleven different plant growth promoting traits *in vitro*. The RP2 isolate of groundnut rhizoplane was positive for most of the traits, besides showed an efficient antibiosis towards diverse phytopathogenic bacteria and fungi. RP2 was identified as *Pseudomonas* spp. (RP2) by 16S rDNA analysis. Selected PGPR isolates including RP2 were screened for groundnut growth promotion. Plants germinated from seeds bacterized with *Pseudomonas* spp. (RP2) and *B. sonorensis* (RS4) had significantly higher plant biomass (including individual shoot length, root length, and dry weight), leaf chlorophyll content, and root morphological parameters (root volume, surface area, and length). Both the isolates efficiently colonized the groundnut root surface and detected with the help of SEM imaging. Apart RP2 and RS4 provided resistance against stem rot pathogen *S. rolfsii*. However, RP2-bacterized groundnut plants were highly able to withstand stem wilt.

Although *B. sonorensis* (RS4) was not isolated from the groundnut habitat, it displayed an efficient growth promotion and root colonization of groundnut along with previous report on tomato and chilli plants. This indicates the non-specific growth promotion ability of RS4 in

many crop plants. The active ingredients for the preparation of RS4 liquid formulation were identified, different combinations of these ingredients were tested for cells viability, and shelf-life. Chitosan- amended liquid formulations were efficient in keeping RS4 cells viable for longer time of storage over other tested compounds. Therefore, chitosan based RS4 liquid bioformulation can facilitate increase in the crop yields.

7.2. Root exudates metabolites specific to groundnut- PGPR interaction

As root colonization is a competitive process influenced by host genotype, the outcome of any plant- microbe interaction depends on the chemical composition of the plant REs. The role groundnut REs in RS4 and RP2 growth, chemotaxis, colonization, and biofilm formation were assessed. Both the PGPR isolates were influenced by 12d and 24d-REs, significantly increased all the physiological processes. RP2 isolate displayed the notable increase of parameters within 12d and 24d-REs, which was not detected by RS4 isolate. Besides, higher chemotaxis of RP2 over RS4 was detected in both 12d and 24d-REs. The growth and biofilm induction of RS4 were highly triggered by groundnut REs over RP2 isolate. This shows the selective role of REs by facilitating different processes of RP2 and RS4, even though both were potent PGPR of the groundnut. Thus REs might modulates the PGPR physiological characteristics by their chemical composition.

An attempt was made to characterize the RS4 and RP2-bacterized groundnut REs to identify the interaction specific metabolites. The bacterized and non-bacterized plant REs showed varied profiles along the plant development that are detected by GC-MS/MS. The number metabolites detected was more in non-bacterized plant REs, though the cumulative levels of specific group of metabolites detected were decreased in control REs. At the same time the levels of all the groups increased or constant in 24d-REs than 12d-REs. Multivariate analysis of the GC-MS/MS data, using PCA, PLS-DA, and clustering analysis clearly segregated the REs profiles. Metabolites like organic acids, sugars, fatty acids, hydrocarbons, alcohols, and amino acids were detected in REs. The cumulative levels of organic acids, sugars, and amino acids were significantly modulated in RP2-bacterized REs, whereas hydrocarbons in RS4-bacterized REs.

Benzoic acid and salicylic acid were identified as interaction specific metabolites of RP2 and detected more in bacterized plant REs. They displayed antifungal activity against *S. rolfsii* and inhibited the mycelia growth. Other metabolites like myristic acid, stearic acid, and palmitic

acid were identified for their RP2 growth promotion, chemotaxis, colonization, and biofilm induction. Numerous other metabolites levels were increased in bacterized plant REs specifically detected in bacterized plant REs. Likewise, threonine and glyoxylic oxime acid specific to RP2 bacterization while 5-methyluridine and 9, 12-octadecadienoic acid specific to RS4. Serine, D-glucopyranoside, pentanoic acid, and 2-pyrrolidinone specific to bacterization by both RS4 and RP2. Proline, valine, urea, benzoic acid, propanedioic acid, tartaric acid, and pentanedioic acid were increased by PGPR bacterization. PGPR bacterization specifically influenced the exudation pattern of plant REs with respect to individual metabolites and concentrations. Therefore groundnut and PGPR modified their metabolite profiles and physiological parameters in interaction to support each other for beneficial association.

7.3. Total root protein changes during groundnut- PGPR interaction

The plant- PGPR interactions take place primarily on the root surface. Understanding the root proteomic changes during PGPR interaction will provide an insight into underlying molecular events. Groundnut total root proteins were evaluated by RS4 and RP2 bacterization after 12 and 24 DAS. In total 28 proteins were differentially expressed and identified through MALDI-TOF MS/MS. Two defense-related proteins pentatricopeptide repeat-containing protein in 12d-RS4 and class II small heat shock protein in 12d-RP2 were up- regulated, while Allergen Ara h 1 was down regulated in 12d-RS4 and 12d-RP2 samples.

In RS4 interaction with groundnut, wound-induced protein WIN2 was down-regulated, whereas proteins including β-1,3-glucanase, Hsp70, chitinase, pleiotropic drug resistance protein 1, thioredoxin, β-fructofuranosidase of defense response, sucrose synthase 4, phospho-2-dehydro-3-deoxyheptonate aldolase of growth related, Ran3 GTP binding protein, nuclear export mediator factor of transporting function, BnaC03g33460D, α-methyl-mannoside-specific lectin, allergen Ara h 1 were up-regulated. While, four proteins annotated as glutathione S-transferase, ascorbate peroxidase of defense response, enolase, and formate dehydrogenase of growth related proteins were up-regulated in 24d-RP2. The higher expression of antifungal enzyme chitinase was detected in both 12d and 24d-RP2 roots. Thaumatin-like protein and SEC14 cytosolic factor family protein were up-regulated in 24d-RP2, but they were down-regulated in 24d-RS4 roots. The simultaneous higher expression of defense and growth related proteins upon PGPR bacterization, illustrate the PGPR triggered plant defense activation and growth promotion in groundnut. This was physiologically evident from the growth promotion and antifungal resistance observed in presence of PGPR.

7.4. Bacterial cell wall proteins changed in presence of groundnut root exudates

PGPR respond to the plant signals passed through the REs by modulating their cellular proteins especially the cell wall related proteins where the interaction takes place. Proteomic approach helps to understand the molecular level variations in PGPR cell wall by an unbiased and comparative way. We identified 24 differentially expressed RS4 proteins that include typical and non-canonical cell wall components apparently related with variety of cellular functions. The differentially expressed proteins were related to amino acids synthesis (aspartate kinase, 2,3,4,5-tetrahydropyridine-2,6 dicarboxylate N-succinyl transferase met-tRNAi formyl transferase, and glutamate 5-kinase 2), signalling (aspartate kinase), antioxidant (alkyl hydroperoxide reductase), carbohydrate metabolism (β-*N*-acetylglucosaminidase and formate acetyltransferase), transporters (ABC transporter, PTS sugar transporter subunit IIA, multidrug transport AcrB, and a putative outer membrane porin), morphological (glutamate decarboxylase and spore coat protein O), and antibiosis- related secondary metabolites synthesizing/transporting proteins (oxalate decarboxylase and polyketide synthase).

All detected proteins of RS4 cell wall fraction were induced in presence of groundnut 12d or 24d-REs. The higher expression of chemotaxis triggering CWP of RS4 in presence of REs and enhanced RS4 chemotaxis observed in presence of REs connects the link between plant elicited RS4 movement for interaction. Many transporter proteins were highly expressed in presence of REs presumably for the uptake of metabolites present in the REs for bacterial cellular functions. Apart, REs also activated the antibiosis related proteins to antagonise pathogens for plant protection.

7.5. Conclusions

- *Pseudomonas* sp. (RP2) and *Bacillus sonorensis* (RS4) are potent groundnut PGPR for improving plant growth and significantly influenced root morphological parameters by efficiently colonizing the roots.
- A higher concentration of chitosan promoted the longer shelf life of the broad spectrum PGPR RS4 liquid formulation. This can be a potent alternative to chemical fertilizers.
- Groundnut REs promoted PGPR growth, biofilm formation and chemotaxis. Simultaneously, PGPR bacterization significantly influenced the plant REs profiles with respect to individual metabolite and their concentration.

- Amino acids, sugars, and organic acids were specific groups influenced by RP2 bacterization in groundnut REs. Hydrocarbons could be a common group of metabolites influenced by PGPR bacterization.
- Significant changes in the RS4 cell wall and groundnut root proteome were detected with respect to growth promotion and defense response.
- Differentially expressed proteins related to transporters, defense, amino acid, and carbohydrate metabolism were enhanced in RS4-groundnut interaction. In RS4, chemotaxis related protein specifically up-regulated in presence of REs.
- RP2-induced changes in REs and root proteins have implications in root colonization, suppression of pathogen, promotion of plant growth and activation of defense response against *S. rolfsii*.

References

Abd-Alla, M.H., El-Enany, A.W., Nafady, N.A., Khalaf, D.M., Morsy, F.M., 2004. Synergistic interaction of *Rhizobium leguminosarum* bv. viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiological Research 169, 49–58.

Afzal, A., Bano, A., 2008. Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). International Journal of Agriculture & Biology 10, 85–88.

Alagawadi, A.R., Gaur, A.C., 1994. Inoculation of Azospirillum brasilense and phosphate-solubilizing bacteria on yield of sorghum (*Sorghum bicolor* L. Moench) in dry land. AGRIS 69, 347–350.

Alstrom, S., Burns, R.G., 1989. Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biology and Fertility of Soils 7, 232–238.

Amborabe, B., Fleurat-lessard, P., Chollet, J., Roblin, G., 2002. Antifungal effects of salicylic acid and other benzoic acid derivatives towards *Eutypa lata* : structure – activity relationship. Plant Physiology and Biochemistry 40, 1051–1060.

Antelmann, H., Engelmann, S., Schmid, R., Hecker, M., 1996. General and oxidative stress responses in *Bacillus subtilis* : cloning, expression, and mutation of the alkyl hydroperoxide reductase operon. Journal of Bacteriology 178, 6571–6578.

Antoun, H., Prevost, D., 2006. Ecology of plant growth promoting rhizobacteria. Springer Netherlands.

Arora, N.K., Khare, E., Maheshwari, D.K., 2010. Plant Growth and Health Promoting Bacteria: constraints in bioformulation, commercialization, and future strategies, in: Maheshwari, D.K. (Ed.), Plant growth and health promoting bacteria, Microbiology. pp. 97–116.

Asadhi, S., Reddy, B.V.B., Sivaprasad, Y., Prathyusha, M., Krishna, T.M., Kumar, K.V.K., 2013. Characterisation, genetic diversity and antagonistic potential of 2, 4-diacetylphloroglucinol producing *Pseudomonas fluorescens* isolates in groundnut-based cropping systems of Andhra Pradesh, India. Archives of Phytopathology and Plant Protection 46, 1966–1977.

Badri, D. V., Chaparro, J.M., Zhang, R., Shen, Q., Vivanco, J.M., 2013. Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. Journal of Biological Chemistry 288, 4502–4512.

Badri, D. V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant, Cell and Environment 32, 666–681.

Bae, H., Herman, E., Bailey, B., Bae, H., Sicher, R., 2005. Exogenous trehalose alters *Arabidopsis* transcripts involved in cell wall modification, abiotic stress, nitrogen metabolism, and plant defense. Physiologia Plantarum 125, 114–126.

Bais, H.P., Park, S.W., Weir, T.L., Callaway, R.M., Vivanco, J.M., 2004. How plants communicate using the underground information superhighway. Trends in Plant Science 9, 26–32.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57, 233–266.

Banaei-asl, F., Bandehagh, A., Uliaei, E, D., Farajzadeh, D., Sakata, K., Mustafa, G., Komatsu, S., 2015. Proteomic analysis of canola root inoculated with bacteria under salt stress. Journal of Proteomics 124, 88–111.

Bano, A., Muqarab, R., 2017. Plant defence induced by PGPR against *Spodoptera litura* in tomato (*Solanum lycopersicum* L.). Plant biology 19, 406–412.

Barnawal, D., Pandey, S.S., Bharti, N., Pandey, A., Ray, T., Singh, S., Chanotiya, C.S., Kalra, A., 2017. ACC deaminase-containing plant growth promoting rhizobacteria protect *Papaver somniferum* from downy mildew. Journal of Engineering and Applied Sciences 12, 3218–3221.

Baroja-Fernandez, E., Munoz, F.J., Li, J., Bahaji, A., Almagro, G., Montero, M., Etxeberria, E., Hidalgo, M., Sesma, M.T., Pozueta-Romero, J., 2012. Sucrose synthase activity in the sus1/sus2/sus3/sus4 *Arabidopsis* mutant is sufficient to support normal cellulose and starch production. Proceedings of the National Academy of Sciences 109, 321–326.

Barratt, D.H.P., Derbyshire, P., Findlay, K., Pike, M., Wellner, N., Lunn, J., Feil, R., Simpson, C., Maule, A.J., Smith, A.M., 2009. Normal growth of *Arabidopsis* requires cytosolic invertase but not sucrose synthase. Proceedings of the National Academy of Sciences 106, 13124–13129.

Bashan, Y., Bashan, L.E., Prabhu, S. R., Hernandez, J.P., 2014. Advances in plant growth-promoting bacterial inoculant technology : formulations and practical perspectives (1998–2013). Plant and Soil 378, 1–33.

Beauregard, P.B., Chai, Y., Vlamakis, H., Losick, R., Kolter, R., 2013. *Bacillus subtilis* biofilm induction by plant polysaccharides. Proceedings of the National Academy of Sciences 110, 1621–1630.

Becker, J.O., Cook, R.J., 1988. Role of siderophores in supression of *Pythium* species and production of increased growth response of wheat by fluorescent Pseudomonads. Phytopathology 78, 778-782.

Belitsky, B.R., Brill, J., Bremer, E., Sonenshein, A.L., 2001. Multiple genes for the last step of proline biosynthesis in *Bacillus subtilis*. Journal of Bacteriology 183, 4389–4392.

Berger, L.R., Stamford, N.P., Santos, C.E.R.S., Freitas, A.D.S., Franco, L.O., Stamford, T.C.M., 2013. Plant and soil characteristics affected by biofertilizers from rocks and organic matter inoculated with diazotrophic bacteria and fungi that produce chitosan. Journal of Soil Science and Plant Nutrition 13, 592–603.

Berger, L.R.R., Stamford, N.P., Willadino, L.G., Laranjeira, D., Lima, M.A.B., Malheiros, S.M.M., Oliveira, W.J., Stamford, T.C.M., 2016. Cowpea resistance induced against *Fusarium oxysporum* f.sp. tracheiphilum by crustaceous chitosan and by biomass and chitosan obtained from *Cunninghamella elegans*. Biological Control 92, 45–54.

Bergmeyer, H.U., Bernt, E., 1963. Lactate dehydrogenase. In: HU Bergmeyer. Methods of Enzymatic Analysis. London: Academic Press. pp. 574-579.

Bertin C., Yang, X., Weston, L.A., 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256, 67–83.

Bhardwaj, D., Ansari, M.W., Sahoo, R.K., Tuteja, N., 2014. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories 13, 1–10.

Biase, D.D., Pennacchietti, E., 2012. Glutamate decarboxylase-dependent acid resistance in orally acquired bacteria: function, distribution and biomedical implications of the gadBC operon. Molecular Microbiology 86, 770–786.

Bieniawska, Z., Barratt, D.H.P., Garlick, A.P., Thole, V., Kruger, N.J., Martin, C., Zrenner, R., Smith, A.M., 2007. Analysis of the sucrose synthase gene family in *Arabidopsis*. The Plant Journal 49, 810–828.

Böhme, K., Li, Y., Charlot, F., Grierson, C., Marrocco, K., Okada, K., Laloue, M., Nogué, F., 2004. The *Arabidopsis COW1* gene encodes a phosphatidylinositol transfer protein essential for root hair tip growth. The Plant Journal 40, 686–698.

Boothby, D., Daneo-moore, L., Higgins, M.L., Coyette, J., Gerald, D., Shockman, G.D., 1973. Turnover of bacterial cell wall peptidoglycans turnover of bacterial cell. Journal of Biological Chemistry 248, 2161–2169.

Brown, A.E., Swinburne, T.R., 1971. Benzoic acid : an antifungal compound formed in Bramley's Seedling apple fruits following infection by *Necfria galligena* Bres. Physiologocal Plant Pathology 1, 469–475.

Butcher, B.G., Helmann, J.D., 2006. Identification of Bacillus subtilis σ^W-dependent genes that provide intrinsic resistance to antimicrobial compounds produced by *Bacilli*. Molecular Microbiology 60, 765–782.

Campos, M.K.F., Schaaf, G., 2017. The regulation of cell polarity by lipid transfer proteins of the SEC14 family. Current Opinion in Plant Biology 40, 158–168.

Capitani, G., Biase, D.D., Aurizi, C., Gut, H., Bossa, F., Grutter, M., 2003. Crystal structure and functional analysis of *Escherichia coli* glutamate decarboxylase. The EMBO Journal 22, 4027–4037.

Cappellari, L.R., Santoro, M.V., Reinoso, H., Travaglia, C., Giordano, W., Banchio, E., 2015. Anatomical, morphological, and phytochemical effects of inoculation with plant growth-promoting rhizobacteria on peppermint (*Mentha piperita*). Journal of Chemical Ecology 41, 149–158.

Chaloupka, J., Kreckova, P., 1971. Turnover of mucopeptide during the life cycle of *Bacillus megaterium*. Folia Microbiologoca. 16, 372–382.

Chanda, G.K., Bhunia, G., Chakraborty, S.K., 2011. The effect of vermicompost and other fertilizers on cultivation of tomato plants. Journal of Horticulture and Forestry 3, 42–45.

Chandrababu, S., Raj, A.D.S., 2009. Rhizosphere microflora of wild yams at Agasthyamalai ranges in southern part of western ghats in India. Journal of Root Crops 35, 182–195.

Chandrasekhar, K., Dileep, A., Lebonah, D.E., Kumari, J.P., 2014. A short review on proteomics and its applications. International Letters of Natural Sciences 17, 77–84.

Chaparro, J.M., Badri, D. V, Bakker, M.G., Sugiyama, A., Manter, D.K., Vivanco, J.M., 2013. Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. Plant Physiology and Biochemistry 72, 169–177.

Charoenlap, N., Shen, Z., Mcbee, M.E., Muthupalani, S., Wogan, G.N., Fox, J.G., Schauer, D.B., 2012. Alkyl hydroperoxide reductase is required for *Helicobacter cinaedi* intestinal colonization and survival under oxidative stress in BALB/c and BALB/c interleukin-$10^{-/-}$ mice. Infection and Immunity 80, 921–928.

Chauhan, H., Bagyaraj, D.J., Selvakumar, G., Sundaram, S.P., 2015. Novel plant growth promoting rhizobacteria- Prospects and potential. Applied Soil Ecology 95, 38–53.

Chen, J., 2006. The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use, Bangkok, 1-11.

Chen, L., Xie, Q.W., Nathan, C., 1998. Alkyl hydroperoxide reductase subunit C (AhpC) protects bacterial and human cells against reactive nitrogen intermediates. Molecular Cell 1, 795–805.

Chen, W.P., Chen, P.D., Liu, D.J., Kynast, R., Friebe, B., Velazhahan, R., Muthukrishnan, S., Gill, B.S., 1999. Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. Theoretical and Applied Genetics 99, 755–760.

Chen, Z., Chen, B., Guo, Q., Shi, L., He, M., Qin, Z., Li, L., He, P., Wang, Z., Hu, D., Yang, S., 2015. A time-course proteomic analysis of rice triggered by plant activator BTH. Journal of Plant Growth Regulation 34, 392–409.

Chrispeels, M.J., Raikhel, N. V, 1991. Lectins, lectin genes, and their role in plant defense. The Plant cell 3, 1–9.

Clore, G.M., Venditti, V., 2013. Structure, dynamics and biophysics of the cytoplasmic proteinprotein complexes of the bacterial phosphoenolpyruvate:sugar phosphotransferase system. Trends in Biochemical Sciences 38, 265–275.

Cohen, A.C., Bottini, R., Piccoli, P.N., 2008. *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in *Arabidopsis* plants. Plant Growth Regulation 54, 97–103.

Coleman, H.D., Ellis, D.D., Gilbert, M., Mansfield, S.D., 2006. Up-regulation of sucrose synthase and UDP-glucose pyrophosphorylase impacts plant growth and metabolism. Plant Biotechnology Journal 4, 87–101.

Coleman, H.D., Yan, J., Mansfield, S.D., 2009. Sucrose synthase affects carbon partitioning to

increase cellulose production and altered cell wall ultrastructure. Proceedings of the National Academy of Sciences 106, 13118–13123.

Collavino, M.M., Sansberro, P.A., Mroginski, L.A., Aguilar O.M., 2010. Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biology and Fertility of Soils 46, 727–738.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Alfaro-Cuevas, R., López-Bucio, J., 2014. *Trichoderma* spp. Improve growth of *Arabidopsis* seedlings under salt stress through enhanced root development, osmolite production, and Na^+ elimination through root exudates. Molecular Plant-Microbe Interactions 27, 503–514.

Cosgrove, K., Coutts, G., Jonsson, I.M., Tarkowski, A., Kokai-Kun, J.F., Mond, J.J., Foster, S.J., 2007. Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in *Staphylococcus aureus*. Journal of Bacteriology 189, 1025–1035.

Crouzet, J., Trombik, T., Fraysse, Å.S., Boutry, M., 2006. Organization and function of the plant pleiotropic drug resistance ABC transporter family. FEBS Letters 580, 1123–1130.

Dadarwal, K.R., 1997. Biotechnological approaches in soil microorganisms for sustainable crop production, in: Dadarwal, K.R (Ed.), Scientific Publishers, pp. 351.

Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil 245, 35–47.

Dam, N.M. Van, Bouwmeester, H.J., 2016. Metabolomics in the rhizosphere : tapping into belowground chemical communication. Trends in Plant Science 21, 256–265.

Dardanelli, M.S., Manyani, H., González-Barroso, S., Rodríguez-Carvajal, M.A., Gil-Serrano, A.M., Espuny, M.R., López-Baena, F.J., Bellogín, R.A., Megías, M., Ollero, F.J., 2010. Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinum* Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. Plant and Soil 328, 483–493.

Das, S., Chou, M., Jean, J., Yang, H., Kim, P.J., 2017. Arsenic-enrichment enhanced root exudates and altered rhizosphere microbial communities and activities in hyperaccumulator *Pteris vittata*. Journal of Hazardous Materials 325, 279–287.

Das, S.N., Dutta, S., Kondreddy, A., Chilukoti, N., Pullabhotla, S.V.S.R.N., Vadlamudi, S., Podile, A.R., 2010. Plant growth-promoting chitinolytic *Paenibacillus elgii* responds positively to tobacco root exudates. Journal of Plant Growth Regulation 29, 409–418.

De Biase, D., Pennacchietti, E., 2012. Glutamate decarboxylase-dependent acid resistance in orally acquired bacteria: Function, distribution and biomedical implications of the *gadBC* operon. Molecular Microbiology 86, 770–786.

De-la-Peña, C., Lei, Z., Watson, B.S., Sumner, L.W., Vivanco, J.M., 2008. Root-microbe communication through protein secretion. Journal of Biological Chemistry 283, 25247–25255.

Denancé, N., Szurek, B., Noël, L.D., 2014. Emerging functions of nodulin-like proteins in non-nodulating plant species. Plant and Cell Physiology 55, 469–474.

Devi, K.K., Seth, N., Kothamasi, S., Kothamasi, D., 2007. Hydrogen cyanide producing rhizobacteria kill subterranean termite *Odontotermes obesus* (Rambur) by cyanide poisoning under in vitro conditions. Current Microbiology 54, 74–78.

Dey, R., Pal, K.K., Bhatt, D.M., Chauhan, S.M., 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiological Research 159, 371–394.

Dias, B.B.A., Cunha, W.G., Morais, L.S., Vianna, G.R., Rech, E.L., De Capdeville, G., Aragão, F.J.L., 2006. Expression of an oxalate decarboxylase gene from *Flammulina* sp. in transgenic lettuce (*Lactuca sativa*) plants and resistance to *Sclerotinia sclerotiorum*. Plant Pathology 55, 187–193.

Dinesh, R., Anandaraj, M., Kumar, A., Bini, Y.K., Subila, K.P., Aravind, R., 2015. Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. Microbiological Research 173, 34–43.

Dowling, D.N., Gara, F.O., 1994. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends in Biotechnology 12, 133–141.

Dubrovskaya, E., Pozdnyakova, N., Golubev, S., Muratova, A., Grinev, V., Bondarenkova, A., Turkovskaya, O., 2017. Peroxidases from root exudates of *Medicago sativa* and *Sorghum bicolor*: Catalytic properties and involvement in PAH degradation. Chemosphere 169, 224–232.

Dutta, S., Kaur, M., Podile, A.R., 2011. Crop Health Improvement with Groundnut Associated Bacteria in: Maheshwari, D.K. (Ed.), Bacteria in agrobiology: Crop Productivity, Springer-Verlag Berlin Heidelberg, pp. 407–430.

Dutta, S., Podile, A.R., 2010. Plant Growth Promoting Rhizobacteria (PGPR): the bugs to debug the root zone. Critical Reviews in Microbiology 36, 232–244.

Dutta, S., Rani, T.S., Podile, A.R., 2013. Root exudate-induced alterations in *Bacillus cereus* cell wall contribute to root colonization and plant growth promotion. PLoS ONE 8, 1–12.

Entus, R., Poling, M., Herrmann, K.M., 2002. Redox regulation of *Arabidopsis* 3-deoxy- D -arabino- heptulosonate 7-phosphate synthase. Plant Physiology 129, 1866–1871.

Faiza, B., Halima, Z., Nour-Eddine, K., 2011. Physiological responses of salt stress and osmoprotection with proline in two strains of lactococci isolated from camel's milk in Southern Algeria. African Journal of Biotechnology 10, 19429–19435.

Falke, J.J., Bass, R.B., Butler, S.L., Chervitz, S.A., Danielson, M.A., 1997. The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. Annual Review of Cell and Developmental biology 13, 457–512.

Falke, J.J., Hazelbauer, G.L., 2001. Transmembrane signaling in bacterial chemoreceptors. Trends in Biochemical Sciences 26, 257–265.

Fan, B., Carvalhais, L.C., Becker, A., Fedoseyenko, D., Wirén, N., Borriss, R., 2012.

Transcriptomic profiling of *Bacillus amyloliquefaciens* FZB42 in response to maize root exudates. BMC Microbiology 12, 116.

Feehily, C., Karatzas, K.A.G., 2013. Role of glutamate metabolism in bacterial responses towards acid and other stresses. Journal of Applied Microbiology 114, 11–24.

Feussner, I., Polle, A., 2015. What the transcriptome does not tell- proteomics and metabolomics are closer to the plants' patho-phenotype. Current Opinion in Plant Biology 26, 26–31.

Foerster, C.W., Foerster, H.F., 1973. Glutamic acid decarboxylase in spores of *Bacillus megaterium* and its possible involvement in spore germination. Journal of Bacteriology 114, 1090–1098.

Friedrich, L., Moyer, M., Ward, E., Ryals, J., 1991. Pathogenesis-related protein 4 is structurally homologous to the carboxy-terminal domains of hevein, Win-1 and Win-2. Molecular and General Genetics 230, 113–119.

Garcia J.A.L., Barbas C., Probanza A., Barrientos, M.L., Mañero F.J.G., 2001. Low molecular weight organic acids and fatty acids in root exudates of two *Lupinus cultivars* at flowering and fruiting stages. Phytochemical Analysis 12, 305–311.

García-Cristobal, J., García-Villaraco, A., Ramos, B., Gutierrez-Mañero, J., Lucas, J.A., 2015. Priming of pathogenesis related-proteins and enzymes related to oxidative stress by plant growth promoting rhizobacteria on rice plants upon abiotic and biotic stress challenge. Journal of Plant Physiology 188, 72–79.

Geigenberger, P., Fernie, A.R., 2014. Metabolic control of redox and redox control of metabolism in plants. Antioxidants and Redox Signaling 21, 1389–1421.

Geigenberger, P., Thormählen, I., Daloso, D.M., Fernie, A.R., 2017. The unprecedented versatility of the plants thioredoxin system. Trends in Plant Science 22, 249–262.

George, E., Kumar, S.N., Jacob, J., Bommasani, B., Lankalapalli, R.S., Morang, P., Kumar, B.S.D., 2015. Characterization of the bioactive metabolites from a plant growth-promoting rhizobacteria and their exploitation as antimicrobial and plant growth-promoting agents. Applied Biochemistry and Biotechnology 176, 529–546.

Gholami, A., Shahsavani, S., Nezarat, S., 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. International Journal of Agricultural and Biosystems Engineering 3, 9–14.

Ghosh, A., Dutta, S., 2016. Investigation on arbuscular mycorrhizal colonization on the roots of some members of Fabaceae and selection of suitable hosts for mass multiplication of VAM. International Journal of Advanced Research in Biological Sciences 3, 193–197.

Goswami, D., Dhandhukia, P., Patel, P., Thakker, J.N., 2014. Screening of PGPR from saline desert of Kutch : Growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. Microbiological research 169, 66–75.

Goswami, D., Thakker, J.N., Dhandhukia, P.C., 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture 2, 1–19.

Goteti, P.K., Emmanuel, L.D.A., Desai S, Shaik, M.H.A., 2013. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays* L.). International Journal of Microbiology 2013, 1–7.

Gourion, B., Rossignol, M., Vorholt, J.A., 2006. A proteomic study of *Methylobacterium extorquens* reveals a response regulator essential for epiphytic growth. Proceedings of the National Academy of Sciences 103, 13186–91.

Gray, E.J., Smith, D.L., 2005. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant-bacterium signaling processes. Soil Biology and Biochemistry 37, 395–412.

Gregorio, P.R.D., Michavila, G., Muller, L.R., Borges, C.S., Sa, E.L.S., Pereira, C., Vincent, P.A., 2017. Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. PLoS ONE 12, 1–22.

Gregorová, Z., Kováčik, J., Klejdus, B., Maglovski, M., Kuna, R., Hauptvogel, P., Matušíková, I., 2015. Drought-induced responses of physiology, metabolites, and PR proteins in *Triticum aestivum*. Journal of Agricultural and Food Chemistry 63, 8125–8133.

Gupta, G., Parihar, S.S., Ahirwar, N.K., Snehi, S.K., and Singh, V., 2015. Plant Growth Promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. Journal of Microbial & Biochemical Technology 7, 96–102.

Gyaneshwar P, Kumar G.N, Parekh, L.J., Poole, P.S., 2002. Role of soil microorganisms in improving P nutrition of plants. Plant and Soil 245, 83–93.

Hahne, H., Mäder, U., Otto, A., Bonn, F., Steil, L., Bremer, E., Hecker, M., Becher, D., 2010. A comprehensive proteomics and transcriptomics analysis of *Bacillus subtilis* salt stress adaptation. Journal of Bacteriology 192, 870–882.

Haizel, T., Merkle, T., Pay, A., Fejes, E., Nagy, F., 1997. Characterization of proteins that interact with the GTP-bound form of the regulatory GTPase Ran in *Arabidopsis*. The Plant Journal 11, 93–103.

Haldar, S., Sengupta, S., 2015. Plant-microbe cross-talk in the rhizosphere: insight and biotechnological potential. The Open Microbiology Journal 9, 1–7.

Hamid, R., Khan, M.A., Ahmad, M., Ahmad, M.M., Abdin, M.Z., Musarrat, J., Javed, S., 2013. Chitinases: an update. Journal of Pharmacy and Bioallied Sciences 5, 21–29.

Han, H.S., Lee, K.D., 2005. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Research Journal of Agriculture and Biological Sciences 1, 210–215.

Hao, W., Ren, L., Ran, W., Shen, Q., 2010. Allelopathic effects of root exudates from watermelon and rice plants on *Fusarium oxysporum* f.sp. *niveum*. Plant and Soil 336, 485–497.

Heidari, M., Golpayegani. A., 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). Journal of the Saudi Society of Agricultural Sciences 98, 57–61.

Herman, P.L., Ramberg, H., Baack, R.R.D., Markwell, J., Osterman, J.C., 2002. Formate dehydrogenase in *Arabidopsis thaliana* : overexpression and subcellular localization in leaves. Plant Science 163, 1137–1145.

Herrmann, K.M., 1995. The shikimate pathway as an entry to aromatic secondary metabolism. Plant Physiology 107, 7–12.

Hirsch, A.M., Bauer, W.D., Bird, D.M., Cullimore, J., Tyler, B., Yoder, J.I., 2003. Molecular signals and receptors: Controlling rhizosphere interactions between plants and other organisms. Ecology 84, 858–868.

Holtmann, G., Bremer, E., 2004. Thermoprotection of *Bacillus subtilis* by exogenously provided glycine betaine and structurally related compatible solutes: involvement of Opu transporters. Journal of Bacteriology 186, 1683–1693.

Hourton-Cabassa, C., Ambard-Bretteville, F., Moreau, F., Virville, J.D., Rémy, R., Francs-Small, C.C, 1998. Stress induction of mitochondrial formate dehydrogenase in potato leaves. Plant Physiology 116, 627–635.

Huang, G., Guo, G., Yao, S., Zhang, N., Hu, H., 2016. Organic acids, amino acids compositions in the root exudates and Cu-accumulation in castor (*Ricinus communis* L.) under Cu stress. International Journal of Phytoremediation 18, 33–40.

Huang, J., Kim, C.M., Xuan, Y., Park, S.J., Piao, H.L., Je, B.I., Liu, J., Kim, T.H., Kim, B.K., Han, C.D., 2013. *OsSNDP1*, a Sec14-nodulin domain-containing protein, plays a critical role in root hair elongation in rice. Plant Molecular Biology 82, 39–50.

Ibort, P., Imai, H., Uemura, M., Aroca, R., 2018. Proteomic analysis reveals that tomato interaction with plant growth promoting bacteria is highly determined by ethylene perception. Journal of Plant Physiology 220, 43–59.

Idriss, E.E., Makarewicz, O., Farouk, A., Rosner, K., Greiner, R., Bochow, H., Richter, T., Borriss, R., 2002. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiology 148, 2097–2109.

Ikegami, A., Yoshimura, N., Motohashi, K., Takahashi, S., Romano, P.G.N., Hisabori, T., Takamiya, K.I., Masuda, T., 2007. The CHLI1 subunit of *Arabidopsis thaliana* magnesium chelatase is a target protein of the chloroplast thioredoxin. Journal of Biological Chemistry 282, 19282–19291.

Ippólito, S.D., Mendieta, J.R., Terrile, M.C., Tonon, C.V., Mansilla, A.Y., Colman, S., Albertengo, L., Rodríguez, M.S., Casalongué, C.A., 2017. Chitosan as source for pesticide formulations, in: Shalaby, E.A. (Ed.), Biological activities and application of marine polysaccharides. pp. 3–15.

Jain, A., Singh, A., Singh, S., Singh, H.B., 2015a. Phenols enhancement effect of microbial consortium in pea plants restrains *Sclerotinia sclerotiorum*. Biological Control 89, 23–32.

Jain, A., Singh, A., Singh, S., Singh, H.B., 2015b. Biological management of *Sclerotinia sclerotiorum* in pea using plant growth promoting microbial consortium. Journal of Basic Microbiology 55, 961–972.

Jha, C.K., Saraf, M., 2015. Plant growth promoting rhizobacteria (PGPR): a review. Journal of Agricultural Research and Development 5, 108–119.

Jiang, S.C., Mei, C., Liang, S., Yu, Y.T., Lu, K., Wu, Z., Wang, X.F., Zhang, D.P., 2015. Crucial roles of the pentatricopeptide repeat protein SOAR1 in *Arabidopsis* response to drought, salt and cold stresses. Plant Molecular Biology 88, 369–385.

Jin-Woo, K., Kim, J., Park, B.K., Choi, O., Park, C.S., Hwang, I., 2003. Identification of genes for biosynthesis of antibacterial compound from *Pseudomonas fluorescens* Bl6, and Its activity against *Ralstonia solanacearum*. Journal of Microbiology and Biotechnology 13, 292–300.

Jones, D.L., 1998. Organic acids in the rhizosphere– a critical review. Plant and Soil 205, 25–44.

Joo, G., Kim, Y., Lee, I., Song, K., Rhee, I., 2004. Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. Biotechnology Letters 26, 487–491.

Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., Greenberg, J.T., 2009. Priming in systemic plant immunity. Science 324, 89–91.

Jung, I.L., Kim, I.G., 2003. Polyamines and glutamate decarboxylase-based acid resistance in *Escherichia coli*. Journal of Biological Chemistry 278 , 22846–22852.

Jydegaard-Axelsen, A.M., Hoiby, P.E., Holmstrom, K., Russell, N., Knochel, S., 2004. CO_2-and anaerobiosis-induced changes in physiology and gene expression of different *Listeria monocytogenes* Strains. Applied and Environmental Microbiology 70, 4111–4117.

Kachhap, S., Chaudhary, A., Singh, S.D., 2015. Response of plant growth promoting rhizobacteria (PGPR) in relation to elevated temperature conditions in groundnut (*Arachis hypogaea* L.). The Ecosan 9, 771–778.

Kachlany, S.C., Fine, D.H., Figurski, D.H., 2000. Secretion of RTX leukotoxin by *Actinobacillus actinomycetemcomitans*. Infection and Immunity 68, 6094–6100.

Kamilova, F., Kravchenko, L.V., Shaposhnikov, A.I., Makarova, N., Lugtenberg, B., 2006. Effects of the tomato pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici* and of the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugars in tomato root exudate. Molecular Plant-Microbe Interactions 19, 1121–1126.

Kandasamy, S., Loganathan, K., Muthuraj, R., Duraisamy, S., Seetharaman, S., Thiruvengadam, R., Ponnusamy, B., Ramasamy, S., 2009. Understanding the molecular basis of plant growth promotional effect of *Pseudomonas fluorescens* on rice through protein profiling. Proteome Science 8, 1–8.

Kang, S., Khan, A.L., You, Y., Kim, J., Kamran, M., Lee, I., 2014. Gibberellin production by newly isolated strain *Leifsonia soli* SE134 and its potential to promote plant growth. Journal of Microbiology and Biotechnology 24, 106–112.

Kang, S.M., Khan, A.L., Hamayun, M., Shinwari, Z.K., Kim, Y.H., Joo, G.J., Lee, I.J., 2012. *Acinetobacter Calcoaceticus* ameliorated plant growth and influenced gibberellins and functional biochemicals. Pakistan Journal of Botany 44, 365–372.

Kanzaki, H., Saitoh, H., Ito, A., Fujisawa, S., Kamoun, S., Katou, S., Yoshioka, H., Terauchi, R., 2003. Cytosolic HSP90 and HSP70 are essential components of INF1-mediated hypersensitive response and non-host resistance to *Pseudomonas cichorii* in *Nicotiana benthamiana*. Molecular Plant Pathology 4, 383–391.

Kavino, M., Harish, S., Kumar, N., Saravanakumar, D., Samiyappan, R., 2010. Effect of chitinolytic PGPR on growth, yield and physiological attributes of banana (*Musa* spp.) under field conditions. Applied Soil Ecology 45, 71–77.

Khorassani, R., Hettwer, U., Ratzinger, A., Steingrobe, B., Karlovsky, P., Claassen, N., 2011. Citramalic acid and salicylic acid in sugar beet root exudates solubilize soil phosphorus. BMC Plant Biology 11, 1–8.

Kim, H., Hahn, M., Grabowski, P., McPherson, D.C., Otte, M.M., Wang, R., Ferguson, C.C., Eichenberger, P., Driks, A., 2006. The *Bacillus subtilis* spore coat protein interaction network. Molecular Microbiology 59, 487–502.

Kim, J., Lee, J., Lee, C., Woo, S.Y., Kang, H., Seo, S., Kim, S., 2015. Activation of Pathogenesis-related Genes by the *Rhizobacterium*, *Bacillus* sp. JS, which induces systemic resistance in tobacco plants. Plant Pathology Journal 31, 195–201.

Kishore, G.K., Pande, S., Podile, A.R., 2005a. Chitin-supplemented foliar application of *Serratia marcescens* GPS5 improves control of late leaf spot disease of groundnut by activating defence-related enzymes. Journal of Phytopathology 153, 169–173.

Kishore, G.K., Pande, S., Podile, A.R., 2005b. Biological control of collar rot disease with broad- spectrum antifungal bacteria associated with groundnut. Canadian Journal of Microbiology 51, 123–132.

Kishore, G.K., Pande, S., Podile, A.R., 2005c. Phylloplane bacteria increase seedling emergence, growth and yield of field-grown groundnut (*Arachis hypogaea* L.). Letters in Applied Microbiology 40, 260–268.

Kishore, G.K., Pande, S., Podile, A.R., 2005d. Biological control of late leaf spot of peanut (*Arachis hypogaea*) with chitinolytic bacteria. Phytopathology 95, 1157–1165.

Kost, T., Stopnisek, N., Agnoli, K., Eberl, L., Weisskopf, L., 2013. Oxalotrophy, a widespread trait of plant-associated *Burkholderia* species, is involved in successful root colonization of lupin and maize by *Burkholderia phytofirmans*. Frontiers in Microbiology 4, 1–9.

Krishi, B.C., Medinipur, P., 2014. Effect of biofertilizer on productivity of groundnut (*Arachis hypogaea* L.) in red and laterite zone of West Bengal. Karnataka Journal of Agricultural Sciences 27, 230–231.

Kuan, K.B., Othman, R., Rahim, K.A., Shamsuddin, Z.H., 2016. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PLoS ONE 11, 1–19.

Kumar, A., Singh D.R., Latha, S., and Johri, R.M., 2017. Effect of arbuscular mycorrhizal fungi on plant growth and essential oil and artemisinin content of *Artemisia annua* L. International Journal of Pharma and Bio Sciences 11, 33–44.

Kumar, A., Bahadur, I., Maurya, B.R., Raghuwanshi, R., Meena, V.S., Singh, D.K., Dixit, J., 2015. Does a plant growth promoting rhizobacteria enhance agricultural sustainability? Journal of Pure and Applied Microbiology 9, 715–724.

Kumar, V., Chattopadhyay, A., Ghosh, S., Irfan, M., Chakraborty, N., Chakraborty, S., Datta, A., 2016. Improving nutritional quality and fungal tolerance in soya bean and grass pea by expressing an oxalate decarboxylase. Plant Biotechnology Journal 14, 1394–1405.

Kumar, V., Singh, P., Jorquera, M.A., Sangwan, P., Kumar, P., Verma, A.K., Agrawal, S., 2013. Isolation of phytase-producing bacteria from Himalayan soils and their effect on growth and phosphorus uptake of Indian mustard (*Brassica juncea*). World Journal of Microbiology and Biotechnology 29, 1361–1369.

Kwon, Y.S., Lee, D.Y., Rakwal, R., Baek, S.B., Lee, J.H., Kwak, Y.S., Seo, J.S., Chung, W.S., Bae, D.W., Kim, S.G., 2016. Proteomic analyses of the interaction between the plant-growth promoting rhizobacterium *Paenibacillus polymyxa* E681 and *Arabidopsis thaliana*. Proteomics 16, 122–135.

Laluk, K., AbuQamar, S., Mengiste, T., 2011. The *Arabidopsis* mitochondria-localized pentatricopeptide repeat protein PGN functions in defense against necrotrophic fungi and abiotic stress tolerance. Plant Physiology 156, 2053–2068.

Lee, C.R., Cho, S.H., Yoon, M.J., Peterkofsky, A., Seok, Y.J., 2007. *Escherichia coli* enzyme IIANtr regulates the K^+ transporter TrkA. Proceedings of the National Academy of Sciences 104, 4124–4129.

Lee, J., Lim, Y.P., Han, C.T., Nou, I.S., Hur, Y., 2013. Genome-wide expression profiles of contrasting inbred lines of Chinese cabbage, Chiifu and Kenshin, under temperature stress. Genes and Genomics 35, 273–288.

Lee, K.D., Han, H., 2005. Physiological Responses of Soybean - Inoculation of Bradyrhizobium japonicum with PGPR in Saline Soil Conditions. Research Journal of Agriculture and Biological Sciences 1, 216–221.

Leibig, M., Liebeke, M., Mader, D., Lalk, M., Peschel, A., Götz, F., 2011. Pyruvate formate lyase acts as a formate supplier for metabolic processes during anaerobiosis in *Staphylococcus aureus*. Journal of Bacteriology 193, 952–962.

Lennen, R.M., Politz, M.G., Kruziki, M.A., Pfleger, B.F., 2013. Identification of transport proteins involved in free fatty acid efflux in *Escherichia coli*. Journal of Bacteriology 195, 135–144.

Li, B., Li, Y., Wu, H., Zhang, F., Li, C., Li, X., Lambers, H., Li, L., 2016. Root exudates drive interspecific facilitation by enhancing nodulation and N_2 fixation. Proceedings of the National Academy of Sciences 113, 6496–6501.

Li, J., McConkey, B.J., Cheng, Z., Guo, S., Glick, B.R., 2013a. Identification of plant growth-promoting bacteria-responsive proteins in cucumber roots under hypoxic stress using a proteomic approach. Journal of Proteomics 84, 119–131.

Li, X., Zhang, T., Wang, X., Hua, K., Zhao, L., Han, Z., 2013b. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-

borne pathogen. International Journal of Biological Sciences 9, 164–173.

Li, Z., Chang, S., Lin, L., Li, Y., An, Q., 2011. A colorimetric assay of 1-aminocyclopropane-1-carboxylate (ACC) based on ninhydrin reaction for rapid screening of bacteria containing ACC deaminase. Letters in Applied Microbiology 53, 178–185.

Lim, J., Kim, S., 2013. Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in Pepper. Plant Pathology Journal 29, 201–208.

Ling, N., Raza, W., Ma, J., Huang, Q., Shen, Q., 2011. Identification and role of organic acids in watermelon root exudates for recruiting *Paenibacillus polymyxa* SQR-21 in the rhizosphere. European Journal of Soil Biology 47, 374–379.

Litzinger, S., Duckworth, A., Nitzsche, K., Risinger, C., Wittmann, V., Mayer, C., 2010. Muropeptide rescue in *Bacillus subtilis* involves sequential hydrolysis by β-N-acetylglucosaminidase and N-Acetylmuramyl-L-Alanine Amidase. Journal of Bacteriology 192, 3132–3143.

Liu, W., Hou, J., Wang, Q., 2015. Collection and analysis of root exudates of *Festuca arundinacea* L. and their role in facilitating the phytoremediation of petroleum-contaminated soil. Plant and soil 389, 109–119.

Liu, W., Zhou, X., Li, G., Li, L., Kong, L., Wang, C., Zhang, H., Xu, J.R., 2011. Multiple plant surface signals are sensed by different mechanisms in the rice blast fungus for appressorium formation. PLoS Pathology 7, 1–13.

Liu, Y., Chen, L., Wu, G., Feng, H., Zhang, G., Shen, Q., Zhang, R., 2017a. Identification of root-secreted compounds involved in the communication between cucumber, the beneficial *Bacillus amyloliquefaciens*, and the soil-borne pathogen *Fusarium oxysporum*. Molecular Plant Microbe Interactions 30, 53–62.

Liu, Y., Jiang, X., Guan, D., Zhou, W., Ma, M., Zhao, B., 2017b. Transcriptional analysis of genes involved in competitive nodulation in *Bradyrhizobium diazoefficiens* at the presence of soybean root exudates. Scientific Reports 7, 1–11.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real- time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 408, 402–408.

Long, H.H., Schmidt, D.D., Baldwin, I.T., 2008. Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. PLoS ONE 3, 1–10.

Lopez-Bucio, J., Nieto-Jacobo, M. F., Ramirez-Rodriguez, V., Herrera-Estrella, L., 2000. Organic acid metabolism in plants: From adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Science 160, 1–13.

López-Ráez, J.A., Shirasu, K., Foo, E., 2017. Strigolactones in plant interactions with beneficial and detrimental organisms: the yin and yang. Trends in Plant Science 22, 527–537.

Loyola-Vargas V.M., Broeckling C.D., Badri, D., Vivanco, J.M., 2007. Effect of transporters on the secretion of phytochemicals by the roots of *Arabidopsis thaliana*. Planta 225, 301–310.

Lu, H., Sun, J., Zhu, L., 2017. The role of artificial root exudate components in facilitating the degradation of pyrene in soil. Scientific Reports 7, 1–10.

Luo, Q., Wang, S., Sun, L., Wang, H., 2017. Metabolic profiling of root exudates from two ecotypes of *Sedum alfredii* treated with Pb based on GC-MS. Scientific reports 7, 1–9.

Luo, T., Fan, T., Liu, Y., Rothbart, M., Yu, J., Zhou, S., Grimm, B., Luo, M., 2012. Thioredoxin redox regulates ATPase activity of magnesium chelatase CHLI subunit and modulates redox-mediated signaling in tetrapyrrole biosynthesis and homeostasis of reactive oxygen species in pea plants. Plant Physiology 159, 118–130.

Mahdi, S.S., Hassan, G.I., Samoon, S.A., Rather, H.A., Dar, S.A., Zehra, B., 2010. Bio-fertilizers in organic agriculture. Journal of Phytology 2, 42–54.

Maillet, F., Poinsot, V., André, O., Puech-Pagès, V., Haouy, H., Gueunier, M., Cromer, L., Giraudet, D., Formey, D., Niebel, A., Martinez, E.A., Driguez, H., Becard, G., Denarie, J., 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469, 58–63.

Majeed, A., Abbasi, M.K., Hameed, S., Imran, A., Rahim, N., 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in Microbiology 6, 1–10.

Mallick, M.A., 2016. Phosphate solubilizing microbes : an effective and alternative approach as biofertilizers. International Journal of Pharmacy and Pharmaceutical Sciences 8, 37–40.

Manjula, K., Podile, A.R., 2005. Production of fungal cell wall degrading enzymes by a biocontrol strain of *Bacillus subtilis* AF1. Indian Journal of Experimental Biology 43, 892–896.

Mark, G.L., Dow, J.M., Kiely, P.D., Higgins, H., Haynes, J., Baysse, C., Abbas, A., Foley, T., Franks, A., Morrissey, J., O'Gara, F., 2005. Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. Proceedings of the National Academy of Sciences 102, 17454–17459.

Masalha, J., Kosegarten, H., Elmaci, Ö., Mengel, K., 2000. The central role of microbial activity for iron acquisition in maize and sunflower. Biology and Fertility of Soils 30, 433–439.

Mathivanan, S., Chidambaram, A.A., Sundramoorthy, P., Baskaran, L., Kalaikandhan, R., 2014. Effect of combined inoculations of plant growth promoting rhizobacteria (PGPR) on the growth and yield of groundnut (*Arachis hypogaea* L.). International Journal of Current Microbiology and Applied Sciences 3, 1010–1020.

Mauck, J., Chan, L., Glaser, L., 1971. Turnover of the cell of gram-positive bacteria. Journal of Biological Chemistry 246, 1820–1827.

Mcpherson, D.C., Kim, H., Hahn, M., Wang, R., Grabowski, P., Eichenberger, P., Driks, A., 2005. Characterization of the *Bacillus subtilis* spore morphogenetic coat protein CotO. Journal of Bacteriology 187, 8278–8290.

Meierhoff, K., Felder, S., Nakamura, T., Bechtold, N., Schuster, G., 2003. HCF152 , an

Arabidopsis RNA binding pentatricopeptide repeat protein involved in the processing of chloroplast psbB-psbT-psbH-petB-petD RNAs. The Plant Cell 15, 1480–1495.

Meng, L., Wong, J.H., Feldman, L.J., Lemaux, P.G., Buchanan, B.B., 2010. A membrane-associated thioredoxin required for plant growth moves from cell to cell, suggestive of a role in intercellular communication. Proceedings of the National Academy of Sciences 107, 3900–3905.

Michalet, S., Rohr, J., Warshan, D., Bardon, C., Roggy, J., Domenach, A., Czarnes, S., Pommier, T., Combourieu, B., Guillaumaud, N., Bellvert, F., Comte, G., 2013. Phytochemical analysis of mature tree root exudates in situ and their role in shaping soil microbial communities in relation to tree N-acquisition strategy. Plant Physiology and Biochemistry 72, 169–177.

Mirza, M.S., Mehnaz. S., Normand, P., Prigent-Combaret, C., Moënne-Loccoz, Y., Bally, R., Malik, K.A., 2006. Molecular characterization and PCR detection of a nitrogen-fixing *Pseudomonas* strain promoting rice growth. Biology and Fertility of Soils 43, 163–170.

Mishra, Y., Chaurasia, N., Rai, L.C., 2009. AhpC (alkyl hydroperoxide reductase) from *Anabaena* sp. PCC 7120 protects *Escherichia coli* from multiple abiotic stresses. Biochemical and Biophysical Research Communications 381, 606–611.

Moir, A., 1990. The genetics of bacterial spore germination. Annual Review of Microbiology 44, 531–553.

Monchgesang, S., Strehmel, N., Schmidt, S., Westphal, L., Taruttis, F., Muller, E., Herklotz, S., Neumann, S., Scheel, D., 2016. Natural variation of root exudates in *Arabidopsis thaliana*-linking metabolomic and genomic data. Scientific Reports 6, 1–11.

Morona, R., Reeves, P., 1982. The *tolC* locus of *Escherichia coli* affects the expression of three major outer membrane proteins. Journal of Bacteriology 150, 1016–1023.

Mostafa, H., Amir, G., 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). Journal of the Saudi Society of Agricultural Sciences 98, 57–61.

Mwita, L., Chan, W.Y., Pretorius, T., Lyantagaye, S.L., Lapa, S.V., Avdeeva, L.V., Reva, O.N., 2016. Gene Expression regulation in the plant growth promoting *Bacillus atrophaeus* UCMB-5137 stimulated by maize root exudates. Gene 590, 18–28.

Nadal, M. and Paszkowski, U., 2013. Polyphony in the rhizosphere: presymbiotic communication in arbuscular mycorrhizal symbiosis. Current Opinion in Plant Biology 16, 473–479.

Nagler, K., Krawczyk, A.O., De Jong, A., Madela, K., Hoffmann, T., Laue, M., Kuipers, O.P., Bremer, E., Moeller, R., 2016. Identification of differentially expressed genes during *Bacillus subtilis* spore outgrowth in high-salinity environments using RNA sequencing. Frontiers in Microbiology 7, 1–15.

Nagahashi, G., Douds, D.D., 2000. Partial separation of root exudates components and their effects upon the growth of germinated spores of AM fungi. Mycological Research 104, 1453-1464.

Nardi, S., Concheri, G., Pizzeghello, D., Sturaro, A., Rella, R., Parvoli, G., 2000. Soil organic matter mobilization by root exudates. Chemosphere 5, 653–658.

Natalia, D., Jumadila, O., Anggraini, I.D., Meutia, F., Puspasari, F., Hasan, K., 2016. Alkyl hydroperoxide reductase from *Bacillus aquimaris* MKSC6.2 protects *Esherichia coli* from oxidative stress. Journal of Basic Microbiology 56, 834–837.

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters 170, 265–270.

Nayak, S., Prasanna, R., Pabby, A., Dominic, T.K., Singh, P.K., 2004. Effect of urea, blue green algae and *Azolla* on nitrogen fixation and chlorophyll accumulation in soil under rice. Biology and Fertility of Soils 40, 67–72.

Naz, I., Bano, A., Hassan, T.U., 2009. Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L. African Journal of Biotechnology 8, 5762–5766.

Neal, A.L., Ahmad, S., Gordon-Weeks, R., Ton, J., 2012. Benzoxazinoids in root exudates of maize attract *Pseudomonas putida* to the rhizosphere. PLoS ONE 7, 1–10.

Neto A.C.R., Maraschin, M., Piero, R.M.D., 2015. Antifungal activity of salicylic acid against *Penicillium expansum* and its possible mechanisms of action. International Journal of Food Microbiology 215, 64–70.

Neubauer U, Nowack B, Furrer G, Schulin, R., 2000. Heavy metal sorption on clay minerals affected by the siderophore desferrioxamine B. Environmental science and technology 34, 2749–275.

Neumann, G., Bott, S., Ohler, M.A., Mock, H.P., Lippmann, R., Grosch, R., Smalla, K., 2014. Root exudation and root development of lettuce (*Lactuca sativa* L.cv. Tizian) as affected by different soils. Frontiers in Microbiology 5, 1–6.

Neumann, G., Römheld, V., 2000. The release of root exudates as affected by the plant's physiological status., in: Willig, S., Varanini, Z., Nannipieri, P., (Ed.), The rhizosphere, biochemistry and organic substances at the soil–plant interface. Marcel Dekker, New York, NY, USA, pp. 41–93.

Nobrega, F.M., Santos, I.S., Cunha, M.D., Carvalho, A.O., Gomes, V.M., 2005. Antimicrobial proteins from cowpea root exudates : inhibitory activity against *Fusarium oxysporum* and purification of a chitinase-like protein. Plant and Soil 272, 223–232.

Nosheen, A., Bano, A., 2014. Potential of plant growth promoting rhizobacteria and chemical fertilizers on soil enzymes and plant growth. Pakistan Journal of Botany 46, 1521-1530.

Oburger, E., Dell'mour, M., Hann, S., Wieshammer, G., Puschenreiter, M., Wenzel, W.W., 2013. Evaluation of a novel tool for sampling root exudates from soil-grown plants compared to conventional techniques. Environmental and Experimental Botany 87, 235–247.

Oburger, E., Schmidt, H., 2016. New methods to unravel rhizosphere processes. Trends in Plant Science 21, 243–255.

Oku, S., Komatsu, A., Tajima, T., Nakashimada, Y., Kato, J., 2012. Identification of chemotaxis sensory proteins for amino acids in *Pseudomonas fluorescens* Pf0-1 and their involvement in chemotaxis to tomato root exudate and root colonization. Microbes and Environments 27, 462–469.

Olayinka, B.U., Etejere, E.O., 2015. Growth analysis and yield of two varieties of groundnut (*Arachis hypogaea* L.) as influenced by different weed control methods. Indian Journal of Plant Physiology 20, 130–136.

Olczak, A.A., Seyler, R.W., Jonathan, W., Maier, R.J., Olson, J.W., 2003. Association of *Helicobacter pylori* antioxidant activities with host colonization proficiency. Infection and Immunity 71, 580–583.

Ordal, G.W., Villani, D.P., Rosendahl, M.S., 1979. Chemotaxis towards sugars by *Bacillus subtilis*. Journal of General Microbiology 115, 167–172.

Ortíz-castro, R., Valencia-cantero, E., López-bucio, J., 2008. Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. Plant Signaling & Behavior 3, 263–264.

Oswald, C., Tam, H.K., Pos, K.M., 2016. Transport of lipophilic carboxylates is mediated by transmembrane helix2 in multidrug transporter AcrB. Nature Communications 7, 1–10.

Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J., Dowling, D.N., 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Frontiers in Microbiology 6, 1–9.

O'Toole, G.A., 2011. Microtiter dish biofilm formation assay. Journal of Visualized Experiments 47. 10–11.

Panhwar, Q.A., Naher, U.A., Jusop, S., Othman, R., Latif, M.A., Ismail, M.R., 2014. Biochemical and molecular characterization of potential phosphate-solubilizing bacteria in acid sulfate soils and their beneficial effects on rice growth. PLoS ONE 9, 1–14.

Park, C.J., Seo, Y.S., 2015. Heat shock proteins: a review of the molecular chaperones for plant immunity. The Plant Pathology Journal 31, 323–33.

Park, J.H., Bolan, N., Megharaj, M., Naidu, R., 2011. Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. Journal of Hazardous Materials 2, 829–836.

Parray, J.A., Jan, S., Kamili, A.N., Qadri, R.A., 2016. Current perspectives on plant growth-promoting rhizobacteria. Journal of Plant Growth Regulation 35, 877–902.

Paul, D., Nair, S., 2008. Stress adaptations in a Plant Growth Promoting Rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. Journal of Basic Microbiology 48, 378–384.

Paungfoo-Lonhienne, C., Lonhienne, T.G.A., Yeoh, Y.K., Donose, B.C., Webb, R.I., Parsons, J., Liao, W., Sagulenko, E., Lakshmanan, P., Hugenholtz, P., Schmidt, S., Ragan, M.A., 2016. Crosstalk between sugarcane and a plant-growth promoting *Burkholderia* species. Scientific Reports 6, 1–14.

Persello-Cartieaux, F., Nussaume, L., Robaglia, C., 2003. Tales from the underground: Molecular plant-rhizobacteria interactions. Plant, Cell and Environment 26, 189–199.

Pétriacq, P., Williams, A., Cotton, A., McFarlane, A.E., Rolfe, S.A., Ton, J., 2017. Metabolite profiling of non-sterile rhizosphere soil. The Plant Journal 92, 147–162.

Piccini, D., Azcon, R., 1987. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi on the utilization of Bayovar rock phosphate by alfalfa plants using a sand-vermiculite medium. Plant and Soil 101, 45–50.

Pinedo, C.A., Bringhurst, R.M., Gage, D.J., 2008. *Sinorhizobium meliloti* mutants lacking phosphotransferase system enzyme HPr or EIIA are altered in diverse processes, including carbon metabolism, cobalt requirements, and succinoglycan production. Journal of Bacteriology 190, 2947–2956.

Pino, N.J., Muñera, L.M., Peñuela, G.A., Pino, N.J., Muñera, L.M., Root, G.A.P., 2016. Root exudates and plant secondary metabolites of different plants enhance polychlorinated biphenyl degradation by rhizobacteria. Bioremediation Journal 20, 108–116.

Pinto, J.E., Dyer, W.E., Weller, S.C., Herrmann, K.M., 1988. Glyphosate induces 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase in potato (*Solanum tuberosum* L.) cells grown in suspension culture. Plant Physiology 87, 891–893.

Piršelová, B., Kuna, R., Libantová, J., Moravčíková, J., Matušíková, I., 2011. Biochemical and physiological comparison of heavy metaltriggered defense responses in the monocot maize and dicot soybean roots. Molecular Biology Reports 5, 3437–3446.

Podile, A.R., Kishore, G.K., 2002. Biological Control of Peanut Diseases, in: Gnanamanickam, S.S. (Ed.), Biological control of crop diseases. pp. 30.

Podile, A.R., Kishore, K., 2006. Plant growth-promoting rhizobacteria. Annual Review of Microbiology - Annual Review of Microbiololgy 63, 195-230.

Podile, A.R., Prakash, A.P., 1996. Lysis and biological control of *Aspergillus niger* by *Bacillus subtilis* AF1. Canadian Journal of Microbiology 42, 533–538.

Podile, A.R., Vukanti, R., Sravani, A., Kalam, S., Dutta, S., Durgeshwar, P., Papa Rao, V., 2014. Root colonization and quorum sensing are the driving forces of plant growth promoting rhizobacteria (PGPR) for growth promotion. Proceedings of the National Academy of Sciences 80, 407–413.

Powell, B.S., Court, D.L., Inada, T., Nakamura, Y., Michotey, V., Cui, X., Reizer, A., Saier, M.H., Reizer, J., 1995. Novel proteins of the phosphotransferase system encoded within the rpoN operon of *Escherichia coli*: Enzyme IIANtr affects growth on organic nitrogen and the conditional lethality of an erats mutant. Journal of Biological Chemistry 9, 4822-4839.

Pramanik, M.H.R., Nagai, M., Asao, T., Matsui, Y., 2000. Effects of temperature and photoperiod on phytotoxic root exudates of cucumber (*Cucumis sativus*) in hydroponic culture. Journal of Chemical Ecology 26, 1953–1967.

Pühler, A., Arlat, M., Becker, A., Göttfert, M., Morrissey, J.P., O'Gara, F., 2004. What can bacterial genome research teach us about bacteria–plant interactions. Current Opinion in Plant

Biology 7, 137–147.

Qiu, M., Xu, Z., Li, X., Li, Q., Zhang, N., Shen, Q., Zhang, R., 2014. Comparative proteomics analysis of *Bacillus amyloliquefaciens* SQR9 revealed the key proteins involved in in situ root colonization. Journal of Proteome Research 13, 5581–5591.

Raikhel, N.V., Lee, H.I., 1993. Structure and function of chitin-binding proteins. Annual Review of Plant Physiology and Plant Molecular Biology 44, 591–615.

Rajaee, S., Alikhani, H.A., Raiesi, F., 2007. Effect of plant growth promoting potentials of *Azotobacter chroococcum* native strains on growth, yield and uptake of nutrients in wheat. Journal of Water and Soil Science 11, 285–297.

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V., Samiyappan, R., 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protection 20, 1–11.

Rani, T.S., Podile, A.R., 2014. Extracellular matrix-associated proteome changes during non-host resistance in citrus - Xanthomonas interactions. Physiologia Plantarum 150, 565–579.

Raupach, G.S., Kloepper, J.W., 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Biological Control 88, 1158–1164.

Richard, H., Foster, J.W., 2007. Sodium regulates *Escherichia coli* acid resistance, and influences GadX- and GadW-dependent activation of gadE. Microbiology 153, 3154–3161.

Rijavec, T., Lapanje, A., 2016. Hydrogen cyanide in the rhizosphere : not suppressing plant pathogens, but rather regulating availability of phosphate. Frontiers in Microbiology 7, 1–14.

Roitsch, T., González, M.C., 2004. Function and regulation of plant invertases: Sweet sensations. Trends in Plant Science 9, 606–613.

Romero, I., Fernandez-Caballero, C., Goñi, O., Escribano, M.I., Merodio, C., Sanchez-Ballesta, M.T., 2008. Functionality of a class I beta-1,3-glucanase from skin of table grapes berries. Plant Science 174, 641–648.

Rosenberg, E.Y., Bertenthal, D., Nilles, M.L., Bertrand, K.P., Nikaido, H., 2003. Bile salts and fatty acids induce the expression of *Escherichia coli* AcrAB multidrug efflux pump through their interaction with Rob regulatory protein. Molecular Microbiology 48, 1609–1619.

Ruan, L., Pleitner, A., Gänzle, M.G., McMullen, L.M., 2011. Solute transport proteins and the outer membrane protein NmpC contribute to heat resistance of *Escherichia coli* AW1.7. Applied and Environmental Microbiology 77, 2961–2967.

Rudrappa, T., Czymmek, K.J., Pare, P.W., Bais, H.P., 2008. Root-Secreted malic acid recruits beneficial. Plant physiology 148, 1547–1556.

Rudrappa, T., Splaine, R.E., Biedrzycki, M.L., Bais, H.P., 2008. Cyanogenic pseudomonads influence multitrophic interactions in the rhizosphere. PLoS ONE 3(4): e2073.

Ryu, C., Farag, M.A., Hu, C., Reddy, M.S., Kloepper, J.W., Pare, P.W., 2004. Bacterial

volatiles induce systemic resistance in *Arabidopsis*. Plant Physiology 134, 1017–1026.

Saha, D., Prasad, A.M., Srinivasan, R., 2007. Pentatricopeptide repeat proteins and their emerging roles in plants. Plant Physiology and Biochemistry 45, 521–534.

Sanchez, L., Weidmann, S., Brechenmacher, L., Batoux, M., Tuinen, D.V., Lemanceau, P., Gianinazzi, S., Gianinazzi-Pearson, V., 2004. Common gene expression in *Medicago truncatula* roots in response to *Pseudomonas fluorescens* colonization, mycorrhiza development and nodulation. New Phytologist 161, 855–863.

Sankar, B., Karthishwaran, K., Somasundaram, R., 2013. Photosynthetic pigment content alterations in *Arachis hypogaea* L. in relation to varied irrigation levels with growth hormone and triazoles 5, 7–13.

Santos, V.D.C., Rey, P., 2006. Plant thioredoxins are key actors in the oxidative stress response. Trends in Plant Science 11, 329–34.

Sanz-Barrio, R., Corral-Martinez, P., Ancin, M., Segui-Simarro, J.M., Farran, I., 2013. Overexpression of plastidial thioredoxin f leads to enhanced starch accumulation in tobacco leaves. Plant Biotechnology Journal 11, 618–627.

Saravanakumar, D., Samiyappan, R., 2007. ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogaea*) plants. Journal of Applied Microbiology 102, 1283–1292.

Saravanan, R.S., Rose, J.K.C., 2004. A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues. Proteomics 4, 2522–2532.

Saravanan, V.S., Subramoniam, S.R., Raj, S.A., 2004. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. Brazilian Journal of Microbiology 35, 121–125.

Sarma, B.K., Yadav, S.K., Singh, S., Singh, H.B., 2015. Microbial consortium-mediated plant defense against phytopathogens: Readdressing for enhancing efficacy. Soil Biology and Biochemistry 87, 25–33.

Sarwar, M., Kremer, R.J., 1995. Determination of bacterially derived auxins using a microplate method. Letters in Applied Microbiology 20, 282–285.

Sasse, J., Martinoia, E., Northen, T., 2018. Feed your friends : do plant exudates shape the root microbiome ? Trends in Plant Science 23, 25–41.

Savci, S., 2012. Investigation of effect of chemical fertilizers on environment. APCBEE Procedia 1, 287–292.

Sayyed, R.Z., Gangurde, N.S., Patel, P.R., Joshi, S.A., Chincholkar, S.B., 2010. Siderophore production by *Alcaligenes faecalis* and its application for growth promotion in *Arachis hypogaea*. Indian Journal of Biotechnology 9, 302–307.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C., Manners, J.M., 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proceedings of the National Academy of Sciences 97, 11655–11660.

Schwyn, B., Neilands, J.B.,1987. Universal chemical assay for the detection determination of siderophores. Analytical Biochemistry 160, 47–56.

Sharma, 2007. Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. Journal of Herbal Medicine and Toxicology 1, 61–63.

Sharma, A., Johri, B.N., 2003. Combat of iron-deprivation through a plant growth promoting fluorescent *Pseudomonas* strain GRP3A in mung bean (*Vigna radiata* L. Wilzeck). Microbiological Research 158, 77–81.

Sharma, K., Anjaiah, V., 2000. An efficient method for the production of transgenic plants of peanut (*Arachis hypogaea* L.) through *Agrobacterium tumefaciens*-mediated genetic transformation. Plant Science 159, 7–19.

Sharma, R.P., Datt, N., Chander, G., 2009. Effect of vermicompost, farmyard manure and chemical fertilizers on yield, nutrient uptake and soil fertility in okra (*Abelmoschus esculentus*) - onion (*Allium cepa*) sequence in wet temperate zone of Himachal Pradesh. Journal of the Indian Society of Soil Science 57, 357–361.

Sharma, S., Kulkarni, J., Jha, B., 2016. Halotolerant rhizobacteria promote growth and enhance salinity tolerance in peanut. Frontiers in Microbiology 7, 1600.

Sharma, S., Kumar, V., Tripathi, R.B., 2011. Isolation of phosphate solubilizing microorganism (PSMs) from soil. Journal of Microbiology and Biotechnology Research 1, 90–95.

Sheehan, D., Meade, G., Foley, V.M., Dowd, C.A., 2001. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochemical Journal 16, 1–16.

Sherathia, D., Dey, R., Thomas, M., Dalsania, T., Savsani, K., Pal, K.K., 2016. Biochemical and molecular characterization of DAPG-producing plant growthpromoting rhizobacteria (PGPR) of groundnut (*Arachis hypogaea* L.). Legume Research 39, 1–9.

Shi, S., Richardson, A.E., Callaghan, M.O., Deangelis, K.M., Jones, E.E., Stewart, A., Firestone, M.K., Condron, L.M., 2011. Effects of selected root exudate components on soil bacterial communities. FEMS Microbiology Ecology 77, 600–610.

Shweta, B., Maheshwari, D.K , Dubey, R.C., Arora, D.S., Bajpai, V.K., Kang, S.C., 2008. Beneficial effects of fluorescent *Pseudomonads* on seed germination, growth promotion, and suppression of charcoal rot in groundnut (*Arachis hypogea* L.). Journal of Microbiology and Biotechnology 18, 1578–1583.

Sial, R.A., Chaudhary, E.H., Hussain, S., Naveed, M., 2008. Effect of organic manures and chemical fertilizers on grain yield of maize in rainfed area. Pakistan Journal of Soil Science 26, 130–133.

Siddikee, M.A., Chauhan, P.S., Anandham, R., Han, G.H., Sa, T., 2010. Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. Journal of Microbiology and Biotechnology 20, 1577–1584.

Siebold, C., Flükiger, K., Beutler, R., Erni, B., 2001. Carbohydrate transporters of the bacterial phosphoenolpyruvate: Sugar phosphotransferase system (PTS). FEBS Letters 504, 104–111.

Silva, M.N., Carvalho, V.G.B., Sarmento, B., Stamford, N.P., Pintado, M.E., Vasconcelos M.W., 2014. Effect of a chitosan enriched biofertilizer on induced resistances against the pinewood nematode, *Bursaphelenchus xylophilus*, in: 11th International Conference of the European Chitin Society, at Porto, Portugal.

Singh, N.K., Raja, K., Kumar, R., Kumar, D., Shukla, P., Kirti, P.B., 2013. Characterization of a pathogen induced thaumatin-like protein gene AdTLP from *Arachis diogoi*, a Wild Peanut. PLoS ONE 8, 1–18.

Singh, P., Kumar, V., Agrawal, S., 2014. Evaluation of phytase producing bacteria for their plant growth promoting activities. International Journal of Microbiology 2014.

Slavov, S., Onckelen, V.H., Batchvarova, R., Atanassov, A., Prinsen, E., 2004. IAA production during germination of *Orobanche* spp. seeds. Journal of Plant Physiology 161, 847–853.

Smith, T.J., Blackman, S.A., Foster, S.J., 2000. Autolysins of *Bacillus subtilis*: Multiple enzymes with multiple functions. Microbiology 146, 249–262.

Somers, E., Vanderleyden, J., Srinivasan, M., 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. Critical Reviews in Microbiology 30, 205–240.

Song, J.Y., Kim, H.A., Kim, J.S., Kim, S.Y., Jeong, H., Kang, S.G., Kim, B.K., Kwon, S.K., Lee, C.H., Yu, D.S., Kim, B.S., Kim, S.H., Kwon, S.Y., Kim, J.F., 2012. Genome sequence of the plant growth-promoting rhizobacterium *Bacillus* sp. Strain JS. Journal of Bacteriology 194, 3760–3761.

Sood, S.G., 2003. Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular-arbuscular mycorrhizal tomato plants. FEMS Microbiology Ecology 45, 219–227.

Storz, G., Imlayt, J.A., 1999. Oxidative stress. Current Opinion in Microbiology 2, 188–194.

Strehmel, N., Böttcher, C., Schmidt, S., Scheel, D., 2014. Phytochemistry profiling of secondary metabolites in root exudates of *Arabidopsis thaliana*. Phytochemistry 108, 35–46.

Stukkens, Y., Bultreys, A., Trombik, T., Vanham, D., Boutry, M., 2005. NpPDR1, a pleiotropic drug resistance-type ATP-binding cassette transporter from *Nicotiana plumbaginifolia*, plays a major role in plant pathogen defense. Plant Physiology 139, 341–352.

Sturm, A., 1999. Invertases. primary structures, functions, and roles in plant development and sucrose partitioning. Plant Physiology 121, 1–7.

Sturm, A., Tang, G.Q., 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. Trends in Plant Science 4, 401–407.

Sturrock, J.L.R., Ekramoddoullah, A.K.M., 2010. The superfamily of thaumatin-like proteins : its origin, evolution, and expression towards biological function. Plant Cell Reports 29, 419–436.

Sun, L., Lu, Y., Kronzucker, H.J., Shi, W., 2016. Quantification and enzyme targets of fatty

acid amides from duckweed root exudates involved in the stimulation of denitrification. Journal of Plant Physiology 198, 81–88.

Szoboszlay, M., White-Monsant, A., Moe, L.A., 2016. The effect of root exudate 7, 4′-dihydroxyflavone and naringenin on soil bacterial community structure. PLoS ONE 11(1): 1–16.

Tan, S., Yang, C., Mei, X., Shen, S., Raza, W., Shen, Q., Xu, Y., 2013. The effect of organic acids from tomato root exudates on rhizosphere colonization of *Bacillus amyloliquefaciens* T-5. Applied Soil Ecology 64, 15–22.

Tanzubil, P.B., Yahaya, B.S., 2017. Assessment of yield losses in groundnut (*Arachis hypogaea* L.) due to arthropod pests and diseases in the Sudan savanna of Ghana. Journal of Entomology and Zoology Studies 5, 1561–1564.

Taurian, T., Anzuay, M.S., Angelini, J.G., Tonelli, M.L., Ludueña, L., Pena, D., Ibáñez, F., Fabra, A., 2010. Phosphate-solubilizing peanut associated bacteria: Screening for plant growth-promoting activities. Plant and Soil 329, 421–431.

Tauzin, A.S., Giardina, T., 2014. Sucrose and invertases, a part of the plant defense response to the biotic stresses. Frontiers in Plant Science 5, 1–8.

Thilagar, G., Bagyaraj, D.J., Podile, A.R., Vaikuntapu, P.R., 2016. *Bacillus sonorensis*, a novel plant growth promoting rhizobacterium in improving growth, nutrition and yield of chilly (*Capsicum annuum* L.). Proceedings of the National Academy of Sciences, India Section Biological Sciences 1, 1-6.

Tisdale, S.L., Nelson, W.L., 1975. Soil Fertility and Fertilizers, 3rd ed. Macmillan Publishing, New York, USA.

Tjamos, S.E., Flemetakis, E., Paplomatas, E.J., Katinakis, P., 2005. Induction of resistance to *Verticillium dahliae* in *Arabidopsis thaliana* by the biocontrol agent K-165 and pathogenesis-related proteins gene expression. Molecular Plant-Microbe Interactions 18, 555–561.

Tripura, C., Sashidhar, B., Podile, A.R., 2007. Ethyl methanesulfonate mutagenesis – enhanced mineral phosphate solubilization by groundnut-associated *Serratia marcescens* GPS-5. Current Microbiology 54, 79–84.

Vaikuntapu, P.R., Dutta, S., Samudrala, R.B., Rao, V.R.V.N., Kalam, S., Podile, A.R., 2014. Preferential promotion of *Lycopersicon esculentum* (Tomato) growth by plant growth promoting bacteria associated with tomato. Indian Journal of Microbiology 54, 403–412.

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., Boyce, A.N., 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-A review. Molecules 21, 1–17.

Venturi, V., Keel, C., 2016. Signaling in the Rhizosphere. Trends in Plant Science 21, 187–198.

Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255, 571–586.

Vigers, A.J., Wiedemann, S., Roberts, W.K., Legrand, M., 1992. Thaumatin-like pathogenesis-

related proteins are antifungal. Plant Science 83, 155–161.

Vold, B., Szulmajster, J., Carbone, A., 1975. Regulation of dihydrodipicolinate synthase and aspartate kinase in *Bacillus subtilis*. Journal of Bacteriology 121, 970–974.

Volkmar, K.M., Bremer, E., 1998. Effects of seed inoculation with a strain of *Pseudomonas fluorescens* on root growth and activity of wheat in well-watered and drought-stressed glass-fronted rhizotrons. Canadian Journal of Plant Science 78, 545–551.

Vranova, V., Rejsek, K., Skene, K.R., Janous, D., Formanek, P., 2013. Methods of collection of plant root exudates in relation to plant metabolism and purpose: A review. Journal of Plant Nutrition and Soil Science 176, 175–199.

Wakatsuki, T., 1995. Metal oxidoreduction by microbial cells. Journal of Industrial Microbiology and Biotechnology 14, 169–177.

Walker, T.S., Bais, H.P., De, E., Schweizer, H.P., Rahme, L.G., Fall, R., Vivanco, J.M., 2004. *Pseudomonas aeruginosa*-Plant root interactions. pathogenicity, biofilm formation, and root exudation. Plant Physiology 134, 320–331.

Walker, V., Bertrand, C., Bellvert, F., Moënne-Loccoz, Y., Bally, R., Comte, G., 2011. Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*. New Phytologist 189, 494–506.

Wang, Z., Zou, Y., Li, X., Zhang, Q., Chen, L., Wu, H., Su, D., Chen, Y., Guo, J., Long, Y., Zhong, Y., Liu, Y., 2006. Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes *via* distinct modes of mRNA silencing. The Plant Cell 18, 676–687.

Wasim, M., Bible, A.N., Xie, Z., Alexandre, G., 2009. Alkyl hydroperoxide reductase has a role in oxidative stress resistance and in modulating changes in cell-surface properties in *Azospirillum brasilense* Sp245. Microbiology 155, 1192–1202.

Watanabe, I., Espinas, C.R., Berja, N.S., Alimagno, B.V., 1977. The utilization of the *Azolla - Anabaena* complex as a nitrogen fertilizer for rice. Monograph. International Rice Research Institute, Manila, Philippines.

Wu, H., Raza, W., Fan, J., Sun, Y., Bao, W., Liu, D., 2008. Chemosphere Antibiotic effect of exogenously applied salicylic acid on in vitro soilborne pathogen, *Fusarium oxysporum* f.sp. *niveum*. Chemosphere 74, 45–50.

Xia, J., Wishart, D.S., 2016. Using metaboanalyst 3.0 for comprehensive metabolomics data analysis. Current Protocols in Bioinformatics 55:14.10.1-14.10.91.

Xiang, N., Lawrence, K.S., Kloepper, J.W., Donald, P.A., McInroy, J.A., 2017. Biological control of *Heterodera glycines* by spore-forming plant growth-promoting rhizobacteria (PGPR) on soybean. PLoS ONE 12, 1–19.

Xie, J., Shi, H., Du, Z., Wang, T., Liu, X., Chen, S., 2016. Comparative genomic and functional analysis reveal conservation of plant growth promoting traits in *Paenibacillus polymyxa* and its closely related species. Scientific Reports 6, 1–12.

Xie, S., Wu, H., Chen, L., Zang, H., Xie, Y., Gao, X., 2015. Transcriptome profiling of *Bacillus subtilis* OKB105 in response to rice seedlings. BMC Microbiology 15, 1–14.

Xu, Y., Guo, J., Wang, L., Jiang, R., Jin, X., Liu, J., Fan, S., Quan, C.S., Ha, N.C., 2016. The crystal structure of the YknZ extracellular domain of ABC transporter YknWXYZ from *Bacillus amyloliquefaciens*. PLoS ONE 11, 1–14.

Yamada, Y., Tikhonova, E.B., Zgurskaya, H.I., 2012. YknWXYZ is an unusual four-component transporter with a role in protection against sporulation-delaying-protein-induced killing of *Bacillus subtilis*. Journal of Bacteriology 194, 4386–4394.

Yan, Z., Reddy, M.S., Ryu, C., Mcinroy, J.A., Wilson, M., Kloepper, J.W., 2002. Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Biologican Control 92, 1329–1333.

Yano, A., Kodama, Y., Koike, A., Shinya, T., Kim, H.J., Matsumoto, M., Ogita, S., Wada, Y., Ohad, N., Sano, H., 2006. Interaction between methyl CpG-binding protein and Ran GTPase during cell division in tobacco cultured cells. Annals of Botany 98, 1179–1187.

Yatazawa, M., Tomomatsu, N., Hosoda, N., 1980. Soil Science and plant nutrition nitrogen fixation in *Azolla-Anabaena* symbiosis as affected by mineral nutrient status. Soil Science and Plant Nutrition 26, 415–426.

Yeole, R.D., Dube, H.C., 2000. Siderophore-mediated antibiosis of rhizobacterial fluorescent *Pseudomonads* against certain soil-borne fungal plant pathogens. Journal of Mycology and Plant Pathology 30, 335–338.

Yi, Y., de Jong, A., Frenzel, E., Kuipers, O.P., 2017. Comparative transcriptomics of *Bacillus mycoides* root exudates reveals different genetic adaptation of endophytic and soil isolates. Frontiers in Microbiology 8, 1–14.

Yu, J.Q., Ye, S.F., Zhang, M.F., Hu, W.H., 2003. Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochemical Systematics and Ecology 31, 129–139.

Yuan, J., Zhang, N., Huang, Q., Raza, W., Li, R., Vivanco, J.M., 2015. Organic acids from root exudates of banana help root colonization of PGPR strain *Bacillus amyloliquefaciens* NJN-6. Scientific Reports 5, 1–8.

Zahid, M., Abbasi, M.K., Hameed, S., Rahim, N., 2015. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). Frontiers in Microbiology 6, 1–10.

Zaidi, A., Ahemad, M., Oves, M., Ahmad, E., Khan, M.S., 2017. Role of phosphate-solubilizing bacteria in legume improvement, in: Khan M.S., Musarrat J., Zaidi A. (Ed.), Microbes for Legume Improvement. pp. 175–197.

Zelcbuch, L., Lindner, S.N., Zegman, Y., Slutskin, I.V., Antonovsky, N., Gleizer, S., Milo, R., Bar-Even, A., 2016. Pyruvate formate-lyase enables efficient growth of *Escherichia coli* on acetate and formate. Biochemistry 55, 2423–2426.

Zgurskaya, H.I., Weeks, J.W., Ntreh, A.T., Nickels, L.M., Wolloscheck, D., 2015. Mechanism of coupling drug transport reactions located in two different membranes. Frontiers in Microbiology 6, 1–13.

Zhang, F., Meng, X., Yang, X., Ran, W., Shen, Q., 2014. Quantification and role of organic acids in cucumber root exudates in *Trichoderma harzianum* T-E5 colonization. Plant Physiology and Biochemistry 83, 250–257.

Zhang, L., Alfano, J.R., Becker, F., 2015. Proline metabolism increases katG expression and oxidative stress resistance in *Escherichia coli*. Journal of Bacteriology 197, 431–440.

Zhang, N., Wang, D., Liu, Y., 2014. Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains. Plant and Soil 374, 689–700.

Zhang, N., Yang, D., Wang, D., Miao, Y., Shao, J., Zhou, X., Xu, Z., Li, Q., Feng, H., Li, S., Shen, Q., Zhang, R., 2015. Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. BMC Genomics 16, 1–20.

Zhou, D., Huang, X.F., Chaparro, J.M., Badri, D. V., Manter, D.K., Vivanco, J.M., Guo, J., 2016. Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects. Plant and Soil 401, 259–272.

Ziegler, J., Schmidt, S., Chutia, R., Müller, J., Böttcher, C., Strehmel, N., Scheel, D., Abel, S., 2015. Non-targeted profiling of semi-polar metabolites in *Arabidopsis* root exudates uncovers a role for coumarin secretion and lignification during the local response to phosphate limitation. Journal of Experimental Botany 67, 1421–1432.

Ziegler, M., Engel, M., Welzl, G., M.S., 2013. Development of a simple root model to study the effects of single exudates on the development of bacterial community structure. Journal of Microbiolog 94, 30–36.

Zuo, F.L., Yu, R., Khaskheli, G.B., Ma, H.Q., Chen, L.L., Zeng, Z., Mao, A.J., Chen, S.W., 2014. Homologous overexpression of alkyl hydroperoxide reductase subunit C (ahpC) protects *Bifidobacterium longum* strain NCC2705 from oxidative stress. Research in Microbiology 165, 581–589