1. INTRODUCTION

Eukaryotes maintain close interactions with the surrounding microorganisms in nature, which are counted as meta-organisms or holobionts. This is essential as a natural adaptation mechanism for their fitness and survival. Microbes associated with rhizo-microbiome are enormously diverse and is mostly dependent on plant species and the environment (Hariprasad et al., 2014; Sarma et al., 2015). Due to this close association, rhizosphere microbiome is considered as the second-genome of the plant (Berg et al., 2014).

Plants spend an enormous portion of their photosynthetically-fixed carbon sources (viz., sugars, organic acids, amino acids, mucilage, dead epidermal cells) for maintaining their rhizo-microbiome. In return, microbes play a major role in nutrient uptake, maintenance of root architecture and deliver protection against phytopathogens (Pieterse et al., 2016). There are five types of alleochemicals produced by PGPR, which includes siderophores, antibiotics, mycolytic enzymes, volatile metabolites and natural systemic fungicides such as phosphinothricin and bialaphos (Saraf et al., 2014; Khan et al., 2016). Many PGPR produce exocellular enzymes which hydrolyze chitin, cellulose, proteins, hemicellulose etc., enabling them to contribute towards direct suppression of plant pathogens. Enzymatic digestion or deformation of cell wall components of fungal pathogens by these enzymes is one of the significant mechanisms of managing soil-borne pathogens (Saraf et al., 2014).

In order to impart a positive effect on the host plant (such as in biocontrol agents), the rhizosphere microbes must be highly competent (Parray et al., 2016), involving effective root colonization, survivability and successful proliferation in the host rhizosphere in presence of native rhizosphere microflora (Haas and Defago, 2005; Mendes et al., 2013). The denitrification process and synthesis of the siderophores (hydroxamates) are involved in the ability of Pseudomonas to compete in rhizosphere. Studies have shown that the bacterial mutants, which are unable to produce nitrate reductase and hydroxamates exhibited less competition than the wild-type leading to the hypothesis that denitrification by rhizosphere microbes could work as a selective advantage in the root environment (Philippot et al., 2013).

Communication between bacteria enabled in a cell density-dependent manner controls the expression of specific functions and is termed as quorum sensing, which
is made possible through complex chemical signaling. In gram negative bacteria it is mediated through N-acetyl-L-homoserine lactone (N-AHL) molecules. This communication allows bacteria to monitor their population density through responding to the concentration of quorum sensing molecules they produce (Schuster et al., 2013). N-AHL molecules are produced by many gram-negative PGPR, which are reported to play a major role in their biocontrol activity (Perez-Montano et al., 2014).

The biocontrol potential of pseudomonads has a direct correlation with its capability to produce antimicrobial compounds and lytic enzymes (Upadhyay and Srivastava, 2011). Most of the PGPRs especially the fluorescent pseudomonads produce a wide array of antibiotics. Whole genome sequencing of P. fluorescens Pf-5 showed that ~ 5.7 % of genome is dedicated only for synthesis of secondary metabolites (Paulsen et al., 2005). Such metabolites synthesised by pseudomonads are closely associated with fungal inhibition. Therefore the role of pseudomonad genetic elements on plant growth regulation is stronger than previously thought. Major antibiotics produced by fluorescent pseudomonads include 2, 4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, 2-hydroxy phenazines, and phenazine-1-carboxamide, of which DAPG is the most studied antibiotic (Choudhary et al., 2016). DAPG, at lesser concentrations, acts as signal molecule in the host plants and stimulates root exudation, root branching and induce systemic resistance (Vacheron et al., 2013). P. chlororaphis Q2-87 induces DAPG mediated signaling of ISR mechanism in Arabidopsis which was shown to be independent of jasmonic acid but occurred through the ethylene responsive gene (Vleesschauwer and Hofte, 2009).

The present study was designed to analyse the parameters of rhizosphere competence of shortlisted fluorescent pseudomonads such as sorbitol fermentation, nitrate reduction and production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, mycolytic enzymes (protease, lipase, chitinase, cellulose and amylase), phenazine antibiotics and N-AHL molecules.
2. MATERIALS AND METHODS

2.1. Sorbitol Fermentation and Nitrate Reduction

For sorbitol fermentation test, overnight cultures of fluorescent pseudomonads were inoculated into phenol red sorbitol broth containing peptone -10g L⁻¹ (w/v), beef extract - 1g L⁻¹ (w/v), NaCl - 5g L⁻¹ (w/v), sorbitol - 5g L⁻¹ (w/v) and phenol red indicator - 0.018 g L⁻¹ (w/v) and incubated at 37 °C for 24 h. The sorbitol fermentation resulted in acid production and the resulting drop in the media pH, can be identified by the colour change.

The isolates were then inoculated in to broth containing potassium nitrate containing broth and incubated at 37 °C for 48 h. The formation of a red colour within two minutes of adding of N,N-dimethyl-α-naphthylamine and sulphanillic acid indicate nitrate reduction. A red colour development on addition of a pinch of zinc powder confirms nitrate reduction (Engelkirk and Duben-Engelkirk, 2008).

2.2. Production of ACC Deaminase

All isolates were tested for their ability to degrade ACC as per Li et al. (2011) using ACC (Sigma-Aldrich) as the sole substrate. Briefly, isolates were grown on Dworkin and Foster (DF) medium (Dworkin and Foster, 1958) amended with ACC (3 mM), under shaking conditions (120 rpm). After 72 h, cells were separated by centrifugation and the cell free culture supernatant was diluted with ACC-DF medium (in 1:10 ratio). 2 ml of ninhydrin reagent (HiMedia) was added to the supernatant, followed by incubation in bath for 30 min under boiling condition. Purple colour development was read spectrophotometrically at 570 nm, where DF medium without ACC served as blank. The amount of ACC deaminase was indirectly calculated as difference in the ACC left in ACC-DF medium (mM L⁻¹) with and without incubation.

2.3. Estimation of Mycolytic Enzymes

2.3.1. Protease: All shortlisted fluorescent pseudomonads were tested for their capability to produce protease as per Anand and Kulothungan (2010). One millilitre of 72 h culture supernatant was added to the reaction mixture [1 % (w/v) casein in 0.05 M citrate phosphate buffer (pH 7)] and incubated for 1 h at 37 °C. The reaction was stopped by adding 10 % (w/v) TCA and was allowed to stand for another 20 min
at the same temperature. The absorbance was read at 275 nm and the results were expressed as Unit mL\(^{-1}\) against tyrosine standard graph.

**2.3.2. Lipase:** For quantitative lipase estimation, 2 ml of the fresh pseudomonad culture supernatant was diluted with 8 ml of water, followed by addition of 0.5 ml vegetable oil. The mixture was kept at 37 °C for 2 h and ethanol was added to make the final concentration of 30 % (v/v). Free fatty acid was extracted with petroleum ether, evaporated and dissolved in 15 ml ethanol. The mixture was then titrated against 0.05N NaOH using phenolphthalein indicator. The results were expressed as Unit mL\(^{-1}\) lipolytic units (Anand and Kulothungan, 2010).

**2.3.3. Chitinase:** 0.1 mL colloidal chitin (10 mg mL\(^{-1}\)) substrate was added to 0.4 mL cell free culture supernatants of fluorescent pseudomonads in 1 M sodium acetate buffer (pH 4). It was incubated for 1 h at 37 °C, followed by the addition of 6 mL of 1 % (w/v) para-dimethyl aminobenzaldehyde. The reaction mixture was centrifuged and the colour change of the supernatant was measured at 585 nm. Chitinase production was expressed as µg mL\(^{-1}\) against standard N-acetyl glucosamine (Subramanyam and Rao, 1987).

**2.3.4. Cellulase:** Production of cellulase was investigated as per Patagundi et al. (2014). Isolates were inoculated in a broth containing 0.2 % (w/v) K\(_2\)HPO\(_4\), 0.03 % (w/v) MgSO\(_4\), 1 % (w/v) peptone and 0.025 % (w/v) NH\(_4\)SO\(_4\) (w/v) and incubated for 72 h. The supernatant was mixed with 0.05 M phosphate buffer containing 1 % (w/v) carboxymethyl cellulose. It was further incubated for 30 min at 50 °C, followed by addition of 1.5 ml dinitrosalicylic acid (DNS) reagent followed by incubation (10 min at 100 °C). Absorbance of colour change was read at 540 nm and expressed in Unit mL\(^{-1}\) against a standard glucose graph (50-500 µg mL\(^{-1}\)).

**2.3.5. Amylase:** For quantitative estimation of amylase produced, fluorescent pseudomonad isolates were grown in medium containing peptone (10 g L\(^{-1}\) w/v), beef extract (1 g L\(^{-1}\) w/v), NaCl (5 g L\(^{-1}\) w/v) and starch (5 g L\(^{-1}\) w/v), incubated for 72 h at 37 °C. The cell free culture supernatant (0.1 mL) was added to 1 mL substrate [1 % (w/v) soluble starch in 15 mM buffer of pH 7] and was incubated at 37 °C for 10 min, followed by addition of 2 mL of DNS and heated at 100 °C for 10 min, cooled and optical density was read at 540 nm (Sjofjan and Ardyati, 2011). Amylase production results were expressed as Unit mL\(^{-1}\) against standard glucose (50-500 µg mL\(^{-1}\)).
2.4. Qualitative Detection of N-AHL Production

Cross-feeding plate assay for the detection of N-AHL using reporter strains was performed. Here, 0.8 % (w/v) LB agar plates were streaked with fluorescent pseudomonads and the reporter strain *Chromobacterium violaceum* (CV) 026 (Obtained from Prof. Robert McClean, University of Nottingham and Prof. Paul Williams, Texas State University as gift) 1 cm apart, as per the methodology suggested by McClean *et al.* (1997). N-AHL producing strain of *Agrobacterium veroni* MCC 2031 obtained from National Centre for Cell Sciences (NCCS), Pune served as reference strain.

2.5. Qualitative Screening for Antibiotics

To determine the phenazine antibiotics synthesised by fluorescent pseudomonads, isolates were grown in 10 mL of KB broth for 4 days at 28 ± 2 °C. Cell free culture supernatants were acidified to pH 2.0 with 1 % (v/v) trifluoroacetic acid (Himedia) and the phenazine compounds were extracted twice with double volume of ethyl acetate. Organic fractions were separated, pooled, evaporated to dryness and resuspended to 2 mL methanol (Ghirardi *et al.*, 2012).

The samples were centrifuged at 14,000 rpm for 230 min and 20 µL supernatant was injected in the column. Phenazines were separated by HPLC, on a C18 column (Diameter 4.6 mm and 250 mm length reverse phase column from Agilent Technologies™) fitted onto a Waters™ HPLC 510 system with UV-visible detector. The culture supernatant extracts were tested at 248 & 367 nm for phenazines, at 285 & 330 nm for monoacetylphloroglucinol at 270 & 330 nm for 2,4-diacetylphloroglucinol and at 255 & 308 nm for pyoluteorin. The solvent system was retained at 100 % (v/v) acetonitrile (Merck) and 0.1 % (v/v) trifluoroacetic acid as per Bonsall *et al.* (1996) and the pressure was maintained at 1300 psi.

2.6. Statistical Analyses

All the experiments were performed in triplicates, mean and standard error was calculated from the experimental data. Graphs were generated using Graph Pad Prism 6.1 software.
3. RESULTS AND DISCUSSION

It was summarized that there are three major signaling mechanisms which occur in plant rhizosphere.

(i) Microbial inter-species and intra-species signaling, which occur mainly through quorum sensing molecules.

(ii) Signals from host plants to microorganisms through plant-secreted molecules.

(iii) Signaling from microorganisms to plants by microbially synthesised molecules affecting plant defence response, gene expression and root composition (Venturi and Keel, 2016).

Generally when a new biocontrol agent is introduced into a new host environment, plants recognize them as non-self and the establishment of the mutually beneficial relationship often fails. This competition is highly aggressive in terms of colonization by eliminating non-competent rhizosphere microbes (Khan et al., 2016; Venturi and Keel, 2016). Therefore, in order to establish an efficient biocontrol mechanism by any microbe, its rhizosphere competency is an essential factor (Pieterse et al., 2016). The present objective was to detect the rhizosphere competence of shortlisted fluorescent pseudomonads, which were characterized and shortlisted as mentioned in Chapter I.

3.1. Biochemical Variations Associated with Rhizosphere Competence of the Shortlisted Fluorescent Pseudomonads

In the present experiment, sorbitol fermentation and nitrate utilization were studied with all the eleven shortlisted isolates. It was observed that six were positive for sorbitol fermentation and nitrate reduction (Table 10). Sorbitol fermentation is considered as a major contributor to the rhizosphere competence of PGPR (Ghirardi et al., 2012). Delorme et al. (2003) observed that Pseudomonas strains with both the denitrification process genes (narG and nosZ) survived better in the soil environment and total denitrifiers showed better rhizosphere competence (Choudhary et al., 2009). P. fluorescens impaired in nitrate or nitrite reductase activity were deficient in rhizosphere colonization and furthermore, it has been shown that denitrification is associated with rhizosphere competence in rhizosphere-isolated fluorescent pseudomonads (Redondo-Nieto et al., 2013). Downstream reactions that lead to
nitrogen contribute to further increase in the energetic yield and global metabolic efficiency of bacteria (Ghirardi et al., 2012). However, interestingly in comparison to non-denitrifiers, strains harboring only nitrate reductase activity were not significantly better disease control agents in the present study. Nitrate - nitrite reduction results in high energy yield and is expected to contribute to rhizosphere competence. A study using metagenomics and transcriptomic approach has clearly shown that the higher expression of denitrifying genes is a signature signal in root colonization (Ofek-Lalzar et al., 2014). The activation of denitrification mechanism results in significant divergence of levels of oxygen availability between roots and presence of nitric oxide in host plant. Hence, in the light of the present biochemical observations, it is presumed that denitrifying gene expression must be either absent or significantly low in tested bacterial isolates.

3.2. Variations Associated with Production of ACC Deaminase

In order to establish a link between the ACC utilization ability and disease suppression, levels of ACC deaminase were estimated in the eleven fluorescent pseudomonad isolates. The isolates were checked for their ability to utilize ACC as the sole nitrogen source. Among all fluorescent pseudomonad isolates, highest ACC production was shown by M137 (2.31 ± 0.36 mM), followed by the isolate M139 (2.29 ± 0.04 mM) and M80 (2.21 ± 0.06 mM). ACC deaminase activity was not detected in the isolates R69 and M140 under test conditions (Table 11).

Higher levels of ethylene in the host plants are shown to have an inhibitory effect on root elongation in plant seedlings (Glick, 2014). A number of PGPR produce the enzyme ACC deaminase and this cleaves the plant ethylene precursor ACC and optimizes the level of ethylene in the process. ACC deaminase activity of the rhizobacterial species has significant positive effects on the host plant by facilitating plant growth under different types of stress (Rodriguez et al., 2008). Ali et al. (2013) reported the use of ACC deaminase producing fluorescent pseudomonads showed plant growth promotion traits along with drought tolerance. In a study on community compositions of Bacillus in the commercial tomato rhizosphere showed that only 6% of the Bacillus isolates were able to produce ACC deaminase, which also indicated potential plant growth promoting traits on tomato (Xu et al., 2014). Seed treatment with ACC deaminase producing Pseudomonas pseudoalcaligenes isolates from paddy
rhizosphere helped the plants to reduce *Magnaporthe grisea* infection by production of β-1,3-glucanase and chitinase and also showed improved vigour in rice plants (Jha and Subramanian, 2014). Overall, the present observations shows that ACC deaminase producing pseudomonad isolates can act as potential biocontrol candidates.

### 3.3. Mycolytic Enzyme Production

Mycolytic enzymes are the key players in many groups of fungi and bacteria and are being exploited widely for crop disease management (Ajit et al., 2006). Analysis of protease production showed that highest production was in M80 (10.73 ± 0.46 U mL$^{-1}$), followed by M96 (10.69 ± 0.18 U mL$^{-1}$) and by R78 (8.14 ± 0.44 U mL$^{-1}$). Highest lipase production was shown by the isolates M135 (2.83 ± 0.15 µM NaOH mg$^{-1}$ protein$^{-1}$ h$^{-1}$) followed by R78 (1.73 ± 0.07 µM NaOH mg$^{-1}$ protein$^{-1}$ h$^{-1}$) and R69 (0.53 ± 0.03 µM NaOH mg$^{-1}$ protein$^{-1}$ h$^{-1}$), whereas the highest chitinase production was shown by the isolate M140 (117.50 ± 10.20 U mL$^{-1}$) followed by M96 (106.00 ± 9.54 U mL$^{-1}$) and R69 (67.17 ± 3.77 U mL$^{-1}$). Highest cellulase production was by isolate M132 (15.33 ± 0.04 U mL$^{-1}$), trailed by M127 (15.10 ± 0.00 U mL$^{-1}$) and M135 (15.09 ± 0.03 U mL$^{-1}$). Highest amylase production was by R69 (0.27 ± 0.07 U mL$^{-1}$) trailed by M132 and M80 (0.17 ± 0.04 U mL$^{-1}$). Collectively results of the present study reveals that all the fluorescent pseudomonad isolates were capable of producing extracellular mycolytic enzymes at varying levels (Table 11).

The lytic enzyme production by the PGPR attributes to their efficiency in fungal cell wall degradation, which may account for improved disease management. Biocontrol strain *Bacillus subtilis* AF1, positive for the production of β - 1,3 and 1,4 glucanases suppressed >90 % growth of *Aspergillus niger*, a fungal pathogen causing black mould disease in many vegetable crops (Manjula and Podile, 2005). Tomato root treatment with isolates of *Bacillus*, which produced mycolytic enzymes showed increased defence against the fungal pathogen *Rhizoctonia solani* (Solanki et al., 2012). Furthermore, the plant showed a higher production of phenolic contents, defence-specific enzymes, chitinase, glucanase, polyphenoloxidase, peroxidase and phenylalanine ammonia lyase in leaves followed by root treatment.

Chitinases comprise the second largest group of antifungal proteins, which are found in a large group of organisms. Chitinases inhibit growth of fungal pathogens by hydrolyzing the chitin cell wall and generate chitin oligosaccharides that act as
eliciters for ISR (Hamid et al., 2013). Chitinase producing B. subtilis CRB20 isolate has got the potential fashion not only to augment plant growth but also to protect the tomato seedlings from F. oxysporum infection (Hariprasad et al., 2014). In another study by Babu et al. (2015) identified glucanase and chitinase producing PGPR can manage early blight disease caused by Alternaria solani in tomato. Illakiam et al. (2013) reported that the protease producing P. aeruginosa isolate which acts as an antagonist against the charcoal rot plant pathogen Macrophomina phaseolina. In another interesting study by Alamri (2014) used Bacillus subtilis JF419701 produced α-1,3 and β-1,3-glucanase, protease and chitinase. In vitro studies showed that the biocontrol strain depicted efficient inhibition against F. oxysporum, Alternaria alternata, Pythium ultimum, Exserohilum rostratum, Macrophomina phaseolina and Rhizoctonia solani through the production of various mycolytic enzymes.

Mycolytic enzyme production by PGPR can independently act as a defence mechanism. Technically, since the priming site of bacteria and fungus are spatially separated in the experiment set up, it is apparently sure that defence mechanism is not necessarily associated with mycolytic enzyme production (Tonelli et al., 2011). Nevertheless, in the natural environment the fungal spore inoculation need not imitate the experimental setup. In this context, the above mentioned mycolytic enzyme production results can be considered as an added rhizospheric advantage for biocontrol strains while shortlisting the potential candidates.

3.3. N-AHL Production by Fluorescent Pseudomonads

Since microbes in rhizospheric niche live in colonies, it is hypothesized that every microbial component provides specific benefit to the host plant (Sarma et al., 2015). Bacteria have complex communication processes to control the expression of functions in a cell density-reliant manner, termed as quorum sensing (Podile et al., 2014). This promotes the formation of rhizo-microbiome and synchronise their behavior in a competitive environment (Venturi and Keel, 2016). Many of the pathogens also communicate in a similar manner. It has been reported that biocontrol agents such as pseudomonads inhibit the quorum sensing of phyto-pathogens by breaking down the N-AHLs and reducing their virulence (Reddy and Saravanan, 2013).
In the present study all the tested isolates were positive for N-AHL in the qualitative assay carried out using reporter strain CV026. N-AHL are the most common and studied signal of communication in gram negative bacteria. It has been reported that N-AHL release has a direct correlation with secondary metabolite release such as antibiotics, which ultimately end up as antagonistic mechanism (McClean et al., 2004). It is also established that bacterial N-AHL can act as a major signal in rhizosphere competition (Ghirardi et al., 2012) and in the induction of ISR in various plant species including tomato (Barriuso et al., 2008).

Many PGPR members were able to reduce plant ethylene levels through chemical deamination of ACC, especially Pseudomonas. Whole genome sequences of many PGPR proved that they contain ACC deaminase coding gene (acds), which can degrade ACC into ammonia and α-ketobutyrate (Prigent-Combaret et al., 2008). AcdS activity decreases root ethylene production and alleviate the suppressing effect on host root development (Glick, 2014). A recent study by Rajesh and Rai (2016) clearly showed the virulence inhibition of two major phyto-pathogens, P. aeruginosa PA01 and Pectobacterium carotovorum, by the N-AHL molecules secreted by an endophytic biocontrol strain Bacillus cereus VT96. The conclusive evidence on activation of ISR in tomato through N-AHL signal was shown by Schuhegger et al. (2006). The study used two N-AHL producing strains, Serratia liquefaciens MG1 and Pseudomonas putida IsoF to prime tomato plants against the fungal pathogen Alternaria alternate. Northern blot and microarray results evidently showed an increased expression of defence specific genes in the host.

Uptake of quorum sensing signaling molecules by the surrounding bacteria has two consequences. Firstly, they regulate characteristics such as secretion of diverse exoproducts. These exoproducts have various functions such as virulence factors that damage host, nutrient scavenging molecules (siderophores) and compounds for delivering support for the growth in biofilms and surfactants for facilitating movement. Second, this may leads to an enhanced production of signal molecules (termed autoinduction), which often leads to positive feedback at high cell densities and an increase in cooperative production of exoproducts (Diggle et al., 2007). In the current study, all isolates were better colonizers through rhizosphere competence in the context of N-AHL production (Figure 23).
3.4. Production of Antibiotics

All the eleven isolate extracts were tested for their absorption peaks for detection of four common antibiotics. Out of eleven fluorescent pseudomonad isolates six of them showed peak absorption at 248 and 367 nm at the aforementioned HPLC conditions, indicating production of phenazine compounds. Five of the isolate supernatants showed peak absorption at 255 and 308 nm indicating the presence of 2,4-diacytethylphloroglucinol. Only one isolate showed production of mono acetyl phloroglucinol with peak absorption at 285 and 330 nm (Table 12). No fluorescent pseudomonad isolate extracts were observed with specific peaks for pyoluteorin. The representative HPLC absorbance peaks are represented in Figure 24.

Competition is a major strategy among bacterial communities to establish a niche (Haas and Défago, 2005). Antibiotic production is a major interference competition mechanism among bacteria. Activities such as motility and antibiotic production, together can retain their competitive balance (Hibbing et al., 2010). Despite the pathogen inhibitory action, lower concentrations of antibiotics produced from PGPR plays a major role as communication signal (Lareen et al., 2016). For example, the antimicrobial compounds such as phenazine and pyocyanin, produced by P. aeruginosa, have shown to influence on gene expression in several other bacterial species (Hibbing et al., 2010). Furthermore, the capacity of fluorescent pseudomonads to synthesise phenazine antibiotics gives them a competitive advantage under iron-depriving circumstances and contributes towards iron mobilization in the soil (Ghirardi et al., 2012). Mechanism of inhibition of phyto-pathogens by PGPR, mediated through phenazine production is still unclear, nevertheless, proposed mechanisms including DNA replication inhibition, uncoupling of electron transport and disruption of normal membrane functions (Khan et al., 2016).

Besides manipulating the host immune system, rhizo-microbiome protects host against soil-borne pathogens through production of antibiotics, nutrients and niche competition (Pieterse et al., 2014). The major classes of bacterial signals which have been proposed to function as biocontrol agents are antibiotics. Common PGPR-produces antibiotic compounds, include oomycin A, DAPG, hydrogen cyanide and phenazine, which are responsible for the biocontrol mechanism (Lareen et al., 2016). Out of the ~ 6000 known antibiotic compounds containing phenazine, ~ 100 are from
natural origin and are mainly synthesised by *Pseudomonas* spp. (Mavrodi *et al.*, 2006). Gram-negative gamma group Proteobacteria, fluorescent pseudomonads, especially *P. fluorescens*, *P. chlororaphis* and *P. aeruginosa* are the best-studied producers of pyocyanine and other phenazine antibiotics (Haas and Defago, 2005; Mavrodi *et al.*, 2006). The first report on inhibition of *F. oxysporum* by phenazines produced by *P. chlororaphis* was by Thomashow and Weller (1988). Since then, so many compounds from microbial origin are reported as potential inhibitors of pathogens. A large number of PR proteins, mainly enzymes such as peroxidase, lipoxygenase, β-1,3-glucanase and chitinase are linked with the activation of ISR (Waewthongrak *et al.*, 2014).

The metabolite 2,4-diacetylphloroglucinol contributes to antifungal activity in fluorescent pseudomonads. A study on 230 fluorescent pseudomonads collected from around globe showed that 2,4-diacetylphloroglucinol production in the biocontrol strains were associated with improved disease suppression activity in *Fusarium* – tomato and *Pythium* – cucumber host-pathogen interaction (Rezzonico *et al.*, 2007). In a noted study by Agaras *et al.* (2015) used characteristics such as *in vitro* antifungal activity, root growth promotion, AHL signaling, biofilm formation, production of mycolytic enzymes (Phospholipase and Exoprotease), antibiotics (DAPG, phenazine, pyoluteorin and pyrrolnitrin), hydrogen cyanide, IAA, ACC and siderophores, for shortlisting the best fluorescent *Pseudomonas* spp.

PGPR agents produce wide variety of metabolites and enzymes and their biocontrol traits rely mostly on their emission to manage phyto-pathogens (Saraf *et al.*, 2014). The present study results highlight the metabolic profiling of fluorescent pseudomonads pertaining to antibiotics, mycolytic enzymes and their ability to metabolize various substrates. This functional diversity presumably leads to different results in antagonizing fungal pathogen under green-house conditions. In any case there are substantial literature supports on the action of allelochemicals to induce resistance at the green-house level. Thus, there is every reason to hypothesise that the aforesaid behavior will improve the capability of fluorescent pseudomonads to substantiate the pathogen invasion in host.