New Method for Isolation of Plant Probiotic Fluorescent Pseudomonad and Characterization for 2,4-Diacetylphloroglucinol Production under Different Carbon Sources and Phosphate Levels

Sumant Chaubey, Malini Kotak and Archana G*

Department of Microbiology & Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390 002, Gujarat, India

Abstract

**Aims:** Present work describes the new enrichment method for the isolation of effective root colonizing and rhizosphere competent strains of genus fluorescent Pseudomonad and study of metabolic regulation of 2,4-DAPG biosynthesis in them under carbon sources and Pi levels.

**Methods and Results:** Three rounds of plant assay was performed using root tip attached microorganism mixtures for the next round of root treatment followed by phenotypical separation of fluorescent colonies to isolate fluorescent pseudomonad strains from different crop and vegetables rhizospheres. Isolated strains were characterized for their Plant Growth Promoting Rhizobacteria (PGPR) traits viz phosphate solubilisation, production of siderophore, IAA, HCN, 1-aminocyclopropane-1-carboxylate/L-methionine utilization pathway and antifungal metabolites production. Isolated strains have shown high 2,4-diacetylphloroglucinol production and strain G2 has shown 4.6 fold high production than Pf CHAO. Conclusions: Strain G1 and G8 supported 2,4-DAPG production under sucrose and found to be suitable biocontrol for succrose rich rhizosphere. Strain G1 and G2 showed good 2,4-DAPG production at high Pi and will perform well in phosphate fertilizer supplemented soils.

**Significance of Study:** Identification of factors favorable for bio-control will facilitate the targeted application of specific strains to plant rhizosphere/soil type/fertilizer supplemented suitable to their biocontrol activity i.e. "prescription" controls.

**Keywords:** Fluorescent pseudomonad; PGPR traits; PhID; 2,4-DAPG; Carbon sources; Pi

Introduction

Fluorescent pseudomonad represents a major group of the plant beneficial rhizobacteria present in various crop rhizospheres [1-3]. Fluorescent *Pseudomonas* sp. control plant diseases by antibiotics [4-6], competition for niches and nutrients i.e. effective root colonization [7]. Competitive root tip colonization by *Pseudomonas* strains can play an important role in the efficient control of soil borne crop diseases caused by fungi [8-10]. Three major types of molecules found to be involved in the antagonism towards soil borne fungal pathogens: siderophores, antibiotics and HCN [11-13]. Among the antibiotics the polyketide-2,4-diacylphloroglucinol (2,4-DAPG) has received particular attention because of its broad-spectrum antifungal, antibacterial and antihemminthic activity [6,12,14,15]. *P. fluorescens* CHAO isolated from a Swiss soil naturally suppressive to black root rot of tobacco caused by *Chalara elegans* (synanamorph *Thielaviopsis basica*). *Pf* CHAO reduces the extent of disease caused by several root-pathogenic fungi such as *Thielaviopsis basicola, Gaeumannomyces graminis* var. *tritici* (*Ggt*), *Pythium ultimum, Rhizoctonia solani*, and *Fusarium oxysporum* [16]. In many of these studies, production of 2,4-DAPG has emerged as a key factor in the biological control activity of *Pf* CHAO [17]. *Pf* CHAO has been used as a model organism to identify biosynthetic genes of HCN and 2,4-DAPG and to study their regulation [16,17].

Certain plant growth promoting rhizobacteria (PGPR) contain the enzyme ACC deaminase to lower endogenous levels of ethylene by hydrolyzing ACC into a keto-butyrate and ammonia, which affects plant growth [18], while majority of soil microorganisms produce ethylene from methionine (L-MET) via the 2-keto-4-methylthiobutyric acid (KMBA) pathway. Methionine is deaminated to produce 2-keto-4-methylthiobutyric acid (KMBA), which is then oxidized to produce ethylene by *Escherichia, Pseudomonas, Bacillus, Acinetobacter, Aeromonas, Rhizobium,* and *Corynebacterium* species [19]. Some bacterial strains have either ACC deaminase activity (*Pseudomonas Putida* biotype A, A7), or the ability to produce ethylene from L-MET (*Acinetobacter calcoaceticus*, M9) or both (*Pseudomonas fluorescens*, AM3) [18].

 Sugars constitute a major component in root exudates and a very labile source of carbon for microorganisms [20]. Jaeger et al. [21] has reported that sucrose availability was highest at the tip section of the grass root and decreased in progressively older sections. Effective root colonizer and plant growth promoting strains of fluorescent pseudomonas have isolated worldwide and it was found to be a time consuming isolation method. Method of isolation can be made easy by the enrichment of effective root colonizers and plant growth promoting fluorescent pseudomonad using specific enriching conditions. Kuiper et al. [22] described a method to select enhanced grass root tip colonizing bacteria. In this method a mixture of rhizosphere bacteria is applied on a sterile seedling. After plant growth in a gnotobiotic system

*Corresponding author: Archana G, Department of Microbiology and Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390 002, Gujarat, India. Tel: 0991-265-2794398; Fax: 0991-265-2792508; E-mail: sumant.msu@gmail.com

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those bacteria that have reached the root tip are isolated. These are subsequently used to inoculate a fresh sterile seedling, which again is allowed to grow [23]. After three of these enrichment cycles, excellent competitive root tip colonizers were obtained [15]. Kamimoto et al. [15] used this method to select enhanced tomato root tip colonizers. To our knowledge, no procedures have been described, which facilitate the selection of effective root colonizing and plant beneficial fluorescent pseudomonad.

2,4-DAPG producing pseudomonad are commonly found in the rhizosphere of important crops such as cucumber, maize, pea, tobacco, tomato, and wheat and protect from severe phytopathogens [3,9,19,22,24-26]. In the fungus Pythium ultimum var. sporangiiferum, 2,4-DAPG causes alterations of the plasma membrane, vacuolization, and the disintegration of cell contents [24], suggesting that it impedes the maintenance of membrane integrity. In bacteria, 2,4-DAPG may cause lysis by a novel antibiotic mechanism. For instance, mexitillin-resistant Staphylococcus aureus may lyse within 2 h of exposure to 0.5 μM 2,4-DAPG [27], whereas Vibrio paradoxus lyses more slowly and in response to higher 2,4-DAPG concentrations of 114 μM [28]. The biosynthetic locus for 2,4-DAPG includes phlA, phlC, phlB, and phlD, which are transcribed as an operon from a promoter upstream of phlA [29]. PhlD is responsible for the production of monoacetylphloroglucinol (MAPG), and PhlA, PhlC, and PhlB are necessary to convert MAPG to 2,4-DAPG. PhlD is especially interesting because of its homology to members of the highly conserved chalcone and stilbene synthase family of plant enzymes, which is suggestive of a common evolutionary origin [29]. Probes and primers specific for sequences in phlD have been used in combination with colony hybridization and polymerase chain reaction (PCR) to quantify population sizes of 2,4-DAPG producers in the rhizosphere environment [1,14,21,30].

Important obstacle to commercial application of efficient 2,4-DAPG producers is the inconsistency of their performance [31]. Although its ability to reduce the severity of diseases caused by soil borne fungal pathogens under laboratory conditions has been reported in several studies, inconsistent performance in commercial settings and field trials tends to be disappointing [25]. Understanding the sources of variability is key to overcoming this obstacle. Because a primary mechanism of disease suppression available to fluorescent pseudomonad is antibiosis [25], it is thought that variable performance might result from variation in production of antimicrobial compounds like 2,4-DAPG production. Variable performance might be because of variability in 2,4-DAPG production due to variations in environmental conditions, abiotic and biotic, that might confront bacterial metabolite production in the rhizosphere. Inorganic phosphate inhibited PHL production in different ARDRA groups of fluorescent pseudomonad to various degrees in the study by Duffy and Defago, 1999. PHL production by CHA0 was almost abolished by 100 mM phosphate [31]. Pf CHA0 was used as a model organism. This study deals with isolation of rhizospheric fluorescent pseudomonad from various plants and ecologically diverse locations, characterization of their plant growth promoting traits and detail study on 2,4-Diacetyl phloroglucinol production in the isolates.

Materials and Methods

Isolation of efficient root colonizing and plant growth promoting fluorescent pseudomonad

Seed sterilization: Equal size Vigna radiata seeds were thoroughly washed with sterile distilled water. Seed were further treated with 1% HgCl₂ (For 2 minute) followed treatment of 70% ethanol (For 2 minute). Final wash of sterile distilled water to remove traces of HgCl₂ and seeds were transferred to sterile petri plates containing wet filter paper. Sterile seeds on soaked filter paper were incubated 30 ± 2°C and kept in dark. Seeds were allowed to germinated up to radicle size of 1 cm.

Enrichment method for isolation of fluorescent pseudomonad: Isolation of fluorescent pseudomonad involved the three successive round of plant inoculation. Root samples of were washed with distill water for two to three times to remove all the superficially attached bacteria. The suspensions of tightly attached bacteria were prepared in sterile 0.85% saline after vigorously vortex of root tip samples in 20 ml saline sample for nearly 30 minutes. In the method, a mixture of rhizospheric bacteria was applied on a germinated seedling and seeds were transferred to sterile petri plates containing 0.8% agar-agar containing 0.8% agar-agar as solidifying agent. The germinated seedlings were allowed to grow at 30°C under maintained light-dark period. After 7 day, the plants growth was monitored in term of shoot and root weight. Plants showing the enhanced growth compare to uninoculated and/or Pf CHA0 were selected out for further study. The roots were washed twice with sterile distill water so that only those bacteria that have colonized efficiently remain attached. One cm of root tips were sliced from main and lateral roots to resuspended in 1.5 ml of 0.85% sterile saline and vortex vigorously for 30 minutes. Serial dilutions was performed and dilutions were spreaded evenly on the King’s B Agar Medium (KMB). Plates were incubated at 30°C for 24 hours and were observed for the fluorescent colonies as fluorescent pseudomonad produces water soluble green fluorescence pigment when subjected to UV exposure. Screening of microorganisms was done on the basis of fluorescence of the colony. Whole zone/patches of fluorescent colonies was collected by wire loop and resuspended in 1.5 ml of 0.85% sterile saline and vortexes for making it uniform suspension. The suspensions of fluorescent colonies were used to inoculate sterile germinated seedlings and repeated for two more cycles of plant inoculation study. Fluorescent bacteria were purified by repeated streaking on KMB plates and were believed to be good plant growth promoting and root colonizing bacteria.

Identification bacterial cultures by biochemical methods: For the identification of fluorescent pseudomonad biochemical test like catalase test, oxidase Test, Hugh-Leifson’s Oxidation-Fermentation test, Gram staining and arginine dihydrolase test was performed using protocol as described in Bergey’s manual.
Identification bacterial cultures by molecular methods: Modified CTAB method was used for the extraction of genomic DNA. 1.5 ml of overnight grown cultures was centrifuged at 10,000 rpm for 5 minutes at 4°C. The supernatant was drained off and the pellet was resuspended in 200 µl of T. E. Pellet was vortexed vigorously to resuspend the pellet and then was kept at 60°C for 30 minutes. 100 µl of 3M NaCl was added to it followed by 80 µl of 10% CTAB. It was mixed properly and then again kept at 60°C for 10 minutes. Equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added and centrifuged at 10,000 rpm for 12 minutes at 4°C. Aqueous phase was collected and 2-3 volume of chilled 100% ethanol was added and kept for 1 hour. Further it was centrifuged at 10,000 for 10 minutes at 4°C and supernatant was drained off and the pellet was again washed with 70% ethanol. The ethanol was allowed to evaporate and dried DNA was resuspended in sterile distilled water.

Identification of fluorescent pseudomonad by molecular method involved the amplification of region including the 3' half of the 16S rDNA with the whole 16S-23S rRNA Internal Transcribed Spacer (ITS) sequence using specific primers [11]. Primer sequences ITS1F-5’-AGTCGTAACTAGGTAG-3’ and ITS2R-5’-GACCATATATAACCCCAAG-3’ was used to get amplicon size of 560 bp.

PGPR traits of fluorescent Pseudomonas strains

Phosphate solubilization: Phosphate solubilization ability was checked on Pikovskaya’s agar medium (Hi–Media Ltd., India) which contain insoluble dicalcium phosphate. Overnight grown cultures in 1.5 ml centrifuge tube and with equalized OD 0.30±0.01. One loopful of each strain was spotted on Pikovskaya’s agar medium and incubated at 30°C for 24 hours and was observed for zone of clearance/colony size.

Antifungal activity: Antifungal activity was checked on potato dextrose agar (Hi–Media Ltd.). Fluorescent Pseudomonas strains were grown in King’s B broth for 24 hrs. Overnight grown cultures in 1.5 ml centrifuge tube and with equalized OD 0.30±0.01 were centrifuged at 6,000 rpm for 5 minute and further washed with normal saline (0.80% NaCl). 50 µl of concentrated pellet were inoculated on the four corners of the plate and R. bataticola inoculated at the centre of potato dextrose agar plate. The inhibition of R. bataticola was observed after 48-72 hours of growth. Percentage fungal inhibition was calculated by the formula. Percentage inhibition=(Radial growth of fungus in absence of inoculants) – (Radial growth of fungus in presence of inoculants/ (Radial growth of fungus in absence of inoculants) × 100.

Siderophore production: For detection of siderophores, overnight grown culture washed with saline and spotted on the Chrome Azurol-S (CAS) agar plates and observed for the colour change from greenish-blue to yellowish orange halo around culture pellet [4]. The halo due to chelation of Fe²⁺ from the CAS-Fe²⁺ conjugate and the diameter of halo zone/colony size was calculated which indirectly represent the ability of siderophore production by isolates. The siderophore production of isolates was compared to the bio-control and siderophore producing standard strain Pf/CHA0.

HCN production: HCN production by isolates was checked by method of Bakker and Schipper, 1987 on Kings B Medium. King’s B agar amended with 4.4 g/l glycine is used in HCN estimation single isolates were streaked in each plate. Whatman no. 1 filter paper disc (9 cm in diameter) was soaked in 0.5% Picric acid in 2% sodium carbonate. Soaked disc was placed in the lid of each petriplate. Petriplates were sealed with parafilm and incubated at 30°C for 4 days. An uninoculated medium with the soaked filter paper was kept as control for comparison of results.

IAA estimation: IAA estimation was done using Salkowsky method. Overnight grown 100 µl culture was inoculated in 2 ml minimal media amended with 50 µg/ml tryptophan. Incubated at shaking condition for 48 hours at 30°C at 200 rpm. Growth culture was centrifuged at 10, 000 g for 15 minutes. 1 ml of supernatant was taken fresh tube and 2 to 3 drops of ortho-phosphoric acid added to the supernatant followed by addition of 2 ml of reagent (1 ml of 0.5 M FeCl₃ in 50 ml of 35% HClO₄). Samples were incubated for 25 minutes and absorbance was measured at 530 nm. Concentration of IAA was measured against standard graph plot of pure IAA (Hi-media, India) at the range of 10-100 µg/ml.

Characterization for ACC deaminase/ KMBA pathway: Plate technique using salt minimal medium containing ACC as sole nitrogen source (enrichment technique) was used to characterize the strains for ACC deaminase activity. The composition of salt minimal media containing ACC as sole nitrogen source in g L⁻¹ is as follows, KH₂PO₄, 1.36; NaHPO₄, 2.13; MgSO₄.7H₂O, 0.2; CaCl₂.2H₂O, 0.7; FeSO₄.7H₂O, 0.2; CuSO₄.5H₂O, 0.04; MnSO₄.7H₂O, 0.02; ZnSO₄.7H₂O, 0.02; H₂BO₃, 0.003; CoCl₂.6H₂O, 0.007; Na₂MoO₄.2H₂O, 0.004; Substrate ACC, 5 mM; Glucose, 1.0% dissolved in 1000 ml of distilled water.

The presence of KMBA was determined by precipitation with 2,4-dinitrophenylhydrazine according to Primrose [32]. The culture medium was separated and 0.1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2 M HCl was added to 1 ml of culture filtrate and vortextes at room temperature in darkness. Presence of KMBA was confirmed by formation of a yellow precipitate after 30 min.

Effect of nutritional factors on 2,4-DAPG biosynthesis by fluorescent pseudomonad

Quantification of 2,4-DAPG biosynthesis: Each strain was grown in 1/5 diluted 20 ml King’s B medium at shaking condition at 130 rpm at 26°C for 72 hours. Culture supernatant was acidified by addition of 1N HCl to make pH equal to 2. 10 ml ethyl acetate was added to supernatant and vigorously vortex for 2 minute and allowed to separate in two layers. Upper ethyl acetate phase was extracted and was allowed to evaporate and antibiotic preparation was dissolved in 1 ml methanol and stored in -20°C for Bioassay for 2,4-DAPG was performed using methicillin resistant Staphylococcus aureus 6538 as a sensitive strain and phytopathogen R. bataticola. HPLC analysis. A mixture of 30% ACN: 25% Methanol: 45% MQ water was used as mobile phase using C18 reverse phase column (250 x 4.6 mm) and flow rate at 1.0 ml/min at wavelength 272 nm. 2,4-DAPG was quantified using the standard plot of peak area and concentration (10-100 µg/ml).

Effect of carbon sources and Pi level on 2,4-DAPG biosynthesis: Each strain was grown in 1/5 diluted 20 ml King’s B medium with the supplementation of phosphate to the final concentrations of 0, 8, 12, 17, 50 and 100 mM. Each strain was grown for 72 hours and OD was observed at 600 nm to monitor cell growth. Ethyl acetate extraction and HPLC for 2,4-DAPG was carried out in similar manner as discussed earlier.

Each strain was inoculated in King’s B broth (without glycerol) and supplementation of 1% carbon sources: glucose, sucrose, fructose, mannitol and arabinose. Each strain was grown for 72 hours and OD was observed at 600 nm to monitor cell growth. Ethyl acetate extraction and HPLC for 2,4-DAPG was carried out in the similar manner as discussed earlier.
Plant inoculation study

The seed sterilization and germination was done as described earlier. The germinated seedlings were incubated for 45 minutes in 1.5 ml of overnight grown cultures. Germinated seedlings were inoculated in Murashige and Skoog media supplemented with 0.8% agar as solidifying agent. Plants were allowed to grow for 10 days. At the end of 10 day roots and shoot weight was measured.

Results

Isolation and characterization of fluorescent Pseudomonas strains

The mixtures of rhizosphere bacteria from cotton, sugarcane, groundnut, brinjal, rice, banana and tobacco rhizospheres used to inoculate seedlings of *Vigna radiata* and enhanced competitive root tip colonizers were enriched as described in the material and methods (Figure 1). At the end of each cycle the colony diversity in terms of morphology, colour and opacity get decreased and the number of fluorescent colony get increased. After the third cycle of enrichment, 12 fluorescent colonies were selected and checked by fluorescent pseudomonad specific biochemical tests and PCR based method (Table 1). Four newly isolated strains G1, G2, G8 and C2 appeared positive for fluorescent pseudomonad specific biochemical (catalase, oxidase, oxidative/fermentative and arginine dihydrolase test) and molecular identification methods (ITS amplification). All strains were gram negative and rod shaped and has showed amplification of 560 bp using fluorescent pseudomonad specific ITS primers (Figure 2).

PGPR traits of fluorescent *Pseudomonas* strains

Strain G1, G8 and C2 has shown higher IAA production than *Pf* CHA0. Strain G1, G2, G8, C2 and *Pf* CHA0 did not showed growth on minimal medium containing ACC as sole nitrogen source but showed growth on L-methionine. Further confirmation for the presence of KMBA pathway in the strains were done by the analysis of precipitate after addition of 2,4-dinitrophenyl hydrazine to the culture supernatant (Figure 3).

All strains showed higher phosphate solubilization than *Pf* CHA0. Strain C2 showed higher siderophore production than *Pf* CHA0 while other strains showed siderophore production similar to *Pf* CHA0. Strain G2, G8 and C2 have shown higher antifungal activity than model bio-control strain *Pf* CHA0. Except C2, strain G1, G2 and G8 showed HCN biosynthesis ability similar to *Pf* CHA0.

Effect of nutritional factors on 2,4-DAPG biosynthesis by fluorescent pseudomonad

Amplification of PhlD and biosynthesis of 2,4-DAPG: All selected strain has shown amplification of *phl D* of 726bp (Figure 4) as previously reported by Raaijmakers, et al. [33]. 2,4-DAPG production by G1, G2, G8 and C2 was significantly high than *Pf* CHA0 (Table 1) and correlated well with bioassay against *S. aureus* and *R. bataticola* (Table 2 and Figure 5).

Effect of carbon sources and Pi level on 2,4-DAPG biosynthesis: Strain G8 has shown remarkably high 2,4-DAPG production in sucrose.

<table>
<thead>
<tr>
<th>Biochemical methods</th>
<th><em>Pf</em> CHA0</th>
<th>G1</th>
<th>G2</th>
<th>G8</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hugh-Leifson's Oxidation/fermentation test</td>
<td>Oxd</td>
<td>Oxd</td>
<td>Oxd</td>
<td>Oxd</td>
<td>Oxd</td>
</tr>
<tr>
<td>Arginine Dihydrolase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gram nature</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Morphology</td>
<td>Cell shape</td>
<td>Rod</td>
<td>rod</td>
<td>rod</td>
<td>rod</td>
</tr>
<tr>
<td>Molecular method</td>
<td>ITS(560bp)Amplification</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1: Identification of fluorescent pseudomonad by biochemical and molecular methods.
on 2,4-DAPG production (Table 3 and Figure 6). The effect of other carbon sources was not much significant and conclusive (Table 3).

As reported previously that inorganic phosphate represses the 2,4-DAPG production and in case of *Pf* CHA0 it get repressed at 10 mM inorganic phosphate level. However strain G2 has resisted the inhibitory effect of Pi on 2,4-DAPG production and has shown good production up to 50 mM supplemented Pi, G1 has shown constant 2,4-DAPG production up to 50 mM (Figure 7). G2, C2, M3 and G8 have shown very much positive effect on plant growth similar to *Pf* CHA0 (Figures 8 and 9).

**Discussions**

Enrichment method developed in present work was the modification of method invented by Kamilova et al. [15] but in such way that it has yielded only plant beneficial, root colonizing fluorescent pseudomonad. Fluorescent pseudomonads strain G1, G2, G8 and C2 showed the presence of specific biochemical enzymes/pathway (arginine dihydrolase, catalase, oxidase, oxidative respiration) and further confirmed by the Internal Transcribed Spacer (ITS) rRNA coding sequence using specific primers as used by Locatelli et al. [22] which was considered to be more effective rather than the identification by 16S rDNA sequencing. These strains have proved to be efficient PGPR strains as they possess the high IAA production, phosphate solubilization, siderophore production and antifungal activity than model bio-control and PGPR strain *Pf* CHA0. The utilization of L-methionine as a nitrogen source by these strains confirms the presence of KMBA pathway. G1, G2 and G8 showed HCN biosynthesis which has been reported to be effective mechanism of bio-control by fluorescent pseudomonad [20]. Strain G1, G2, G8 and C2 have shown high 2,4-DAPG production and correlated well with bioassay against *S. aureus* and *R. bataticola*.

Quantitative and qualitative differences in the sugar composition of root exudates determine the bio-control mechanism by fluorescent pseudomonad strains in given crop-pathogen systems [8,24]. As earlier reports says that glucose but not glycerol and sucrose has enhanced 2,4-DAPG production [8,24]. As earlier reports says that glucose but not glycerol and sucrose has enhanced 2,4-DAPG production up to 50 mM (Figure 7). G2, C2, M3 and G8 have shown very much positive effect on plant growth similar to *Pf* CHA0 (Figures 8 and 9).

Table 2: PGPR traits of fluorescent *Pseudomonas* strains.

<table>
<thead>
<tr>
<th>PGPR Traits</th>
<th>Pf CHA0</th>
<th>G1</th>
<th>G2</th>
<th>G8</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytostimulation</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IAA production (ug/ml)</td>
<td>11 ± 1.2</td>
<td>20 ± 0.53</td>
<td>9.3 ± 0.74</td>
<td>14.8 ± 0.75</td>
<td>22 ± 0.73</td>
</tr>
<tr>
<td>Growth on ACC (sole N-source)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth on L-methionine (sole N-source)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>KMBA pathway</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mineralization</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P-Solubilisation (C2/Cs)</td>
<td>1.3</td>
<td>1.9</td>
<td>2.1</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Siderophore activity (C2/Cs)</td>
<td>2</td>
<td>1.8</td>
<td>1.7</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Antibiosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal Inhibition (%)</td>
<td>38.4</td>
<td>38.4</td>
<td>50</td>
<td>57.6</td>
<td>41.6</td>
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<tr>
<td>HCN production</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2,4-DAPG production (ng/ml)</td>
<td>740 ± 9.21</td>
<td>2340 ± 87</td>
<td>3350 ± 117.5</td>
<td>965 ± 10.27</td>
<td>995 ± 25.13</td>
</tr>
<tr>
<td>Bactericidal against <em>S. aureus</em></td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<td>+++</td>
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<tr>
<td>Bactericidal against <em>S. aureus</em></td>
<td>+++</td>
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reported previously that inorganic phosphate represses the 2,4-DAPG production and in case of PfCHA0 it gets repressed at 10 mM inorganic phosphate level. However strain G2 has resisted the inhibitory effect of Pi on 2,4-DAPG production and has shown good production up to 50 mM supplemented Pi. Differential influence of carbon and mineral sources on 2,4-DAPG production was suggested due to various degrees of adaptation of strains to given rhizospheric nutrient composition.

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