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# Plant Growth-Promoting Chitinolytic *Paenibacillus elgii* Responds Positively to Tobacco Root Exudates

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**Abstract** Bacterial strains from chitin/chitosan-rich soils, from two industries, were screened for their chitinolytic, antifungal, and mineral phosphate solubilization abilities. The isolate SMA-1-SDCH02, positive for all three properties, was selected and identified as *Paenibacillus elgii* based on morphological and biochemical characters and supported by 16S rRNA gene sequence analysis. *P. elgii* enhanced the growth of groundnut in terms of shoot height, root length, total chlorophyll, and fresh and dry weight when applied alone or in combination with chitosan. The plant growth-promoting activity of *P. elgii* was seen in tobacco in a specially designed gnotobiotic setup indicating its capability to promote growth of at least groundnut and tobacco. Metabolite changes in the bacteria, studied using attenuated total reflectance-infrared (ATR-IR) spectroscopy, revealed split bands of amide I at the 1659- and 1636-$cm^{-1}$ regions when grown in minimal media amended with tobacco root exudates. The difference in ATR-IR bands in the presence of tobacco root exudates indicated production of compounds with differences in functional groups.

**Keywords** Amide I band · Attenuated total reflectance-infrared spectroscopy · Chitin/chitosan-rich soils · Gnotobiotic setup · Metabolite changes · *Paenibacillus elgii* · PGPR

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## Introduction

Biological means for plant growth promotion and disease control are preferred over synthetic chemicals as they are ecofriendly and cost effective. Plant growth-promoting rhizobacteria (PGPR) exert beneficial effects on plant growth and development that are exploited for improving plant growth and yield besides controlling diseases (Kloepper and others 1980). PGPR and their interactions with plants are exploited commercially (Podile and Kishore 2006) and hold great promise for sustainable agriculture. A number of PGPR strains have been identified and applied for use as plant growth promoters but the search for better PGPR strains suitable for development of effective formulations (longer shelf life) continues. Different carrier-based chitin/chitosan-supplemented formulations (Manjula and Podile 2001, 2005) were effective against several phytopathogens and also significantly increased plant growth.

Chitin is a major structural polysaccharide and is abundant in the cell walls of the majority of fungi. The $\beta$ $1 \rightarrow 4$ glycosidic bonds in the chitin are responsible for cell wall integrity and, therefore, are a sensitive target for chitin-degrading enzymes. Chitin degradation is an important attribute of several of the successful microbial agents used in biological control of fungal pathogens. We have shown the extensive damage caused to major fungal pathogens of groundnut by chitinolytic biocontrol strains and the partially purified enzymes (Podile and Prakash 1996; Manjula and others 2004; Manjula and Podile 2005), and exploited the chitinolytic potential of the biocontrol PGPR strains to improve both the shelf life and effectiveness of the formulations (Manjula and Podile 2001, 2005; Kishore and others 2005a, b). The chitinolytic bacterial strains isolated from the phylloplane of groundnut were effective as PGPR when applied on the seeds (Kishore and

others 2005c). To further exploit the chitinolytic potential of bacteria as biocontrol PGPR, we have selected chitin-rich soil samples from two industries to isolate chitin-degrading bacteria that might promote plant growth.

Plant roots exude a wide range of potentially valuable small-molecular-weight compounds into the rhizosphere which play a crucial role in determining the outcome of plant-microbe interactions. On the other hand, the presence of particular microbes in the root region of plants has different effect(s) on plant growth. The quality of the root exudates often is responsible for the host-specific effect. The signal compounds largely responsible for the specific host-microbe relationships belong (but are not restricted) to flavonoids in the legume-*Rhizobium* interaction. Flavonoids are perceived as aglycones, which induce rhizobial *nod* genes by interacting with NodD protein that results in a conformational change in the NodD to bind to *nod* box elements in the *nod* genes (Perret and others 2000). The concerted expression of these genes leads to the synthesis of Nod factor molecules, lipochitooligosaccharides that usually consist of four or five $\beta$-1,4 *N*-acetylglucosamines, with the terminal nonreducing sugar *N*-acylated by a 16–18-carbon fatty acid. The assemblage of these substitutions results in a specific Nod factor that is recognized by the host legume. Thus, chemicals exuded from the roots determine the host-microbe specificity affecting both partners. Detectable changes in the chemistry of bacterial partners in the plant-PGPR interaction, in response to root exudates, could be related to the host specificity of PGPR strains and may help in understanding the mechanism of interaction to manipulate the host range of the microsymbiont. Changes in the bacterial strains at the metabolite level were studied using methods of vibrational spectroscopy, including Fourier transformed infrared (FTIR) and attenuated total reflectance infrared (ATR-IR) (Garip and others 2009).

We report on the isolation, selection, and identification of chitinolytic bacteria from chitin-rich soil. Furthermore, the growth-promoting activity of *Paenibacillus elgii*, selected through a screening process, was also studied for characteristic PGPR activities, including its ability to solubilize mineral phosphates and chitinolytic and in vitro antifungal activities. The gnotobiotic setup designed to study the influence of root exudates on bacteria and detect the metabolic changes in the bacterial isolate in response to the aseptically prepared tobacco root exudates is also discussed.

## Materials and Methods

### Isolation of Bacteria

Soil samples were collected from the premises of a chitin/chitosan-producing company, Mahatani Chitosan Pvt. Ltd., Gujarat, India, and from a mushroom production firm, S. M. Agritech Pvt. Ltd., Hyderabad, India, which were rich in chitin/chitosan. Serially diluted soil samples were plated in Luria-Bertani (LB) agar and M9 media with colloidal chitin as the sole source of carbon as described by Kishore and others (2005b). Plates were incubated at 30°C for 24-96 h. Colonies showing zones of clearance in the colloidal chitin-containing plates were screened for antifungal and mineral phosphate-solubilizing activities.

### Characterization of Isolate SMA-1-SDCH02

Identification of the bacterial isolate was done based on the morphological and biochemical tests described by Benson (1990) and further confirmed by 16S rRNA gene sequence analysis.

The bacterial isolate was grown in LB broth at 30°C. Cells were harvested after 36 h and processed immediately for isolation of genomic DNA using the standard procedure (Sambrook and Russell 2001). One hundred nanograms of genomic DNA was used as the template and amplified the 16S rRNA gene in a thermocycler (Eppendorf Mastercycler Gradient, Germany) using universal primers: forward primer 27F (5′GTTTGATCCTGGCTCAG3′) and reverse primer 1489R (5′TACCTTGTTACGACTTCA3′). Each 50-µl reaction contained 0.1 mM of each primer, 1.5 mM of $MgCl_2$ (Sigma-Aldrich, USA), 10 mM of each dNTPs (Fermentas), and 2 U of *Taq* DNA polymerase (Sigma-Aldrich, USA). The thermal cycling was performed with initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 10 min. The PCR product was analyzed on 1% agarose gel and eluted for sequencing at MWG Pvt. Ltd., Bangalore, India. The sequence ($\sim$1470 bp) was matched with the nucleotide database available at GenBank using the BLAST tool in NCBI. Phylogenetic analysis was done by using MEGA 4 (Kumar and others 2008).

### Antifungal Activity

The antifungal activity of the bacterial isolates was checked by the dual-plate method. Spores of *Botrytis cinerea* and *Rhizoctonia solani* (fungal plant pathogens) were grown at the center of a plate containing potato dextrose agar (PDA). Test bacteria were spot-inoculated at the periphery of the plate and incubated at 27 $\pm$ 2°C for 5 days. Inhibition zones were measured as distance (in cm) between the bacterial strain and the fungal pathogen after 7 days of growth.

### Qualitative and Quantitative Estimation of Phosphate (P) Solubilization

Qualitative and quantitative estimation of the phosphate-solubilizing activity of isolates was carried out as described by Tripura and others (2007). One milliliter of bacterial suspension was inoculated in 50 ml of National Botanical Research Institute Phosphate (NBRIP) broth in Erlenmeyer flasks and incubated for 7 days at 30°C. At the end of the incubation period the cell suspension was centrifuged at 8200×*g* for 6 min. The cell-free supernatant was used for inorganic phosphate (iP) estimation. A known concentration of $KH_2PO_4$ served as the standard. To 0.3 ml of the appropriately diluted cell-free supernatant was added 0.7 ml of the reaction mixture (one part 10% ascorbic acid to six parts 0.42% ammonium molybdate in concentrated sulfuric acid). After incubation at 37°C for 1 h, the absorbance was measured at 820 nm.

### Evaluation of PGPR Activity in Groundnut under Greenhouse Conditions

#### *Seed Bacterization*

Bacterial isolate SMA-1-SDCH02, identified as *Paenibacillus elgii*, was selected through the screening process for evaluation of its plant growth-promoting activity with and without chitosan [Mahatani Chitosan Pvt. Ltd., 90% degree of deacetylation (DD)] on groundnut under greenhouse conditions as described by Kishore and others (2005c) with minor modifications. Seeds of groundnut cv. JL 24 were surface sterilized with 0.02% (w/v) $HgCl_2$ and washed three to five times with sterile distilled water to remove traces of $HgCl_2$. For inoculum preparation, pure bacterial isolates were grown in 50 ml of LB broth for 24-48 h at 30°C and the cell pellet was collected by centrifugation at 4600×*g* for 10 min. The cell pellet was washed twice with buffer containing 20 mM glucose and 20 mM of potassium phosphate and finally dissolved in the same buffer. Chitosan (1%) and the surface-sterilized seeds were suspended in this cell suspension for 30 min and dried overnight under a flow of sterile air, resulting in $1 \times 10^6$ to $1 \times 10^7$ cfu/seed. Seed treatments with *P. elgii* alone, *P. elgii* with chitosan, and chitosan alone were compared with sterile water treatment.

#### *Growth Promotion Studies*

Bacterized groundnut seeds were sowed (3 seeds/pot) in 30-cm-diameter plastic pots filled with red soil, black soil, and manure (2:2:1). The temperature in the greenhouse was maintained at 28 ± 2°C and the pots were adequately watered daily. The emergence of seedlings was recorded 6 days after sowing (DAS). The plants were uprooted at 25 DAS to measure the shoot height, root length, fresh weight, and total chlorophyll content. The dry weight of the plants was recorded after drying in an oven at 80°C for 24 h. For estimation of total chlorophyll content, 1.0 g of fresh leaf sample was cut into small pieces and crushed in 80% (v/v) acetone. Chlorophyll content was measured spectrophotometrically using the specific absorption coefficients for chlorophyll *a* at 663 nm and of chlorophyll *b* at 645 nm. Total chlorophyll content was estimated according to Graan and Ort (1984). The experiment was conducted in a completely randomized block design with five replicates of each treatment and repeated twice.

### Plant Growth-Promoting Activity on Tobacco under Gnotobiotic Conditions

The system consisted of two autoclavable toothpick boxes connected one on top of the other (Fig. 1). The upper chamber was filled with 30 g of sand with a round hole at the bottom through which a 200-µl micropipette tip was inserted. Hoffland's plant nutrient solution (PNS) consisting of 5 mM $Ca(NO_3)_2$, 5 mM $KNO_3$, 2 mM $MgSO_4$, 1 mM $KH_2PO_4$, and micronutrients (g/l of $MnSO_4$, 0.61; $ZnSO_4{\cdot}7H_2O$, 0.1; $H_3BO_3$, 1.27; $Na_2MoO_4{\cdot}2H_2O$, 0.40; and $CuSO_4$, 0.04) was used as the nutrient solution for plant growth. The bottom of the micropipette tip was blocked with cotton and connected the upper chamber with the lower chamber containing PNS. The system was autoclaved and cooled before use. Tobacco seeds were surface sterilized by placing them in 1% sodium hypochlorite solution for 30 s followed by rinsing three times with sterile distilled water. *P. elgii* was grown overnight in 10 ml of LB broth, and cells were collected by centrifugation at 3200×*g* for 5 min. Sterilized distilled water was added to the cell pellet to yield a bacterial suspension of $1 \times 10^7$ cfu/ml. Surface sterilized seeds were steeped in this bacterial suspension for 30 min and dried for 1 h, whereas treatment with sterile water served as the control. Seeds with respective treatments were placed separately in the chamber containing sand of the gnotobiotic setup. The shoot height, root length, and dry weight of the plants were recorded after 30 days of growth. The experiment was conducted in a completely randomized block design with six replicates of each treatment and repeated thrice.

### Preparation of Tobacco Root Exudates

Tobacco root exudates (TRE) were collected from tobacco (*Nicotiana tabacum* L. cv Xanthi) plants according to the procedure of Slavov and others (2004). Tobacco plants were grown in sterile soil to the 15-leaf stage. All plants were grown in a culture room under controlled conditions

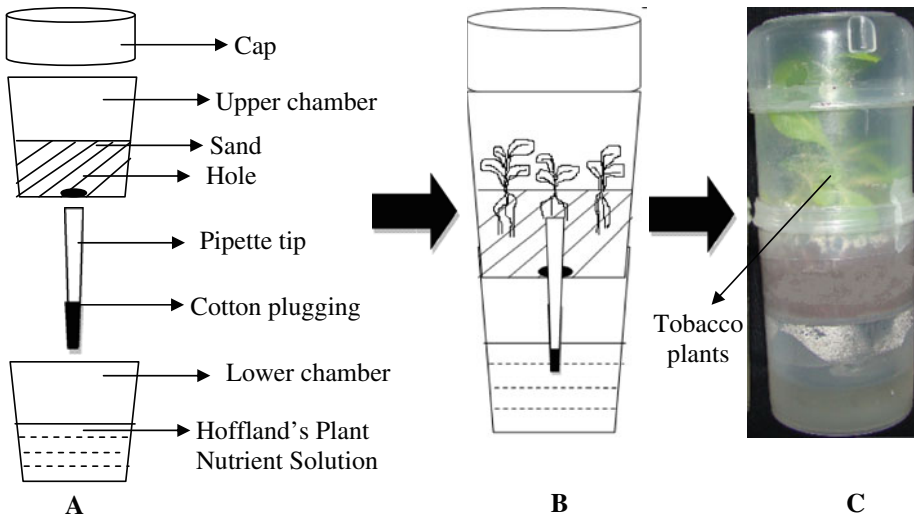


**Fig. 1** Gnotobiotic setup was designed using toothpick bottles. **a** Detailed labeling of the setup. **b** Assembled setup. **c** Growth of tobacco plants in the gnotobiotic setup

(25°C and 16/8 h light/dark period) and watered on a regular basis with sterile distilled water. At the 15-leaf stage, four tobacco plants were transferred to flasks containing 200 ml of sterile distilled water after carefully washing the roots of soil. The flasks were kept for 2 weeks and sterile distilled water was added to maintain a constant liquid level. After this period, the aqueous media containing the compounds released by the roots were collected and filter sterilized (0.22-μm filter). Finally, the volume was adjusted to 100 ml/g fresh root weight and stored at 4°C until further use.

### Preparation of *P. elgii* SMA-1-SDCH02 for ATR-IR Studies

Samples were prepared according to Kamnev and others (1997). *P. elgii* was grown to an OD of 0.5-0.6 in LB, minimal media (MM), and minimal media amended with TRE, 1:1 (v/v). Cells were separated from the supernatant by centrifugation at 8200×*g* for 8 min at 4°C, washed three times with 0.85% NaCl solution, and finally washed with double distilled water and dried in air at 50°C for 8 h prior to measurements. The spectra of the air-dried *P. elgii* cell pellet were recorded with a total of 64 scans at a resolution of 4 $cm^{-1}$ in the transmission mode (mid-infrared region, 4000–400 $cm^{-1}$) using a Nicolet 5700 FTIR spectrometer. Analysis of spectral data was performed using Origin 6.1 software (OriginLab Corporation, Northhampton, MA, USA). The spectra were normalized by using the same software program for visual representation of the differences.

### Statistical Analysis

All the data obtained were subjected to statistical analysis as described by Gomez and Gomez (1984). The effect of the PGPR treatments under greenhouse conditions was determined according to Duncan's multiple-range test (DMRT) by the magnitude of the $F$ value ($P < 0.05$). When a significant $F$ test was obtained for treatments, separation of means was accomplished based on the critical difference at the 5% level. For gnotobiotic studies, Student's $t$ test was performed and significance was determined at the 1% level ($t < 0.01$).

## Results

### Isolation of Bacteria

A total of 53 pure culture isolates of culturable bacteria were isolated from the soil samples collected from Mahatani Chitosan Pvt. Ltd., Gujarat, India, and S. M. Agritech Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Ten of 40 bacterial isolates from Mahatani chitosan soils (prefixed as MC) and three of 13 isolates from S. M. Agritech (prefixed as SMA) showed chitinolytic zones of 3 mm or more on colloidal chitin plates (Table 1). Antifungal and mineral phosphate solubilization (MPS) activities of the selected isolates (≥ 3 mm chitinolytic zone) are compared in Table 1. All ten chitinolytic bacteria from MC showed no antifungal or MPS activities. The isolate SMA-1-SDCH02 was chitinolytic and antifungal apart from solubilizing mineral phosphate, whereas SMA-1-SDCH01, although showed chitinolytic and antifungal activities, did not solubilize inorganic phosphate. SMA-1-SDCH03 solubilized mineral phosphate, besides being chitinolytic, but lacked antifungal activity. Therefore, we selected SMA-1-SDCH02 for identification and to test for plant growth-promoting activity.

**Table 1** Details of biological activity of different bacterial isolates from two different locations

| Designation of bacterial isolates | Source of soil sample | Chitinolytic activity[a] | Antifungal activity[b] | Mineral phosphate solubilization[c] |
|---|---|---|---|---|
| MC-1-SDCH6 | Mahatani chitosan | + | − | − |
| MC-3-SDCH10 | Mahatani chitosan | + | − | − |
| MC-1-SDCH7 | Mahatani chitosan | + | − | − |
| MC-2-SDCH8 | Mahatani chitosan | + | − | − |
| MC-3-SDCH12 | Mahatani chitosan | + | − | − |
| MC-3-SDCH14 | Mahatani chitosan | + | − | − |
| MC-2-SDCH9 | Mahatani chitosan | + | − | − |
| MC-1-SDCH8 | Mahatani chitosan | + | − | − |
| MC-1-SDCH9 | Mahatani chitosan | + | − | − |
| MC-1-SDCH11 | Mahatani chitosan | + | − | − |
| SMA-1-SDCH01 | S. M. Agritech | + | + | − |
| SMA-1-SDCH02 | S. M. Agritech | + | + | + |
| SMA-1-SDCH03 | S. M. Agritech | + | − | + |

+ = showing activity; − = showing no activity

[a] Chitinolytic activity of the bacterial isolates 3 mm or greater of the zone of clearance on M9 medium containing colloidal chitin after 5-6 days of incubation

[b] Antifungal activity by dual culture plate measured as zone of inhibition after 96 h of incubation at 27 ± 2°C

[c] Mineral phosphate solubilization (MPS) of the bacterial isolates was measured as zone of clearance on NBRIP medium after incubation for 7 days at 30°C

## Identification of the Isolate SMA-1-SDCH02

### *Morphological and Biochemical Characterization*

Morphologically, isolate SMA-1-SDCH02 appeared as a white, circular, sticky, and medium-sized colony with smooth margins on nutrient agar. Microscopic observation revealed that the isolate was rod-shaped, Gram-positive, and formed spores. Biochemical tests of SMA-1-SDCH02 showed a positive reaction for catalase, hydrolysis of starch and nitrate reduction, and failure to produce indole and utilize citrate.

### *Molecular Characterization and Identification*

BLAST results of the 16S rRNA gene sequence of SMA-1-SDCH02 showed maximum identity to the genus *Paenibacillus*. Based on sequence comparison and phylogenetic analysis of the 16S rRNA gene from SMA-1-SDCH02 with 30 sequences of the nearest type species retrieved from EzTaxon (http://147.47.212.35:8080/), it was concluded that the test strain was *Paenibacillus elgii* (Fig. 2). The nucleotide sequence of part of the 16S rRNA gene (~1470 bp) determined in this study was deposited in the GenBank database under accession number GQ266393.

## Plant Growth-Promoting Activity of *Paenibacillus elgii* SMA-1-SDCH02 on Groundnut

### *Effect on Seed Germination*

Seed bacterization with *P. elgii* SMA-1-SDCH02 significantly improved the germination percentage (Fig. 3) of groundnut seeds, both individually (72.30%) and in combination with chitosan (75%) 6 DAS. Only chitosan-treated groundnut seeds also showed an increase in the germination percentage (52.38%). The combined effect of chitosan and *P. elgii* was significant ($P < 0.05$) but not remarkable.

### *Effect on Plant Growth*

The data presented in Fig. 3 suggested that both treatments—*P. elgii* alone and in combination with chitosan—significantly enhanced the growth of groundnut compared to control and chitosan-treated seeds. The greatest increase in root length (31.75%), fresh weight (31.75%), and total chlorophyll content (7.87%) was seen in plants treated with *P. elgii* with chitosan followed by *P. elgii* alone. The maximum increases in shoot height and dry weight (25.33 and 61.32%, respectively) were also recorded in seedlings treated with *P. elgii* and chitosan; however, they were not significantly different from those of *P. elgii*-treated seedlings.

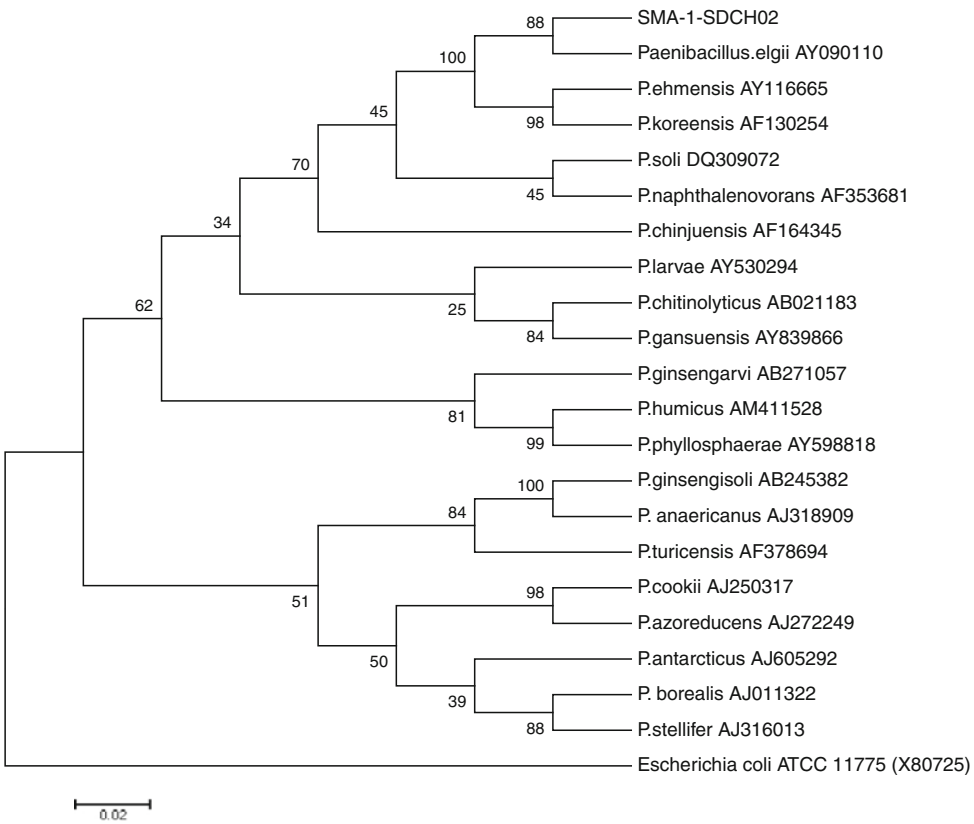


**Fig. 2** Phylogenetic tree, constructed using MEGA v4, showing the position of isolate SMA-1-SDCH02 with other species of the genus *Paenibacillus* based on 16S rDNA full sequences. Scale bar = 0.02 accumulated changes per nucleotide. *E. coli* 16S rDNA sequence was used as the out-group

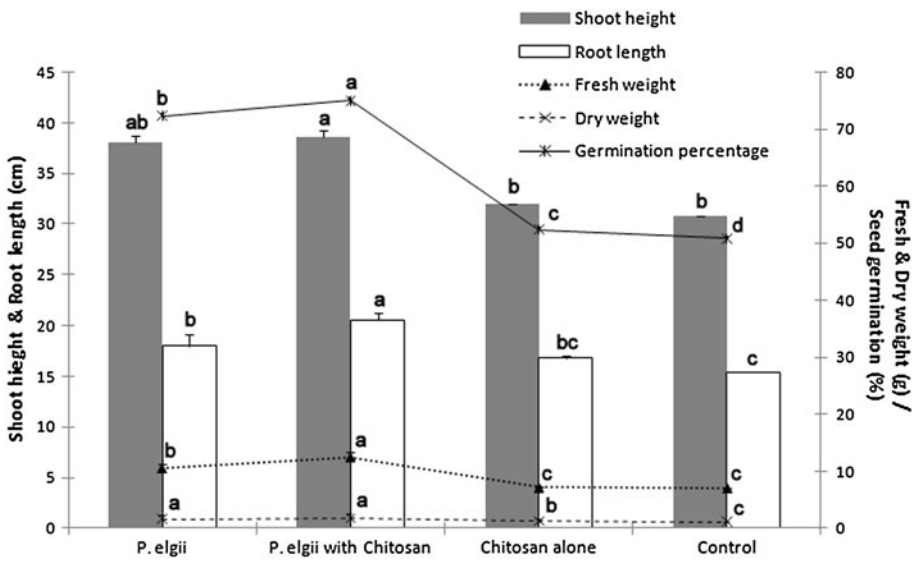


**Fig. 3** Effect of *P. elgii* with or without chitosan on germination and growth of groundnut seeds. Seed germination percentage was recorded at 6 DAS. Shoot height and root length were measured in centimeters, while fresh weight and dry weight were measured in grams at 25 DAS. Groundnut seeds bacterized with *P. elgii* with or without chitosan were raised in plastic pots filled with soil in the greenhouse. Treatments consisted of five replicate pots containing three seeds each and were repeated twice. Data represent the mean ± SD ($n = 15$) of the two independent experiments. Different letters on each bar represent values that are statistically different ($P = 0.05$). The *vertical bars* indicate standard error

### Growth-Promoting Activity of *P. elgii* SMA-1-SDCH02 on Tobacco under Gnotobiotic Conditions

Growth of tobacco seeds bacterized with *P. elgii* SMA-1-SDCH02 was highly significant under gnotobiotic conditions after 25 days of growth. An increase in shoot height (55.90%), root length (72.17%), and dry weight (91.52%) over control was observed (Fig. 4).

### ATR-IR Studies of *P. elgii* SMA-1-SDCH02

The ATR-IR spectra of *P. elgii* SMA-1-SDCH02 grown in different media with or without tobacco root exudates (TRE) showed a difference in bands at around 2900 and 1650 $cm^{-1}$ (Fig. 5). The spectra were normalized with respect to the $CH_2$ asymmetric stretching band. The bands at 2933.2 and 2929.4 $cm^{-1}$ in the spectra of *P. elgii* grown in MM and MM + TRE, respectively, were not detectable in *P. elgii* cells grown in LB broth. The major difference was the amide I band (Fig. 5). A distinct single band was detectable at 1632 $cm^{-1}$ after normalization in *P. elgii* grown in MM, whereas in MM + TRE and LB the amide I band appeared to be split into 1659- and 1636-$cm^{-1}$ bands.

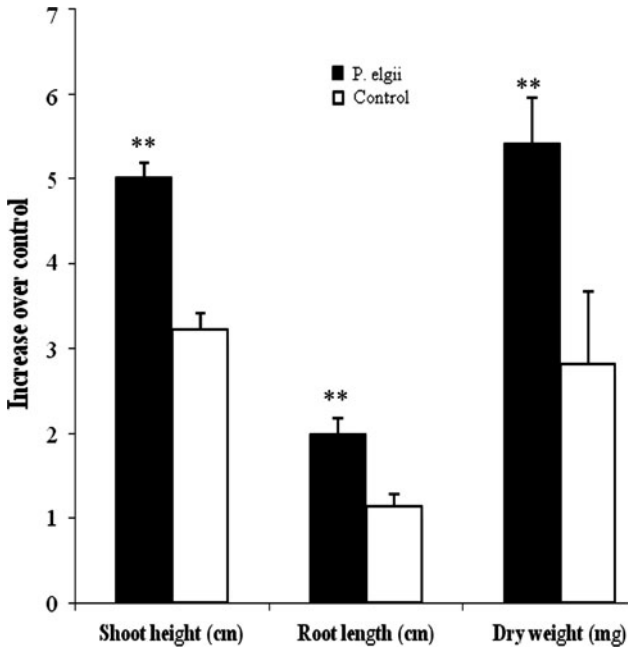


**Fig. 4** Effect of *P. elgii* on tobacco seedlings after 25 days. Shoot height and root length were measured in centimeters, while dry weight was measured in terms of miligrams. Tobacco seeds bacterized with *P. elgii* were grown in gnotobiotic setup. Treatments consisted of six replicates and were repeated thrice. Data represent the mean (± SD, $n = 18$) of the three independent experiments. ** Denotes statistically highly significant at $t < 0.01$

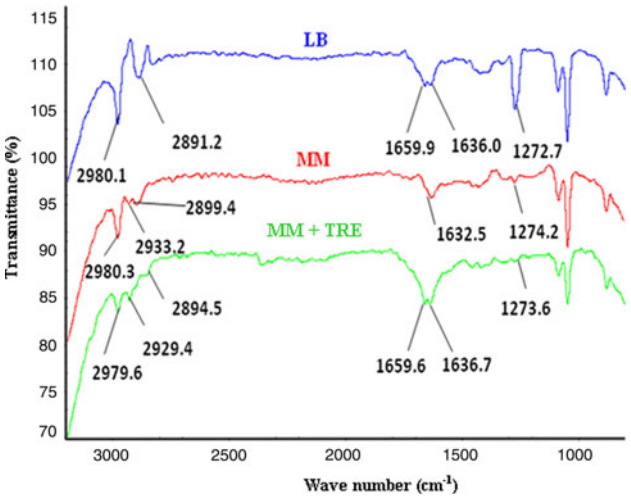


**Fig. 5** ATR-IR spectra (in 3200–800-$cm^{-1}$ region) of *P. elgii* grown in different media. *P. elgii* was grown to OD = 0.5–0.6 in Luria-Bertani medium (LB), minimal media (MM), or MM with tobacco root exudates (TRE), and the cell pellet was used to detect the alteration in metabolite profile. Spectra of the air-dried *P. elgii* cell pellet were recorded with a total of 64 scans at a resolution of 4 $cm^{-1}$ in the transmission mode (mid-infrared region, 4000–400 $cm^{-1}$) using a Nicolet 5700 FTIR spectrometer

## Discussion

The ability of PGPR, isolated from the rhizosphere of crop plants, to promote plant growth and induce resistance in various crops is known (Podile and Kishore 2006). The bacteria isolated from sources other than the rhizosphere also hold potential in improving plant growth when applied as seed treatments (Kishore and others 2005c). Of the 53 bacterial strains isolated from chitin-rich soil, the isolate *Paenibacillus elgii* SMA-1-SDCH02 significantly enhanced the growth of the groundnut in terms of shoot height, root length, and fresh and dry weight besides increasing the total chlorophyll content in the leaves. The growth-promoting effect of *P. elgii* was confirmed in tobacco under gnotobiotic conditions. The *P. elgii* isolate SMA-1-SDCH02 solubilized mineral phosphate apart from its chitinolytic and antifungal activities.

Phosphorus is one of the major nutrients present in soil in the form of insoluble phosphates that plants are unable to utilize as such. PGPRs solubilize precipitated phosphates and enhance phosphate availability to different crops, which could represent a possible mechanism of plant growth promotion under field conditions (Verma and others 2001). *P. elgii*, selected through the screening process, displayed MPS activity and also showed antifungal activity (Table 1), which will enhance its application as a biocontrol PGPR.

Most of the bacterial strains from chitin-rich soils showed good chitinolytic activity. Extracellular chitinolytic

enzymes produced by the biocontrol agents lyse chitin (Podile and Prakash 1996), a major constituent of fungal cell walls, thereby playing a significant role in the management of phytopathogenic fungi and indirectly improving the growth of crop plants. The chitinolytic potential of bacterial inoculants would help in developing formulations with longer shelf lives and greater effectiveness when used in combination with chitin/chitosan.

Although *P. elgii* SMA-1-SDCH02 was not isolated from plant parts, it has tremendous potential in plant growth promotion and antifungal activities similar to other reported PGPR strains. This provides ample evidence for utilizing bacterial strains from unconventional sources, especially chitin-rich soils (with an added quality of chitinolysis), to facilitate plant growth directly or indirectly. Bacteria, including *Bacillus* and *Paenibacillus*, isolated from soils and used as rhizosphere inoculum effectively improved plant growth besides control diseases (von der Weid and others 2003).

A variety of bacterial traits and specific genes contribute to the process of root colonization by PGPR, but only a few have been identified (Benizri and others 2001; Lugtenberg and others 2001). These include motility, chemotaxis to seed and root exudates, production of pili or fimbriae, production of specific cell surface components, ability to use specific components of root exudates, protein secretion, and quorum sensing (Lugtenberg and others 2001). Host-specific effects of root exudates on rhizobacteria occur at a metabolic level. Here we made an attempt to see whether there is a change in the bacterial partner, at the metabolite level, in response to the nutrients in the medium, especially in the presence of tobacco root exudates using a specially designed simple gnotobiotic setup to study the metabolic alterations in *P. elgii*. Root exudates are known to influence the metabolism of rhizobacteria. Van Overbeek and van Elsas (1995) found that the expression of the $\beta$-galactosidase gene in a transgenic *Pseudomonas fluorescens* mutant was induced by proline, but not by 125 other substrates, and by root exudates from three monocot species, but not white clover. Miller and others (1989) believed that the specificity of bacterial isolates in the rhizosphere are due to the quantity and composition of the root exudates, which may vary from one plant species to another and even from one cultivar to another. Thus, differences between isolates in the use of substrates may be related to differences in the composition of the respective root exudates. The present study showed that there was a difference in the spectra of bacteria grown in the presence of different root exudates. Depending on the root exudate available, bacteria produced different metabolite(s) that could be related to growth-promoting ability. A difference in bands at around 2930 $cm^{-1}$ was observed when the bacterial cells were grown in three different media. The region between 3100 and 2800 $cm^{-1}$ features various C–H stretching modes of methylene and terminal methyl groups of mainly the fatty acid chains of lipopolysaccharide (LPS) and phospholipid constituents of the cell envelope (Kamnev and others 1999). Because the difference in the bands at this region was observed only between enriched media LB and MM but not in MM and MM amended with TRE (except for a slight shift of the band 2980.3 and 2933.2 $cm^{-1}$ in MM and 2979.6 and 2929.4 $cm^{-1}$ in MM amended with TRE), the contribution of compounds, designated at this region, to host-microbe interaction may be ruled out for now. Moreover, it has been reported that the IR position of the $CH_2$ band for some LPS forms may be influenced by externally added cations or by reduced water content, indicating an increase in the acyl chain packing density (Brandenburg and others 1999).

A significant distinction in the ATR-IR spectra, recorded in this study, was at the amide I region. A split in the band at this region was detected when *P. elgii* was grown in enriched LB and MM amended with TRE, whereas a single band was visible when grown in MM. The band at 1657 $cm^{-1}$ was attributed to the amide I vibrations of structural proteins (Cakmak and others 2006) corresponding mainly to the C=O stretching modes of polypeptides and protein backbones. The position of amide I absorption is sensitive to protein conformation and implies structural and conformational differences in cell proteins (Haris and Severcan 1999). It has been a determining factor in classifying bacteria based on IR studies. A difference in this region shows the specificity of the bacteria for specific root exudates. Similar studies have been done in synthetic media using bacteria isolated from the rhizosphere of plants under different environmental conditions (Kamnev 2008). Most of these studies basically were done to identify and characterize different bacterial strains based on the production of their metabolites (Filip and others 2004).

The splitting of the amide I band was spectroscopically detected in *Azospirillum* when grown in the presence of nanomolars of wheat germ agglutinin, which was considered to be a molecular signal in plant-microbe interactions (Antonyuk and Evseeva 2006). The $\alpha$-helix type of protein secondary structure features a single narrow band at about 1655–1658 $cm^{-1}$, whereas different $\beta$ sheets exhibit bands from 1637 to 1623 $cm^{-1}$. Bacteria under normal growth conditions showed the amide I band at about 1650–1660 $cm^{-1}$, reflecting the predominance of $\alpha$ helices among secondary-structure components of bacterial cellular proteins; however, for cells grown under nitrogen deficiency, the amide I band appeared to be split, with an additional band at 1632 $cm^{-1}$ corresponding to an enhanced proportion of $\beta$-structure components (Kamnev 2008). However, our observation showed the presence of the band at 1632.5 $cm^{-1}$ for bacteria grown in minimal

media, whereas in the presence of enriched nutrients in media (LB or TRE), the splitting occurs. This study suggests that in MM, $\beta$ components are predominant, and in the presence of additional nutrients, both the $\alpha$ helix and $\beta$ structure of protein secondary structures can be observed. These spectroscopic changes may be related to alterations in the surface proteins of the bacterial cell and facilitate adaptation to the varying environmental conditions (Kamnev 2008). Cell surface proteins, in turn, evidently are involved in stress resistance and plant root colonization capabilities of bacteria (Burdman and others 2001).

The metabolite analysis in the present study is a start of the utilization of the metabolomics approach and is being continued to identify the specific changes and to link them to the events in plant-PGPR interaction. The changes in functional groups observed when *P. elgii* was grown in the presence of TRE indicate that the isolate may produce different compounds in response to specific root exudates. We are further analyzing the metabolic changes in *P. elgii* from the gnotobiotic setup with different host plants to identify some key compounds produced during their interaction.

The present work established *P. elgii*, isolated from chitin-rich soil, as a potential PGPR strain that showed mineral phosphate solubilizing, antifungal, chitinolytic, and growth-promoting activities. The ATR-IR spectra of *P. elgii* showed a difference at 1650 $cm^{-1}$ region with root exudate-amended media, indicating the production of compounds having amide bonds during the interaction. The efficiency of *P. elgii* in this study demonstrated that unconventional sources could be a potential PGPR sink for development of bioformulations to increase crop yields and reduce disease.

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