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# Impact of plant growth-promoting rhizobacteria on yield and disease control of *Nicotiana tabacum*

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**Abstract:** An unexplored soil microbial community associated with the root system of *Nicotiana tabacum* was isolated to analyze its impact on growth and yield of the crop. A total of nine isolates out of 180 were biochemically screened and characterized as potential plant growth-promoting rhizobacteria due to the expression of growth-promoting traits. All isolates were positive for ammonia production, 8 were positive for phosphate solubilization but none for auxin production. The majority of the isolates were also found positive for hydrogen cyanide, siderophore and hydrolytic/degradative enzymes production, enabling them to restrict the growth of *Fusarium oxysporum* in an *in vitro* assay. Although all tested isolates enhanced tobacco growth significantly, Baj-ER-01 and CD-RS-03 were found to be the most promising in enhancing all aspects of growth. This study provides evidence for the enhancement of growth and yield of inoculated tobacco plants through an adequate supply of nutrients and/or controlling phytopathogens.

**Key words:** rhizobacteria; soil; tobacco; phytopathogens; plant protection

## INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are a diverse group of free-living soil bacteria colonized in the rhizosphere, rhizoplane and endorhizosphere of the plant root system [1]. This indigenous soil microbial community is extensively investigated as an alternative to chemical fertilizers for maintaining soil ecosystems and sustainable crop production. Although the exact mechanism of plant-growth enhancement by these bacteria is unknown, it is believed that these bacteria involve a number of direct and indirect mechanisms that, either singly or synergistically, affect the growth of colonized plants. Direct mechanisms include the release of growth-stimulating phytohormones, such as indole acetic acid (IAA), cytokinin and gibberellins [2], whereas indirect processes include an increase in biological nitrogen fixation activity, solubilization of inorganic phosphates and other nutrients, production of compounds that inhibit phytopathogenic growth, improvement of soil structure and elimination of toxic heavy metals or other xenobiotic compounds, all of which help the survival of plants under saline conditions and enhanced mineral absorption [3-7].

The successful isolation and application of PGPR such as *Chryseobacterium palustre* and *Chryseobacterium humi* to *Zea mays* [2], *Bacillus subtilis* and *Pseudomonas fluorescence* to *Pelargonium graveolens* [8] and *Pseudomonas putida, Azotobacter chroococum* to *Lycopersicon esculentum* [9] have not only demonstrated positive effects on growth and yield of inoculated plants but also provided documented proof of reduced chemical fertilizer use per hectare [10]. Such reports have fueled the interest of many investigators to isolate and study the role of PGPR from the root system of previously unexplored plant types, or from

the same type, but under different geographically and ecologically conditions.

The inclusion of newer and previously unexplored areas and the knowledge of indigenous rhizobacterial populations associated with a specific crop of a particular region are needed to widen the existing pool of potential PGPR. The search and understanding of the diversity of the microbe populations in soil in a distinct/distant area is not only important for the discovery of novel rhizobacterial strains but also in the search of region-specific growth-promoting microbial strains [11]. The soil microbiological data and the information about PGPR colonizing crops in the study area (Mansehra, Northern Pakistan) are still lacking. Tobacco, the major cash crop of this area in Khyber Pakhtunkhwa (KPK), is grown over a wide area. Currently, chemical fertilizers and fungicides are the only choice for the provision of nutrients and to control fungal diseases of tobacco plants in order to enhance growth and yield of the crop. The information concerning the use of PGPR to increase the growth and yield of tobacco and to reduce the cost and environmental hazards of applied chemicals is not only a limitation of this area but worldwide.

Therefore, the aim of this study was to isolate the indigenous soil microbial community from the rhizosphere, rhizoplane and endorhizosphere of tobacco (*Nicotiana tabacum* L.) plants grown on various soils of a previously unexplored hilly area. The isolated bacteria were biochemically characterized and their role in growth enhancement of tobacco plant seedlings was evaluated through different plant-growth parameters with the aim of assessing their potential in growth enhancement and inhibition of fungal pathogens.

## MATERIALS AND METHODS

### Study area

Samples were collected from the tobacco fields of the Pakhal Valley district Mansehra. The study site lies in northwest of Pakistan at a latitude 34°19′59″ N, longitude 73°12′00″ E, and elevation of 1088 m (WGS 84 coordinate reference system) above sea level. The area is located at the foothills of the great Himalayan range that extends from Kashmir and comprises of plains, valleys and lakes. The climate of the area is warm to temperate with significant rainfall (1445 mm per annum) and precipitation even in the driest months. The average temperature per annum is ~18.5-19°C.

### Soil sampling and isolation of bacteria

Tobacco rhizospheric soil samples (n=10) along with roots of the plant were collected from ten different locations in the Mansehra region. Each sample was kept in a separate, sterile and labeled polythene bag and brought to the Land Resource Research Institute (LRRI), National Agriculture Research Center (NARC), Islamabad. Bacteria from soil samples were isolated from the rhizosphere, rhizoplane and endorhizosphere by the serial dilution method on Lauria Bertini (LB) agar media [12]. For the rhizospheric sample, 1 g of soil was suspended in 9 mL of autoclaved distilled water and mixed by a vortex. Serial dilutions (up to $10^{-9}$ dilution) were made by taking 1 mL from this suspension and pouring it into another tube containing 9 mL of distilled water. For the rhizoplane samples, the root-adhered soil particles were removed and immersed in 9 mL of sterile distilled water and serially diluted, as described for the rhizosphere sample. Lastly, to prepare the endorhizosphere dilutions, 1 g of root was taken and surface-sterilized using Clorox. The samples were washed three times with distilled water and crushed using a mortar and pestle. The crushed sample (1 g) was mixed in 9 mL of distilled water and serially diluted. A suspension of 0.1 mL was obtained from $10^{-4}$, $10^{-5}$ and $10^{-6}$ dilutions of each sample and plated on LB agar plates separately. Numerous colonies grown on media after 24 h of incubation at 28°C were purified by restreaking on the same media and stored at 4°C for further analysis.

### Morphological and microscopic characterization of isolated bacteria

All the bacterial isolates were examined for their colony morphology, including shape, size, color, margins, opacity and elevation on LB agar plates as described [12]. The bacteria were examined for Gram staining as described previously [13]. Based on the morphological features and Gram staining, the isolates were grouped, and representative isolates were selected for further analyses.

### *In vitro* analysis of plant growth-promoting ability of isolates

Plant growth-promoting characteristics such as the production of ammonia, phosphate solubilization index and indole acetic acid production (auxin) by isolates were analyzed using methods described for each character/ability [14-17]. The experiments for each characteristic were repeated twice to obtain consistent results.

### *In vitro* analysis of bacterial factors antagonistic to phytopathogens

The ability of isolates to produce hydrogen cyanide (HCN) was tested by streaking bacteria on nutrient agar medium plates modified with 4.4 g glycine $L^{-1}$ [41]. Whatman filter paper no 1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed inside the lid of Petri plates. The plates were incubated for 4 days at 36±2°C and development of orange color was considered as indicating the presence of HCN-positive bacteria. Similarly, siderophore production was detected as a change of the color from blue to orange on chrome azurol S (CAS) agar described previously [18].

### Bioassays for the production of toxic/degradative enzymes

Catalase, amylase, pectinase and protease production by bacterial isolates was determined using standard methods [19].

### Determination of antifungal activity of isolates

The antagonistic activity of bacterial isolates was tested against *Fusarium oxysporum*. This fungal plant pathogen was inoculated in potato dextrose agar (PDA) from a previously pure culture stored in the Land Resource Research Institute, NARC, Islamabad, Pakistan. After seven days of incubation, a 5-mm fungal mycelial disk was taken from the fungal culture and placed on one side of a fresh PDA plate. Using a sterile toothpick, a colony of bacteria was picked from an overnight-grown pure culture and inoculated into the same plate on the opposite side. A fungal disk placed on the Petri plate without any bacterial isolate served as a control. The inoculated plates were incubated for 7 days at 28°C and mycelial growth was compared with that of the control. The experiment was repeated three times for each treatment. Zones of inhibition were measured by calculating the fungal mycelial growth toward the antagonistic bacteria. The percentage growth inhibition of the fungal pathogen was calculated as described [20] using the formula:

$$\text{Percentage Growth Inhibition (PGI)} = [1 - (\text{Fungal growth/fungal control growth})] \times 100.$$

### Greenhouse/pot experiment to analyze the effect of PGPR inoculation on plant growth

The effect of PGPR on tobacco plants was investigated in the greenhouse of the Land Resource Research institute (LRRI), National Agriculture Research Center (NARC), Islamabad. Ten mL of bacterial culture ($1\times10^9$ CFU/mL) in LB broth medium were prepared for each isolate. Briefly, a bacterial isolate from its pure culture (stored at -80°C) was streaked on LB agar plates and incubated for 24 h at 28°C to confirm its purity. Following this incubation, a single colony was picked from its respective plate and restreaked for overnight incubation under the same conditions. After 24 h of incubation, the bacterial cells from the plate were mixed in LB broth medium poured into 15-mL falcon tubes and the suspensions were adjusted to $1\times10^9$ CFU/mL in a final volume of 10 mL.

The *N. tabacum* L. (cv. K-399) transplants were collected and their roots were washed and soaked in tubes containing representative bacterial isolate culture for 10 to 15 min (inoculation with bacteria) followed by implantation (in triplicate for each isolate) in round-bottomed sterile pots (25 cm×28 cm) containing autoclaved soil. Non-inoculated treatments of plants were also set up as a control. The effect of bacterial isolates on treated tobacco transplants was observed for 90 days and the growth parameters (shoot and root length, number of leaves, shoot and root dry and fresh weight) of inoculated and control plants were measured and compared.

### Statistical analysis

The data for growth parameters are presented as the mean±SD. In order to determine the comparative ef-

fects of different inoculants/isolates, the data were analyzed using ANOVA and *post hoc* analysis using GraphPad prism. A statistically significant difference was considered at $p<0.05$.

## RESULTS

### Isolation and morphological characteristics of isolates

A total 180 bacterial colonies were isolated on LB agar plates from the rhizosphere, rhizoplane and endo-rhizosphere of tobacco plant selected from an unexplored area. Morphological characteristics of all isolates were determined using a multistep strategy for initial screening which helped to reduce the number of isolates with a likely potential of PGPR into 40. A second screening on the basis of Gram staining helped to further reduce the number of potential PGPR isolates to 9 (Supplementary Table S1). These screened isolates were different from one another according to multiple morphological characteristics and Gram staining. Wide variations of colony characteristics were clearly evident. The majority of the isolates were of irregular form with milky white and transparent colonies. The isolated cells were mostly found to be round-shaped with variable elevation and margins of colonies. Six isolates showed Gram-positive reactions while 3 were identified as Gram negative. Further morphological classification revealed that 3 isolates belonged to diplobacillus, 3 to micrococcus, 2 to streptococcus and 1 to diplococcus families.

### Direct and indirect growth-promoting traits of the tested isolates

All 9 isolates were tested *in vitro* to assess their potential to enhance plant growth. Nine different traits involved in plant-growth enhancement, either directly (the production of phytohormones) or indirectly (restriction of the growth of phytopathogens), were investigated. All bacterial isolates, except Baj-ER-01, showed clear zones around the colonies on growth medium containing Pikovskaya's agar, suggesting efficient phosphate solubilization (Table 1). The highest phosphate solubilization potential was recorded for the sample Dhd-RP-07-01. Similarly, all bacterial isolates associated with the tobacco root system were found to be capable of nitrogen fixation with a moderate to strong potential. However, none of the isolates produced auxin.

Production of compounds toxic/inhibitory to the growth of phytopathogens, such as HCN and siderophores, is another important characteristic of PGPR. In this study, both compounds were produced by 7 isolates, and only 2 isolates (Dhd-RS-02 and ITC-RS-01) were found negative for siderophore production and 2 isolates (ITC-06 and BP-RS-01) for HCN production (Table 1). Similarly, for the ability of degradative/inhibitory enzyme production, six isolates were capable of producing amylase, 8 were positive for pectinase, while all isolates produced catalase (Table 1). All isolates were positive for the production of at least two different degradative enzymes.

### Antifungal potential of the tested isolates

To associate the production of hydrolytic enzymes and HCN by PGPR to that of fungal growth inhibition, the antifungal potential of all 9 isolates was tested against *F. oxysporum* by the dual culture assay. The growth of fungi on the control plate spread all over, but when grown with the tested isolates, the bacteria restricted their growth. The restriction of fungal growth was observed for all 9 bacterial isolates. A promising percentage of growth inhibition (>40%) by each inoculant, except BP-RS-01, was evident (Fig. 1). This confirmed that all isolates harbor the potential of restricting fungal growth through the production of degradative enzymes and HCN (Fig. 1, Table 1).

### Effect of PGPR on the growth parameters of inoculated tobacco plants

A direct inoculation of isolates on tobacco roots was also performed in a greenhouse experiment to link the *in vitro* growth characteristics with the actual growth parameters of plants. All isolates, after three months of inoculation, enhanced the growth of inoculated tobacco plants, which resulted in a significant increase in all growth parameters over the non-inoculated control (Fig. 2, Table 2).

The relative percent increase in root and shoot length compared to the control was recorded as being between 34-101% and 47-83%, respectively (Fig. 3A). Isolate CD-RS-03 exerted a maximum positive

**Table 1.** *In vitro* production of direct and indirect plant growth-promoting traits of rhizobacterial isolates.

| Inoculant | Protease | Amylase | Pectinase | Catalase | Phosphate solubilization (Sol. Index) | $NH_3$ | Siderophore | IAA | HCN |
|---|---|---|---|---|---|---|---|---|---|
| Control | | – | – | – | – (0) | – | – | – | – |
| ITC-06 | + | + | + | + | + (1.72) | + | + | – | – |
| CD-RS-03 | + | + | ++ | + | + (2.33) | ++ | + | – | + |
| Dhd-RS-02 | + | + | +++ | + | + (2.33) | ++ | – | – | + |
| BJ-ER-01 | ++ | | + | + | ++ (3.00) | + | + | – | + |
| Dhd-RP-01 | – | + | – | + | ++ (2.60) | ++ | + | – | + |
| BP-RS-01 | – | + | + | + | + (2.25) | +++ | + | – | – |
| ITC-RS-01 | + | + | + | + | ++ (2.85) | +++ | – | – | + |
| Dhd-RP-07-01 | – | – | ++ | + | +++ (4.50) | + | + | – | + |
| Baj-ER-01 | ++ | – | + | + | – (0) | ++ | + | – | + |

Presence, absence and level of activity for a given trait is shown by the type of symbol (- or +) and number of time (+, ++ or +++) it appears against each isolate.
(+) presence of a characteristic/trait with low activity, (++) presence with moderate activity, (+++) presence with strong activity, (–) absence of a characteristic/trait

**Table 2.** Effect of isolated PGPR on various growth parameters of tobacco plants.

| Inoculant | Shoot Length (cm) Mean±SD | Root Length (cm) Mean±SD | No of Leaves ($Plant^{-1}$) Mean±SD | Shoot Fresh Wt. (mg $plant^{-1}$) Mean±SD | Root Fresh Wt. (mg $plant^{-1}$) Mean±SD | Shoot Dry Wt. (mg $plant^{-1}$) Mean±SD | Root Dry Wt. (mg $plant^{-1}$) Mean±SD |
|---|---|---|---|---|---|---|---|
| [a]Control | 15.38±1.02 | 14.33±1.60 | 4.67±0.58 | 12.47±0.96 | 1.64±0.27 | 1.46±0.14 | 0.52±0.16 |
| [b]ITC-6 | 26.31±2.26*a | 25.27±1.61*afh | 9.33±0.58*a | 26.79±4.44*a | 7.61±0.78*aj | 3.60±0.16*a | 2.49±0.17*a |
| [c]CD-RS-03 | 28.21±1.74*ae | 24.38±3.21*a | 13.33±1.53*a | 26.47±3.69*a | 7.56±1.19*aj | 4.09±0.13*ad | 2.55±0.37*a |
| [d]Dhd-RS-02 | 27.14±2.85*a | 24.04±0.83*a | 11.33±1.53*a | 25.82±3.00*a | 6.28±1.54*aj | 3.02±0.59*ac | 2.43±0.42*a |
| [e]BJ-ER-01 | 22.67±1.53*ac | 26.24±1.79*afh | 10.67±1.53*a | 24.50±1.23*a | 7.40±0.62*aj | 3.07±0.39*a | 2.02±0.09*a |
| [f]Dhd-RP-01 | 25.51±0.96*a | 19.67±0.76*abeij | 11.67±1.53*a | 27.26±1.03*a | 8.75±1.68*aj | 3.32±0.34*a | 2.14±0.41*a |
| [g]BP-RS-01 | 27.36±1.47*a | 23.12±0.46 aj | 11.67±2.08*a | 24.67±0.84*a | 9.13±0.46*aj | 3.32±0.38*a | 2.56±0.74*a |
| [h]ITC-RS-01 | 23.83±1.76*a | 19.17±3.18*beij | 10.33±1.53*a | 22.75±6.50*a | 8.88±1.21*aj | 3.99±0.42*a | 2.75±0.62*a |
| [i]Dhd-RP-07-01 | 26.85±1.17*a | 25.97±1.84*afh | 12.33±2.08*a | 21.93±1.12*a | 8.73±1.05*aj | 3.73±0.45*a | 1.80±0.33*a |
| [j]Baj-ER-01 | 25.34±1.12*a | 28.86±0.51*afgh | 11.67±0.58*a | 25.35±4.50*a | 13.84±1.04*abcdefghi | 3.68±0.21*a | 2.18±0.35*a |

*Indicates significant difference at $p<0.05$.
All growth parameters displayed significant enhancement after inoculation with the designated isolates when compared with the non-inoculated control. The alphabet adjacent to asterisk on a given value indicates that in same column, the given value is significantly different from those represented by the indicated letter.

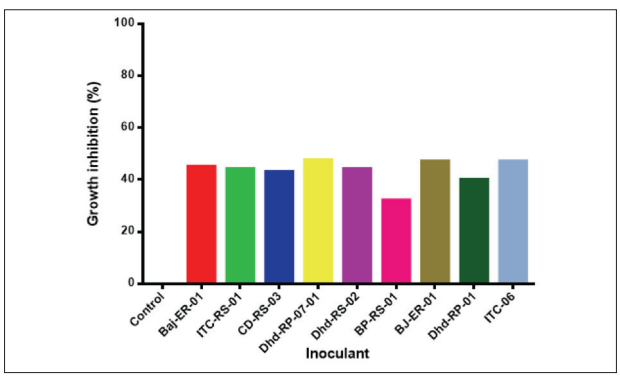


**Fig. 1.** Percentage growth inhibition of *Fusarium oxysporum* by PGPR isolates in a dual culture assay.

influence on shoot length, followed by BP-RS-01. On the other hand, plants treated with Baj-ER-01 showed maximum increase in root length, followed by plants treated with BJ-ER-01. Moreover, the treated plants showed a higher number of leaves than the control (Fig. 3A). Treatment of plants with CD-RS-03 resulted in an 186% increase in the number of leaves over the control. None of the PGPR showed enhancement less than 100% for this parameter.

A relative increase in shoot and root dry weight was recorded from 106-179% and from 246-429%,


**Fig. 2.** Growth of tobacco plants inoculated with selected PGPRs (BJ-ER-01 and CD-RS-03) and the non-inoculated control after 90 days.

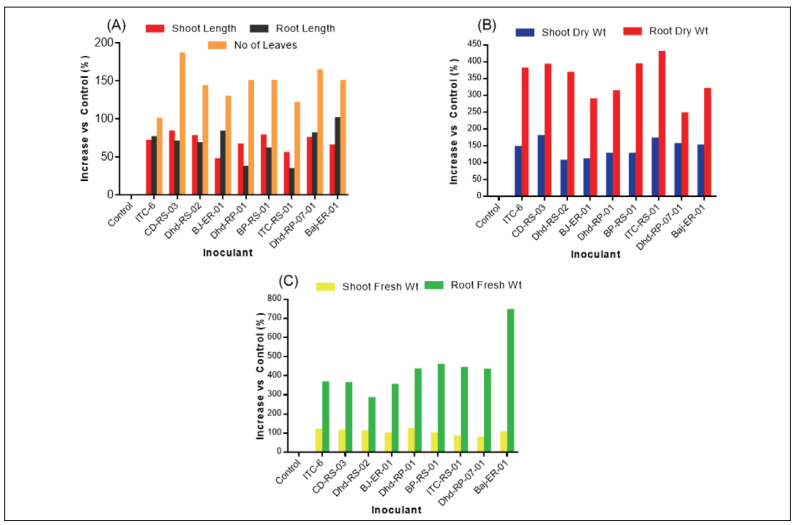


**Fig. 3.** Relative percentage increases in different growth parameters of PGPR inoculated tobacco plants compared with control after 90 days of inoculation. **A** – Lengths of shoots and roots and numbers of leaves; **B** – shoot and root dry weight; **C** – shoot and root fresh weight.

respectively (Fig. 3B). The corresponding shoot and root fresh weights were in the range of 76-119% and 282-742%, respectively (Fig. 3C). The plants treated with Baj-ER-01 showed the highest root fresh weight increase while the inoculant Dhd-RP-01 showed the maximum increase in shoot fresh weight. The root fresh weight enhancement by Baj-ER-01 inoculation was not only significantly different from the control but also from the other treatments (Table 2). Similarly, the shoot and root dry weights clearly demonstrated the significantly higher impact of the treatments as compared to the non-inoculated control. Statistically, all growth parameters showed a significant increase over the control; however, all inoculants showed the highest increase in root fresh weight/dry weight as compared to the other parameters.

## DISCUSSION

The extensive use of fertilizers and chemical fungicides, specifically in hilly areas, has had severe hazardous effects, including the leaching of chemicals that pollute nearby water resources and streams, the killing of beneficial microbes of the soil, making plants susceptible to pathogenic attack, decreasing soil fertility and irreversibly injuring to the soil community system [21]. Hence, alternative strategies with the least threat to human health and the environment but a strong potential to enhance the crop yield are needed. PGPR, the inhabitants of plant rhizosphere, rhizoplane and endorhizosphere, may provide a good alternative for growth and health promotion of plants. Phosphorous and nitrogen are the two important nutrients while auxins and gibberellins are two important groups of hormones that can be provided by these organisms, thus directly influencing plant growth [22-24]. Indirectly, these bacteria enhance plant growth by producing deleterious compounds for phytopathogens, such as enzymes, antibiotics, HCN and siderophores [25].

Among all traits, the ability of microbes to maintain the supply of solubilized phosphates and fixation of nitrogen are of prime importance. Previous studies have reported the beneficial effects of both processes on crop production [23,24,26,27]. All PGPR isolates examined in this study showed a strong potential for phosphate solubilization. The solubilization index for the majority of isolates was recorded as >2, indicating their ability to maintain the level of phosphate in a soil enriched with its insoluble form and acting as a limiting factor for crop production. Previous studies have not reported the isolation of such a large population of phosphate-solubilizing bacteria. In three different reports, phosphate solubilizers were found as 23.5% of the total tested, 5 out of 207 tested and 4 out of 9 tested, respectively [28,30]. Isolation of such a large number may be unique for this first-ever explored

area, and is a positive indicator to further study and explore the microbial community of the region. This will have a strong impact on the yield, sustainable growth and preservation of the ecosystem by reducing the use of chemical fertilizers.

Similar results were recorded for the nitrogen fixation ability of the tested isolates, which is again a strong indication that the application of these microbes can be useful for maintaining an adequate supply of nitrogenous compounds to a tobacco crop, hence lowering the dependency on nitrogenous fertilizers. These results are in accordance with a study performed to investigate the importance of nitrogen fixation through PGPR for growth of plants [31]. Our results suggest that all isolates are capable of supplying adequate amounts of necessary growth nutrients, thus having a positive impact on the growth of the plants in a natural environment.

Surprisingly, none of the tested isolates produced auxin, which is in contrast to many previous reports on PGPR [32-34]. All these studies described auxin as a common growth factor produced by PGPR and associated their production as a vital factor for growth promotion of various crops. However, the experiments performed on tobacco plants in this study indicate that a lack of expression of indole-3-acetic acid (IAA) by PGPR is not a limiting factor for plant growth in the soil under investigation, which may be true for other unexplored soil systems. Thus, our study does not agree with the conclusion of several previous studies that auxin is the only essential factor for attaining plant-growth promotion through PGPR [11,35]. We suggest that multiple traits are required by PGPR for adequate growth promotion and their synergistic effect may compensate the absence or unavailability of a key growth-promoting factor like auxin.

A number of studies have identified the role of HCN production and siderophorogenesis in suppressing the growth of fungal pathogens [36-38]. These antagonistic factors, in coordination with hydrolytic/degradative enzymes, disrupt the fungal cell wall and inhibit fungal growth [39-41], thus indirectly promoting plant growth. The collective presence of these antifungal agents in all isolates suggested their positive role in promoting the growth of tobacco plants. The experimental application of these isolates strongly inhibited the growth of phytopathogenic fungi, which provided indirect evidence for support of tobacco growth. This property is vital for crops that grow in regions (like northern Pakistan) where high annual rainfall is recorded and temperature conditions favor the growth of phytopathogenic fungal strains.

The impact of expression of growth promoting traits by PGPR on plant growth was finally confirmed by inoculating the isolates on plant seedlings in a greenhouse experiment. The observed growth enhancement of inoculated plants clearly demonstrated the beneficial effects of the PGPR on growth and yield. These traits collectively lead to a significant increase in root/shoot biomass, length of roots/shoots and number of leaves of inoculated plants. These findings are in agreement with previously reported studies describing the significant effect of PGPR on growth parameters of various crops [30,42]. In both studies, the majority of isolates enhanced shoot length (by up to 45% and 66%, respectively), root length (52% and 66%, respectively), shoot dry weight (62% and 100%, respectively), root dry weight (172% and 129%, respectively), shoot fresh weight (50% and 79%, respectively) and root fresh weight up to a maximum of 104% and 92%, respectively. Interestingly, the PGPR isolates of this study showed ~2-7-fold enhancement (shoot length by up to 83%; root length by 101%; shoot dry weight by 179%; root dry weight by 429%; shoot fresh weight by 119%, and root fresh weight up to maximum 742%) in all growth.

These results also support the fact that the lack of expression of a key plant growth trait (auxin in this study) *in vitro* does not necessarily affect the potential of growth enhancement. It is likely that growth hormones produced by the host plant are in sufficient quantity, but the nutrient supply and/or phytopathogens act as a limiting factor, restricting growth. Hence, the external support provided by PGPR may help to overcome these inhibitory factors, leading to healthy plants.

This study also describes variable responses of plants to PGPR inoculation. The influence of different inoculants/isolates on growth parameters varied from one parameter to another. For example, the inoculation of CD-RS-03 had a more pronounced effect on one parameter, i.e. the number of leaves, but not on other parameters i.e. shoot and root lengths. This variation may be explained by the fact that despite being of the same species, each individual plant has

specific traits and rhizospheric competencies affecting its response to different isolates [30]. Another likely explanation of the variable response may be different colonizing potentials of different isolates. An improper PGPR colonization would likely lead to variations in the impact on growth parameters. Despite the variations in response to different PGPR, the yields of all inoculated plants were statistically significantly higher as compared to the control, which is a direct indication that all isolates purified and tested in this study have a strong ability to provide nutrients such as phosphorous and nitrogen, and also to protect inoculated plants from phytopathogens.

In conclusion, the findings of our study provide the first information about an indigenous microbial community associated with tobacco plants in a mountainous and unexplored region of Hazara, northern Pakistan, and its positive influence on the growth and yield of tobacco. These unexplored microbial species expressed efficient growth-promoting traits, which enhanced the yield of inoculated tobacco plants. All isolates significantly influenced the growth parameters and have strong potential to be considered as plant growth pomoting agents for tobacco and other crops. Further studies of their molecular characteristics would be extremely beneficial for their application in the field. Their successful application is likely to reduce the use of chemical fertilizers and fungicides, which may have a strong and sustainable impact on the economy of Pakistan, which hugely (10%) relies on its tobacco crop.

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**Author contributions:** Sohail Khan performed the experiments while Fazli Subhan, and Kashif Syed Haleem helped in the preparation of the manuscript. Muhammad Nasir Khan, Ibrar Khan and Tariq Sultan provided technical expertise in the experimentation and data analysis. Isfahan Tauseef designed and supervised the experiments and prepared the final manuscript.

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## Supplementary Data

**Supplementary Table S1.**
Available at: http://serbiosoc.org.rs/NewUploads/Uploads/Supplementary%20Table%20S1_2786.docx