Received: 14 February 2023 | Revised: 30 March 2023 | Accepted: 10 April 2023

NATURE'S SYMPHONY

ISSN-E: 3007-2034

ISSN-P: 3007-2026

Website link: https://www.tsfns.com/

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To cite this article: Abdullah, H., Ahmed, A. and Tariq, A. 2023. Phytostimulatory Impact of Biofilm Producing PGPR on *Triticum Aestivum* L. *Nature's Symphony*, 1(1):10-21.

DOI: https://doi.org/10.69547/NS.11.02

Article QR

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Original Research

Phytostimulatory Impact of Biofilm Producing PGPR on *Triticum Aestivum* L.

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Keywords: PGPR, biofilms, EPS, *Triticum aestivum*, biofertilizers

ABSTRACT

Plant growth promoting rhizobacteria (PGPR) have ability to produce biofilms around host plant roots which help them in efficient colonization and protect them from desiccation and soil borne pathogens. These biofilms act as a shield both for bacteria as well as for plants. Bacteria with biofilms secrete various extracellular polymeric substances (EPS). Exopolysaccharide is the principle component of bacterial biofilm that determine various aspects of biofilms. For the current study, already isolated bacterial strains were screened both qualitatively and quantitatively for their biofilm forming potential. Their growth promoting ability and exopolysaccharide potential analysis was carried out and phytostimulatory impact was studied by plant microbial interaction assay under laboratory conditions using *Triticum aestivum* L. Different growth and biochemical parameters of bacterially treated and untreated plants were recorded and analyzed. Maximum increase (89%) in seed germination was recorded by plants treated with bacterial strain *Burkholderia cepacia* (FS1). Similarly, maximum increase of 111% in total chlorophyll content and 75% in soluble protein content was observed in plants treated with *Pseudomonas* sp. (DS1) and *Pseudomonas* sp. (FS2) respectively. Our study showed that biofilm forming bacteria display significant enhancement in plant growth and health.

1. INTRODUCTION

Plants developed symbiotic relationship with certain rhizospheric bacteria which in turn influence different beneficial effects on their host plant through various activities like phytohormone production (IAA), nitrogen fixation, iron sequestration, siderophore production, phosphate solubilization, etc. The phytohormones help in cell division and plant growth. Nitrogen fixation, iron sequestration and phosphate solubilization help the plants acquire these essential nutrients, thus ensuring healthy growth of the plants. Siderophores also help in iron chelation. These rhizospheric bacteria are generally referred as plant growth promoting rhizobacteria (Raza *et al.*, 2016). Plant growth promoting rhizobacteria (PGPR) have unique ability to form biofilms around roots of associated plants to develop a successful plant-microbial interaction by preventing them from being detached from plant roots. Different environmental factors affect efficient root colonization and thus affect PGPR activity. Biofilm production is considered as an additional strategy that protect rhizobacteria and enable their survival under hostile environmental conditions. This also influences

the plant growth by efficient colonizing of plant roots by these bacteria (Burmølle *et al.*, 2014; Ansari and Ahmad, 2018).

Biofilms are well organized bacterial communities enclosed within self-produced extracellular polymeric matrix (Seneviratne *et al.*, 2011). These can be formed on both biotic and abiotic surfaces. These extracellular polymeric substances (EPS) contain variety of substances including various proteins and exopolysaccharides. EPS play crucial role in determining the structure and function of biofilms. It has been reported that EPS sequester the uptake of various toxins and metal ions and protect the plant roots from different environmental stresses. Besides this, bacteria within biofilms possess many more advantages such as higher population densities, antimicrobial tolerance and competition for nutrients (Mohammed, 2018). Biofilms are thought to improve the soil fertility by releasing various enzymes and nutrients in soil and trigger the development of lateral root hairs which ultimately increase plant production (Velmourougane *et al.*, 2017).

The aim of current study is to screen biofilm forming bacteria and study their phytostimulatory effects on *Triticum aestivum* var. FSD 2008. The plant selected for the desired study is the staple food of Pakistan and chief source of carbohydrate.

2. MATERIALS AND METHODS

Present study was carried out using twenty already isolated bacterial strain which were subjected for determination of biofilm and EPS production potential along with auxin estimation.

2.1 Ring Test Analysis for Biofilm Formation Potential

Both qualitative and quantitative methods were carried out to determine biofilm production of bacterial strains. Qualitative determination of biofilm forming bacteria was carried out through ring test following the methods reported by Qurashi and Sabri (2011) with some modifications. Bacterial cultures were grown for 48 hours at 37 ℃. Following the incubation, the cultures were decanted and biofilms in the test tubes were observed after 20 minutes of staining with 1% crystal violet solution. Quantification of bacterial cells in biofilm was carried out by dislodging and suspending the biofilm by adding acetic acid in stained test tubes and measuring the absorbance at 570nm by using spectrophotometer (Cecil 7200).

2.2 Auxin Production Analysis

Auxin producing ability of selected bacteria was observed following Ahmed and Hasnain (2010). Briefly, the bacteria were cultured in LB-broth supplemented with L-tryptophan for seven days, after which 1.0 mL of the supernatants were mixed with Salkowshi's reagent (2.0 mL) and incubated for 60 minutes. Optical density was measure at 535 nm using a spectrophotometer (Cecil 7200).

2.3 Exopolysaccharide (EPS) Potential Analysis

EPS potential of selected bacterial strains was observed by following Kwon *et al.* (2002). 120 hours old bacterial cultures were centrifuged at 12,000 rpm for 30 minutes. The supernatant was mixed with three volumes of isopropanol slowly along the side wall of the flask and allowed to stand at 4ºC. After 20 minutes, white precipitates of EPS were observed.

2.4 Plant Growth Experiment

Certified seeds of *Triticum aestivum* L. var. FSD 2008 were obtained from Punjab Seed Corporation, Lahore, Pakistan. Plant growth experiment was carried out following Ahmed and Hasnain (2010) under laboratory conditions. The experiment was conducted in

triplicates. After 20 days, plants were harvested and various growth parameters i.e., length of shoot and root, fresh weight and number of leaves per plant of bacterially treated and non-treated plants were recorded.

2.4.1 Biochemical Analysis

Content of chlorophyll a, b as well as total chlorophyll of treated and non-treated plants were estimated by modified method described by Wellburn (1994). The total soluble protein content of inoculated and uninoculated plants was assessed following Lowry *et al.* (1951).

2.5 Statistical analysis

The data obtained were statistically analyzed by applying Duncan's multiple range test using SPSS ver. 16.

3. RESULTS

3.1 Biofilm Formation Potential

Qualitative analysis of biofilm formation was analyzed by observing thick films formed inside the wall and bottom of test tubes. Out of twenty bacterial strains screened, only thirteen were recorded as biofilm formers which were further analyzed for their EPS and auxin production potential. Biofilms were formed at interface by all the bacterial strains except the bacterial strain *Pseudomonas* sp. (DS3) which formed biofilm at the bottom. These bacteria were grouped as very strong, strong, moderate and weak biofilm formers based on quantitative analysis by recording their crystal violet optical density at 570nm (Fig. 1). Bacterial strains *Pseudomonas* sp. (ES2), *Pseudomonas aeruginosa* (DS4), *Pseudomonas* sp. (ES3) and *Shewanella putrefaciens* (FS3) were recorded as highly biofilm formers (Table 1).

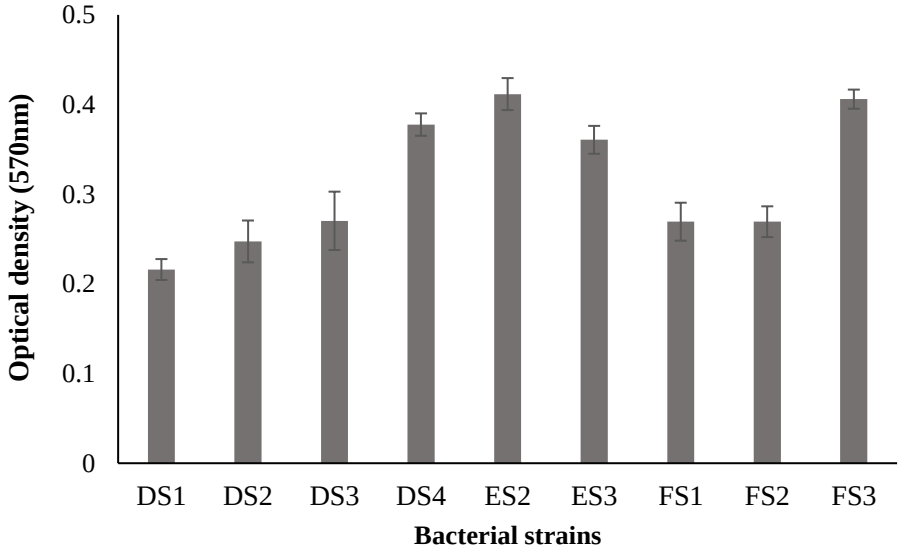

Figure 1. Bacterial cell density in biofilms produced by bacterial strains

Table 1. Biofilm, auxin and EPS production potential of bacterial isolates

Sr. no.	Bacterial isolates	Position of biofilm	Biofilm formation potential	Auxin production potential	EPS production potential
1.	DS1	Interface	+++	++	++++
2.	GR5	-	-	++	-
3.	GS2	Interface	++	++	-
4.	DS2	Interface	+++	++	++++
5.	DS3	Bottom	+++	++++	+++
6.	DS4	Interface	++++	+++	+++
7.	DH4	-	-	+++	-
8.	EH2	-	-	-	-
9.	ES2	Interface	++++	+++	+++
10.	ES3	Interface	++++	++++	++++
11.	ES4	-	-	-	-
12.	EH5	Interface	++	+++	++
13.	HS4	Interface	++	-	-
14.	KH3	-	-	++++	-
15.	FS1	Interface	+++	++	++++
16.	FS2	Interface	+++	++	+++
17.	FS3	Interface	++++	++++	+++
18.	FS4	Interface	++	-	++
19.	MH1	-	-	+++	-
20.	IR1	-	-	-	-

Strong formers (++++), moderate formers (+++), weak formers (++)

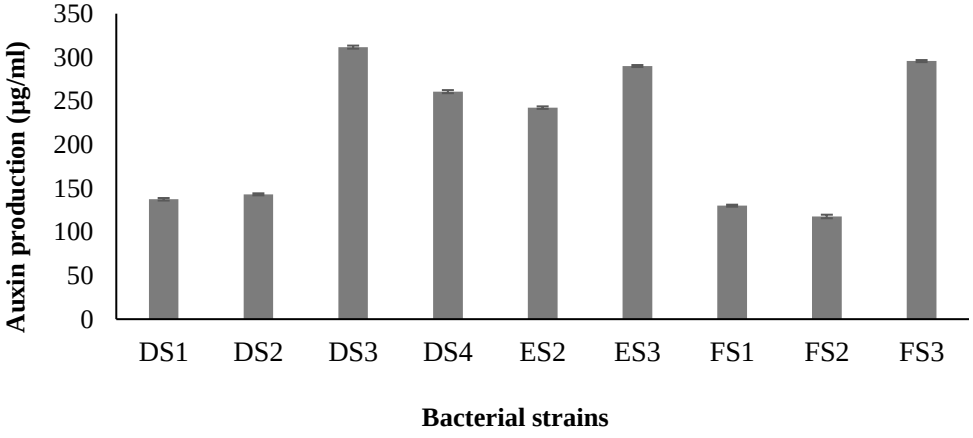

Figure 2. Auxin production potential of bacterial strains

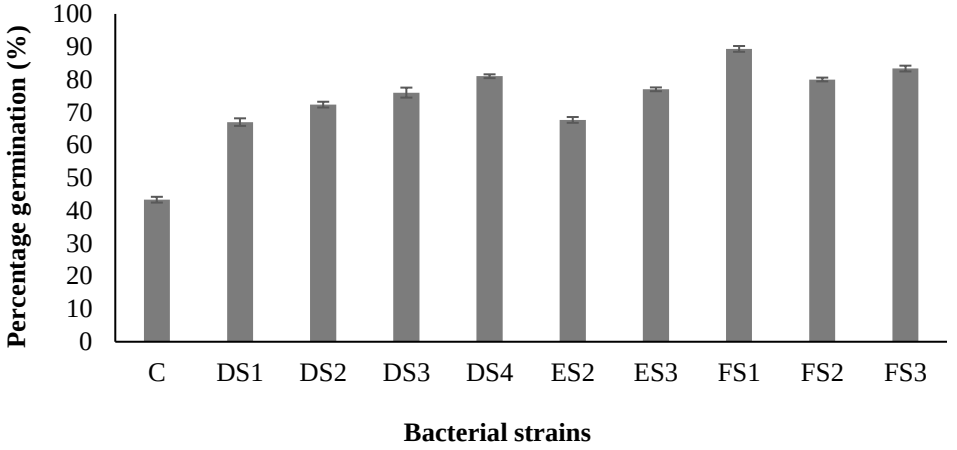

Figure 3. Effect of bacterial inoculations on percentage germination of *Triticum aestivum* (C - control)

3.2 Growth Potential Analysis

These thirteen biofilm forming bacterial strains were further investigated for their auxin production ability and only eleven bacteria were recorded as auxin producers (Table 1). The bacterial strains *Pseudomonas* sp. (DS3), *Pseudomonas* sp. (ES3), *S. putrefaciens* (FS3), *Pseudomonas* sp. (ES2) and *P. aeruginosa* (DS4) were recorded as highly auxin producing whereas bacterial strains *S. putrefaciens* (DS2), *Pseudomonas* sp. (DS1), *Burkholderia cepacia* (FS1) and *Pseudomonas* sp. (FS2) were recorded as moderate auxin producing strains (Fig. 2).

3.3 Exopolysaccharide (EPS) Potential Analysis

Out of eleven biofilm and auxin producing bacterial strains, ten bacterial strains have shown potential to form EPS. *S. putrefaciens* (DS2), *Pseudomonas* sp. (DS1), *Pseudomonas* sp. (ES3) and *B. cepacia* (FS1) have very high EPS forming ability while bacterial strains *Pseudomonas* sp. (DS3), *Pseudomonas* sp. (ES2), *P. aeruginosa* (DS4), *Pseudomonas* sp. (FS2) and *S. putrefaciens* (FS3) have shown strong potential to produce EPS (Table 1).

3.4 Plant Growth Experiment

On the basis of biofilm, auxin and EPS production ability of bacterial strains nine most efficient bacterial strains i.e., *S. putrefaciens* (DS2), *Pseudomonas* sp. (DS1), *Pseudomonas* sp. (DS3), *Pseudomonas* sp. (ES2), *P. aeruginosa* (DS4), *Pseudomonas* sp. (ES3), *B. cepacia* (FS1), *Pseudomonas* sp. (FS2) and *S. putrefaciens* (FS3) were selected for plant growth experiment. Different growth parameters of bacterially inoculated and non-inoculated plants were observed and recorded. Germination percentage of bacterially treated seeds were greater than non-treated seeds. The maximum percentage germination was expressed by *B. cepacia* (FS1) i.e., 89.33%. Seeds treated with bacterial strains *Pseudomonas* sp. (FS3), *P. aeruginosa* (DS4), *Pseudomonas* sp. (FS2), *Pseudomonas* sp. (ES3), *Pseudomonas* sp. (DS3), *S. putrefaciens* (DS2), *Pseudomonas* sp. (ES2) and *Pseudomonas* sp. (DS1) showed 83.33, 81, 80, 77, 76, 72.33, 67.66 and 67% increase in percentage germination (Fig. 3). Similarly, profound increase in length of shoots of bacterially treated plants were also recorded. The increase recorded by inoculating bacterial strains *B. cepacia* (FS1), *Pseudomonas* sp.

(DS3), *Pseudomonas* sp. (DS1), *Pseudomonas* sp. (ES3), *S. putrefaciens* (FS3), *Pseudomonas* sp. (ES2), *S. putrefaciens* (DS2) and *P. aeruginosa* (DS4) were 11.2, 11.17, 11.12, 11.1, 11.1, 11.09, 11.07 and 11% respectively, over the uninoculated plants (Fig. 4). The maximum increase of 4.41% was recorded in root length in case of bacterial strain *Pseudomonas* sp. (DS1) (Fig 4) while maximum increments of 10.81, 10.8 and 10% in number of leaves were recorded when plants were treated with bacterial strains *Pseudomonas* sp. (DS1), *P. aeruginosa* (DS4) and *S. putrefaciens* (FS3) respectively, compared with untreated wheat plants (Fig. 5). Similarly, the enhanced fresh weight of treated plants with bacterial strains *Pseudomonas* sp. (ES3), *S. putrefaciens* (FS3), *Pseudomonas* sp. (DS3), *B. cepacia* (FS1), *Pseudomonas* sp. (FS2), *P. aeruginosa* (DS4), *Pseudomonas* sp. (ES2), *S. putrefaciens* (DS2) and *Pseudomonas* sp. (DS1) was recorded as 165, 161, 152.25, 152, 151, 140, 104, 103 and 95% respectively, as compared to non-inoculated plants (Fig. 5).

3.4.1 Biochemical Analysis

The maximum increase of chlorophyll a was recorded up to 134, 143, 115.5, and 115% in plants treated with bacteria ES3, DS2, DS3 and FS1 respectively when compared to control ones (Fig. 6). Similarly, boost shown by bacteria DS1, DS3, DS2, ES3 and FS1 in the chlorophyll 'b' content of treated plants was 49.48, 43.26, 43.25, 42.3 and 40.24% respectively (Fig. 6). Total chlorophyll content of treated plants was increased with bacteria ES2, DS1, ES3, DS2, FS1 and DS3 up to 171.37, 111.72, 106, 105.26, 93 and 92.16% respectively, over the control ones (Fig. 6). Similarly, increase in the protein content with the bacteria FS2, FS3, DS4, DS3, DS1, DS2 and ES2 was recorded as 75.21, 55.34, 43.42, 37.5, 26.97, 20.39 and 15.78% respectively, compared to untreated plants (Fig. 7).

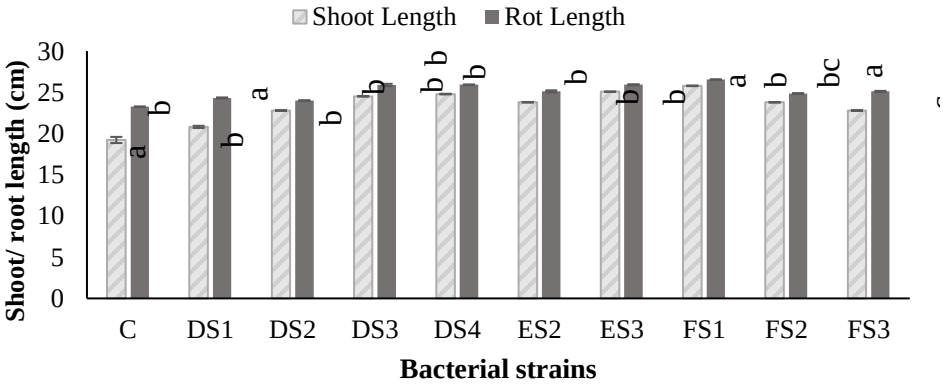

Figure 4. Effect of bacterial inoculations on root and shoot of *Triticum aestivum*. Duncan's multiple range test showed significant differences as indicated by letters (P = 0.05) (C – control)

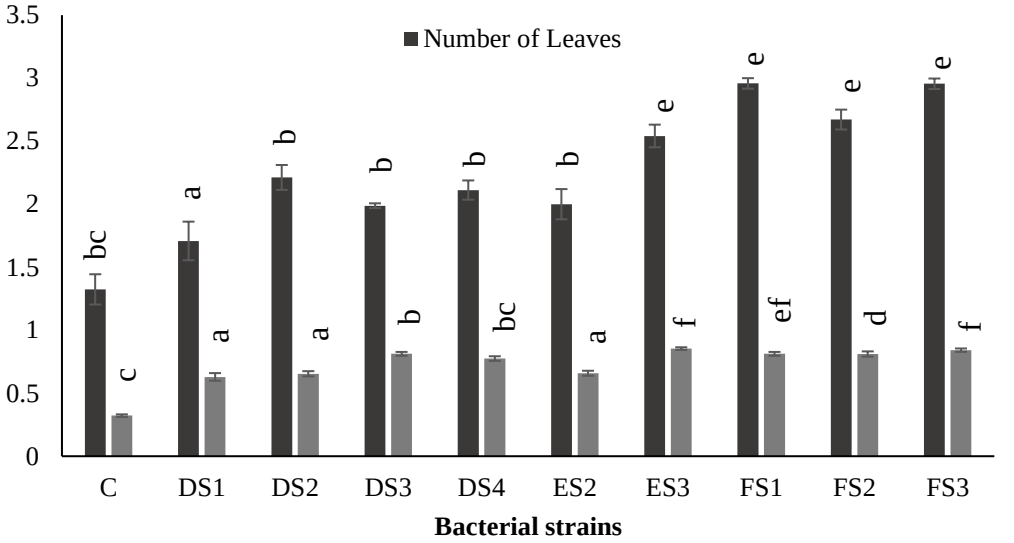

Figure 5. Effect of bacterial inoculations on number of leaves and fresh weight of *Triticum aestivum*. Duncan's multiple range test showed significant differences as indicated by letters (P = 0.05) (C – control)

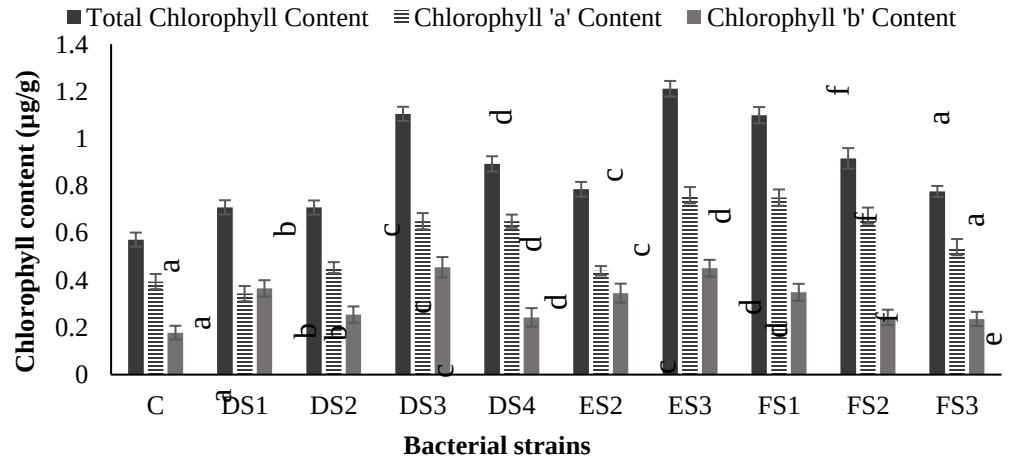

Figure 6. Effect of bacterial inoculations on chlorophyll 'a', 'b' and total chlorophyll content of *Triticum aestivum*. Duncan's multiple range test showed significant differences as indicated by letters (P = 0.05) (C – control)

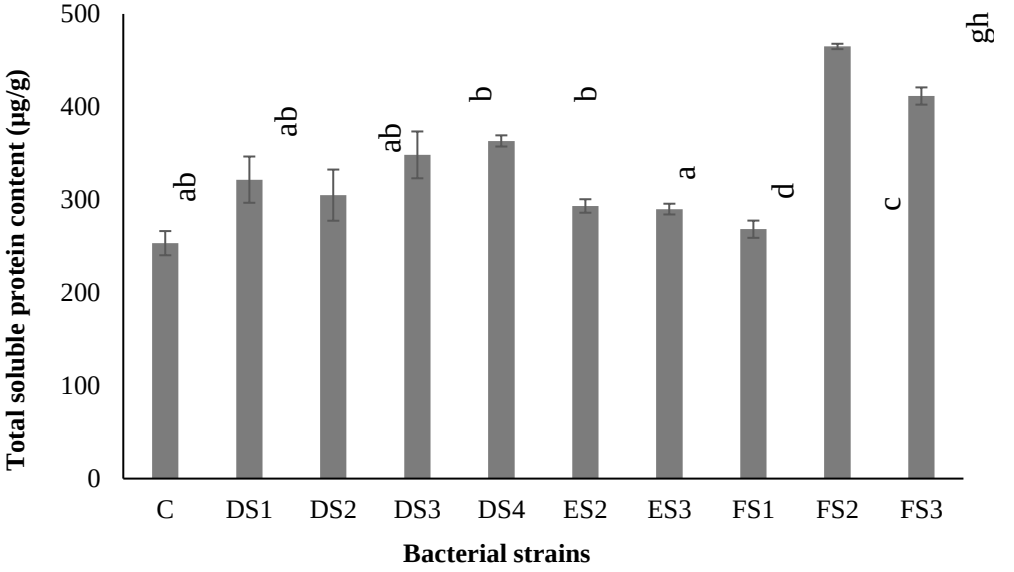

Figure 7. Effect of bacterial inoculations on soluble protein content of *Triticum aestivum*. Duncan's multiple range test showed significant differences as indicated by letters (P = 0.05) (C – control)

4. DISCUSSION

The frequent use of chemical fertilizers reduces crop nutrient values and soil fertility and has hazardous impact on environment. To develop eco-friendly and sustainable strategy, biofertilizers are efficient and cost-effective alternative. Recently, biofilms formation by PGPR has been the key interest for many scientists. Present work deals with the study of phytostimulatory potential of biofilm forming PGPR on *Triticum aestivum* var. FSD 2008. Wheat is among the top cultivated cereal crops of the world as it is staple food for many countries. Various PGPR are reported to form biofilms on biotic and abiotic surfaces. In present study, profound increase in plant growth parameters such as length of root and shoot, number of leaves and fresh weight were recorded in bacterially treated plants. It has been studied from literature that biofilm forming bacteria displayed profound increment in plant growth as compared with non-inoculated plants. Studies of Altaf and Ahmad (2017) showed noticeable increase in growth attributes when plants were inoculated with biofilm-forming PGPR as compared to non-biofilm former inoculants. Biofilms produced by rhizobacteria improves their root colonization by retaining moisture.

In present study, seeds inoculated with biofilm forming bacterial strains expressed profound increase in percentage germination. Habib *et al.* (2019) reported that increased percentage germination of PGPR treated plants is due to efficient exogenous auxin supply by bacterial strains. Present study exhibits a notable increase in root length as well when inoculated with biofilm forming PGPR. Improved root growth of inoculated plants might be due to the developmental changes induced by auxin and biofilm simultaneously by PGPR. These PGPR besides improving root health also affect root-to-shoot hormonal signaling and balance, which plays major role in regulating leaf growth and other physiological processes of plants (Ansari and Ahmad, 2018; Singh *et al.*,

2019). Rhizobacterial strains exhibiting biofilm forming ability beside auxin production potential have been stated to improve the growth and health of different crop plants. These results are in accordance with Ansari and Ahmad (2018) who reported increased in length of root and shoot and spikes dry biomass of wheat plant treated with biofilm-forming PGPR. Findings of Azri *et al.* (2018) also suggested that biofilm forming PGPR associations enhance yield and quality of oil palm seedlings. Similar findings were also reported by Kalam *et al.* (2017).

The strains used in current study were producing exopolysaccharide which is the principle component of biofilm. EPS produced by the biofilm forming PGPR may remain bound or secreted in the soil and form soil aggregates (Saraf *et al.*, 2011). Ahn *et al.* (2007) reported significant increase in shoot length and extensively growing root system in case of EPS forming *Pseudomonas* sp. treated plants. This extensive root system further increased water and nutrient uptake from soil that enhances plant growth. Exopolysaccharides induce the facilitation of the initial attachment of cells to substratum and provide protection (Singh *et al.*, 2017). Different studies have shown that PGPR produce EPS that retain water in soil and protect plant roots from desiccation by forming a rhizosheath around the roots (Zheng *et al.*, 2018). Therefore, exopolysaccharide producing growth promoting bacteria showed significant results in comparison with non-treated plant. Khan *et al.* (2018) studied that EPS forming rhizobacteria showed increased productivity in contrast to non-EPS producing bacterial inoculants. Similar results were also described by Khan *et al.* (2019) .

In present study, improvement in fresh weight of the bacterially treated plants were also recorded indicating enhanced nutrient uptake due to PGPR activities. These results are in accordance with Sapre *et al.* (2018) who also recorded increased fresh weight besides shoot and root length in case of *Avena sativa* plant. Similar findings were also documented by Zhao *et al.* (2018) and Saleem *et al.* (2018) in bacterially treated tomato and sunflower plants respectively. In current study, bacterial strains have significantly enhanced chlorophyll contents (total chlorophyll, chlorophyll a and chlorophyll b) in the inoculated plant leaves. Chen *et al.* (2016) reported that biofilm forming bacteria increased the chlorophyll contents in maize plant. In current study increase in total soluble protein contents of treated plants has been observed. Khan and Bano (2016) also reported that EPS-producing bacteria considerably increased protein content in leguminous plants.

5. CONCLUSION

Biofilm forming ability of PGPR enhances their growth promotional potential by enabling their attachment and survival under harsh environmental conditions. Researchers have great interest in developing biofertilizers using bacterial biofilms. In current study, all the biofilm forming PGPR showed significant increase in growth parameters such as shoot and root length, number of leaves and fresh weight. The chlorophyll as well as the protein content was also increased in the presence of these bacteria. It can be concluded from this prospect, that these bacterial strains can be applied to plants as biofertilizers to enhance yield and nutrient value of the plants.

Acknowledgment
The research work was supported by University of the Punjab, Lahore, Pakistan.

Conflict of Interest
The authors declare no conflict of interest.

REFERENCES

Ahmed, A. and Hasnain, S. 2010. Auxin-producing Bacillus sp.: Auxin

quantification and effect on the growth of Solanum tuberosum. 82:313-319.

Ahn, T. S., Ka, J. O., Lee, G. H. and Song, H. G. 2007. Microcosm study for revegetation of barren land with wild plants by some plant growth-promoting rhizobacteria. *J Microbiol Biotechnol*, 17:52-57.

Altaf, M. M. and Ahmad, I. 2017. In vitro and In vivo biofilm formation by Azotobacter isolates and its relevance to rhizosphere colonization. *Rhizosphere*, 3:138-142.

Ansari, F. A. and Ahmad, I. 2018. Plant growth promoting attributes and alleviation of salinity stress to wheat by biofilm forming Brevibacterium sp. FAB3 isolated from rhizospheric soil. *Saudi Journal of Biological Sciences*.

Azri, M. H., Ismail, S. and Abdullah, R. 2018. An endophytic Bacillus strain promotes growth of oil palm seedling by fine root biofilm formation. *Rhizosphere*, 5:1-7.

Burmølle, M., Ren, D., Bjarnsholt, T. and Sørensen, S. J. 2014. Interactions in multispecies biofilms: do they actually matter? *Trends in Microbiology*, 22:84-91.

Chen, Y., Liu, T., Wang, K., Hou, C., Cai, S., Huang, Y., Du, Z., Huang, H., Kong, J. and Chen, Y. 2016. Baicalein Inhibits Staphylococcus aureus Biofilm Formation and the Quorum Sensing System In Vitro. *PloS One*, 11:e0153468.

Habib, S., Fatima, H.-e.-. and Ahmed, A. 2019. Comparative Analysis of Pre-Germination and Post-Germination Inoculation Treatments of Zea mays L. to Mitigate Chromium Toxicity in Cr-Contaminated Soils. *Polish Journal of Environmental Studies*, 28:597-607.

Kalam, S., Das, S. N., Basu, A. and Podile, A. R. 2017. Population densities of indigenous Acidobacteria change in the presence of plant growth promoting rhizobacteria (PGPR) in rhizosphere. *J Basic Microbiol*, 57:376-385.

Khan, N. and Bano, A. 2016. Modulation of phytoremediation and plant growth by the treatment with PGPR, Ag nanoparticle and untreated municipal wastewater. *Int J Phytoremediation*, 18:1258-1269.

Khan, N., Bano, A., Rahman, M. A., Guo, J., Kang, Z. and Babar, M. A. 2019. Comparative Physiological and Metabolic Analysis Reveals a Complex Mechanism Involved in Drought Tolerance in Chickpea (Cicer arietinum L.) Induced by PGPR and PGRs. *Scientific Reports*, 9:2097.

Khan, N., Bano, A., Shahid, M. A., Nasim, W. and Ali Babar, M. D. 2018. Interaction between PGPR and PGR for water conservation and plant growth attributes under drought condition. *Biologia*, 73:1083-1098.

Kwon, K. K., Lee, H. S., Jung, S.-Y., Yim, J.-H., Lee, J.-H. and Lee, H. K. 2002. Isolation and identification of biofilm-forming marine bacteria on glass surfaces in Dae-Ho Dike, Korea. *Journal of Microbiology*, 40:260-266.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193:265-275.

Mohammed, A. F. 2018. Effectiveness of exopolysaccharides and biofilm forming plant growth promoting rhizobacteria on salinity tolerance of faba bean (*Vicia faba* L.). *African Journal of Microbiology Research*, 12:399-404.

Qurashi, A. W. and Sabri, A. N. 2011. Osmoadaptation and plant growth promotion by salt tolerant bacteria under

salt stress. *African Journal of Microbiology Research*, 5:3546-3554.

Raza, W., Ling, N., Yang, L., Huang, Q. and Shen, Q. 2016. Response of tomato wilt pathogen Ralstonia solanacearum to the volatile organic compounds produced by a biocontrol strain Bacillus amyloliquefaciens SQR-9. *Scientific Reports*, 6:24856.

Saleem, M., Asghar, H. N., Zahir, Z. A. and Shahid, M. 2018. Impact of lead tolerant plant growth promoting rhizobacteria on growth, physiology, antioxidant activities, yield and lead content in sunflower in lead contaminated soil. *Chemosphere*, 195:606-614.

Sapre, S., Gontia-Mishra, I. and Tiwari, S. 2018. Klebsiella sp. confers enhanced tolerance to salinity and plant growth promotion in oat seedlings (Avena sativa). *Microbiological Research*, 206:25-32.

Saraf, M., Jha, C. K. and Patel, D. 2011. The Role of ACC Deaminase Producing PGPR in Sustainable Agriculture. *In*: Maheshwari, D. K. [Ed.] *Plant Growth and Health Promoting Bacteria.* Springer Berlin Heidelberg, Berlin, Heidelberg. pp. 365-385.

Seneviratne, G., Weerasekara, M. L. M. A. W., Seneviratne, K. A. C. N., Zavahir, J. S., Kecskés, M. L. and Kennedy, I. R. 2011. Importance of Biofilm Formation in Plant Growth Promoting Rhizobacterial Action. *In*: Maheshwari, D. K. [Ed.] *Plant Growth and Health Promoting Bacteria.* Springer Berlin Heidelberg, Berlin, Heidelberg. pp. 81-95.

Singh, S., Singh, S. K., Chowdhury, I. and Singh, R. 2017. Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. *Open Microbiol J*, 11:53-62.

Singh, S. K., Singh, P. P., Gupta, A., Singh, A. K. and Keshri, J. 2019. Chapter Twelve - Tolerance of Heavy Metal Toxicity Using PGPR Strains of Pseudomonas Species. *In*: Singh, A. K., Kumar, A. and Singh, P. K. [Eds.] *PGPR Amelioration in Sustainable Agriculture.* Woodhead Publishing. pp. 239-252.

Velmourougane, K., Prasanna, R. and Saxena, A. K. 2017. Agriculturally important microbial biofilms: Present status and future prospects. *Journal of Basic Microbiology*, 57:548-573.

Wellburn, A. R. 1994. The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *Journal of Plant Physiology*, 144:307-313.

Zhao, D., Zhao, H., Zhao, D., Zhu, X., Wang, Y., Duan, Y., Xuan, Y. and Chen, L. 2018. Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. *Biological Control*, 119:12-19.

Zheng, W., Zeng, S., Bais, H., LaManna, J. M., Hussey, D. S., Jacobson, D. L. and Jin, Y. 2018. Plant Growth-Promoting Rhizobacteria (PGPR) Reduce Evaporation and Increase Soil Water Retention. *Water Resources Research*, 54:3673-3687.