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Ochrobactrum, bacillus and Enterobacter isolates of Hot Water Spring Augment the Growth of Zea Mays Seedlings

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OCHROBACTRUM, *BACILLUS* AND *ENTEROBACTER ISOLATES* OF HOT WATER SPRING AUGMENT THE GROWTH OF ZEA MAYS SEEDLINGS

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ABSTRACT

Hot springs situated at high altitudes is a kind of remarkable ecosystem for the exploration of microbial flora. It was hypothesized that hot springs can harbor bacteria with plant growth-promoting and exopolysaccharides (EPS) producing ability that can favour the growth of plants. For the investigation of this hypothesis, seven EPS producing bacterial isolates were isolated from a water sample of hot water spring of Tattapani, Azad Kashmir and characterized morphologically and biochemically. Three out of seven isolates (BE1, BN1 and BN3) showed significant production of EPS (14-15 mg / 100 ml). Growth kinetics study revealed that optimum EPS production was attained at pH 9, with fructose as a carbon source and peptone as a nitrogen source. Inoculation of these isolates caused augmentation in seed germination (27-38 %), shoot length (27-35 %), seedling length (10-14 %), number of roots (12-25 %) of *Zea mays* (variety-MMRI yellow) seedlings and significant rise in auxin (28-51 %) and soluble protein content (50-68 %) as compared to non-inoculated treatment. Alcian blue staining unveiled the good colonization potential of these isolates on inoculated roots. Bacterial isolates were identified as *Ochrobactrum intermedium* (BE1), *Bacillus pumilus* (BN1) and *Enterobacter cloacae* (BN3), respectively through 16S rRNA analysis. Bacterial strain BN3 showed promising results for plant growth promotion along with EPS production. Fourier-transform infrared (FTIR) spectroscopy of EPS produced by strain BN3 revealed the complex composition of EPS. We concluded that hot springs can be the possible home for EPS producing bacteria with plant growth promotion capability.

Keywords: Exopolysaccharides, fourier-transform infrared spectroscopy. hot spring, plant growth promotion, *Zea mays.*

INTRODUCTION

Microbes are ubiquitous in their distribution ranging from hot springs to hydrothermal vents and tropics to the poles. Predominantly, microbes of thermophilic habitats have developed certain physiological alterations to with stand high temperature and chemical stress. Microbial populations of hot springs situated at low elevations have been widely explored universally but lot of these valued gene pools have left uninvestigated because of lack of access for humans. Thermal springs are of much importance in terms of microbial diversity to be explored as a source of unique genes, molecules and hydrolytic enzymes for various biotechnological processes (Sahay et al., 2017). Microorganisms of extreme environment have been known for its importance in the industrial sector due to the ability to play their role under variable conditions. The microbial population of numerous hot springs present in United States, Russia, Iceland, Algeria, New Zealand and India have been explored and identified via 16S rRNA sequence from culture-dependent or culture-independent approaches (Verma et al., 2018). Microbes of the extreme environment have been known as a source of unique metabolites

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such as pigments and enzymes which can be helpful in agricultural sector as inoculants either to stimulate the development of plants or as biocontrol agents (Verma et al., 2017). Hot springs are also an ideal habitat for thermophiles that are basically geothermally hot, found all over the world (Amin et al., 2017). Thermophilic temperature normally damages biomolecules, proteins and nucleic acids of cell. Thermophilic bacteria have evolved strong mechanisms to cope up with the extreme environment and remain active at high temperature (Ali et al., 2023). The first thermophile, *Thermus aquaticus* was identified by Professor Thomas D. Brock in 1960 that could grow at temperature up to 70 ºC and broadly used in molecular biology (Pinto, 2021).

Generally, microbes present in stress environment deal with the extreme conditions by two strategies. The first strategy is through functional properties of cell membrane, periplasmic membrane and secretory proteins of entire cell. The second and most important strategy is through production of extracellular exopolysaccharides (Parrilli et al., 2021). Structurally, EPSs are the carbohydrate polymers of high molecular weight (10-1000kDa) (Malik et al., 2015). It may be either permanently bounded with cell as capsular polysaccharides (CPS) or may be secreted outside the cell into their surroundings as temporarily attached exopolysaccharides (EPS) (Malang et al., 2015).

Amount of exoploysaccharides produced by bacteria depends upon the type of extreme conditions for example temperature, pH, salinity, etc. Four steps are involved in the synthesis of microbial exopolysaccharides with the help of groups of enzymes (Mishra and Jha, 2013). First step of biosynthesis involves substrate uptake such as glucose which is consumed by the bacterial cell by diffusion, active transport or group translocation. Second step encompasses sugar metabolization by phosphorylation of substrate for instance glucose to glucose-6-phosphate by the ‘hexokinase’ enzyme. Glucose-6-phosphate is transformed into glucose-1-phosphate by ‘phosphoglucomutase’. This product may be consumed for energy production or for EPS synthesis. Then, glucose-1-phoshate is converted into a key intermediate uridine diphosphate (UDP) glucose, by group ll enzymes known as ‘glucosepyrophosphorylase’ that can either interconverted in other sugars to continue catabolism process. Then, polymerization occurs with the help of group lll enzymes ‘glycosyltransferases’ that translocate sugar moieties from donor molecules to definite acceptor molecules i.e. repeating units bind on glycosyl carrier lipids known as isoprenoid alcohol (Campbell et al., 1997). This alcohol terminal is attached to each monosaccharide by pyrophosphate connection due to which the polymerization of monomers occurs and polysaccharides are synthesized. After the synthesis of polysaccharides, these are modified by acetylation, acylation, methylation and sulphation with the aid of group lV hydrophobic enzymes such as permease, flippase, or ABC transporters (Liu et al., 1996). These modified polysaccharides are finally moved towards the cell surface and secreted out of the cell as capsular polysaccharides or exopolysaccharides (Mishra and Jha, 2013).

Extracellular substances produced by bacteria can help to promote plant growth at molecular and genetic level. The presence of these type of bacteria has strong capability to increase the plant growth, tolerance and resistance (Rosier et al., 2018). Bacterial EPSs have diverse functions mainly comprising of cell protection, nutrients accumulation, stable biofilm formation, plants roots colonization for nitrogen fixation, antimicrobial activity (Ajijah et al., 2023). Microbial exopolysaccharides play a main role in soil aggregation facilitating water

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movement and water holding capacity. Aeration and temperature effect seed germination and root growth. EPS production by bacteria cause resistance against water stress (Naseem et al., 2018).

Bacterial EPS has diverse applications in the areas such as industries (dairy, food, textile, cosmetics, pharmaceuticals, medicine), environment (flocculation, remediation) and agriculture (herbicides, fungicides and insecticides mobility improvement) (Roberts, 1996). Keeping this in view, aim of the current research was the isolation and characterization of bacterial species form hot springs with plant growth promotion and EPS production for the enhancement of sustainability in agriculture sector.

MATERIALS AND METHODS

EPS Producing Bacterial Strains

Water samples were collected in a sterile screw capped bottle from hot water spring of Tattapani Azad Kashmir, located in Abbottabad, Pakistan. Bacterial isolates were obtained by serial dilution method and grown on Luria Bertani (LB) agar plates at 37, 45 and 60 ℃. L- agar plates which were incubated at 37 ℃ displayed more morphologically diverse bacterial colonies so, they were selected for further study. For morphological characterization, colony morphology, cell morphology and motility were determined. Different tests such as Triple-sugar-iron (TSI), Methyl Red-Voges Proskauer (MR-VP), catalase, oxidase, starch hydrolysis and DNase were performed for biochemical characterization of selected bacterial isolates (Cappuccino and Sherman, 2014). Provasoli medium (P-medium) was used to screen the EPS production ability of the selected strains (Kölbel-Boelke et al., 1988).

Extraction and Analysis of EPS

Bacterial isolates were grown in L-broth for 2-5 days at 37 ℃. Cold ethanol precipitation method was used for the extraction of bacterial EPS (Batool et al., 2015b). Total EPS produced by each isolate was estimated by weighing the freshly precipitated EPS. For the determination of dry weight, EPS were dried at 58 °C for 24 hours and weighed again. EPS produced by these isolates was stored at -20 °C till further use. Quantification of EPS was done in terms of total carbohydrate content by using the phenol-sulfuric acid method (DuBois et al., 1956).

Optimization of EPS Production

Effect of different growth conditions such as variable pH (5, 7, 9), carbon source (glucose, fructose, sucrose, lactose) and nitrogen source (peptone, beef extract, yeast extract, KNO_3) were assessed on EPS production ability of each bacterial isolate. EPS produced by 24 hours old bacterial culture was extracted by cold ethanol precipitation method (Batool et al., 2015b) and estimated by weighing (mg/100 ml).

Screening for Plant Growth Promoting Characteristics

Selected EPS producing bacterial isolates were screened for plant growth promotion characteristics such as phosphate solubilization and hydrogen cyanide (HCN) production. Pikovskaya's agar medium was used for analyzing the bacterial phosphate solubilization ability (Pikovskaya, 1948). Method of Kumari et al., (2018) was applied to determine the Hydrogen Cyanide (HCN) production by bacterial isolates.

Plant Microbe Interaction (PMI) Studies

In order to determine the inoculation impact of these isolates on the growth promotion of *Zea mays*, seeds of *Zea mays* (variety-MMRI yellow) were bought from Punjab Seed Centre, Lahore, Pakistan. Pots were filled with autoclaved sandy

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clay loam textured soil with 0.60 % organic matter content and pH 7.2. Surface sterilization of healthy *Zea mays* seeds was carried out by dipping the seeds in $HgCl_2$ solution (0.1 %) for three to five minutes followed by several washing with autoclaved distilled water to remove the traces of $HgCl_2$. For the preparation of bacterial suspensions, selected strains were cultured in L-broth for 24 hours at 37 ˚C and 150 rpm. Cultures were centrifuged and pellet was re-suspended in autoclaved distilled water. In order to equalize the number of bacterial cells in each suspension, cell density (10^7–10^8 Colony Forming Unit (CFU) per ml^{-1}) was maintained by keeping the optical density of cultures at 0.5 (600 nm). The sterilized seeds were soaked in respective bacterial suspension for 15-20 minutes. Some seeds were dipped in autoclaved distilled water for the similar time period to serve as control. Aseptically, seeds were transferred to respective labeled pots and watered daily. Pots were placed in random design in green house with 12 hours' photoperiod of sunlight. Percentage germination of seeds was observed after three days. After germination, Hoagland's solution (10 ml) was supplemented to provide nutrients to germinated seedlings. General growth of the seedlings was noticed daily and was harvested after six weeks. Different growth parameters such as seedling length, root length, shoot length, number of roots and number of leaves were noted (Batool et al., 2015 a).

Biochemical Analysis

Inoculated and un-inoculated *Zea mays* (variety-MMRI yellow) seedlings were analyzed for auxin and soluble protein content. Extraction and estimation of soluble protein content was done by following Lowry (1951). Khan et al., (2016) method was used for the extraction of auxin of *Zea mays* seedlings.

Bacterial Colonization on Plant Roots

For the analysis of bacterial colonization potential on the roots of inoculated plants, the roots were rinsed three times with autoclaved distilled water in order to remove the soil particles and dried with the help of blotting paper. Long segments (about 5 cm each) of roots were separated and kept in autoclaved distilled water in shaking conditions overnight for the removal of loosely associated bacterial cells. For the quantification of bacterial cells in term of CFUs ml^{-1}, the root segments were vortexed and 0.01 ml was spreaded on the LB agar plates (Qurashi and Sabri, 2012). For each strain, 08 roots were used for the collection of data. For the visualization of associated EPS on rhizoplanes, Alcian blue staining was performed. For this purpose, thin segments of about 5 cm in length were detached from different locations of roots and stained with 0.1 % Alcian blue 8 GX (Sigma) dye. The stained root sections were transferred to glass slides with 2-3 drops of sterile water and observed under light microscope (Labomed, Labo America, Inc.) after placing the cover slips at 100X objective with oil immersion (Qurashi and Sabri, 2012).

Phylogenetic Analysis of Bacterial Strains

16S rRNA gene sequences of selected bacterial strains (BE1, BN1, BN3) were determined by dideoxy Sanger sequencing. For the identification of selected bacterial strains, these obtained sequences were compared with known NCBI GenBank sequences by using NCBI BLAST program (https://www.ncbi.nlm.nih.gov/BLAST).

MEGA Ver. 6.0 software and neighbor-joining method were employed for the construction of phylogenetic trees. In order to get the accession numbers, nucleotide sequences were submitted to NCBI GenBank (Batool et al., 2015b).

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Fourier Transform Infrared spectral (FTIR) Analysis

For FTIR analysis, 20 mg dried and lyophilized EPS fraction produced by bacterial strain BN3 was used to attain its functional groups profile by KBr disc method in 500-4000 cm^{-1} wavenumber range (Batool et al., 2015b).

Statistical Analysis

All the experiments were performed in triplicates. The data was statistically analyzed as mean ± standard error in Microsoft excel.

RESULTS

Isolation and Characterization of Bacterial Strains

Total seven bacterial strains namely, BE1, BE2, BE3, BE4, BN1, BN2 and BN3 were isolated from water sample of Tattapani hot water spring, Azad Kashmir, Pakistan. Physiochemical properties of water sample were pH 7.0, temperature 76-85 °C, colorless with soil like muddy smell. All the bacterial strains were good EPS producers as they showed slimy growth on P-media. But, three morphologically different bacterial strains (BE1, BN1, and BN3) were selected for further analysis due to significant slimy appearance. All bacterial colonies were round in shape except BE1 (irregular), slimy in texture, off-white in color, transparent except BE1 (translucent). BE1 and BN3 were Gram-negative rods, non-spore former and motile however, BN1 was Gram-positive rods, spore-former, and non-motile. Selected EPS producing bacterial isolates (BE1, BN1and BN3) were further biochemically characterized (Table 1).

Extraction and Structural Analysis of EPS

Out of seven isolates, three strains showed the highest EPS producing ability BE1 (14 mg EPS/100 ml LB broth medium), BN1 (14 mg EPS/100 ml), BN3 (15 mg EPS/100 ml) as shown in table 2.

Table 1: Biochemical characterization of isolated bacterial strains named as BE1, BN1 and BN3

Bacterial strains	Biochemical tests						
	TSI	MR	VP	Catalase	Oxidase	DNase	Starch hydrolysis
BE1	R/Y/-/-	+	+	+	-	-	-
BN1	R/R/-/-	+	-	+	+	-	-
BN3	Y/Y/+/-	+	-	+	-	-	-

+ (Positive), - (Negative), R/Y/-/- (Red slant/Yellow butt/ No hydrogen gas production/No H_2S gas production), Y/Y/+/- (Yellow slant/Yellow butt/Hydrogen gas production with no H_2S production).

Table 2: Extraction and quantification of EPS produced by isolated bacterial strains

Bacterial strains	EPS (mg / 100 ml)	Carbohydrate content of EPS (mg/ml)
BE_1	14 ±0.91	28.90±0.55
BE_2	11±1.02	53.80±0.42
BE_3	9±0.78	33.28±0.07
BE_4	10±0.83	44.04±0.17
BN_1	14±0.66	70.85±0.18
BN_2	11±1.09	41.09±0.04
BN_3	15±0.75	60.47±0.28

Mean of triplicates ± Standard errors of the mean.

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In general, all bacterial isolates (BE1, BN1 and BN3) selected for this study showed high amount of carbohydrate (28-70 mg/ml) in their EPS (Table 2).

Optimization of EPS Production

High EPS production (2.3-3.0 mg/100 ml) was shown by these isolated strains at all selected pH but optimum EPS production (3-4.5 mg/100 ml) was found at pH 9. All studied carbon and nitrogen sources had positive impact on EPS producing ability of these strains. But, fructose and peptone were revealed as excellent carbon and nitrogen source, respectively for these bacterial isolates for high EPS production in the range of 4.8-6 mg/100 ml (Figure 1).

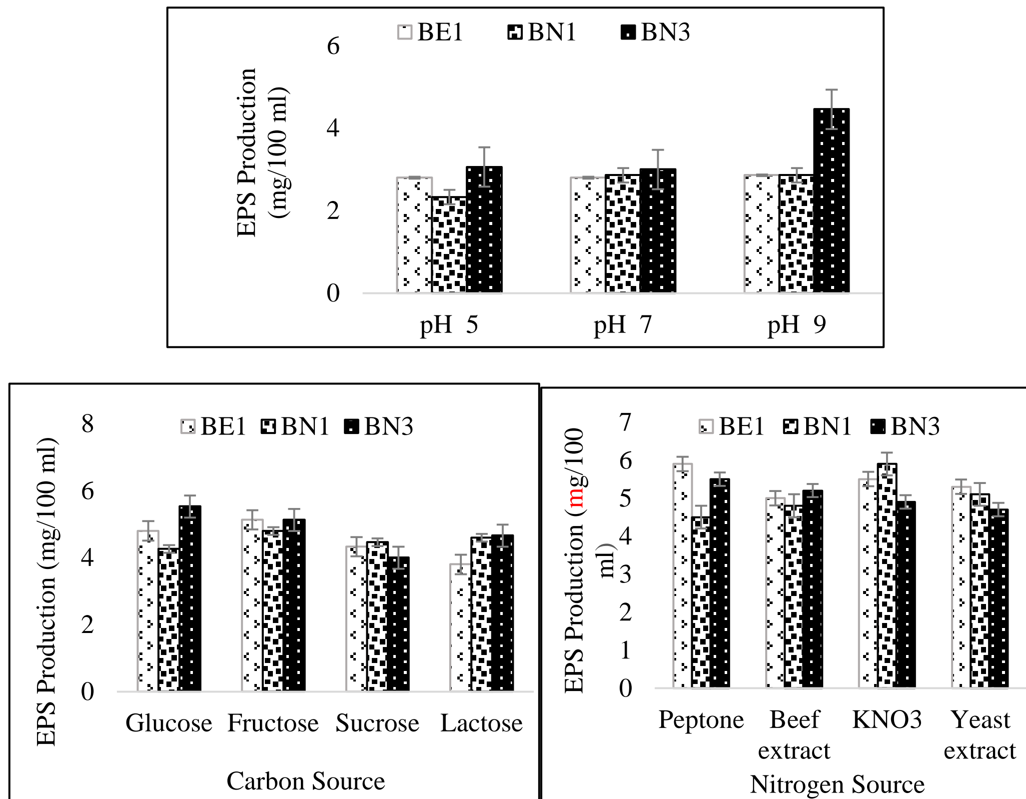

Figure 1: Effect of varying growth parameters (pH, temperature, carbon and nitrogen sources) on EPS production by isolated bacterial strains revealed that pH 9, glucose and peptone were found best for optimum EPS production by selected bacterial isolates. Mean of triplicates and standard errors showed on the top of each bar.

Screening for Plant Growth Promoting Characteristics

Both bacterial strains (BE1 and BN3) showed clear zone on Pikovskaya's agar medium plates that revealed they had phosphate solubilizing ability except strain BN_1. Isolated bacterial strains had high potential of HCN production.EPS production and positive results for growth promoting characteristics indicated that these strains could be further tested for plant growth promotion experiment.

Plant Microbe Interaction (PMI) Studies

In plant microbe interaction experiment, inoculation of these strains showed an increase in all the growth parameters of *Zea mays* (variety-MMRI yellow) seedlings such as percentage germination (27-38 %), seedling length (10-14 %), shoot length (27-35 %), and number of roots (12-25 %) than control (Figure 2). But, inoculation of BN3 showed 38 % increment in seed germination whereas BE1 and BN1 caused

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maximum increase (14 %) in seedling length. Inoculation of BE1 showed significant improvement (35 %) in the shoot length than that of control (Table 3).

Biochemical Analysis

Inoculation of selected bacterial isolates resulted in significantly high soluble protein and auxin content of *Zea mays* seedlings when compared to control. Inoculated seedlings showed 285-514 % rise in auxin content and 43-68 % increase in soluble protein content as compared to non-inoculated treatment (Figure 3). Inoculation of BN3 caused maximum augmentation in soluble protein (1681 mg/g) as well as auxin content (43 mg/g) than that of control.

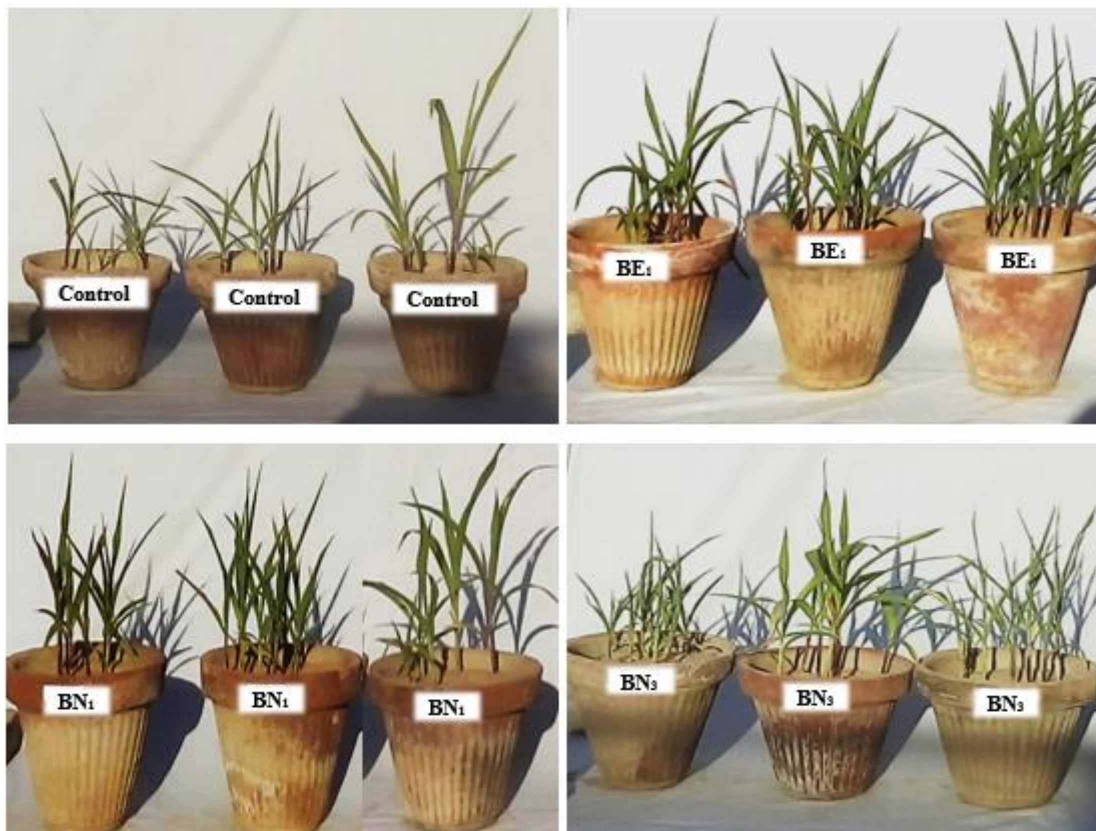

Figure 2: Inoculation effect of isolated EPS producing bacterial strains (BE_1, BN_1 and BN_3) on *Zea mays* seedlings displayed increase in seed germination and studied growth parameters as compared to control.

Table 3: Effect of bacterial inoculation on the growth parameters of *Zea mays* (variety-MMRI yellow) revealed augmentation in all studied parameters.

Bacterial strains	Germination (%)	Seedling Length (cm)	Shoot Length (cm)	Number of roots
Control	60±1.00	33.95±4.58	17.30±3.31	8±0.87
BE_1	76.6±0.88	38.83±2.51	23.37±1.46	9±0.57
BN_1	61±3.00	38.22±1.69	22.51±1.23	10±0.77
BN_3	83.3±0.66	37.48±3.11	22.05±2.05	8±0.54

Mean of triplicates ± Standard errors of the mean.

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Bacterial Colonization on Plant Roots

CFU ml^{-1} is one of the best way to quantify the bacterial growth on plant roots. Generally, this investigation showed that all bacterial isolates (BE1, BN1, BN3) had excellent ability to colonize on the roots with 3.2 to 7.9×10^2 CFU/ml as compared to control (0.5×10^2 CFU/ml). But, strain BN3 exhibited more colonization potential with 7.9×10^2 CFU/ml than that of other two bacterial strains (Table 4).

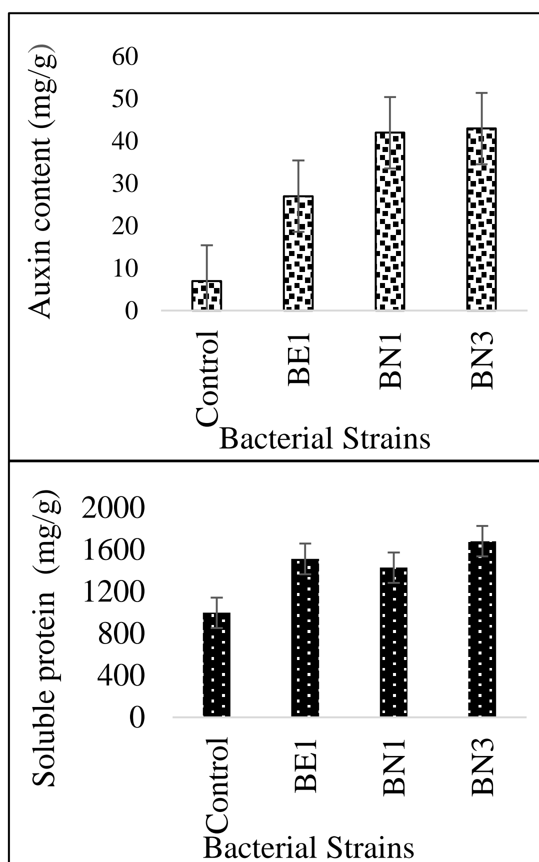

Figure 3: Biochemical analysis of inoculated *Zea mays* seedlings exhibited maximum rise in auxin and soluble protein content as compared to control. Mean of triplicates along with standard error showed at the top of each bar.

Light microscopy in combination with 0.1 % Alcian staining was used to visualize the colonization on *Zea mays* roots by EPS producing bacterial isolates. EPS matrix is stained by Alcian blue stain. Upon microscopic study, more blue color was observed in case of inoculated roots indicating strong biofilm formation capability by these bacterial isolates on roots as compared to un-inoculated root (Figure 4).

Table 4: Determination of CFU ml^{-1} of biofilm formation by isolated bacterial strains on *Zea mays* (variety-MMRI yellow) plant roots.

Bacterial strains	CFU/ml
Control	0.5×10^2
BE_1	7.9×10^2
BN_1	4.1×10^2
BN_3	5.2×10^2

Phylogenetic Analysis of Bacterial Strains

For molecular identification, the nucleotide sequences of bacterial isolates BE1, BN1 and BN3 were obtained from 16S rRNA sequencing. Upon BLAST analysis, it was found that bacterial isolates BE1, BN1, BN3 exhibited 99-100 % similarity with *Ochrobactrum intermedium, Bacillus pumilus* and *Enterobacter cloacae,* respectively. These sequences were deposited in NCBI GenBank under these accession numbers BE1 (MF471478), BN1 (MF471479) and BN3 (MF471480).

Fourier Transform Infrared (FTIR) Spectral Analysis

FTIR spectroscopy is widely applied to find out the different functional groups present on EPS surface. Twelve different peaks were observed in FTIR spectra of EPS of strain BN3 *Enterobacter cloacae* in various absorption ranges Absorption peaks at 685, 759, 821 and 941 cm^{-1} indicated the presence of alkyl halides and aromatic compounds. Absorption peaks at 1023, 1170 cm^{-1} region were indicative of C-O extension of COOH and cyclic structures of carbohydrates. Absorption peaks at 1339, 1394, 1545, and 1603 cm^{-1} wavenumber were particular for amide I and amide II in proteins.

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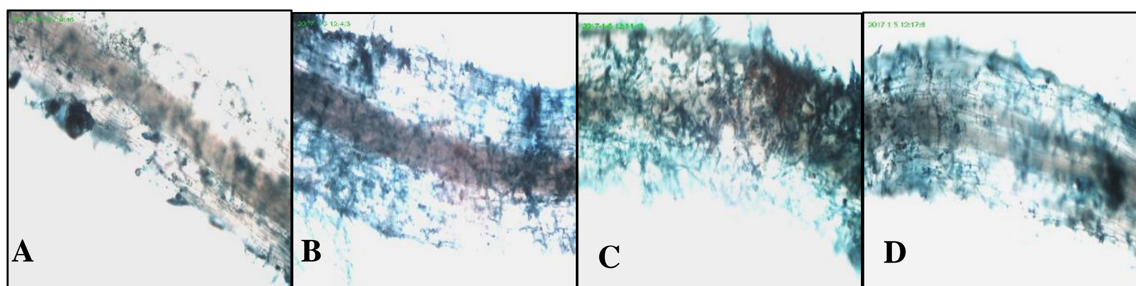

Figure 4: Light microscopy of *Zea mays* roots for analyzing bacterial colonization ability displayed by blue color which showed attachment of isolated EPS producing bacterial strains (B) BE1, (C) BN1, (D) BN3 to inoculated *Zea mays* seedling roots as compared to (A) control.

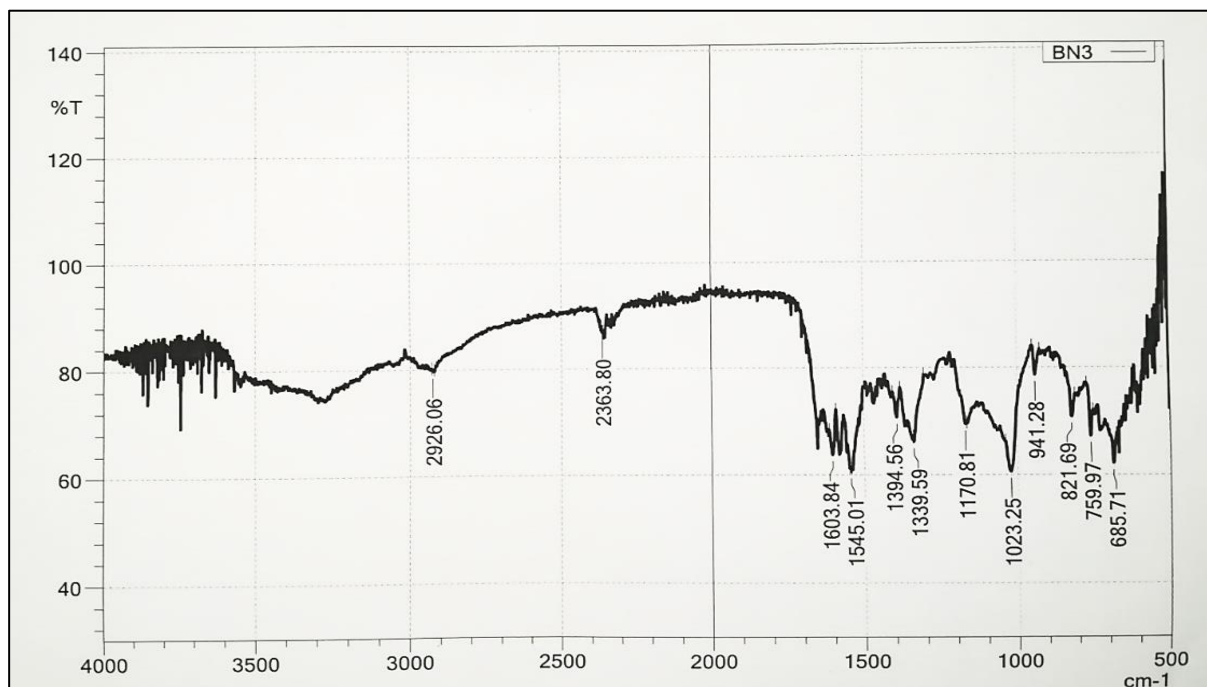

Figure 5: FTIR spectrum of EPS produced by bacterial strain BN3 *Enterobacter cloacae* showed the complex nature of EPS because of the existence of amines, amides, carboxylic acids, lipids and aromatic compounds.

Peak at 2363 cm^{-1} region was because of amines. Peaks in the range of 2926 cm^{-1} wavenumber depicted the presence of symmetrical and asymmetrical vibrations of –CH- in lipids. Three most prominent absorption peaks were appeared in the range of 650-1000 cm^{-1}, 1000-1300 cm^{-1} and 1400-1420 cm^{-1} indicated the presence of alkyl halides, aromatic compounds, carboxylic acids, amines and lipids respectively (Figure 5). Hence, this analysis depicted a variety of functional groups present in the EPS produced by strain BN3 *Enterobacter cloacae*.

DISCUSSION

In the current study, EPS producing strains BE_1 (*Octrobactrum intermedium*), BN_1 (*Bacillus pumilus*), BN_3 (*Enterobacter cloacae*) from Tattapani hot water spring, were screened for plant growth promotion and EPS production potential. Tattapani hot springs are situated on the right bank of the river Poonch (Zahoor et al., 2016). In fact, bacterial isolates of hot springs can show improvement in plant growth due to synthesis of secondary metabolite such as EPS. Hot marine shallow vents were reported as home for the isolation of several high EPS producer such as, *Geobacillus thermodenitrificans, Bacillus*

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licheniformis and *Bacillus thermoantarcticus* (Nicolaus et al., 2002). Many gram-negative EPS producing bacteria such as *Enterobacter, Vibrio, Pseudomonas, Acinetobacter, Alteromonas,* and *E.coli* were also isolated from the extreme environment (Mishra and Jha, 2013). Increase in the yield of EPS under extreme environments is one of the bacterial adaptation to deal with the stress conditions (Dogan et al., 2011). Different bacteria produce variable quantities of EPS such as *Ochrobactrum intermedium* (7 mg/L) (Batool et al., 2015b), *Enterobacter* sp. (>7 g/L), *Enterobacter cloacae* (28 mg/L) (Torres et al. 2012), and *Bacillus subtilis* (1-2.5 mg/100 ml) (Vijayabaskar et al. 2011). Current study described the high amount of EPS production (14-15 mg/100 ml) by selected bacterial strains.

Environmental conditions (pH, temperature) has a great impact on the quantity and quality of EPS produced by bacteria (Osemwegie et al., 2020). Many microorganisms produce high amount of EPS at neutral pH (Morin, 1998). But, there is a variation in optimum environmental conditions for the growth and EPS production by a same bacterium. Usually optimum environmental conditions for EPS production are different from the growth conditions. *Bacillus* sp. have ability to produce high amount of EPS at alkaline pH (8-9) (Achal et al., 2010). Likewise, our bacterial isolates produced high amount of EPS at pH 9 and 28°C. High EPS production also depends on different nutritional conditions such as various types of carbon and nitrogen sources (Morin, 1998 ; Premnath et al., 2021). The carbon and nitrogen source has been supposed to act as substrates in the production of exopolysaccharides (Sutherland, 1994). Fructose revealed to be the best carbon source for high EPS production by bacterial isolates of this study which coincides with the findings of Panosyan et al., (2018). *Bacillus thuringiensis* produced an appreciable quantity of EPS in the presence of peptone as compared to other nitrogen sources such as beef extract, ammonium salts, etc. (Sengupta et al., 2018). Peptone is a mixture of various peptides which provides protein source for EPS production and microbial growth. Similarly, bacterial isolates of current study produced substantial quantity of EPS when the peptone was supplied as a nitrogen source.

Plant growth promoting characteristics are usually found in EPS producing bacteria so, these strains have a strong ability to improve the crop production and plant phosphate solubilization (Etesami and Maheshwari, 2018). Many microbes are also the producer of volatile compounds including HCN that play an important role either in growth promotion or as inhibiting agents (Kai et al., 2009). Likewise, strong phosphate solubilization and HCN production ability was found in our EPS producing bacterial isolates. Thus, these bacterial isolates can be used for growth improvement of crops. In order to enhance the plant growth, EPS producing bacteria exhibited interaction to antimicrobial structures of fungus that influence the movement of bacteria in the rhizosphere (Dubeikovsky et al., 1993). This study has described that *Zea mays* seedlings inoculated with BE1 *Ochrobactrum intermedium*, BN1 *Bacillus pumilus* and BN3 *Enterobacter cloacae* showed maximum increment in all growth parameters due to the strong support of bacterial EPS. *Ochrobactrum intermedium* strain has previously reported to augment the growth and yield of economically important cash crops (Batool et al., 2015a). Plant growth promoting ability by *B. pumilus,* isolated from the *Alnus glutinosa* rhizosphere was proposed by a report (Gutiérrez-Mañero et al., 2001). *Enterobacter cloacae* has reported for its efficient ability of plant growth promotion of alfalfa, citrus, maize and soyabean (Khalifa et al., 2016).

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EPS producing bacteria considerably enhance bacterial colonization to the plant roots due to involvement of the exopolysaccharides (Batool and Hasnain, 2005). Bacterial EPS interaction with roots of inoculated seedling produce stress responses to increase the plant growth in extreme environment (Bramhachari et al. 2018). Current study revealed that EPS produced by *Ochrobactrum intermedium* (Batool et al., 2015a), *Bacillus pumilus* and *Enterobacter cloacae* have an excellent potential for root colonization and subsequently, improve the seedlings growth (Leite et al., 2013).

The plant growth promoting bacteria can mostly yield at least small amount of auxin, if they promote growth of plant roots (Dubeikovsky et al., 1993). Auxin is a plant growth regulating phytohormone responsible for controlling the cell division and root elongation of plant (Kravchenko et al., 2004). Auxin producing bacteria can promote the growth of plant leading to the improvement of crop yield (Khalifa et al., 2016). The thermophilic condition could enhance the soluble protein content of bacteria. Bacterial inoculation also cause enhancement in protein content of plants and promotes plant growth in extreme condition by signifying plant metabolism (Naseem et al., 2018). Inoculated *Zea mays* seedlings showed maximum augmentation in soluble protein as well as auxin content compared to control.

FTIR spectroscopic technique is a quick and profound method for the characterization of the microbial EPS (Huffman et al., 2003). It is used to provide information about molecular compositions of different cell parts such as cell wall, cell membrane and bacterial storage and secretory products (Sheng et al., 2006). Many studies have been majorly concentrated on determining the functional groups of EPS (Sheng et al., 2005). FTIR spectral analysis of EPS of strain BN3 *Enterobacter cloacae* was done in 500-4500 cm^{-1} wavenumber range that stated the presence of different functional groups showing its complex structure. This region is suitable for holding characteristic peaks and microorganism's characterization. Occurrence of charged groups determined EPS production in bacteria and biological functioning of EPS. Various absorption peaks were appeared on FTIR spectra and each peak illustrated the presence of different functional groups. The absorption peaks in the range of 2860-2930 cm^{-1} depicted the existence of symmetrical and asymmetrical CH- vibrations in lipids and peaks at 2400-2300 cm^{-1} because of amines. Absorption peaks near 1690-1400 cm^{-1} were particular for Amide I and Amide II in proteins (Schmitt & Flemming, 1998). The absorption peaks in the range of 1300-1000 cm^{-1} were indicative of C-O extension of COOH in carbohydrates. Absorption peaks in 1100-1000 cm^{-1} range were specified for glycoside groups which is specifically present in cyclic structures of carbohydrates (Rodríguez et al., 2018). fall Absorption peaks in the range of 650-1000 cm^{-1} presented alkyl halides and aromatic compounds (Batool et al., 2015b).

CONCLUSION

This study revealed that isolated bacterial strains *Ochrobactrum intermedium, Bacillus pumilus* and *Enterobacter cloacae* showed significant plant growth promoting capability along with high EPS production which also helps in bacterial as well as plant survival. In this crucial time of population explosion, need of the hour is to develop certain strategies by using microbes or their metabolites to enhance the growth of such plants which are used as a staple crop to avoid the shortage of food in near future.

AUTHORS CONTRIBUTION

Rida Batool and Nazia Jamil designed the research. Noor e Saba Naz conducted the experimental work, analyzed the data, and interpreted the results. All the authors contributed in

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writing, reading and approving the final manuscript.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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