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Efficacy of liquid formulation of versatile rhizobacteria isolated from soils of the North-Western Himalayas on *Solanum lycopersicum*

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In the present study, a total of 53 indigenous diazotrophs and phosphate solubilizing bacteria (PSB) were isolated from the rhizosphere soils of five different crops grown in Palampur, (Himachal Pradesh, India). Out of these, isolates WT-A1 and WT-A2 exhibited significantly higher nitrogenase activity as compared to the reference strain *Azotobacter chroococum* and PT-P2 solubilized more phosphate as compared to the reference strain *Pseudomonas striata*. WT-A2 and PT-P2 showed multiple plant growth promoting traits including indole acetic acid, ammonia and siderophore production. On the basis of morphological, biochemical and 16S rRNA gene sequence analysis, WT-A2 and PT-P2 were identified as *Stenotrophomonas* sp. and *Burkholderia cepacia*, respectively. The liquid formulation of efficient diazotroph and PSB was prepared in sterilized liquid manure, *matka khaad* with the addition of trehalose which maintained their viable counts for one year. Evaluation of growth performance of liquid formulation on *Solanum lycopersicum* (Avtar 7711) *in vivo* concluded that seed bacterization (SB) and seed bacterization, seedling dipping, soil application and foliar spray (SB+SD+SA+FS) exhibited better results in terms of seed germination, fruit quality, dehydrogenase activity and available nitrogen, phosphorus and potassium of soil as compared to the other treatments.

Keywords: *Burkholderia cepacia*, Liquid formulation, *Solanum lycopersicum*, 16S rRNA Gene Sequencing, *Stenotrophomonas* sp.

IPC Int. Cl.[8]: A01, A01C 1/00, C12M, C12N, A01B, A01G 31/00, C05

The rhizosphere is a dynamic entity of the soil habitat, a hub of biological, chemical and physical activities surrounding the living infrastructure of plant roots. The bacteria in rhizosphere zone promote plant growth directly or indirectly and referred as plant growth promoting rhizobacteria, where the direct impact is due to several factors like availability of usable form of nutrients, growth hormones, siderophore production, etc., and indirect impact is due to antifungal metabolites to provide systemic resistance in the plants. Many genera like *Bacillus*, *Burkholderia*, *Herbaspirillum*, *Ochrobactrum*, *Pseudomonas*, *Ralstonia*, *Staphylococcus* and *Stenotrophomonas* are associated with roots of many plants and act as PGPR[1]. Nowadays, researchers have focused on many versatile genera such as *Stenotrophomonas*, *Burkholderia,* etc*.,* for their potential as plant growth promoting rhizobacteria. *Stenotrophomonas* spp. occur ubiquitously in nature but species like *S. maltophilia* and *S. rhizophila* have been frequently isolated from the rhizosphere or the internal plant tissues[2]. Many reports are available in the literature which shows that members of this genus play an important ecological role in the nitrogen and sulphur cycles[3,4]. From last two decades, there is also an increasing concern in *B. cepacia* for its prospective as a phosphate solubilizer and plant growth promoting agent, representing a valid alternative to a variety of chemical fertilizers and pesticides[5]. The members of these genera show plant growth promoting attributes and biocontrol activities against plant pathogens and, therefore utilized for the development of bioinoculants.

Low cost bioinoculant technology has now become a main concern of research in developing countries where, a major role of inoculant formulation is to

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provide a more suitable microenvironment to prevent the rapid decline of introduced bacteria in the soil. In recent years, many of the formulations of the liquid based inoculants are introduced which are less expensive, enhance shelf life of PGPR and tolerate adverse environmental conditions in a better way in comparison to solid carrier based preparations[6,7]. So, liquid formulation with good field performance characteristics that uses low cost materials and are easily attainable by small producers could overcome many problems associated with processing solid carriers. Many researchers have prepared liquid formulation of sludge[8], whey[9] and cow dung, etc.[10] and tested their efficiency on different crops.

Himachal Pradesh, a model state for successful developmental initiatives, is a rich repository of novel kinds of microorganisms possessing useful characteristics. The state besides its location advantage, has unlimited potentials due to the diverse agro-climatic conditions. It is well documented that strains of microbial inoculants identified as potential biofertilizers, do not perform as per expectations when applied in the different geographical locations. So, the present study was aimed to develop a cost effective liquid formulation of sterilized nutrient rich liquid manure, *matka khaad*[11] containing indigenous diazotroph *Stenotrophomonas* sp. and PSB *Burkholderia cepacia* isolated from the rhizosphere soils of North-Western Himalayas and its evaluation for growth performance on *Solanum lycopersicum,* which is the major off-season cash crop of mid-hills of Himachal Pradesh grown under protected cultivation (www.yspuniversity.ac.in/kvk-kandaghat/kgt-achi.htm).

Methodology

Collection of soil samples and isolation of diazotrophs and PSB

Rhizosphere soil samples from 10-15 cm depth of five crops including *Triticum aestivum* (wheat), *Zea mays* (maize), *Solanum tuberosum* (potato) and two medicinal plants *Aloe barbadensis* (*Aloe vera*) and *Bacopa monnieri* (*brahmi*) grown in CSKHPKV, Palampur, 32º6'N, 76º18'E and 1220 m msl, India were collected. Diazotrophs and PSB were isolated using Jensen's and Pikovskaya's media (HiMedia, India), respectively by serial dilution plate technique in triplicates. The reference strains of *Azotobacter chroococum* (MTCC 446) and *Pseudomonas striata* (MTCC 1259) were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. The cultures were preserved in 30 % (v/v) glycerol at -80 °C.

Acetylene reduction assay (ARA)

Nitrogen fixation was determined in nitrogen free medium (Jensen's medium) by the acetylene reduction assay[12]. Ethylene production was measured using a Hewlett Packard gas chromatograph (Model HP Series 5890, USA) fitted with flame ionization detector and a Porapak-N column. The GC oven temperature was adjusted from 72 to 200 °C at 20 °C/min with 2 min hold at 72 °C and 5 min hold at 200 °C. After completion of the ARA, the protein concentration in the cells was determined by the method of Lowry *et al*.[13]. The isolates showed more than 150 nmole C_2H_4 h^{-1} mg^{-1} protein activity were stocked for further study.

Quantitative assay for phosphate solubilizing activity

The quantitative estimation of solubilized phosphate by bacterial isolates was done in NBRIP (National Botanical Research Institute's Phosphate growth medium) broth by the vanadomolybdophosphoric yellow color method[14].

Characterization of multiple plant growth promoting attributes *in vitro*

Indole-3-acetic acid (IAA) production was done by the method of Brick *et al*. (1991)[15]. Siderophore production was detected by the method of Schwyn & Neilands (1987)[16] using agar plates containing the dye chromeazurol sulfate (Merck, India). For ammonia production, the organisms were grown in peptone water at 28±0.1 °C for 4 days and 1 mL of Nessler's reagent was added. Production of ammonia was depicted by development of faint yellow to dark brown color[17].

Phenotypic and genotypic characterization of bacterial isolates

The phenotypic characterization of the bacterial isolates was done on the basis of colony morphology, microscopic observations, and biochemical tests[18]. Total genomic DNA of each isolate was extracted using commercial DNA isolation kit (Real Biogenomics) by following the instruction manual of the manufacturer. The amount of DNA was quantified by recording the absorbance at 260 nm wavelength using UV/VIS spectrophotometer (Bio Rad, SmartSpec 3000). For genomic characterization, 16S ribosomal gene was amplified by using the universal primers i.e. 27F (5' AGAGTTTGATCATGGCTCAG 3') and 1487R (5' TACCTTGTTACGACTTCACC 3')[19]. PCR products of 16S rRNA gene of efficient bacterial isolates were sent for custom sequencing used for the amplification of 16S rRNA gene (Ocimum

Biosolutions, Pvt. Ltd.). These sequences were BLAST (basic local alignment search tool) searched against the sequences of 16S rRNA of bacterial isolates available in the Genbank Nucleotide Database. The sequences were aligned by using Clustal W software and phylogenetic analysis was performed with neighbor-joining method using program in Molecular Evolutionary Genetics Analysis (MEGA) version 6.0[20].

Gene submission and culture deposition

The nucleotide sequences of efficient PGPR isolates WT-A2 and PT-P2 were deposited in GenBank. Further, these isolates were deposited with the National Bureau of Agriculturally Important Microorganisms (Indian Council of Agricultural Research) Kusmaur, Mau Nath Bhanjan, Uttar Pradesh, India.

Preparation of natural medium (*matka khaad*)

Matka khaad, a liquid manure was prepared by mixing cow dung, cow urine, water and jaggery (1:1:1:0.25) in an earthen pot. The mixture was incubated for 10 days at room temperature and used for further studies[11].

Nutrient profile analysis of *matka khaad*

Matka khaad was sterilized twice by autoclaving at 121 ºC with 15 psi for 20 min so as to eradicate its native microflora. Three batches of *matka khaad* were analyzed for their nutrient composition. Determination of organic carbon and total nitrogen in *matka khaad* was done by the digestion and Kjeldahl's methods, repectively[21]. Phosphorous content was measured by Olsen's method[22] and potassium content was determined by flame photometry method[23]. Iron, calcium, manganese, zinc and magnesium contents were measured by DTPA (Diethylene Triamine Penta– acetic acid) method[24].

Preparation of liquid formulation of efficient strains

Efficient PGPR were inoculated in nutrient broth and incubated at 28±0.1 ºC for 48 h with shaking rate (120 rpm). The cells were harvested by centrifugation (10000 rpm) in multifuge XIR (Thermoscientific, Germany) for 10 min at 4 °C and washed twice with 100 mM potassium phosphate buffer solution (*p*H 7) so as to achieve the final concentration of 10^9 CFU/mL. 1 L *matka khaad* was autoclaved twice before use and checked for contamination by serial dilution plate count method on nutrient agar plates. The mother culture was mixed with autoclaved *matka khaad* along with trehalose (10 mM) at the rate of 1 per cent (% w/v) and incubated at room temperature. Further, enumeration of viable cell population was done and the pure cultures were ensured by molecular characterization based on 16S rRNA gene sequencing.

Shelf-life study of efficient PGPR in *matka khaad*

The viable counts of efficient PGPR isolates in liquid carrier were enumerated in terms of Log CFU/mL at monthly intervals up to decline phase by serial dilution technique. The data was recorded after one month interval for 12 months.

***In vivo* growth performance evaluation of PGPR on cash crop**

The growth performance evaluation of efficient PGPR on *Solanum lycopersicum* (Tomato) variety Avtar 7711 using randomized block design (RBD) was carried out in three replications at Vegetable farm, CSKHPKV, Palampur. Different treatments used on cash crop to evaluate growth performance of PGPR *in vivo* were: Treatment 1- seed bacterization (SB), Treatment 2- seedling dipping (SD), Treatment 3- soil application (SA), Treatment 4- foliar spray (FS), Treatment 5- seed bacterization and seedling dipping (SB+SD), Treatment 6- seed bacterization and soil application (SB+SA), Treatment 7- soil application and foliar spray (SA+FS), Treatment 8- soil application and seedling dipping (SA+SD), Treatment 9- seed bacterization and seedling dipping (SB+SD), Treatment 10- seed bacterization, seedling dipping, soil application and foliar spray (SB+SD+SA+FS), Treatment 11- control (basal dose) (BD) and Treatment 12- biofertilizers only (BO). Basal dose i.e. 12:32:16 (375 Kg/ha), MOP (29 Kg/ha) and urea (229 Kg/ha) was used along with each treatment.

Seed bacterization was done by dipping 100 g of tomato seeds in 200 mL (1×10^9 CFU/mL) liquid formulation for 30 min and dried over night at room temperature. 0.5 g seedlings were dipped in 300 mL (1×10^9 CFU/mL) liquid formulation for 30 min and transplanted in soil under protected conditions. 2 mL of the liquid formulation (10^9 CFU/mL) per plant was applied and after 30 days of planting, foliar spray was done by diluting freshly prepared liquid formulation (10^9 CFU/mL) with water (1:1). The data regarding growth and quality parameters of crop was recorded during two years with two annual crops of *Solanum lycopersicum*, while analysis of soil was done after completion of field trials.

Estimation of growth and quality parameters of crop

Seed germination, flowering and fruiting were recorded in different treatments and compared to the control. Average yield was calculated by dividing total weight of tomatoes harvested from each treatment by number of replications and determined in terms of q/ha. Average number of fruits was calculated by dividing total number of tomato harvested from each treatment by number of replications. Total soluble solids (TSS), ascorbic acid and lycopene contents were estimated by the methods of AOAC[25]. Pericarp thickness (mm) of tomato was measured from the equatorial section of the fruit with the help of scale. Fruit firmness was determined by penetrometer (Handpi GY series fruit penetrometer) and recorded in terms of units lbs (pounds). Shelf life of tomato fruits was studied by keeping them at room temperature (15±2 °C) and at refrigerator (4 ºC).

Estimation of soil parameters

Soil samples were collected in three replicates from all the treatments. Colorimetric estimation of dehydrogenase activity was determined by the method of Kumar *et al*. (2013)[26]. Determination of organic carbon, nitrogen, phosphorous and potassium in soil were done by the methods discussed above for *matka khaad* nutrients analysis.

Statistical analysis

Results of the measurements were subjected to analysis of variance (ANOVA) and significance at the 5 % level was tested by critical difference (CD) using Cpcsd (1.0), a programs package for the analysis of commonly used experimental designs. All treatments were in triplicate.

Results

Isolation of diazotrophs and PSB and evaluation for multiple plant growth promoting attributes

In the present study, a total of 53 diazotrophs and PSB were isolated from rhizosphere soils of five different crops. Among those, 28 isolates were obtained on Jensen's medium and 25 were screened on Pikovskaya's media. Two isolates including WT-A1 and WT-A2 showed significantly higher nitrogenase activity as compared to the reference strain of *A. chroococum* (Table 1). Among phosphate solubilizers, PT-P2 solubilized more phosphate as compared to the standard strain *P. striata*. Among all the isolates, 33 showed multiple plant growth promoting attributes such as IAA, siderophore and ammonia production *in vitro*. The efficient diazotrophs WT-A2 and phosphate solubilizer PT-P2 showed significantly higher IAA production than their reference strains *A. chroococcum* and *P. striata*, respectively. The efficient isolates also showed siderophore and ammonia production. Besides that, WT-A1 showed ammonia production, produced IAA but found negative for siderophore production.

Biochemical characterization and phylogenetic relatedness of efficient isolates

Gram negative rods, non-spore former and motile isolates WT-A2 and PT-P2 were positive for catalase, oxidase production, while negative for indole, methyl-red, urease and H_2S production. An attempt was made to identify and decipher the phylogenetic affiliation of efficient bacterial isolates using 16S rRNA gene sequencing. Native diazotrophic isolate WT-A2 revealed maximum homology (99 %) with *Stenotrophomonas maltophilia* (DQ257429) which belongs to γ- proteobacteria, whereas, isolate PT-P2 showed nearly identical sequences (100 %) with *Burkholderia cepacia* (GQ383907) belongs to class β-Proteobacteria (Fig. 1). The accession numbers for 16S rRNA gene sequences of *Stenotrophomonas* sp. (GU371215) and *Burkholderia cepacia* (GU371220) were provided by NCBI, while NBAIM provided accession numbers NAIMCC-B-00877 and NAIMCC-B-00882 to *Stenotrophomonas* sp. and *B. cepacia*, respectively.

Preparation of *matka khaad* and its nutrients analysis

Our studies demonstrated that after 10 days of incubation at room temperature, *matka khaad* was collected as brown colored liquid organic manure. After nutrient analysis it was noticed that *matka khaad* was rich in carbon (1.8 %), nitrogen (2.1 %),

Table 1 — Multiple plant growth promoting attributes of efficient PGPR

Activities*	WT-A1	WT-A2	PT-P2	*A. chroococum*	*P. striata*	CD (5%)
Nitrogenase activity nmol C_2H_4 released h^{-1} mg^{-1} protein	441.58^b	451.45^a	-	372.85^c	-	1.74
Indole-3-acetic acid (µg/mL)	8.65^c	17.45^a	16.68^a	15.51^b	14.40^b	3.23
Phosphate solubilization (µg/mL)	-	-	685.67^a	-	560.61^b	9.24

*Each value represents mean of three replicates. In the same column, significant differences according to CD at $p \leq 0.05$ levels are indicated by different letters. Same letters represent that their values are at par where a, b and c are the significantly higher values.

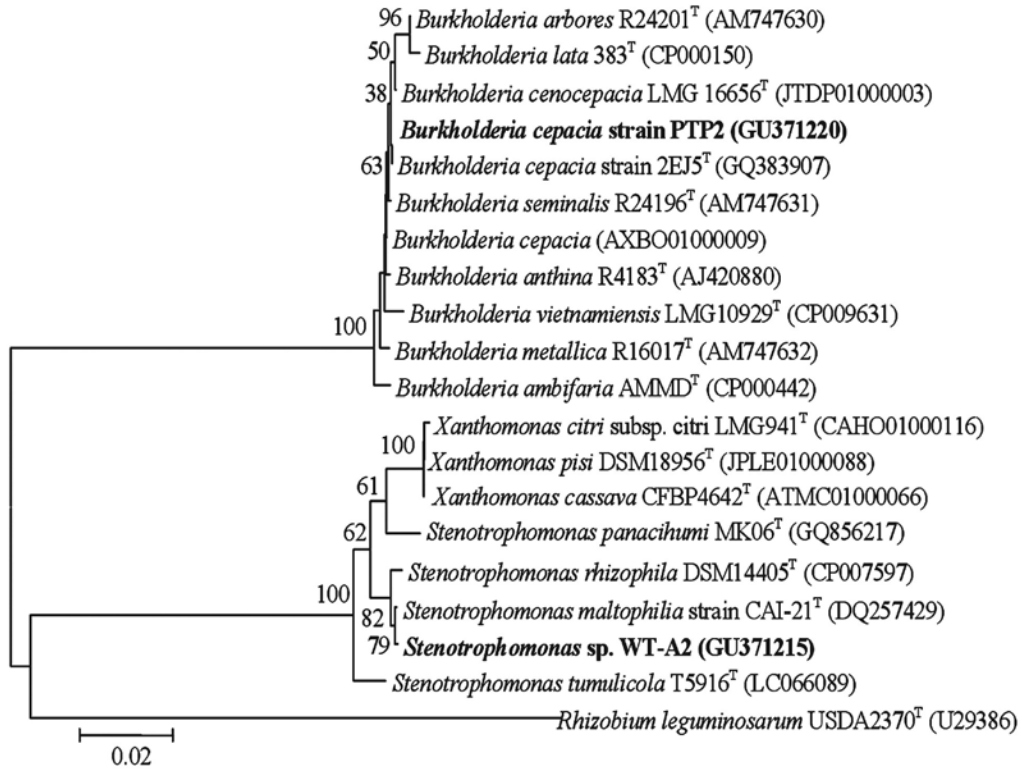

Fig. 1 — Phylogenetic tree constructed by Neighbor-Joining method derived from analysis of the 16S rRNA gene sequence of native isolates and related sequences obtained from NCBI. Scale bar 0.02 substitutions per nucleotide position.

iron (0.26 ppm), calcium (0.43 ppm), manganese (0.03 ppm), zinc (0.021 ppm) and magnesium (1.01 ppm) (Table 2).

One year shelf-life study of efficient PGPR in *matka khaad*

Matka khaad with trehalose as additive maintained the viable counts for *Stenotrophomonas* sp. ranged from (10.98-6.8 Log CFU/mL) and for *Burkholderia cepacia* (10.933-6.77 Log CFU/mL) throughout the year at room temperature (Fig. 2). Data revealed that after 30th day of incubation, the significant increase in the viable counts of *Stenotrophomonas* sp. (10.98 Log CFU/mL) and *B. cepacia* (10.93 Log CFU/mL) was recorded. After 60th day of incubation, a difference in the viable count of *Stenotrophomonas* sp. (10.93 Log CFU/mL) was recorded upto 180th days (10.03 Log CFU/mL) and at 360th day of incubation the count was reduced to 6.8 Log CFU/mL. The viable count of *B. cepacia* was also decreased from 180th days (9.9 Log CFU/mL) to 360th days (6.77 Log CFU/mL) of incubation.

In vivo* studies to evaluate growth performance of liquid formulation on *Solanum lycopersicum

To evaluate the growth performance of efficient PGPR on cash crop under protected conditions,

Table 2 — Nutrient profile of *matka khaad* used in the study

Parameters	Range	Mean Value
1. Carbon (%)	1.6-2.0	1.8
2. Nitrogen (%)	1.6-2.6	2.1
3. Micronutrients (ppm)		
Fe^{2+}	0.22-0.30	0.26
Ca^{2+}	0.40-0.46	0.43
Mn^{2+}	0.026-0.036	0.03
Zn^{2+}	0.017-0.025	0.021
Mg^{2+}	0.81-1.21	1.01

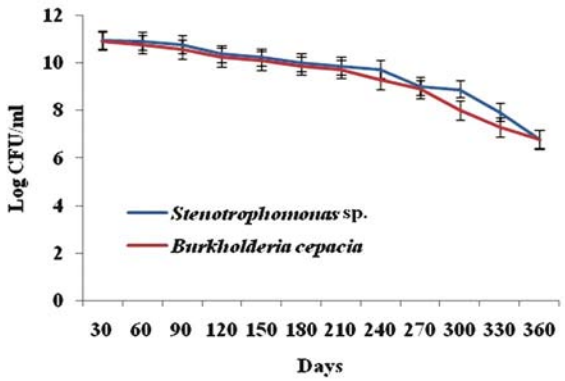

Fig. 2 — One year shelf-life study of efficient PGPR (Log CFU/mL) in *matka khaad* at room temperature. Values represent the average of three replicates. Error bars denote standard error around the mean.

diazotroph and PSB were grown in *matka khaad* amended with trehalose. Data of two years field trials revealed that the seeds inoculated with liquid formulation of PGPR showed earlier germination as compared to the uninoculated seeds. In first year, flowering and fruiting were first recorded in SD treatment, while, in second year it was recorded in SB+SD+SA+FS and FS treatments. During both the years, significantly higher average yields and maximum number of fruits were obtained by SB+SD+SA+FS treatment in comparison to control (BD) (Table 3).

Same table depicts that significantly higher TSS and lycopene contents were also obtained in SB+SD+SA+FS treatment. In case of ascorbic acid, significantly higher content was noticed in SA+FS and SA+SD treatments in both years, respectively. Data clearly shows that liquid formulation also improves the fruit firmness but no significant difference was observed among all the treatments. The composite treatment of SB+SD+SA+FS also contributed in significantly higher shelf life of tomato fruits, i.e., 28 days at room temperature and 52 days in refrigerator, and maximum pericarp thickness as compared to control.

Effect of liquid formulation on soil parameters

Application with SB+SD+SA+FS also contributed in significant increase of soil dehydrogenase activity. Among macronutrients, significant augmentation in nitrogen content was recorded in SB treatment and the reduction in phosphorus and potassium contents were obtained through SB+SD+SA+FS treatment (Table 4). However, in case of organic carbon content, no significant difference was found among all the treatments.

Discussion

Among 53 indigenous isolates, two strains belonging to *Stenotrophomonas* sp.and *Burkholderia cepacia* showing high multiple plant growth promoting activities were used to develop the liquid formulation (Table 1). Although the ability to reduce acetylene is an indirect measure of nitrogen fixation, it is specific for monitoring functional nitrogenase activity, and is indicative of nitrogen fixing potential[27]. Screening of phosphate solubilizers is an important aspect as they are beneficial in solubilizing insoluble complexes of metal cations and clay minerals and making them available to the plants[28]. Phytohormones, siderophore and ammonia production also play a critical role in growth and development of plants. Phenotypic characterization of the efficient isolates WT-A2 and PT-P2 indicated broad similarity to the genus *Azotobacter* and *Pseudomonas*, respectively. Characterization based on 16S rRNA gene sequencing and the neighbor-joining

Table 3 — Effect of liquid formulation on quality parameters of *Solanum lycopersicum* during two years

Treatments	First year									Second year		
	Average yield (q/ha)	Average no. of fruits	Ascorbic acid content (mg/100g)	Average yield (q/ha)	Average no. of fruits	TSS content (ºBrix)	Lycopene content (mg/100g)	Ascorbic acid content (mg/100g)	Fruit firmness (lbs)	Self life (Days)		Pericarp thickness (mm)
										Room temperature	Refrigerator temperature	
T1	150.0^{abc}	272^{ab}	41.9^{ab}	127.5^{cd}	254^{abc}	5.1^{cd}	31.9^{de}	31.2^{bc}	18.6	21^{bc}	29^{bcd}	3.9^{d}
T2	119.0^{f}	199^{bcde}	40.0^{cb}	134.1^{b}	208^{bcde}	4.5^{e}	38.0^{abc}	45.2^{ab}	17.67	19^{bc}	29^{bcd}	5.1^{bc}
T3	128.0^{de}	222^{bcd}	40.0^{cb}	112.0^{e}	218^{bcd}	5.5^{bcd}	29.3^{de}	48.3^{ab}	17.8	21^{bc}	30^{bcd}	4.9^{c}
T4	125.0^{de}	246^{abc}	32.3^{d}	122.0^{d}	247^{abc}	5.0^{cde}	30.0^{de}	37.0^{bc}	15.63	20^{bc}	33^{bc}	4.0^{d}
T5	123.0^{de}	197^{bcde}	40.0^{cb}	112.0^{e}	195^{bcde}	4.9^{cde}	36.3^{abc}	31.2^{bc}	15.67	20^{bc}	32^{bc}	6.3^{a}
T6	144.0^{abc}	258^{ab}	28.6^{e}	132.0^{bc}	282^{ab}	5.5^{bcd}	35.3^{bc}	38.7^{bc}	17.8	19^{bc}	29^{bcd}	5.1^{bc}
T7	127.5^{cde}	207^{bcde}	43.8^{a}	125.0^{bc}	268^{abc}	5.1^{cd}	32.3^{de}	34.4^{bc}	16.6	18^{bc}	29^{bcd}	5.8^{b}
T8	127.5^{cde}	177^{bcde}	32.4^{d}	111.0^{ef}	178^{cd}	6.4^{ab}	39.2^{abc}	39.8^{ab}	17.5	17^{bc}	29^{bcd}	5.0^{bc}
T9	136.0^{bc}	215^{bcd}	34.3^{d}	131.0^{bcd}	212^{bcd}	5.4^{cd}	35.8^{bc}	33.3^{bc}	17.53	21^{bc}	34^{bc}	3.9^{d}
T10	154.0^{ab}	281^{ab}	41.9^{ab}	146.0^{a}	301^{ab}	6.8^{a}	39.8^{abc}	44.1^{ab}	16.0	28^{a}	52^{a}	6.4^{a}
T11	111.0^{h}	152^{cde}	22.6^{f}	104.0^{f}	149^{cde}	5.3^{cd}	31.2^{cde}	27.0^{c}	15.33	16^{c}	25^{d}	3.7^{e}
T12	138.0^{bc}	259^{ab}	34.3^{d}	127.5^{bcd}	266^{abc}	6.0^{b}	35.8^{bc}	44.1^{ab}	18.8	20^{bc}	32^{bc}	5.4^{b}
CD (5%)	(14.63)	(57.94)	(1.49)	(9.82)	(61.48)	(0.52)	(3.75)	(11.54)	(NS)	(4.28)	(5.56)	(0.42)

Value represents the mean of three replicates. In the same column, significant differences according to RBD are indicated by different letters. Same letters represent that their values are statistically at par. *Treatment 1- SB, Treatment 2- SD, Treatment 3- SA, Treatment 4- FS, Treatment 5- SB+SD, Treatment 6- SB+SA, Treatment 7- SA+FS, Treatment 8- SA+SD, Treatment 9- SB+SD, Treatment 10- SB+SD+SA+FS, Treatment 11- control BD and Treatment 12-BO.

Table 4 — Effect of liquid formulation on soil of *Solanum lycopersicum* after two years of field trials

Treatments*	Dehydrogenase activity (μg TPF g^{-1} soil 24 h^{-1})	Available Nitrogen (Kg/ha)	Available Phosphorus (Kg/ha)	Available Potassium (Kg/ha)	Organic Carbon (Kg/ha)
T1	59.0^{ab}	241.0^{a}	24.0^{abc}	584.5^{abc}	10.0
T2	59.0^{ab}	191.0^{b}	20.0^{bc}	451.5^{bcde}	9.3
T3	58.7^{ab}	200.7^{bc}	17.0^{bcd}	425.3^{cde}	8.7
T4	42.3^{cd}	65.0^{h}	21.7^{abc}	482.6^{bcde}	8.7
T5	54.3^{abcd}	151.0^{de}	21.0^{bc}	513.7^{abc}	8.7
T6	59.0^{ab}	149.3^{de}	16.0^{cde}	508.7^{bcd}	8.7
T7	56.0^{abc}	127.0^{f}	21.7^{abc}	528.0^{abc}	8.0
T8	49.3^{abcd}	143.0^{ef}	20.0^{bcd}	449.0^{bcde}	9.3
T9	44.7^{bcd}	134.0^{e}	19.0^{bc}	502.0^{abcd}	8.0
T10	60.0^{ab}	222.0^{bc}	15.5^{cd}	396.5^{de}	9.0
T11	33.7^{cde}	61.0^{h}	27.0^{abc}	596.0^{ab}	7.7
T12	50.7^{abcd}	107.0^{g}	26.0^{abc}	516.7^{abc}	8.3
CD (5%)	(12.53)	(6.10)	(5.35)	(85.82)	(NS)

Value represents the mean of three replicates. In the same column, significant differences according to RBD are indicated by different letters. Same letters represent that their values are statistically at par. *Treatment 1- SB, Treatment 2- SD, Treatment 3- SA, Treatment 4- FS, Treatment 5- SB+SD, Treatment 6- SB+SA, Treatment 7- SA+FS, Treatment 8- SA+SD, Treatment 9- SB+SD, Treatment 10- SB+SD+SA+FS, Treatment 11- control BD and Treatment

phylogenetic tree further confirmed that these isolates phylogenetically related to the *Stenotrophomonas* sp. and *Burkholderia cepacia* (Fig. 1). Therefore, to achieve reliable and accurate identification of bacterial isolates, molecular characterization based on 16S rRNA gene sequencing is an important tool[29].

The present study demonstrated that *matka khaad* was a rich source of carbon, nitrogen and micronutrients which served as a suitable media for adequate growth and maintenance of efficient PGPR (Table 2). As such no information is available on nutritional status of *matka khaad* in the literature, but growth of native isolates is directly attributed to the nutrients present in it. Many cheap and natural substrates such as agro-industrial wastes including molasses[30], whey[9], cow dung extract[10], etc., have been used as carbon and nitrogen sources to increase the population of beneficial microbes. Trehalose amended in *matka khaad* acts as additive and protective agent so maintained the sufficient microbial population upto 180 days. Kalitha *et al*.[31] stated that liquid bioinoculants are special formulations containing not only the desired microorganisms and their nutrients, but also, special cell protectants that improve the longer shelf life of microbes and tolerance to adverse environmental conditions. The liquid formulation developed from *matka khaad* containing efficient PGPR was applied to *Solanum lycopersicum.* Interestingly, no reports regarding the use of *Stenotrophomonas* sp .and *B. cepacia* formulated in liquid carrier were found in literature. Two years data revealed that liquid formulation containing efficient PGPR isolates showed significant effects on growth and biochemical parameters of crop which may be a result of augmented plant growth hormones and continues supply of nitrogen, phosphorus and potassium[32,33] (Table 3). Many workers also have reported the enhancement in growth and yield of lettuce, maize, mung bean and tea by using various PGPR based formulations of *Pseudomonas* sp.[34], *Bacillus* sp.[35], *Enterobacter* sp.[36] and *Viridibacillus* sp.[37]

SB+SD+SA+FS treatment showed a significant increase in dehydrogenase activity which might be due to the soil application of liquid formulation with adequate cell count of diazotroph and phosphate solubilizer (Table 4). Soil bacteria participate in biogeochemical cycles and have been used for crop production for decades. Batra & Manna[38] stated that this activity reflects metabolic ability of the soil and considered to be proportional to the biomass of the microorganisms in soil. Application of PGPR plays a critical role in improving soil health and nutrient uptake of plants through transforming macro-micronutrients of soil either by reducing or oxidizing and thus making them available for the plants[39]. Significant enhancement in nitrogen content obtained through SB+SD+SA+FS treatment could be due to atmospheric nitrogen fixation induced by the diazotroph. The factor that rhizobacteria have capability of altering the insoluble phosphorus compounds to usable forms has facilitate the prospect of inducing microbial solubilization of phosphates in the soil. In our study, significant reduction in phosphorus and potassium contents are correlated to

the results obtained through *in vitro* experiments, where *B. cepacia* could solubilize maximum amount of tricalcium phosphate. The ability of efficient PGPR to fix atmospheric nitrogen and convert insoluble phosphorus and potassium to an accessible form is an important feature for increasing plant yields and improving quality of fruits. From the present study, it is concluded that the native strains *Stenotrophomonas* sp. and *B. cepacia* are efficient PGPR isolated from the rhizosphere soils of North-Western Himalayas and bear potential to serve as broad-spectrum microbial inoculants but further studies may be required if they are to be commercialized or to be used as bioinoculants under different local agro-climatic conditions.

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Conflict of Interest

There is no conflict of interest in the submission of this manuscript. The manuscript does not contain experiments using animals.

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