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Acetylene reductase activity and molecular characterization of plant growth promoting rhizobacteria to know efficacy in integrated nutrient management system

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Isolates from rhizosphere were screened and characterized for their plant growth promotion and biocontrol properties as per standard methods. Five isolates (MK_2, MK_4, MK_5, MK_7 & MK_9) showed the maximum plant growth promoting attributes like P-solubilization, N-fixation etc. Acetylene reduction assay (ARA) method was used to study N_2 fixing ability. The bacterial isolate MK_5 fixed the maximum nitrogen, *i.e.*, 437.26 ηmole C_2H_4 h^{-1} mg^{-1} protein (109 mg of N_2 fixed/ha/d). The isolates MK_5 and MK_7 showed the maximum homology with *Bacillus pumilus* and *B. subtilis*, respectively by 16S rRNA analysis. The bacterial isolate MK_5 in conjunction with 75% level of recommended dose of N and P fertilizers gave increase yield of cauliflower crop by 27.29% over recommended doses of NPK with a saving of 25% N and 25% P fertilizers.

Keywords: Acetylene reduction assay (ARA), *Bacillus* spp., biocontrol, cauliflower, INM, PGPR, 16S rRNA

Introduction

Presently, there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices. Current trends in agriculture are to focus on reduction in the use of inorganic fertilizers and compelling the search for alternatives that enhance soil health and agricultural sustainability. One potential way to decrease negative environmental impacts on sustainable crop production is inoculation of plants with plant growth promoting rhizobacteria (PGPR). PGPR defined as beneficial rhizobacteria that colonize the roots and exert beneficial effects on plant growth and development[1]. PGPR have been applied to various crops to enhance seed emergence, growth and crop yield. Plant growth-promoting bacteria directly stimulate growth by nitrogen fixation, solubilization of nutrients, production of growth hormones and 1-amino-cyclopropane-1-carboxylate (ACC) deaminase; and indirectly by antagonizing pathogenic fungi by production of siderophores, chitinase, β-1,3-glucanase, antibiotics, fluorescent pigments, and cyanide[2].

In recent years, concept of integrated plant nutrient management has been developed, which emphasizes maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality, thus ensuring long term agricultural sustainability. Conjoint use of PGPR and synthetic fertilizers could improve the photosynthetic and metabolic activity, leading to increases in plant metabolites responsible for cell elongation[3]. In the present study, isolation and molecular characterization of two efficient rhizobacterial strains, *Bacillus pumilus* (MK_5) and *B. subtilis* (MK_7), has been done with special reference to their plant growth promoting and N-fixing abilities to increase the cauliflower yield under mid hill conditions of Himachal Pradesh.

Materials and Methods

Sample Collection

Soil samples (up to 15 cm depth) were collected from rhizosphere (with roots) of cauliflower from three (Kangra, Hamirpur & Bilaspur districts) naturally growing agro-climatic zones of Himachal Pradesh. The samples were placed in plastic bags and stored in the Soil Microbiology Laboratory, Department of Soil Science and Water Management, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan, for further isolation and analysis. The isolation of the microorganisms was

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carried out from 8 sites at different locations in Bilaspur (Namhol & Jukhala), Hamirpur (Bhota & Nadaun) and Kangra (Palampur, Dharamshala, Jachh & Pong Dam) area of Himachal Pradesh.

Isolation of Samples and Maintenance of Cultures

Isolation from soil and root samples was carried out by modified replica plating technique. After incubation of 24-48 h, the isolated colonies that developed on enriched medium (master plate) were replica plated onto the selective media: Nitrogen free medium[4] for nitrogen fixing activity and Pikovskaya (PKV) medium[5] for phosphate solubilizing ability. The isolated cultures were purified by streak plate method and maintained on the slants of respective medium at 4°C in a refrigerator. The culture of *Rhizoctonia solani*, *Pythium* sp. and *Fusarium* sp. were procured from the Department of Mycology and Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan. These fungal cultures were maintained on malt extract agar at 4°C.

Screening and Characterization of Bacterial Isolates

The screening of the bacterial isolates for various plant growth promoting activities were performed by adopting the standard methods. Estimation of P-solubilization in liquid PVK medium containing TCP (tri-calcium phosphate) was determined[6]. Phosphate solubilizing activity[5], nitrogen fixing ability[4], production of siderophore[7], HCN[8], IAA[9] and antagonistic activity[10] was also analyzed as per standard methods.

Quantitative Assay of Nitrogenase Activity

Nitrogen fixation capacities of nitrogen fixers were quantified indirectly by acetylene reduction assay (ARA) method.

ARA

Nitrogen fixation of the isolates was determined in nitrogen free medium by the acetylene reduction assay[11]. Pure cultures of all the isolates were inoculated to 100 mL of nitrogen free medium in 250 mL Erlenmeyer flask and were grown to the mid exponential phase at 30°C for 48 h on a rotatory shaker (100 revolutions min^{-1}). The assay vials containing nitrogen free medium were inoculated with aliquots (0.1 OD at 600 nm) as obtained after shaking and incubated till exponential phase. Following incubation, the gas phase in the headspace of vials was replaced with acetylene (10% v/v) and again incubated at appropriate growth temperature for 18 h. 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. After completion of the ARA, the cells were treated with lysozyme and predigested by adding 10% SDS and sonicated briefly. Protein concentration in the resulting distributed mixture of suspension was determined. The nitrogenase activity was calculated by the following formula.

$$\text{Nitrogenase activity } (\eta\text{mole } C_2H_4\ \eta^{-1}\text{mg}^{-1}\text{ protein}) = \frac{C \times P_S \times V}{P_{Std} \times T \times P}$$

Where:

C = conc. of ethylene in ηmoles

P_S = peak height of sample

V = volume of air space in the assay vial

P_{Std} = peak height of standard

T = time of incubation in h

P = protein concentration of bacterial cell in mg

A factor of 4 was used for conversion on nanomoles of C_2H_2 reduced to nanomoles of N_2 fixed mg^{-1} ha^{-1} d^{-1} as an assumption that 3-4 mol acetylene are reduced to ethylene for every mole of N_2 fixed by nitrogenase enzyme[12,13].

Protein Measurement of Bacterial Cells

The protein concentration of the bacterial cell was determined by Lowry method[14]. To 0.2 mL of predigested bacterial cell suspension, distilled water was added to make the volume 1 mL. To this, 5 mL of reagent "C" (copper sulphate-sodium-potassium tartrate solution) was added with immediate vortexing and kept for 10 min at room temperature. Finally, 0.5 mL of reagent "D" (alkaline copper sulphate) was added and incubated in the dark for 30 min. The intensity of blue colour developed was read on spectrophotometer (UV-VIS Spectrophotometer-SL-159, Elico, India) at 660 nm and the amount of protein was extrapolated from the standard curve. Standard curve was prepared by plotting absorbance *vs* concentration of protein.

Molecular Characterization of Selected Isolate

Molecular characterization of selected isolates was conducted to investigate the species taxa of each potential isolate based on molecular assay. All potential isolates were chosen for this analysis. The isolation of genomic DNA was done with CTAB method[15]. Amplification of 16S rRNA gene was carried out by PCR using specific primer (Bf-5′-GCAAGTCGAGCGGACAGATGGGAGC-3′)

and (Br-5′-AACTCTCGTGGTGTGACGGGCGGTG-3′). Amplification would give yield of DNA fragments (approx 1409 & 1375 bp). The PCR condition was carried out for 30 cycles including pre-denaturation step for 2 min at 94°C, denaturation for 30 sec at 92°C, annealing for 30 sec at 55°C, polymerization for 1 min at 75°C and post PCR for 10 min at 75°C. All the PCR products were purified using Real Genomic DNA Extraction Kit and sequenced using ABI 310 (Perkin Elmer, USA). Similarity of each 16S-rRNA sequence was aligned against GenBank database by using BLASTN program. All the sequences were also aligned with ClustalW program for constructing a phylogenetic tree.

Field Experimentation

On the basis of *in vitro* antagonistic activities against phytopathogens and other plant growth promoting traits and N_2-fixing abilities, only three isolates (MK_5, MK_7 & MK_9) at three different levels (50, 75 & 100% of the recommended doses) of N and P were selected in field trial to study the influence of these strains on the growth and yield parameters of cauliflower. The experiment was conducted in the field situated at 32° N latitude and 77° E longitude. The treatments: T_1 [Uninoculated control (recommended doses on NPK fertilizers)], T_2 (MK_5+50% NP), T_3 (MK_5+75% NP), T_4 (MK_5+100% NP), T_5 (MK_7+50% NP), T_6 (MK_7+75% NP), T_7 (MK_7+100% NP), T_8 (MK_9+50% NP), T_9 (MK_9+75% NP) and T_{10} (MK_9+100% NP) were arranged in randomized complete block design (RCBD) and replicated thrice. Sources of nitrogen and phosphorus were calcium ammonium nitrate (25% N) and single super phosphate (16% P_2O_5), respectively. Bacterial cell suspension (at OD_{540nm} 1.00) of 72 h old cultures, grown in 10% nutrient broth, was used as inoculum. Seeds were treated with bacterial inoculum for 8 h, while untreated seeds were placed in sterilized distilled water for 8 h (control). To produce transplants, seeds were sown in a nursery having soil, sand and FYM (1:1:1) mixture, irrigated once a day to maintain moisture at about the field capacity of mixture. Nursery was sown in the second week of March in both years having 10 square meter area for transplanting in 1/10th of hectare area. 30 kg well rotten FYM, 5 g sodium molybdate ($NaMoO_4$), 10 g borax and 1 kg fertilizers having (12:32:16, N:P:K rate) was added at the time of preparation of nursery bed. No fungicides/insecticides were used for raising nursery except seed treatment with PGPR culture. 1-month-old seedlings were transplanted to the field at the spacing of 60×45 cm^2. All of the P was applied at the transplanting time. A split application with 1/3rd of the N was applied at transplanting and equal split applications applied one month later and at curd formation. Booster doses of bacterial inoculum were added at 1-month interval until harvest. Weeding was done manually and the crop was irrigated twice a week to keep moisture level near to the field capacity. Observations on cauliflower yield were recorded after harvest of the crop. The statistical design used in the study was RCBD and data were analyzed[16].

Results

Isolation and Enumeration of Rhizospheric and Endophytic Rhizobacteria

Microbial Population in Rhizosphere Soil

The rhizosphere soil had great variation in total microbial counts at different locations. Among various sites, Bhota had the highest (291.0×10^4 cfu/g soil) and Nadaun had the minimum (180.3×10^4 cfu/g soil) bacterial counts. The population on the Jensen's medium, which is specific for N-fixation, was the highest (72.3×10^4 cfu/g soil) at Jachh and the lowest (56.0×10^4 cfu/g soil) at Jukhala. The percentage of phosphate solubilizing microorganisms to total PVK count ranged from 61.30 (Jukhala, Bilaspur) to 75.60% (Nadaun, Hamirpur).

Microbial Population in Roots of Cauliflower Plants

Cauliflower roots collected from different location/ sites harboured variable microbial populations. The highest (101.7×10^1 cfu/g root) total bacterial count was observed at Nadaun but the lowest (62.3×10^1 cfu/g root) was recorded at Jukhala. The maximum (47.7×10^1 cfu/g root) endophytic bacterial population capable of N-fixation was recorded at Nadaun and the minimum (33.3×10^1 cfu/g root) was observed at Namhol. Percentage of phosphate solubilizers to the total population on PVK medium at different location/sites also varied from 45.3% (at Namhol, Bilaspur) to 61.3% (at Nadaun, Hamirpur).

Screening and Characterization of Bacterial Isolates

Qualitative and Quantitative Estimation of Tricalcium Phosphate (TCP) Solubilization by Bacterial Isolates

All the five isolates (MK_2, MK_4, MK_5, MK_7 & MK_9) were found to solubilize TCP in PVK agar medium. But the maximum solubilising capacity was observed with isolate MK_5 (Table 1).

Indole-3-Acetic Acid Production by Bacterial Isolates

The data presented in Table 2 revealed that isolate MK_5 produced a significantly higher concentration of IAA (29.67 µg/mL) corresponding the maximum (60.67×10^5 cfu/mL) viable count as compared to other isolates tested.

Siderophore Production by Selected Bacterial Isolates

In the present study, all the five bacterial isolates produced a bright zone with yellowish colour around the bacterial colony on Chrome-azurol-S (CAS) medium. The maximum (14.67 mm) bright zone with yellowish colour was observed in case of MK_9. The quantitative estimation of siderophore using CAS liquid assay revealed that bacterial isolate MK_5 produced the maximum (51.36%) siderophore unit, while isolate MK_4 produced the maximum (52.67×10^5 cfu/mL) viable counts after 72 h of incubation (Table 3).

HCN Production by Bacterial Isolates

All the bacterial isolates were screened for HCN production on King's B medium. However, only isolates MK_5, MK_7 and MK_9 caused change in the colour of filter paper from yellow to orange and then to dark brown, showing HCN production.

Antifungal Activity of Bacterial Isolates

The antifungal activity of individual isolates was compared using dual culture method (Table 4). Bacterial isolate MK_4 showed the maximum growth inhibition against *Fusarium* sp. (90.38%), while isolate MK_5 showed the maximum growth inhibition against *R. solani* (84.09%) and *Pythium* sp. (86.04%).

Nitrogenase Activity of Isolates

The comparison of nitrogenase activity of 3 efficient nitrogen-fixing isolates obtained from rhizosphere and roots of cauliflower is shown in Table 5. All the isolates (MK_5, MK_7 & $MK_{9)}$ showed higher nitrogenase activity, *i.e.*, 402.91-437.26 ηmole C_2H_4 h^{-1} mg^{-1} protein (101-109 mg of N_2 fixed/ha/d), as compared to standard strain (procured from the Institute of Microbial Technology, Chandigarh) of *Azotobacter chroococcum*, *i.e.*, 372.85 ηmole

Table 1—Solubilization of tricalcium phosphate (TCP) by selected bacterial isolates in solid and liquid (PKV) medium

Bacterial isolates	P-solubilization in solid medium		
	Phosphate solubilization index (PSI)	% P-solubilization efficiency (%SE)	P-solubilization in liquid medium (µg/mL)
MK_2	2.67	151.36 (12.27)*	444.33 (2.64)**
MK_4	2. 47	147.22 (11.90)	567.67 (2.73)
MK_5	2.76	172.21 (13.06)	664.33 (2.82)
MK_7	2.77	166.67 (12.87)	640.33 (2.80)
MK_9	2.75	158.33 (12.54)	604.00 (2.77)
lsd	0.28	(0.78)	(0.06)

*Figures in parentheses are square root transformed values
**Figures in parentheses are log transformed values

Table 2—Indole-3-acetic acid production by selected bacterial isolates in Luria Bertani Broth

Bacterial isolates	Viable count ($\times10^5$ cfu/mL)	Final pH of supernatant	Indole-3-acetic acid (µg/mL)
MK_2	47.67	5.10	24.83
MK_4	58.33	5.20	24.67
MK_5	60.67	5.13	29.67
MK_7	56.67	5.18	28.33
MK_9	58.67	5.22	25.50
lsd	3.19	0.09	3.26

Table 4—Antifungal activity of selected bacterial isolates against fungal pathogens using dual culture technique

Bacterial isolates	Antifungal activity (% growth inhibition)		
	Rhizoctonia solani	*Pythium* sp.	*Fusarium* sp.
MK_2	81.81 (9.04)	83.72 (9.14)	83.33 (9.12)
MK_4	78.40 (8.85)	81.39 (9.02)	90.38 (9.50)
MK_5	84.09 (9.16)	86.04 (9.26)	89.28 (9.44)
MK_7	77.27 (8.78)	81.39 (9.02)	85.71 (9.25)
MK_9	81.81 (9.04)	77.90 (8.82)	88.09 (9.38)
lsd	(0.21)	(0.26)	(0.23)

Figures in parentheses are square root transformed values

Table 3—Qualitative and quantitative estimation of siderophore activity by selected bacterial isolates on CAS medium

Bacterial isolates	Siderophore activity			
	Viable count ($\times10^5$ cfu/ml)	Final pH of supernatant	Qualitative estimation (Zone size, mm)	Quantitative estimation (% siderophore unit)
MK_2	51.63	5.10	8.67	19.86 (26.37)
MK_4	52.67	5.22	11.33	19.40 (26.01)
MK_5	48.33	5.12	12.67	51.36 (45.78)
MK_7	48.67	5.23	13.33	33.03 (35.03)
MK_9	49.67	5.14	14.67	31.14 (33.90)
Lsd	3.29	0.12	2.77	15.94

Table 5—Nitrogenase activity of native isolates of nitrogen fixers isolated from cauliflower plants

No.	Isolate (*Bacillus* spp.)	Nitrogenase activity*	N_2 fixation (mg/ha per day)**
1	MK_5	437.26	109
2	MK_7	418.45	105
3	MK_9	402.91	101

*ηmol C_2H_4 released h^{-1} mg^{-1} protein
**A factor of 4 was used for conversion of ηmoles of C_2H_2 reduced to ηmoles of N_2 fixed

C_2H_4 h^{-1} mg^{-1} protein (93.0 mg of N_2 fixed/ha/d), whereas nitrogenase activity of MK_2 and MK_4 was observed to be the least.

Molecular characterization (16S rRNA Geneene Sequencing) of Efficient Native PGPR Isolates

An attempt was made to characterize the efficient bacteria isolated from the rhizosphere of cauliflower plants using 16S rRNA gene sequencing to identify and decipher their phylogenetic affiliation. The sequence data of 16S rRNA gene of 2 efficient strains revealed 1409 and 1375 bp partial sequences in isolates MK_5 and MK_7, respectively (Fig. 1).

Nucleotide Sequence Analysis

Nucleotide sequence analysis of 2 test isolates using ClustalW program revealed that isolate MK_5 showed the maximum homology (100%) with *B. pumilus*, while isolate MK_7 showed the maximum homology (99%) with *B. subtilis* (Fig. 2).

Field Experimentation

In general, conjoint application of PGPR isolates and varying doses N and P fertilizers significantly increased plant growth parameters (number of non-wrapper leaves, curd diameter, curd wt, curd depth and curd yield) over uninoculated control. The maximum number (10.70) of non-wrapper leaves was recorded with treatment T_4, followed by with treatment T_3 (10.20) in comparison to uninoculated control (T_1, 9.46). Similarly, the maximum (15.31 cm) curd diameter was recorded with T_4, followed by T_3 (14.82) and the minimum (10.84 cm) curd diameter was recorded with T_1. Further, the maximum curd depth was noticed with T_4 (9.75 cm) and T_3 (9.62 cm) and the minimum (6.87 cm) with T_1. Similarly, the maximum curd wt was observed with T_4 and T_3 (*c.* 965.0 g) and the minimum (750.8 g) with T_1. The curd yield increased significantly under all the treatments over uninoculated control (T_1; 285.1 q/ha) (Fig. 3). The maximum (376.5 q/ha) curd yield was

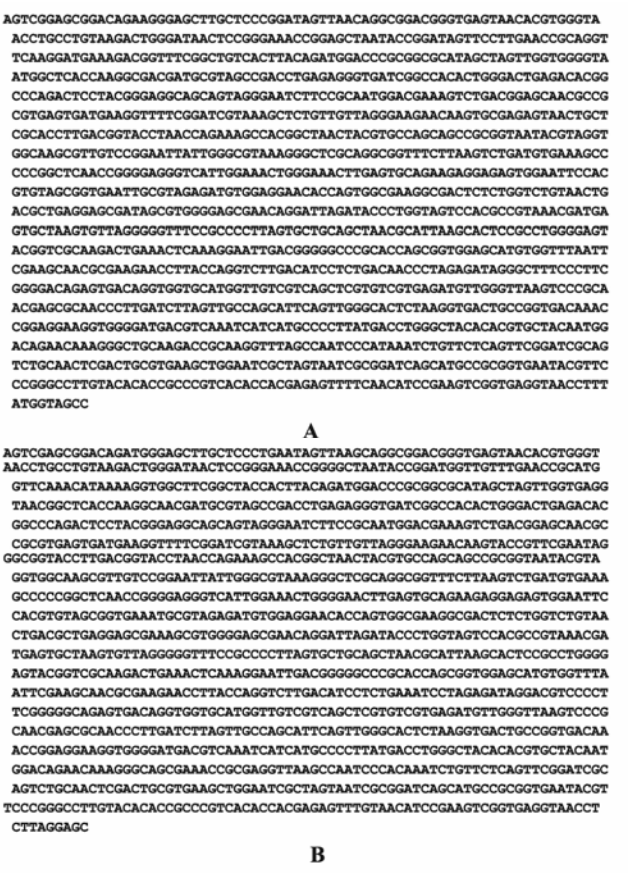

Fig. 1 (A & B)—Partial nucleotide sequence of 16S rRNA gene of efficient isolates: (A) *B. pumulis* (MK_5), & (B) *B. subtilis* (MK_7).

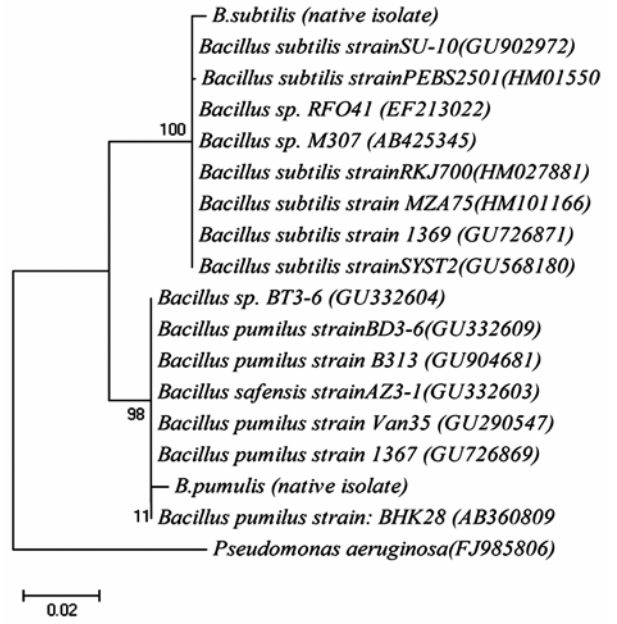

Fig. 2—Phylogenetic tree constructed by Neighbor-Joining method derived from analysis of the 16S rRNA gene sequences of native isolates (MK_5 & MK_7) and related sequences.

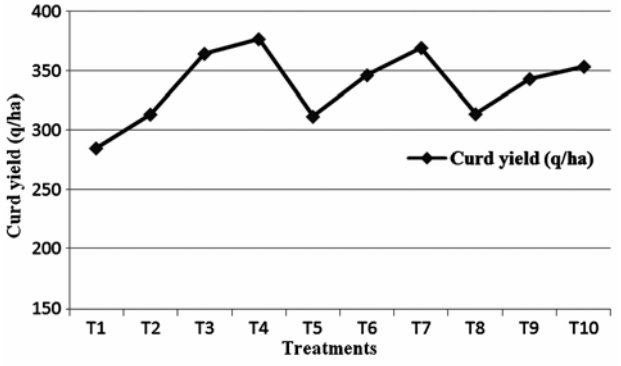

Fig. 3—Effect of PGPR and varying levels of chemical fertilizers on yield of cauliflower.

recorded with T_4, which was also statistically at par with T_7 (369.1 q/ha) and T_3 (364.2 q/ha). Thus the highest percentage increase in curd yield over control (T_1) was recorded in T_4 (32.6%), T_7 (29.46%) and T_3 (27.65%). However, T_3 had the benefit of saving 25% of N (31 kg ha^{-1}) and P_2O_5 (19 kg ha^{-1}) as T4 and T7 had the full dose of NPK fertilizers, while T3 had only 75% of NPK. Therefore, T3 could be the highly recommended treatment for the cauliflower

Discussion

The bacterial isolates capable of growth on PVK medium and N-free medium were screened for the production of siderophore, auxin, HCN and antagonism against *Pythium* sp., *Fusarium* sp. and *R. solani*, pathogens of cauliflower.

Phosphorus is one of the major nutrients, second only to nitrogen, in the requirement of plants. Most of the phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants. PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to cauliflower that represents a possible mechanism of plant growth promotion under field conditions. In the present study, all isolates were able to solubilize phosphate in the rhizosphere soil. The selected five isolates (MK_2, MK_4, MK_5, MK_7 & MK_9) were capable of hydrolyzing tri-calcium phosphate (TCP) in liquid as well as in solid PVK medium. The per cent P-solubilization efficiency shown by different isolates had great variation with the value ranging from 147.22-172.21% and the maximum was observed for MK_5 isolate in solid as well as liquid PVK medium (Table 1). The P-solubilization efficiency was more on solid medium than in liquid medium. The P-solubilization in the rhizosphere of chickpea, mustard and wheat by native bacterial population was also reported[17]. All the five bacterial isolates produced IAA, the amount ranged from 24.67 to 29.67 µg/mL and the maximum by MK_5 (Table 2). Present results are in agreement with other studies where *Bacillus* sp. was reported to produces IAA[18]. The maximum (14.67 mm) bright zone was observed in case of MK_9 isolate, which was statistically at par with yellow zone produced by other isolates MK_5 and MK_7 (Table 3). Bacteria produce low mol wt, high affinity iron (III) chelating substances that transplant iron into bacterial cell called siderophores[19]. It is evident from the results that MK_5 and MK_7 isolates produce HCN under *in vitro* conditions. The selected bacterial isolates showed marked antagonism against *Pythium* sp., *Fusarium* sp. and *R. solani* (Table 4). Bacterial isolate MK_5 showed maximum (84.09%) growth of inhibition against *R. solani* and 86.04% against *Pythium* sp, while MK_4 showed the maximum inhibition against *Fusarium* sp. The culture supernatant of *B. cereus* was shown to inhibit growth of various pathogenic fungi of Chinese cabbage like *F. oxysporum, F. solani* and *P. ultimum*, and *B. subtilis* was shown to be an antagonist of *F. graminearum*, a pathogen of canola[20].

Nitrogen is an essential nutrient for all forms of life on earth. The prokaryotic microbes play an important role in conversion of atmospheric nitrogen (N_2) to available forms for plant and microbial growth. The nitrogenase enzyme catalyzes the reductive breakage of the very strong triple bond of N_2 to generate NH_3. The reduction of acetylene to ethylene (ARA) is proposed as an indirect method to assay for nitrogenase activity[11]. In the present study, the most efficient nitrogen fixing isolate was MK_5, isolated from cauliflower rhizosphere, which showed the highest nitrogenase activity (437.26 ηmole C_2H_4 h^{-1} mg^{-1} protein or about 109 mg of N_2 fixed/ha/d) (Table 5). The ARA of free living bacteria were also reported in the range of 1.8 to 2,844.7 26 ηmole C_2H_4 h^{-1} mg^{-1} protein[21].

Phylogenetic 16S rRNA gene analysis revealed that isolate MK_5 is closely related to *B. pumilus*, while MK_7 displayed maximum homology with *B. subtilis* (Fig. 2). The 16S rRNA gene serves as molecular chronometer, since it is the most conserved part during evolution. 16S rRNA gene sequencing is used and accepted worldwide for identification and phylogenetic analysis of the bacterium. Therefore, to get reliable and accurate identification of bacterial isolates, molecular characterization (16S rRNA gene sequencing) is an important tool.

In present study, the conjoint applications of PGPR and chemical fertilizers significantly increased yields per hectare over recommended chemical fertilizers alone (Fig. 3). An average 6 to 32% increase in yields was noticed by various treatment combinations over uninoculated control. Although the highest curd yield was recorded with treatment T_4 (100% NP+MK_5 isolate), the most economical treatment was T_3 (75% NP+MK_5 isolate), showing 27.65% increase in cauliflower yield with a saving of 25% NP fertilizers and sustenance of soil health. In cabbage, more yield was also observed through dual inoculation of biofertilizers along with full dose of chemical fertilizers[22].

Conclusion

Overall, the results suggest that PGPR are able to induce the production of IAA, solubilization of phosphorus and resistance to pathogens and pests, thereby improving the growth of cauliflower. Thus, use of PGPR (MK_5 at 75% doses of N and P fertilizers) as biofertilizers is an efficient approach to reduce chemical fertilizers and pesticides as an integrated nutrient management (INM) for not only to increase cauliflower yield but also to sustain soil health under mid hill conditions of Himachal Pradesh.

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