Impact assessment of cold-adapted *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 on *Phaseolus vulgaris* yield and soil health

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The poor agriculture practices, fragmented land holdings, fluctuating climatic conditions, and minimal external inputs lead to nutrient deficiency in the Himalayan agroecosystems. Because of the risks associated with chemical fertilizers, their implication is a big question mark. Therefore, two previously characterized plant growth-promoting rhizobacteria *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 were employed to enhance kidney bean productivity and soil health at farmer's fields of Harsil and Chakrata regions of Uttarakhand Himalayas. The study revealed that MP1 and N26 treatment resulted in 25.62% and 37.23% higher grain yield than respective uninoculated controls at the trial fields of Harsil and Chakrata regions, respectively. Further, the bacterial treatments have significantly increased nitrogen, phosphorus, and potassium levels in the soils. The soil diversity analysis revealed the dominance of Proteobacteria and Actinobacteria at Harsil and Chakrata, respectively. Further, the MP1 treatment had increased Firmicutes percentage over uninoculated control at both locations. Conclusively, the application of cold adaptive *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 improved the grain yield and soil health status of the Himalayan agroecosystems. Therefore, they can be explored as an eco-friendly alternative for the commercial production of kidney beans.

KEYWORDS

community structure, soil metagenome, cold adaptive microorganisms, kidney bean, bioinoculants

Introduction

Agriculture is a prodigious private enterprise in India and will continue to be the lifeline of the Indian economy in the future. It is estimated that overall food demand will rise in proportion to the world population. The global population is continuously increasing at the rate of above 1.8 percent annually and it will reach the point from today's calculated 7.4 billion to an anticipated demographical data of 9.6 billion by 2050 (United Nations, 2013). Current agricultural practices are neither economically nor environmentally sustainable. After the green revolution, chemical-based fertilizers and pesticides have enormously boosted agricultural production. However, their indiscriminate use, besides imposing a detrimental effect on the atmosphere has developed resistance in insects against common pesticides. The fertilizer consumption in India is increasing day by day and has reached 133 Kg ha^{-1} in the year 2019-20 in comparison to 94 Kg ha^{-1} in 2004-2005 (Agricultural Statistics, 2019). These trends can't be neglected because they will lead to serious agricultural, environmental, and health issues.

Kidney bean is a high-value cash crop in the Indian Himalayan regions. The local cultivar is known for its premium quality, unique taste, and nutritional value. However, due to nutritional deficiency, habitat degradation, and anthropogenic contamination, the commercialization of kidney beans could not be done properly. Therefore, an eco-friendly and cost-effective farming approach is urgently needed to enhance kidney bean productivity and achieve sustainable agriculture goals. In this regard, plant growth-promoting rhizobacteria (PGPR) seem better alternatives than others (Sahu et al., 2021). As compared to chemical fertilizers, they are safer with reduced environmental damage, have more targeted activity, and are effective in smaller quantities. Therefore, the present study employs previously characterized cold adaptive PGPR strains for the production of *Phaseolus vulgaris* (Kidney bean or Rajmash). These strains have already been tested on several pulse crops under net-house and natural field conditions (Suyal et al., 2017; Rajwar et al., 2018; Joshi et al., 2019; Rawat et al., 2019). Therefore, taking a step forward they were applied in the farmers' fields at two different locations in Uttarakhand Himalaya. This study discusses the impact of the above-mentioned bioinoculants on the Rajmash crop yield and soil bacterial diversity during the first year of field trials. Moreover, the participation of the local farmers in the study has motivated them for adopting this technology. Therefore, besides promoting sustainable agriculture, this study will contribute to the socio-economic upliftment of the local marginal farmers. Although, consecutive studies are recommended to increase the efficiency and persistence of these bio-formulations under natural field conditions.

Materials and methods

Bacterial strain and growth conditions

Cold adaptive bacterial strains *P. jesenii* MP1 and *P. palleroniana* N26 were obtained from the culture collection of Department of Microbiology, GBPUAT Pantnagar, Uttarakhand [Table 1 near here]. Both the strains have already been characterized for their nitrogen fixation as well as phosphate-solubilizing potential (Suyal et al., 2017; Tomer et al., 2017; Rajwar et al., 2018; Joshi et al., 2019; Rawat et al., 2019). The strains were grown aerobically in a nutrient agar medium at 28 ± 1° C during the experiment.

Experimental sites and field trials

A field experiment was performed on farmer's fields in the Indian Central Himalayan region at Harsil (31.0383˚N, 78.7377˚E) and Chakrata (30.7016˚N, 77.8696˚E) villages of Uttarakhand, India during 2019. In order to conduct the experiment in a such hilly region where agriculture fields are fragmented and terraced, a farmer's field with a dimension area equal to or higher than 150m^2 was selected at both sites. Afterward, potential bioinoculants containing bio-formulations (1x10^9 CFU g^{-1}) were prepared. At the time of sowing, bioformulation was mixed in the Jaggrey solution subsequently kidney bean seeds were treated at the rate of 10 g Kg^{-1} seeds (Germination efficiency- >86%) followed by shade drying for 30 min. Subsequently, seeds were sown in the fields at both Harsil and Chakrata locations. Apart from this untreated seeds sown in the fields served as uninoculated farmers' practices or control. With respect to each treated field, a control was set up. Both the sites belong to a temperate zone with an average temperature of 8-29˚C from May to September. A total of 3 treatments (*P. jesenii* MP1, *P. palleroniana* N26, and uninoculated controls) in each site with triplicates were laid down.

A total of six treatments comprising *P. jesenii* MP1, *P. palleroniana* N26, and uninoculated controls in both Harsil and Chakrata soils were laid out in triplicates.

Seed bacterization and sowing

The seeds of the local variety of Rajmah were surface sterilized by soaking in 0.1% HgCl$_2$ for 2 min and then thoroughly washed with autoclaved distilled water. Later, the seeds were mixed with

TABLE 1 Details of the bacterial strains used in the study.

S. No.	Bacterial strains	NCBI accession no.	Functional attributes	References
1.	*Pseudomonas jesenii* MP1	JX310329	N$_2$ fixation & P solubilization	Rajwar et al., 2018; Joshi et al., 2019
2.	*Pseudomonas palleroniana* N26	JN055435	N$_2$ fixation & P solubilization	Tomer et al., 2017; Rawat et al., 2019

0.1% charcoal and overnight grown culture having 10^9 CFU mL^{-1}. The seeds were then dried for 1 h followed by the sowing in respective fields with plant to plant distance of 10 cm and row to row distance of 15 cm.

Soil sampling, soil nutrient analysis, and crop yield

The soil samples were collected before sowing and after harvesting the crop by using a sterilized trowel at a depth of 0-20cm. All the soil samples were collected in triplicates within sterilized polybags and transported to the laboratory under cold and sterile conditions. Soil samples were analyzed for available nitrogen (Subbiah and Asija, 1956), phosphorous (Olsen, 1954), and potassium (Jackson, 1973) content. Moreover, the crop yields were analyzed to compare the treatments with the respective controls.

Soil DNA extraction and quantification of gene copy numbers

Rhizospheric soil composite was subjected to total DNA extraction through Mo Bio's Power Soil DNA isolation kit. The total genomic DNA present in the soil was extracted from 0.5gm composite as per the manufacturer's protocol. The quantity and quality of extracted DNA were checked through NanoDrop at 260nm, following storage at -80°C till further analysis. Copy numbers of 16S rRNA were quantified using iCycleriQ^{TM} Multicolor (Bio-Rad Lab, Hercules, USA) qPCR machine as per the earlier reports (Suyal et al., 2015; Tomer et al., 2017; Rajwar et al., 2018; Suyal et al., 2019).

Metagenome sequencing and Bioinformatic analysis

The bacterial diversity was determined by sequencing V3-V4 segments using Illumina Hiseq sequencing machine along with Agilent Technologies 2100 Bioanalyzer. The quality of the raw sequences was analyzed through FastQC (v0.11.7) and TrimGalore (v0.5.0). Further, the QIIME software package (v. 1.9.0) was used for removing singletons, assigning operational taxonomy units (OTU) to the remaining sequences, and preparation of the Venn diagram (Kumar et al., 2019; Suyal et al., 2021a). The Rarefaction curve and diversity indices were analyzed by using Mothur (v.1.21.1) software.

Statistical analysis

The results were statistically analyzed through one-way ANOVA using R 3.6.1 at $P<0.005$ level of significance.

Results

Agronomical parameters and grain yield

The relative performance of cold adaptive bacteria i.e., *P. jesenii* MP1 and *P. palleroniana* N26 over altitudinal variation i.e. Chakrata and Harsil was established through field trial experiment. Overall results of the experiment demonstrated the positive consequence of both bacteria on agronomical and associated attributes of kidney beans over uninoculated control. At the Harsil site, the effect of *P. jesenii* MP1 on agronomical parameters such as root, shoot, and pod length, no. of gains per pod were found prominent. Similarly, at the Chakarta site, all agronomical parameters were highest positively influenced by *P. jesenii* MP1 [Table 2 near here]. Both the bacterial strains showed a significant increase in grain yield over respective controls at each experimental site. At Harsil, the maximum percent average grain yield was observed in the seeds inoculated with *Pseudomonas jesenii* MP1 (25.62% higher than the uninoculated control). Further, treatment of N26 had improved by 19.28% higher grain yield over the control. Contrary to it, at Chakrata, N26 treatment had provided maximum grain yield (37.23% higher than uninoculated control) followed by MP1 (11.70%). It indicates remarkable growth promotion potential of *P. jesenii* MP1 and *P. palleroniana* N26 at higher altitudes (Table 2).

Soil nutrient status

Under pre-sowing conditions, the soil nitrogen content of Harsil and Chakrata was 246.76 ± 48.23 kg ha^{-1} and 206.19 ± 27.44 kg ha^{-1}, respectively [Table 3 near here]. After harvesting it became 264.09 ± 39.83 kg ha^{-1} (Harsil) and 207.04 ± 27.48 kg ha^{-1} (Chakrata). Further, the application of *Pseudomonas jesenii* MP1 improved it to 274.21 ± 38.46 kg ha^{-1} and 212.49 ± 27.00 kg ha^{-1} at Harsil and Chakrata, respectively. Similarly, *Pseudomonas palleroniana* N26 also enhanced soil nitrogen content to 296.34 ± 21.52 kg ha^{-1} and 225.48 ± 4.12 kg ha^{-1} at Harsil and Chakrata, respectively.

In the case of Phosphorus, Harsil, and Chakrata had 28.80 (± 7.86)kg ha^{-1} and 66.63 (± 24.47) kg ha^{-1} P_2O_5 in pre-sowing soils. After harvesting, it was found 31.30 (± 7.78) kg ha^{-1}, and 70.03 (± 23.92) kg ha^{-1}, respectively. When treated to the MP1, it reached 42.58 (± 8.09) kg ha^{-1} and 84.26 (± 17.25) kg ha^{-1} in Harsil and Chakrata, respectively. Furthermore, N26 treatment had improved it to 61.93 ± 20.63 and 78.51 ± 17.99, respectively.

Similarly, potassium content was 415.44 ± 66.18 ppm (Harsil) and 635.22 ± 236.50 ppm (Chakrata) in pre-sowing soils and 433.89 ± 90.36 ppm (Harsil) and 686.33 ± 239.31 ppm (Chakrata) in post-harvested soils. After treatment with MP1 and N26, it reached 501.29 ± 125.77 ppm and 536.93 ± 126.45 at Harsil and 928.58 ± 167.15 and 278.53 ± 11.87 ppm at Chakrata.

TABLE 2   Effect of *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 on grain yield of Kidney bean.

Experimental sites	Latitude and Longitude	Elevation (m)	Treatment	Plant height (cm)	Pod length (cm)	No. of grain/Pod	1000 grain weight (g)	Yield (qha^{-1})
Harsil	31.0383˚N, 78.7377˚E	2,500	Control	84.67 ± 2.33[a]	10.63 ± 0.18[a]	3.67 ± 0.33[a]	622.91 ± 6.15[a]	10.42 ± 0.63[a]
			MP1	112.00 ± 5.13[c]	12.20 ± 0.17[b]	5.00 ± 0.58[b]	688.53 ± 5.82[c]	13.09 ± 0.54[b] (25.62)
			N26	107.33 ± 3.17[b]	11.90 ± 0.12[b]	4.67 ± 0.33[b]	649.30 ± 7.27[b]	12.43 ± 0.56[b] (19.28)
			SEm	4.069	0.150	0.451	3.553	0.548
Chakrata	30.7016˚N, 77.8696˚E	2,118	Control	34.33 ± 1.20[a]	9.97 ± 0.09[a]	3.00 ± 0.00[a]	476.74 ± 12.10[a]	0.94 ± 0.12[a]
			MP1	40.33 ± 0.67[c]	12.03 ± 0.35[b]	4.33 ± 0.58[b]	523.79 ± 18.38[b]	1.05 ± 0.09[a] (11.70)
			N26	37.33 ± 0.88[b]	11.67 ± 0.15[b]	4.00 ± 0.00[b]	509.28 ± 10.92[b]	1.29 ± 0.14[b] (37.23)
			SEm	1.054	0.255	0.192	5.896	0.066

Data were analyzed at P<0.05 level of significance. Mean ± SE is shown in the table; each value is the mean of six replicates. Values in brackets indicate percent increase over control. The values in parentheses indicate the percent increase over respective control.

Bacterial diversity composition

The 16S rRNA gene abundance was maximum in MP1-treated soils at both sites followed by N26-treated soils with respect to the controls (Table 3). Based on the preliminary results obtained through gene abundances and grain yield, MP1-treated soils were selected for metagenome sequencing. Thus, four soil metagenomes viz. Uninoculated post-harvest soil of Harsil (HRC), Uninoculated post-harvest soil of Chakrata (CHC), MP1-treated soil of Harsil (HRM), and MP1-treated soil of Chakrata (CHM) was sequenced and analyzed. A sum of 31, 04,626.00 purified 16S rRNA amplicon sequences were retrieved from the sequencing. Filtered sequences were allocated to 85041 OTUs. A Venn diagram was plotted to find out the distribution of unique and shared bacterial species among

TABLE 3   Nutrientstatus and gene abundances in the soil samples under study.

Experimental sites	Treatments	Nutrient status			Copy number of the genes (per g of soil)
		N(Kgha^{-1})	P$_2$O$_5$ (Kgha^{-1})	K$_2$O (ppm)	16SrRNA
Harsil	Control (Pre-sowing)	246.76 (± 48.23)	28.80 (± 7.86)	415.44 (± 66.18)	5.21× 10^8 (± 2.65×10^2)
	Control (Post-harvest)	264.09 (± 39.83)	31.30 (± 7.78)	433.89 (± 90.36)	3.31× 10^{10} (± 1.92×10^2)
	Pseudomonas jesenii MP1	274.21 (± 38.46)	42.58 (± 8.09)	501.29 (± 125.77)	6.73× 10^{11} (± 2.68× 10^2)
	*Pseudomonas palleroniana*N26	296.34 (± 21.52)	61.93 (± 20.63)	536.93 (± 126.45)	2.52× 10^{11} (± 1.43× 10^2)
Chakrata	Control (Pre-sowing)	206.19 (± 27.44)	66.63 (± 24.47)	635.22 (± 236.50)	1.02× 10^9 (± 3.02×10^2)
	Control (Post-harvest)	207.04 (± 27.48)	70.03 (± 23.92)	686.33 (± 239.31)	2.11× 10^{11} (± 1.92×10^2)
	*Pseudomonas jesenii*MP1	212.49 (± 27.00)	84.26 (± 17.25)	928.58 (± 167.15)	4.51× 10^{11} (± 1.18× 10^2)
	*Pseudomonas palleroniana*N26	225.48 (± 4.12)	78.51 (± 17.99)	278.53 (± 11.87)	3.21 × 10^{11} (± 1.02× 10^2)

Each value is the mean of three replicates. Values in brackets indicate standard error.

the groups [Figure 1 near here]. The number of species groups present in CHC, CHM, HRC, and HRM is 38559, 35768, 36437, and 34644 respectively. The number of OTUs shared between CHC and CHM was 13231, while the common number of OTUs present between HRC and HRM was 11202. Control groups of both sites shared 13633 OTUs and treated groups had 11724 common OTUs. The total number of OTUs shared by all four groups was only 4318.

The diversity indices revealed the lower value of Simpson in bioinoculant treated soils (0.42 and 0.45 in CHM and HRM, respectively) in comparison to their respective controls (0.69 and 0.57, respectively) (Table 4 near here). Further, Shanaon values were higher in CHM (4.71) and HRM (3.6) than in respective controls i.e. CHC (3.12) and HRC (2.87). Similarly, species richness estimates- Chao and ACE were higher in CHM (3.61×10^4 and 5.62×10^4, respectively) and HRM (3.48×10^4 and 4.87×10^4, respectively) than in CHC (1.41×10^3 and 3.92×10^3, respectively) and HRC (2.93×10^3 and 2.68×10^4, respectively).

Taxonomic distribution and effect of treatment on soil diversity

Chakrata soil

The major phyla in CHC soil belonged to Actinobacteria (23%), Proteobacteria (21%), Cyanobacteria (14%), Acidobacteria (10%), and Planctomycetes (8%). While, dominating phyla of CHM soil were Actinobacteria (27%), Proteobacteria (23%), Firmicutes (20%), Acidobacteria (8%), and Planctomycetes (7%) [Figure 2 near here]. Further, CHC and CHM metagenomes had shown 8% and 6% of assigned OTUs, respectively. At the family level, Bacillaceae dominated in CHM (9%) in comparison to CHC (2%). The other major family was Hyphomicrobiaceae having 4% and 5% of OTUs in CHC and CHM, respectively. Furthermore, the dominating genera in CHC and CHM were *Rhodoplanes* (3%) and *Bacillus* (9%), respectively [Figure 3 near here].
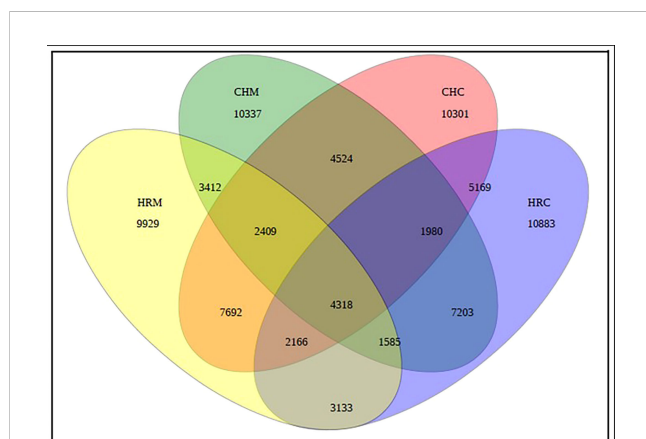
TABLE 4 Diversity indices observed in the soil metagenomes.

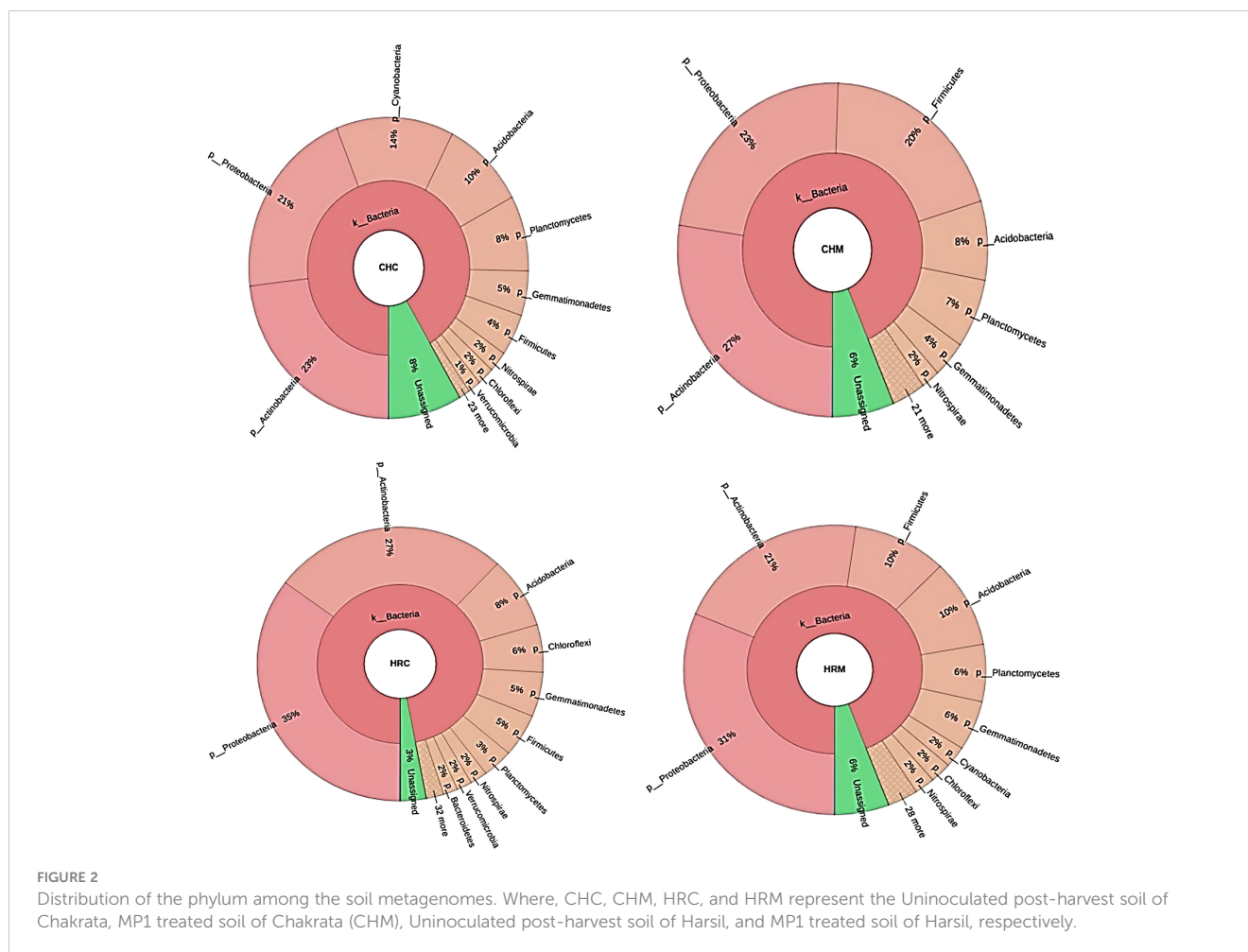| Diversity indices	CHC	CHM	HRC	HRM
Simpson	0.69	0.42	0.57	0.45
Shannon	3.12	4.71	2.87	3.6
Chao	1.41×10^3	3.61×10^4	2.93×10^3	3.48×10^4
ACE	3.92×10^3	5.62×10^4	2.68×10^3	4.87×10^4

Harsil soil

Proteobacteria (35% and 31%) was the major phylum in HRC and HRM followed by Actinobacteria (27% and 21%), respectively (Figure 2). Other abundant phyla in HRM are Acidobacteria (10%), Firmicutes (10%), Planctomycetes (6%), and Gemmatimonadetes (6%); while HRC comprised of OTUs assigned with Acidobacteria (8%), Chloroflexi (6%), Gemmatimonadetes (5%) and Firmicutes (5%). Moreover, both had 6% and 3% assigned OTUs, respectively. More specifically, the dominant members in HRC and HRM were from Nocardioidaceae and Sphingomonadaceae families, respectively. The most abundant genus (2% in both) was *Rhizoplane* in both soils. The other abundant genus was *Bacillus* in both HRM (2%) and HRC (1%) (Figure 3).

Discussion

The cold adaptive *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 were originally isolated from the Himalayan soils and have already been tested in several pulse crops (Tomer et al., 2017; Rajwar et al., 2018; Joshi et al., 2019; Rawat et al., 2019). Both potential bacterial isolates i.e., *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 initially isolate from kidney bean rhizosphere and further their plant nutrient solubilizing potential under *in vitro* conditions on various mediums were explored by Tomer et al. (2017). Further, Tomer et al. (2017) and Rawat et al. (2019) determined bacterial seed germination and yield-enhancing potential through *in vitro* and greenhouse experiments. Afterward, Rajwar et al. (2018) and Joshi et al. (2019) documented the nutrient solubilizing potential and thereby crop growth and yield-improving potential of *Pseudomonas jesenii* MP1 for Chickpea under field conditions at the Crop research center, Pantnagar. Apart from this, Khan et al. (2023) also documented the yield, soil health, and nutrient biofortifying ability of both bacterial isolates under field conditions. Both bacterial isolates have significantly established their crop-improving potential therefore in the present study, they were directly applied to the farmer's field to evaluate their real-time performance.

Nutrient status is the well-recognized health indicator of the soils. The present study revealed that inoculation of *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 significantly improved the nitrogen and phosphorus content in the soils. Moreover, a remarkable difference was observed in the case of potassium content which was found higher in bioinoculant-treated soils at both Harsil and Chakrata (23% and 20% higher, respectively). These results indicate that the unavailable nutrients

FIGURE 2

Distribution of the phylum among the soil metagenomes. Where, CHC, CHM, HRC, and HRM represent the Uninoculated post-harvest soil of Chakrata, MP1 treated soil of Chakrata (CHM), Uninoculated post-harvest soil of Harsil, and MP1 treated soil of Harsil, respectively.

of the soils were made available to the plants by the bio-inoculants. Further, it is also evident from the grain yield data, that showed MP1 and N26 treatment had improved 25.62 and 19.28% higher crop yield at Harsil and 11.70 and 37.23% higher yield at Chakrata over their respective controls. It shows that bioinoculants were able to sustain and perform better at higher altitudes. Recently, our group analyzed the agronomical parameters and soil microbial diversity for the second consecutive cropping year too of the present study (Khan et al., 2023). They have observed similar results in which MP1 and N26 have enhanced approx 23% of grain yield during the second consecutive year of cropping. Moreover, a significant increase in zinc and protein content has also been reported by them.

The metagenome sequencing revealed that both soils were rich in bacterial diversity as revealed through the presence of diversified taxa. It can be evident from the values of diversity indices too. Further, a higher Shannon index indicates additional unique species (Shannon, 1948) and a lower Simpson index indicates heterogeneous ecosystems (Simpson, 1949). Similarly, higher values of species richness indicate higher diversity in a given system. Therefore, in the present study, bioinoculant-treated soils have higher bacterial diversity with respect to their respective untreated controls. A large number of sequences were unassigned OTUs in each of the samples which confirms the existence of novel

microbes and suggests the need for culture-dependent studies in the future. The metagenome analysis showed the dominance of phylum Proteobacteria at Harsil while phylum Actinobacteria at Chakrata. These results are in agreement with the previous studies that showed that higher altitude soils are dominated by proteobacteria, actinobacteria, and acidobacteria (Lugtenberg and Kamilova, 2009; Dai et al., 2018; Kumar et al., 2019). In addition, it has been observed that MP1 treatment altered the microbial diversity with an increment in Firmicutes percentage over uninoculated control at both locations. Previous studies have positively correlated the proteobacterial and firmicute abundance with higher nutrient mobilization and improved soil fertility(Pattnaik et al., 2021; Suyal et al., 2021a). Further, in accordance with the present study, cold adaptive Cyanobacteria, Acidobacteria, Planctomycetes, Gemmatimonadetes, Firmicutes, and Nitrospirae phylum were also reported from higher altitude agroecosystems (Suyal et al., 2015; Suyal et al., 2021b).

Conclusion

The present study revealed that the bioinoculant's application has improved soil health and kidney bean production in the Himalayan farmer's field. Besides promoting organic farming
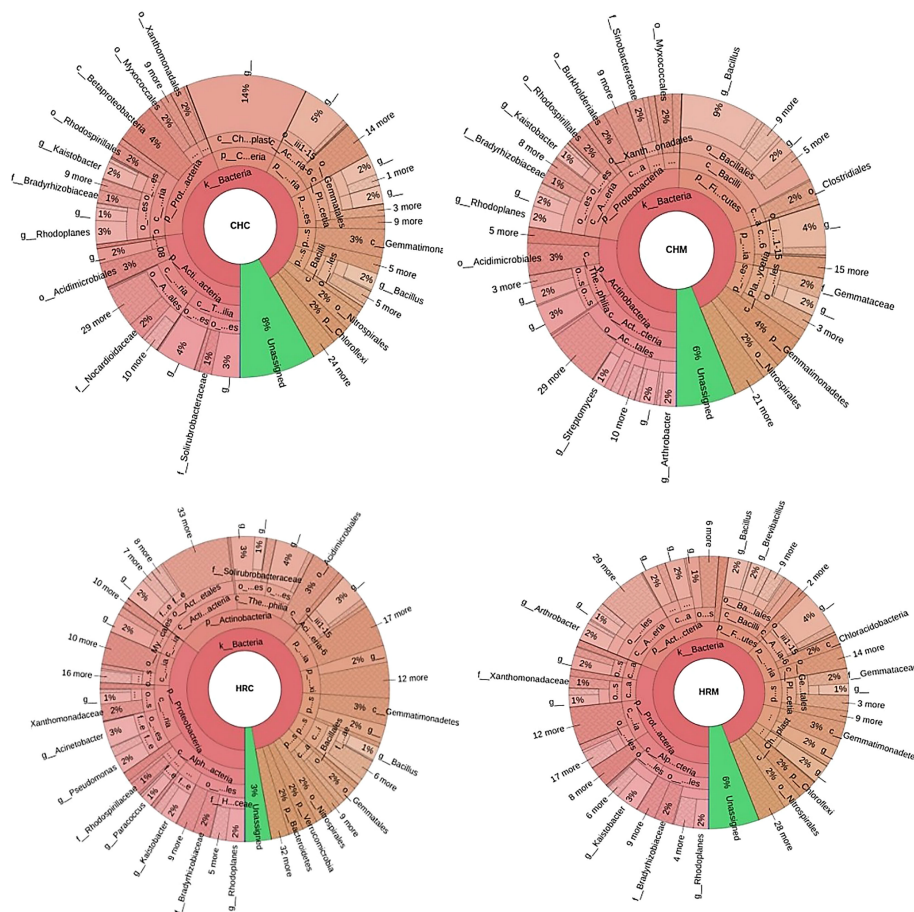
FIGURE 3
The abundance of the genera among the soil metagenomes. Where, CHC, CHM, HRC, and HRM represent the Uninoculated post-harvest soil of Chakrata, MP1 treated soil of Chakrata (CHM), Uninoculated post-harvest soil of Harsil, and MP1 treated soil of Harsil, respectively.

among marginal farmers, the study will help in the commercial production of the crop. Conclusively, *Pseudomonas jesenii* MP1 and *Pseudomonas palleroniana* N26 are strongly recommended to attain agro-environmental sustainability in the Himalayas and the socio-economic development of the associated farmers.

Data availability statement

The metagenomic data presented in this article has been submitted to the NCBI database under the accession number PRJNA607339.

Author contributions

DS: Experimental work, analysis of the results. AK: Experimental work, analysis of the results. AVS: Conceptualization, field arrangements. AA: Analysis of the results. NP: Conceptualization, field arrangements. VS: Conceptualization, field arrangements. RG: Conceptualization, manuscript editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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