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RESEARCH ARTICLE

Potential psychrotrophic bacteria isolated from Tawang, Arunachal Pradesh, India for use as biofertilizer

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Abstract

Nitrogen and phosphorus molecules, being an integral part of various physiological and biochemical processes, are indispensable for the growth and maturation of plant tissues. It is well-accepted that these molecules play a significant role in regulating agricultural productivity. This study aims to isolate and characterise Psychrotrophic microorganisms for use as biofertilizers using biochemical and molecular techniques. The main goal is to find microbial strains that can solubilize phosphorus and fix nitrogen under the harsh environmental conditions found in high-altitude areas. The research involved isolating bacteria soil samples collected from the Tawang region in Arunachal Pradesh and subjecting them to a comprehensive biochemical analysis to determine their phosphate solubilizing and nitrogen-fixing potential. The study discovered 54 bacterial isolates, 44 of which can solubilize phosphate and 10 strains capable of nitrogen fixation. Based on 16S rRNA gene sequence analysis, the isolates were found to belong to the genera *Pseudomonas, Burkholderia, Azotobacter* and *Azospirillum*.

Keywords: Psychrotrophic Bacteria; Biofertilizer; Tawang; High Altitude; *Pseudomonas; Burkholderia; Azotobacter; Azospirillum*

1. Introduction

Sustainable agriculture is the need of the hour owing to the demerits linked to the use of various agro-chemicals in the Indian farming system. The overuse and haphazard dispersal of nutrients within agroecosystems have resulted in environmental issues across the entire food production chain (Kennedy and Choudhury, 2005). Supplementing crops with nutrients, particularly nitrogen and phosphorus, without using artificial fertilizers, is a challenging task.

Phosphorus (P) stands as a fundamental macronutrient crucial for the growth and development of plants. Essential metabolic activities such as respiration, photosynthesis, and cellular energy storage depend on it (Sanz-Sáez et al., 2017; Balemi and Negisho, 2012; Malhotra et al., 2018; Razaq et al., 2017). Phosphorus is frequently the main growth-limiting element, depending on a wide range of biological and environmental conditions (Rajkumar and Kurinjimalar, 2021; Alok et al., 2013). Soil generally has a low concentration of soluble P, ranging from 0.4 to 1.2 gkg^{-1}, despite its significance (Fernández et al., 2014; Joe et al., 2018) in crop production. Approximately 95–99% of the phosphorus in soil remains in insoluble forms, which limits its uptake by the plants (Mara et al., 2014).

For centuries, agricultural practices have employed both organic and inorganic fertilizers to address nutrient deficiencies and uphold a well-balanced soil nutrient composition (Tiwari et al., 2020; Liu et al., 2020). In contrast to other vital nutrients like potassium or nitrogen, phosphorus in the soil has restricted mobility. Conventional plant nutrition models constantly highlight the tremendous difficulty presented by inorganic phosphate's (Pi) sluggish diffusion in limiting plant uptake of P (Barber, 1995). Given that roots can only reach barely any of the total soil P content, this emphasises the necessity for additional mechanisms to provide an appropriate supply of P, particularly in low P content in soil.

Rock phosphate (RP) is the primary source of P_2O_5 used in the production of most phosphate fertilizers. However, the world's supply of rock phosphate is finite, which presents a problem as demand keeps rising (Suleman et al., 2018). Examining alternatives to conventional phosphate fertilizers, the utilization of microorganisms to break down insoluble phosphate compounds has garnered attention of the agricultural scientists. This approach offers a promising avenue for addressing nutrient deficiencies in agriculture sustainably. Through the action of these microorganisms, insoluble phosphate compounds can be converted into forms that are readily accessible to plants, enhancing nutrient uptake efficiency, while minimizing environmental impact. This innovative method signifies a potential shift towards more eco-friendly and efficient agricultural practices (Adnan et al., 2020).

Nitrogen (N_2) is crucial for amino acid and protein formation, constituting 78% of the atmosphere as inert N_2 gas. Plants play a key role in incorporating nitrogen into the food chain, with atmospheric nitrogen transformed into ammonia in the soil through fixation. Microbial processes transform atmospheric nitrogen into organic forms in the soil through fixation. Biological fixation is influenced by environmental factors like water stress and aeration levels, resulting in relatively small amounts of fixed nitrogen under natural conditions (Albrecht et al., 1984). Heterotrophic microorganisms then convert organic nitrogen into reactive inorganic forms (NH_4^+ and NO_3^-) through mineralization and nitrification (Binkley et al., 1989).

These inorganic forms are crucial for plant assimilation, initiating protein and amino acid formation. Within terrestrial ecosystems, the scarcity of natural nitrogen cycle activities makes nitrogen the most limiting nutrient for plant development (Vitousek et al., 1997).

Table 1. Physicochemical parameters of soil samples collected from agricultural fields in Tawang.

Sample code	Moisture Content (%)	Electrical conductivity (µS)	Total Dissolve Solid (ppm)	Salinity (ppm)	Resistivity (KΩ)	pH
DDT1	9.3	158.9	183.7	180.6	2.721	7.21
DDT2	8.7	89.49	103.5	106.3	4.831	7.60
DDT3	9.8	104.1	132.9	119.5	4.099	7.35
DDT4	9.1	96.96	112.1	110.2	4.459	7.21
DDS1	10.2	75.89	69.29	70.13	7.216	7.55
DDS2	10.5	115.9	105.8	102.7	4.694	7.09
DDS3	9.7	68.51	62.53	63.73	7.995	7.27

* DDT = DRL Dettachment Tawang
* DDS = DRL Dettachment Salari

Table 2. Nutrient content of soil samples collected from agricultural fields in Tawang.

Soil sample Code	Mn (mg/100ml)	Mg (mg/100ml)	Fe (mg/100ml)	Ca (mg/100ml)	N (gm/100gm)	P (mg/100ml)	K (mg/100ml)
DDT1	1.47	0.18	17.67	0.18	0.51	0.29	5.27
DDT2	0.96	0.48	23.83	0.29	0.44	0.21	4.25
DDT3	1.19	0.57	23.01	0.09	0.42	0.25	4.87
DDT4	2.14	0.22	21.16	0.24	0.30	0.33	11.31
DDS1	0.58	0.33	34.81	4.96	0.42	0.36	41.92
DDS2	0.37	0.56	21.79	6.77	0.34	0.40	45.83
DDS3	0.32	0.37	20.92	5.69	0.41	0.39	45.37

* DDT = DRL Detachment Tawang
* DDS = DRL Detachment Salari

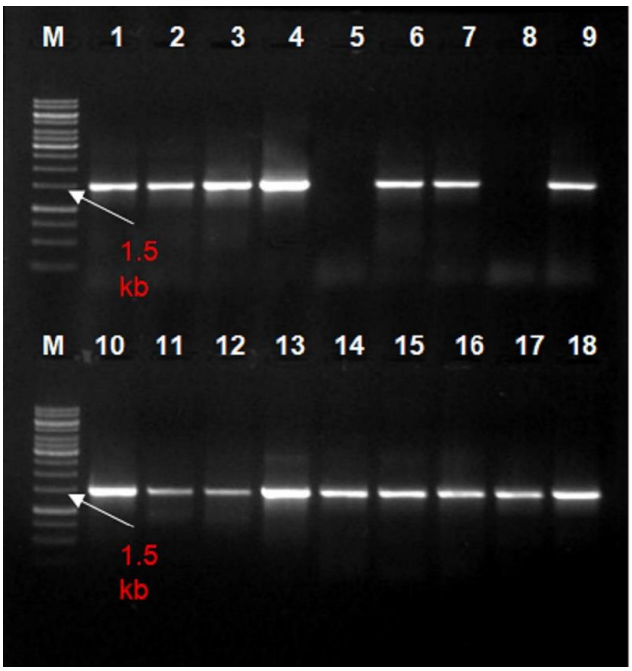

Figure 1. Electrophoretic separation of PCR amplicon of 16S rRNA gene of bacterial isolates isolated from soil samples of Tawang

In tropical, subtropical, and temperate soils, nitrogen-fixing bacteria, like those in the genus *Azospirillum* and *Azotobacter*, are common and form symbiotic partnerships with a variety of wild and cultivated plants (Tyler et al., 1979; Steenhoudt et al., 2000). The focus of research over the last few decades has mostly been on comprehending the molecular aspects of these microorganism's interactions with plants. The primary focus has been on advancing current biotechnological applications, which include agricultural and genetic uses (Burdman et al., 2001; Fedonenko et al., 2001). Intriguingly, investigations into the distribution and ecological roles of nitrogen-fixing microbes have received comparatively fewer spotlights (Tejera et al., 2005). Because of the drawbacks of conventional fertilizers such as soil erosion, environmental impacts, and decreased fertilizer efficiency, implementation of biological nitrogen fixation (BNF), especially with associative nitrogen-fixing bacteria like *Azotobacter* spp., is thought to be a more prudent choice (Choudhury and Kennedy, 2004; Kannan and Ponmurugan, 2010).

The biogeochemical cycles of the soil are controlled by microorganisms, which also manage the cycles of iron, zinc, manganese, nitrogen, sulphur, phosphorus, and potassium. To support general plant growth and development, they metabolise a variety of chemicals, including poisons, inhibitors, and plant growth factors (Sahrawat, 2000; Subba Rao, 2007). Biological nitrogen fixation, as a sustainable and environment friendly means of nitrogen supply to crops, addresses the problems associated with the use of chemical fertilizers. As a result, bacteria that recycle nutrients can be used as biofertilizers, and are an essential part of integrated nutrient supply systems that increase crop yields by providing nutrients in an environmentally friendly manner (Marozsán et al., 2009). According to Robert et al (2015), *Azotobacter* spp. represents a recognized group of heterotrophic, free-living nitrogen-fixing bacteria associated with plants, which enhance the yield of non-leguminous crops. *Azospirillum* is classified as a diazotroph that promotes plant growth, meaning that it fixes atmospheric nitrogen in a way that can be used effectively by plants. It is a kind of free-living bacteria, are usually oblong-rod shaped and do not produce spores. They thrive in environments with limited oxygen, as they are characterized as microaerophilic, indicating their preference for aerobic conditions, but can adjust and live in environments with limited oxygen as well. However, BNF's efficacy varies depending on the environment, and hence utilising native strains is preferable for the optimum results (Kannan and Ponmurugan, 2010).

Arunachal Pradesh, a state in the North Eastern region of India, has an agrarian economy and the farmers follows the traditional system of cultivation, either jhum or terrace farming, which is primarily organic as they do not use fertilizers (Gupta, 2005). The

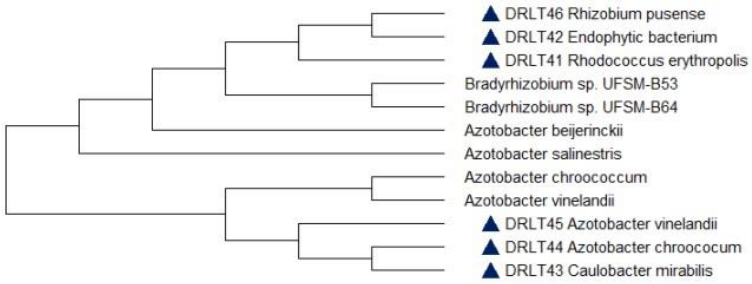

Figure 2. Dendrogram of *Azotobacter* isolates (DRL codes) showing relationship with homologous sequences.

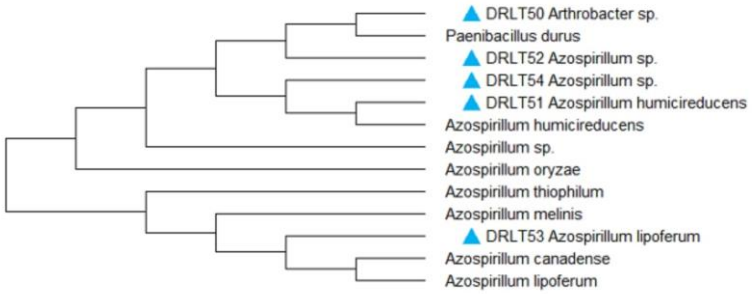

Figure 3. Dendrogram of *Azospirillium* isolates (DRL codes) showing relationship with homologous sequences.

state has huge potential for organic temperate horticulture as a majority of its area falls under the temperate zone owing to the mountainous Himalayan terrain. Due to the increasing demand for organic products, there is great scope for using biofertilizers for organic farming to boost productivity, without compromising soil health and the environment. However, conventional biofertilizers may not exhibit their full potential at high altitude areas owing to the low temperature. Hence, an attempt was made to isolate and screen soil bacteria from agricultural fields of Tawang for use as biofertilizer candidates for phosphate solubilization and nitrogen fixation.

2. Materials and methods

2.1. Sample Collection

Soil samples were collected from agricultural fields located in the Tawang region of Arunachal Pradesh. The samples were collected using a soil auger, from a depth of 30 cm and stored in a sterile polyethylene bag, which were transferred to the laboratory in an ice box for physicochemical and microbiological examinations. For the physicochemical analysis, the soil samples were air-dried and sieved through a 2mm soil sieve to evaluate their physicochemical attributes. For the isolation of PSB, Pikovskaya media was used, while Jensen's agar medium was prepared for isolating nitrogen-fixing bacteria. The single colonies were established using the serial dilution agar plate technique. The cultures were incubated at a temperature of 4°C.

2.2. Identification of bacterial isolates

2.2.1. Colonial morphology of bacterial isolates

The colony morphology was examined on a specific medium. The methodical evaluation of the colonies' characteristics involved considerations such as size, shape, and colour.

2.2.2. Biochemical test

Biochemical assessments, which included tests for hydrogen sulfide production (HS), catalase, oxidase, urease, triple sugar iron (TSI), nitrate reduction, Methyl red (MR), citrate, and others, were conducted. The isolates were subjected to various media for biochemical characterization. Their capabilities in utilizing citrate, reducing nitrate, and producing urease and catalase, were examined (Aneja 2007).

2.2.3. Calculation of Phosphate Solubilization Index (PSI)

The ability of the isolates to solubilize phosphate was assessed by inoculating 10 µL of the cultures onto phosphate-containing media, with each condition replicated five times. After an incubation period of one week at 4°C, the efficiency of phosphate solubilization was assessed. The Phosphate Solubilization Index (PSI) was computed utilizing the following formulae (Edi-Premono et al., 1996).

PSI = Total diameter (colony + clear zone)/diameter of colony

2.2.4. Estimation of Nitrogen-fixing activities

100 µL of an isolate, with a concentration of 10^5 CFU/ml was inoculated into atest tube containing Nfb liquid medium. The mixture underwent continuous stirring at 4 °C during the incubation process for 48 hours. The tubes were centrifuged at 5000 rpm and the absorbance of the supernatant was assessed at 610 nm using a spectrophotometer (Rodriguez et al., 2022).

2.3. Molecular identification

2.3.1. DNA extraction

5 ml of bacterial broth, incubated at 4°C for 2-3 days, was used for extraction of genomic DNA according to the protocol of Moore et al (2004).
Cells were extracted by centrifugation of bacterial culture at 5,000 x g. After resuspending the cell pellet in Tris-HCl buffer, it was disrupted at 37°C after adding EDTA, lysozyme, SDS and RNase A and at 55°C after addition of Proteinase K. Phenol:Chloroform:Isoamylcohol (25:24:1) was added to the pellet to extract the DNA and the supernatant was transferred to a 1.5 ml vial. The process was repeated twice and the pooled supernatant was centrifuged to collect the DNA pellet. The pellet was cleaned with 70% ethanol and was allowed to air dry, which was then dissolved in nuclease free water. Each sample was subjected to three separate replications for accuracy and consistency in the analysis. The concentration and purity of DNA was determined at 260/280 nm using a microvolume spectrophotometer (Biospectrometer, Eppendorf).

2.3.2. 16S rRNA amplification

The genomic DNA was subjected to PCR amplification of 16S rRNA gene, using specific Forward (5′-GAGTTTGATCCTGGCTCA-3′) and reverse (5′-CGGCTACCTTGTTACGACTT-3′) primers (Alam et al., 2006). The PCR reaction mixture of 25µL comprised of template DNA (80 ng), Taq polymerase (1.5 U/µl), 10X Taq polymerase buffer with 100 mM Tris (pH 9), 500 mM KCl, 15 mM $MgCl_2$, dNTP mix (10 mM), and 10 µm of each primer, ensuring precise conditions for amplification.

PCR was set up in a C1000 Thermal Cycler (Bio-Rad), with a program comprising initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 5 minutes, annealing at 48°C for 1 minute, and extension at 72°C for 45 seconds, culminating in a final extension at 72°C for 10 minutes. Visualization of the reaction products involved electrophoresis of 5 µL of the PCR mixture on a 0.8% (w/v) agarose gel, followed by staining with ethidium bromide and visualization using a gel doc system (G Box, Syngene), ensuring accurate detection of amplified DNA fragments. The amplicons were subsequently subjected to Sanger sequencing (Agri Genome Services).

2.3.3. Phylogenetic analysis

The selected isolates, exhibiting promising beneficial traits, were prioritized for further examination *via*. 16S rRNA gene sequencing. Subsequently, the obtained sequences were subjected to BLAST analysis, retrieving homologous sequences from the National Center for Biotechnology Information (NCBI) database. These sequences were then aligned using ClustalW, and their

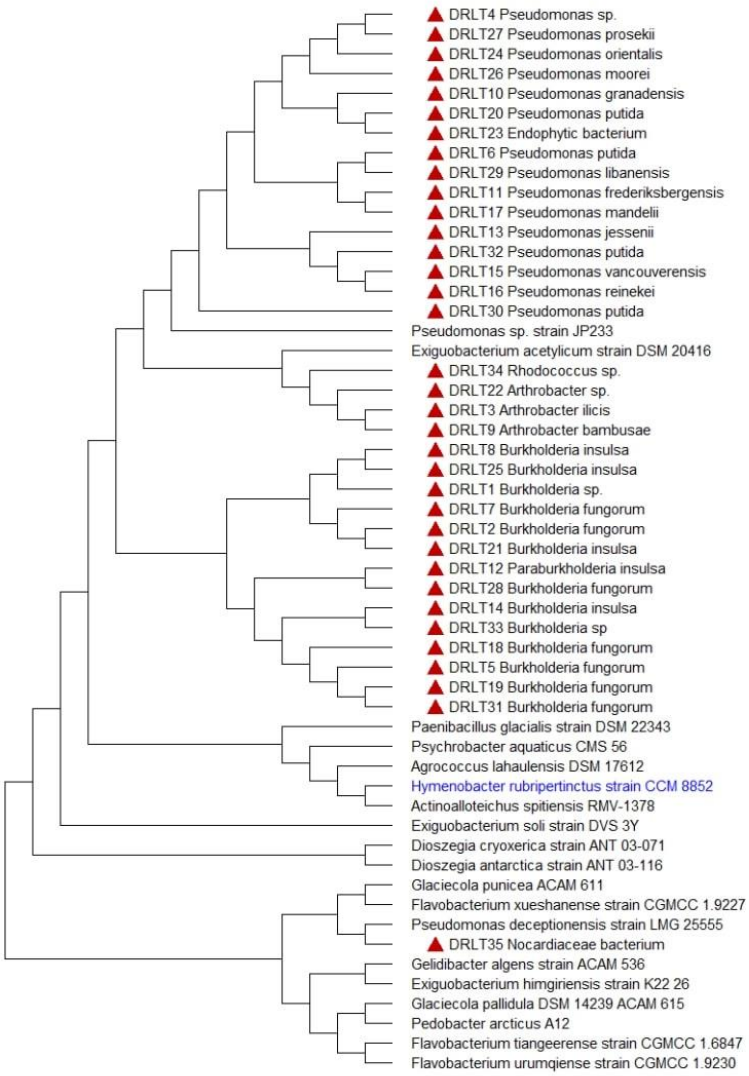

Figure 4. Dendrogram of PSB isolates (DRL codes) showing relationship with homologous sequences.

evolutionary relationships were elucidated using the Neighbor-Joining method in MEGA X software, ensuring a comprehensive analysis of genetic similarities and evolutionary patterns (Chung et al., 2005).

3. Result

3.1. Soil Physicochemical analysis

The representative samples obtained from diverse locations were assessed to evaluate their physicochemical characteristics, and the results are stated in Table 1 and 2.

3.2. Identification of bacterial isolates

A total of 54 bacterial isolates were derived from soil samples collected from Tawang. Through morphological and biochemical analysis, it was determined that a few of the bacterial isolates were gram-positive, with the predominant ones being gram-negative. Notably, the genus *Pseudomonas* emerged as the most prevalent among the isolates. The morphological properties and biochemical characteristics of the bacterial isolates are presented in Tables 3 and 4 respectively.

3.2.1. Phosphate Solubilizing Index (PSI)

54 phenotypically distinct bacterial colonies were isolated from soil samples collected from agricultural fields in the Tawang district of Arunachal Pradesh. Among these, 44 colonies exhibited phosphate solubilization activity, evident through the formation of halo zones on the Pikovskaya agar plate medium. In the Pikovskaya agar medium, the solubilization index of eighteen selected PSBs varied from 2.3 to 4.5 in qualitative experiments. Seven bacterial strains displayed a higher solubilizing zone based on the solubilizing index (Table 5).

3.2.2. Nitrogenase Activity (NA)

10 distinct bacterial isolates displayed nitrogen-fixing activities, ranging from 17 to 53 µg/ml, of which, two isolates displayed higher nitrogenase activity (Table 6).

3.3. Molecular identification

PCR amplification of 16S rRNA gene fragment of the isolates generated a 1.5 kb size amplicon (Figure 1). On sequence analysis of the PCR products, 54 isolates were found to belong to phosphate solubilizing genera such as *Pseudomonas*, *Nocardiacae* and *Burkholderia*, 06 isolates were identified as *Azotobacter* spp., and 05 isolates were classed as *Azospirillum* spp. (Figure 2, Figure 3 and Figure 4).

4. Discussion

The physicochemical examination of the soil samples collected from Tawang showed that the moisture content varied (8.7% in DDT2 to 10.5% in DDS2), while remaining at levels that supported microbial life (Chamizo et al., 2013). DDT1 had the maximum electrical conductivity (68.51 µS to 158.9 µS), along with the corresponding TDS and salinity, indicating a moderate ionic composition (Eigenberg et al., 2002). Bacterial growth was encouraged by the neutral to slightly alkaline soil pH (7.09–7.60). Significant differences in nutrient content were observed, with potassium peaking at 45.83 mg/100ml (DDS2), Phosphorus at 0.40 mg/100g (DDS2), nitrogen at 0.51 g/100g (DDT1), and manganese at 2.14 mg/100ml (DDT4), indicating varied fertility profiles (Johnson et al., 2022; Khan et al., 2023; Zayed et al., 2023; Millaleo et al., 2010).

The morphological and biochemical characterization of the bacterial isolates revealed a preponderance of Gram-negative bacteria along with positive oxidase and catalase activity, which suggested efficient respiration systems. Different metabolic pathways were shown by the diversity of biochemical tests (TSI, urease, and nitrate reductase). With a phosphate solubilization index ranging from 2.3 to 4.5, DRL T 30 showed the highest value. With a peak nitrogenase activity of 53.51 µg/ml, DRL T 52 demonstrated a strong capacity for nitrogen fixation.

The molecular identification of the isolated was conducted through PCR amplification of 16S rRNA gene (Figure 4.7). PSB isolates were shown to cluster with *Pseudomonas* species (*P. putida, P. orientalis*, and *P. mandelii*) in phylogenetic tree analysis (Figure 4.8), indicating their roles in phosphate solubilization through the formation of organic acid and enzymes. The alignment of the isolates such as DRLT6, DRLT23, and DRLT29 with *Pseudomonas* supports their utility in improving phosphate availability to plants.

The phylogenetic analysis of the nitrogen-fixing isolates (Figure 4.9) revealed that DRLT44 and DRLT45 were homologous to the nitrogen-fixing-contributing *Azotobacter chroococcum* and *A. vinelandii, Rhizobium pusense* was associated with DRLT46, indicating symbiotic nitrogen fixation and highlighting the isolates' varied strategies. The ecological significance of Tawang's psychrophilic bacterial isolates for use as biofertilizer is highlighted by these identifications.

Table 3. Morphological properties of the bacterial isolates isolated from agricultural fields in Tawang

Bacterial isolates	Shape	Size	Colour
DRL T 1	Round	Big	Cream
DRL T 2	Irregular	Big	Cream
DRL T 3	Round	Medium	Cream
DRL T 4	Round	Medium	Cream
DRL T 5	Round	Medium	Cream
DRL T 6	Irregular	Medium	White
DRL T 7	Rhizoid	Medium	White
DRL T 8	Round	Medium	White
DRL T 9	Round	Small	Cream
DRL T 10	Irregular	Medium	Cream
DRL T 11	Round	Medium	Cream
DRL T 12	Round	Medium	Cream
DRL T 13	Round	Medium	Cream
DRL T 14	Round	Medium	Cream
DRL T 15	Round	Small	Cream
DRL T 16	Irregular	Big	Cream
DRL T 17	Round	Medium	Cream
DRL T 18	Irregular	Medium	Cream
DRL T 19	Round	Small	Cream
DRL T 20	Round	Big	Cream
DRL T 21	Rhizoid	Medium	Cream
DRL T 22	Round	Medium	Cream
DRL T 23	Round	Medium	White
DRL T 24	Round	Medium	White
DRL T 25	Round	Medium	White
DRL T 26	Round	Medium	Cream
DRL T 27	Round	Big	Cream
DRL T 28	Round	Small	Cream
DRL T 29	Round	Big	Cream
DRL T 30	Round	Big	Cream
DRL T 31	Round	Big	Cream
DRL T 32	Irregular	Medium	Cream
DRL T 33	Irregular	Medium	Pink
DRL T 34	Round	Small	Cream
DRL T 35	Round	Medium	Cream
DRL T 36	Round	Medium	Cream
DRL T 37	Rhizoid	Medium	Cream
DRL T 38	Round	Small	Cream
DRL T 39	Irregular	Small	Cream
DRL T 40	Round	Big	Cream
DRL T 41	Rhizoid	Big	Cream
DRL T 42	Round	Small	Cream
DRL T 43	Round	Small	Cream
DRL T 44	Round	Medium	Cream
DRL T 45	Round	Small	Cream
DRL T 46	Irregular	Small	Cream
DRL T 47	Round	Small	Cream
DRL T 48	Round	Small	White
DRL T 49	Round	Medium	White
DRL T 50	Round	Medium	Cream
DRL T 51	Irregular	Medium	Yellow
DRL T 52	Round	Medium	Cream
DRL T 53	Round	Medium	Cream
DRL T 54	Rhizoid	Medium	White

*DRL T = Defence Research Laboratory Tezpur.
Bacteria were isolated form Tawang soil samples.

The phylogenetic analysis of the *Azospirillum* based nitrogen-fixing isolates showed genetic relationships between *A. humicireducens, A. oryzae,* and *A. lipoferum,* which are grouped into Figure 4.10. This indicates that they share evolutionary features and nitrogen-fixing abilities. Clade A, which included *Paenibacillus durus* and *Arthrobacter* sp, demonstrated phylogenetic differentiation. The branching of Clade B comprised *A. humicireducens* and DRLT51 in one subgroup and other *Azospirillum* species in another, highlighting the role of these bacteria in growth promotion and nutrient cycling and suggesting a shared ancestry and functional similarity.

5. Conclusion

The current study's findings showcased the presence of potential bacterial strains in high altitude region of Tawang for use as biofertilizer candidates in cold regions due to their remarkable resilience to low-temperature conditions. This property is especially important in locations with colder climates, where typical microbial strains may struggle to sustain and function properly. The strains were identified based on 16S rRNA marker sequences and dendogram studies for their relationship with the homologous sequences revealed the distinctiveness of these isolates from microbial populations found in other parts of India. A distinct microbial population tailored to the environmental conditions of the investigated region is suggested by this genetic uniqueness.

In addition, the bacterial strains showed important characteristics for the agriculture use, such as their capacity to solubilize phosphate and fix nitrogen. The ability to solubilize phosphate is crucial, as it indicates that these bacteria possess the capability to enhance phosphorus availability in the soil, a pivotal nutrient essential for optimal plant growth. Moreover, the capacity to fix nitrogen is beneficial, because it allows atmospheric nitrogen to be transformed into a form which plants can easily use for their growth.

These isolates are excellent candidates for the development of cold biofertilizers due to their capacity to flourish in low-temperature zones, phosphate solubilization, and nitrogen fixation. Cold biofertilizers are critical for tackling the unique

Table 4. Biochemical characteristics of bacterial isolates isolated from agricultural fields in Tawang.

Bacterial isolates	Gram strain	Oxidase test	Catalase test	Citrate test	Sucrose test	TSI	HS	Urease	Nitrate reductase	Methyl red
DRL T 01	-ve	-	-	-	-	+	+	-	+	-
DRL T 02	-ve	+	+	+	-	-	-	-	+	-
DRL T 03	-ve	-	-	-	+	+	-	+	+	+
DRL T 04	-ve	-	-	-	+	-	+	+	+	+
DRL T 05	-ve	+	+	+	-	-	-	-	+	-
DRL T 06	-ve	-	-	-	+	-	-	+	+	-
DRL T 07	-ve	-	-	-	-	+	+	-	+	+
DRL T 08	-ve	+	+	+	+	-	+	+	+	-
DRL T 09	-ve	-	-	-	-	-	+	-	+	-
DRL T 10	-ve	-	-	-	-	+	-	-	+	+
DRL T 11	-ve	-	-	-	+	+	-	+	+	-
DRL T 12	-ve	-	-	-	+	-	+	+	+	-
DRL T 13	-ve	+	+	+	-	-	-	-	+	+
DRL T 14	-ve	-	-	-	-	+	-	-	+	-
DRL T 15	+ve	+	+	+	+	+	+	+	-	-
DRL T 16	-ve	-	-	-	-	+	+	-	+	+
DRL T 17	-ve	-	-	-	+	-	+	+	-	-
DRL T 18	-ve	+	+	+	-	-	-	-	+	-
DRL T 19	+ve	+	+	+	-	+	-	+	+	+
DRL T 20	+ve	-	-	-	+	-	+	+	+	-
DRL T 21	+ve	+	+	+	-	+		-	+	-
DRL T 22	-ve	+	-	+	-	-	-	+	-	+
DRL T 23	-ve	-	-	+	+	+	+	-	+	+
DRL T 24	+ve	-	+	-	-	-	-	-	+	+
DRL T 25	+ve	+	+	-	-	-	-	+	-	+
DRL T 26	-ve	+	+	+	-	+	+	-	-	-
DRL T 27	-ve	+	+	+	+	+	-	-	-	-
DRL T 28	-ve	-	+	-	-	-	-	+	+	+
DRL T 29	+ve	+	-	-	+	+	+	+	+	-
DRL T 30	+ve	-	+	+	+	-	-	-	+	-
DRL T 31	-ve	+	-	-	+	+	+	+	+	+
DRL T 32	-ve	+	+	+	-	-	-	-	-	+
DRL T 33	+ve	+	-	-	-	-	-	-	+	+
DRL T 34	-ve	+	-	-	+	+	+	+	-	-
DRL T 35	-ve	+	-	+	+	+	-	-	+	-
DRL T 36	-ve	+	+	+	+	+	-	-+	+	+
DRL T 37	+ve	+	-	-	+	+	+	+	-	+
DRL T 38	-ve	-	-	-	+	-	+	+	-	+
DRL T 39	+ve	+	+	+	+	-	+	-	-	+
DRL T 40	-ve	-	-	+	+	+	-	+	+	+
DRL T 41	-ve	-	+	-	+	+	+	+	+	+
DRL T 42	-ve	+	-	-	-	-	+	-	+	-
DRL T 43	-ve	-	-	+	-	-	-	+	+	-
DRL T 44	-ve	-	+	+	+	+	+	+	+	+
DRL T 45	-ve	+	-	-	-	+	+	-	-	+
DRL T 46	-ve	-	-	-	+	-	+	-	+	-
DRL T 47	-ve	-	+	-	+	+	-	+	-	+
DRL T 48	-ve	+	+	+	-	+	+	-	-	+
DRL T 49	-ve	-	-	-	+	+	+	+	+	-
DRL T 50	-ve	+	-	-	+	-	-	-	+	+
DRL T 51	-ve	-	+	+	-	+	+	-	-	+
DRL T 52	-ve	-	-	-	+		+	+	+	-
DRL T 53	+ve	+	-	-	+	+	-	+	+	-
DRL T 54	-ve	-	+	+	+	+	+	-	+	+

*DRL T = Defence Research Laboratory Tezpur.
Bacteria were isolated form Tawang soil samples.

challenges that colder regions provide to agriculture. These bacterial strains provide a sustainable and eco-friendly way to increase agricultural production in cold climates by increasing nutrient availability and aiding plant growth in low-temperature environments.

Given the growing desire for efficient and environmentally friendly agricultural methods worldwide, the adoption of these strains as candidates for cold biofertilizers offers hope for sustainable agriculture techniques in high-altitude regions. To evaluate the effectiveness and usefulness of these bacterial strains as biofertilizers for high-altitude agriculture, field trials need to be conducted which is being planned.

Table 5. Phosphate Solubilizing Index (PSI) of bacterial isolates isolated from agricultural fields in Tawang.

Isolates	PSI	Isolates	PSI	Isolates	PSI
DRL T 3	3.7	DRL T 4	2.3	DRL T 7	3.2
DRL T 9	2.5	DRL T 10	3.3	DRL T 11	2.7
DRL T 13	2.8	DRL T 15	2.3	DRL T 16	2.7
DRL T 17	3.1	DRL T 20	4.2	DRL T 21	3.6
DRL T 22	3.1	DRL T 26	3.3	DRL T 29	3.5
DRL T 30	4.5	DRL T 33	3.4	DRL T 34	3.9

*DRL T = Defence Research Laboratory Tezpur.
Bacteria were isolated form Tawang soil samples.

Table 6. Nitrogenase Activity (NA) of bacterial isolates isolated from agricultural fields in Tawang.

Isolates	N Concentration (µg/ml)	Isolates	N Concentration (µg/ml)	Isolates	N Concentration (µg/ml)
DRL T 43	17.33	DRL T 44	48.74	DRL T 45	28.19
DRL T 51	19.65	DRL T 52	53.51	DRL T 53	31.72

*DRL T = Defence Research Laboratory Tezpur.
Bacteria were isolated form Tawang soil samples.

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Conflict of interest
The authors declare that there are no conflicts of interest.

Contributions of the authors
[Alok Somvanshi]: Collected field samples and conducted laboratory experiments. Performed data analysis and interpretation of the results. Composed the manuscript.
[Ajitabh Bora]: Conceptualized the research idea, designed experiments, and supervised the project. Assisted in the development of the manuscript. Participated in data visualization and formatting for publication. Reviewed and edited the manuscript for important intellectual content and ensured accuracy of results.
[Baikunth Jyoti Gogoi]: Provided technical support during the experiment and forwarded critical insights into the interpretation of the results.

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