

RESEARCH ARTICLE

The Diversity of *Pseudomonas* species from the Accumulation Zone of Kanchengayao Glacier, North Sikkim, India

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Abstract

Glaciers are the cryospheric niches which support concealed microbial life. They inhabit broad-spectrum culturable and non-culturable bacterial diversity. There is virtually very little information on the psychrophilic/psychrotolerant bacterial diversity found in the glaciers in India. Indian Himalayas are regarded as the world heritage of flora and fauna. As it houses many largest glaciers in its lap, a new venture into glaciers has been started. Microbiological investigation of the glaciers in North-East India will help us to have an insight into the hidden treasure of microflora. We are providing the first report on the *Pseudomonas* sp. diversity from Kanchengayao glacier, North Sikkim, India. It is one of the most dominant genera isolated from glacier ice samples. This genus is one of the most medically and ecologically important groups of Gamma-proteobacteria present in environment. In the present study, the diversity of *Pseudomonas* species isolated from ice core sample was carried out based on the phenotypic and genotypic analysis. It was found that the glacier was abundant in *Pseudomonas azotoformans*; *Pseudomonas poae*; *Stenotrophomonas maltophilia*; *Pseudomonas fluorescens*; *Pseudomonas reactants*; *Pseudomonas hibiscicola* and *Pseudomonas synxantha*. Interestingly, the antibiotic susceptibility test showed that all the isolates were resistant to Ampicillin (10mcg) but all were sensitive to Streptomycin (10mcg), 19 isolates were resistant to Vancomycin (30mcg) and six were resistant against Tetracycline (30mcg) whereas majority of the isolates showed intermediate response. The antibiotic resistance found in this unexplored area is an important study and first of its kind reported from this glacier.

Keywords: *Pseudomonas*, Kanchengayao glacier, Sikkim, 16S rRNA, antibiotic resistance.

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INTRODUCTION

In 1887 Forster had discovered bacteria which can replicate at 0°C. After a few years, Schmidt-Nielsen for the first time coined the term 'psychrophilic' to define the bacteria living under extremely cold conditions^{1,2}. As only minimum growth temperature was believed in this early definition some confuse arose due to the lack of comprehensible definition between cold-loving and cold-tolerant adaptation types³. After the isolation of true psychrophiles in 1960s, cold adapted bacteria were placed into the steno-thermic and eurythermic category and finally Morita coined them into psychrophilic and psychrotrophic / psychrotolerant micro-organisms². True psychrophilic bacteria shows growth at or below 0°C, optimally grows around 15°C to 20°C, whereas psychrotolerant can grows and tolerate temperature above 20°C².

Temperature governs the abiotic and biotic factors which in turn directly dictate the protein machinery and adaptive features of the cryospheric microflora⁴⁻⁶. Cold-dwelling microbes have evolved many such traits that adapt their growth at low temperatures⁷⁻⁸. Both the psychrophilic and psychrotolerant micro-organisms influence the ecology of cryosphere portions of the earth⁹. They play major roles in the biodegradation and recycling of organic matter as well as inorganic nutrients⁹.

The potential for biotechnological applications of psychrophilic and psychro-tolerant bacteria are receiving escalating attention nowadays. Psychrophiles are potential source of novel enzymes for industrial applications and some pigments produced by these microbes are also used as food additive¹⁰. One of the important habitats of psychrophiles are glaciers¹¹. However, the majority of the glaciers studies demonstrate only broad-spectrum bacterial diversity on the surface of the galciers, and virtually very little information exists on which particular psychrophilic or psychrotolerant bacterial species diversity actually reside in those glaciers in the depth zone.

Pseudomonas is one of the most dominant genera reported from many glaciers¹²⁻¹⁴. Along with glaciers, they are present in other cold environments and very often in intimate association with plants and animals. It is one of

the most versatile and ecologically important groups of gamma-proteobacteria present in many environments and it plays a very significant role in nitrogen and carbon cycles¹⁵. Therefore their species diversity studies in glacier ecosystems are crucial in relation to climate change because if there is a sudden deficit of these species diversity in a glacier, then we can co-relate the effects of climate change in glaciers⁹.

In this paper, the diversity of psychro-tolerant *Pseudomonas* species isolated from Kanchengayao glacier ice, North Sikkim, India was studied based on the phenotypic and genotypic analysis. This is the first ever report of *Pseudomonas* species diversity from Kanchengayao glacier of Himalayan valley located at North Sikkim, India.

MATERIALS AND METHODS

Sampling Site

GPS MAP 78S, which is an automated Global Positioning device GPS was used for mapping and to determining the coordinates and land elevation of glacier study sites¹⁶.

Sample collection and preparation

The ice core samples from the Accumulation zone of Kanchengayao glacier was aseptically collected in a sterile thermal cryo sampling box. These sterile sampling containers were sealed tightly and were immediately brought to the laboratory¹⁶⁻¹⁹. For microbiological analysis, the ice core samples were cut aseptically with a sterilized sawtooth knife and around 5 mm annulus of glacial ice core was discarded²⁰. The remaining inner core was first rinsed with cold ethanol (95%) and later on washed with cold sterile water. The ice samples were allowed to melt in the sterile beaker placed at 4°C cold incubator²¹. As described by Zhang all the handling procedures carried out aseptically below 20°C using positive pressure laminar flow hood²².

Physicochemical analysis of glacial ice core samples

The glacial ice core samples were melted at 4°C cold incubator. Physical parameters such as temperature, conductivity, pH, dissolved oxygen (DO) and turbidity were measured with the help of Multi Water Quality Checker U-50 Series Instrument (Horiba, Japan). Chemical constituents like magnesium, calcium, nitrite, nitrate, sulfate,

fluoride, alkalinity, ortho-phosphate, total dissolved solids, silica, iron, and sodium was analyzed by Multi-Parameter Water Testing Kit, WTO15 (Himedia, India). Piper diagram was plotted to draw the analysis²³.

Isolation of bacteria

Bacterial isolation were carried on *Pseudomonas* Isolation Agar (Peptic digest of animal tissue 20g/L; MgCl₂ 1.4g/L; K₂SO₄ 10g/L; Triclosan 0.025g/L; Glycerol 20mL; distilled water 1L). Briefly, 200μL of thawed inner ice core samples were spread plated and incubated at 15°C for 3 weeks. Also, bacterial enrichment was carried out by incubating 100μL water sample in 50 mL *Pseudomonas* isolation broth in 250mL conical flask and then incubated at 15°C for 14 days in incubator cum shaker at 120rpm. After enrichment, bacterial isolation was carried on *Pseudomonas* isolation agar as mentioned above. Morphologically different colonies were selected and sub-cultured by streak plate method¹⁶⁻¹⁹. A total of 22 pure bacterial isolates were isolated from Kanchengayao glacier samples and stored at -80°C in 40% glycerol.

Characterization of bacterial isolates

The morphological, physiological and biochemical characteristics of the selected isolates were evaluated¹⁶. The morphological characters such as colony morphology, Gram-staining, spore staining were performed. The growth of isolates were checked in LB broth at different temperature i.e., 5°C, 10°C, 15 °C, 20°C, 30°C and 40°C in an incubator. Similarly, NaCl dependent growth profile was determined by incubating bacterial isolates in LB media containing different concentrations of NaCl (0%, 1% 2%, 4%, 6%, 8% and 10%) at 15°C incubator. For 0% NaCl concentration in LB media following media composition was used (Casein enzymic hydrolysate: 10g/L; Yeast Extract: 5g/L; d.w. 1L) and pH dependent growth profile were also checked at different range pH (4, 6, 8 and 10) at 15°C in an incubator¹⁶⁻¹⁹.

For protease enzymatic activities SM Agar (Skim Milk powder 28g/L; Tryptone 5g/L; Yeast Extract 2.5g/L; Dextrose 1g/L; Agar 25g/L; distilled water 1L) was used for the demonstration of coagulation and proteolysis²⁴. The proteolytic bacteria hydrolyzed casein to form soluble nitrogenous compounds indicated as clear zone surrounding the colonies. Amylase enzyme

activities were detected by flooding the surface of 48 hours old pure culture on Starch Agar media (Meat extract 3g /L; Peptic digest of animal tissue 5g /L; Starch 2g/L; Agar 25g/L; distilled water 1L) with Iodine solution (Iodine crystals 0.34g; KI 0.66g; distilled water 100mL)²⁵. Amylase positive organisms showed clear halo zone around the colony. Size of the clear halo zone is directly proportional to the starch hydrolyzing activity of the strain under study. Gelatinase hydrolysis was detected on Gelatin Agar (Gelatin 30g/L; Casein enzymic hydrolysate 10g/L; NaCl 10g/L; Agar 25g/L; distilled water 1L). Gelatin positive organisms showed clear halo zone of inhibition around the colony. Size of the clear zone is directly proportional to the gelatin hydrolyzing activity of the strain under study.

Antibiotic resistance profiling of the bacterial isolates

All the bacterial isolates were tested for their response to various antibiotics by disc diffusion method²⁶. The isolates were tested using antibiotic discs for their response to four antibiotics: Ampicillin (10mcg), Vancomycin (30mcg), Tetracycline (30mcg) and Streptomycin (10mcg). All the antibiotic tests were performed in Muller Hinton Agar (MHA) at 15°C incubator. The results were recorded as diameter of the inhibition zone formed which was interpreted from the Zone Size Interpretative Chart.

PCR amplification, sequencing, and analysis of 16S rDNA

The 16S rRNA gene was amplified using universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTACGACTT-3')¹³. For amplification of PCR product, following cycle conditions were used: 94°C for 5 min; 35 cycles of 1min at 95°C, 1min at 55°C, and 2 min at 72°C; and a final extension step of 10 min at 72°C. QIAquick PCR purification kit (Qiagen, Germany) was utilized for purifying the PCR products and subsequently PCR products were sequenced by ABI Applied Biosystems (3500 Genetic Analyzer) using each universal primer i.e., 27F and 1492R. The 16S rRNA gene sequences were aligned with reference gene sequences of related taxa using Clustal W software²⁷. A phylogenetic tree was constructed by MEGA 7 software²⁸ using the neighbor-joining method²⁹.

RESULTS

Physicochemical analysis

The physicochemical analysis of Kanchengayao glacier ice core samples, suggested that all the elements were present in very low concentration (Table 1). The physicochemical parameters were plotted as piper diagram for classification on the basis of chemical composition²³. Piper diagram divides water into four basic types conferring to their location near the four corners of the diamond. Water samples which are rich in (Ca²⁺ + Mg²⁺) and (Cl⁻ + SO₄²⁻) are considered as permanent hardness and placed at the top of diamond, whereas water samples which are rich in (Ca²⁺ + Mg²⁺) and HCO₃⁻ are considered as temporary hardness and placed near

the left corner of diamond. Water samples mainly composed of alkali carbonates (Na⁺ + K⁺) and (HCO₃⁻ + CO₃²⁻) are plotted at the lower corner whereas near the right-hand side of the diamond may be reflected as saline (Na⁺ + K⁺) and (Cl⁻ + SO₄²⁻). The piper diagram suggested that the Kanchengayao glacier ice was Ca-Cl type (Fig. 1).

Table 1. Physicochemical Analysis of glacier sample

Name of the Chemical test	Millipore Water (Control)	Glacier sample
pH	6.9	7.2
Total Dissolved Solid (TDS) (mg/L)	BDL	16
Fluoride Test (ppm)	BDL	0.5
Sulfate Test (ppm)	BDL	10
Nitrate Test (ppm)	BDL	10
Chloride Test (ppm)	BDL	20
Total Hardness Test (ppm)	BDL	100
Orthophosphate Test (ppm)	BDL	0.0
Arsenic Test (ppm)	BDL	0.05
Alkalinity Test (ppm)	BDL	200
Nitrite Test (ppm)	BDL	15
Iron Test (ppm)	BDL	0.3
Silica Test (ppm)	BDL	0.0
Free Chlorine Test (ppm)	BDL	0.0
Turbidity (NTU)	BDL	≥ 10
Dissolve Oxygen (DO) (ppm)	BDL	0.36
Fluoride (ppm)	BDL	0.5
Conductivity (μS/cm)	BDL	0.12
Magnesium (ppm)	BDL	05
Sodium (ppm)	BDL	07
Calcium (ppm)	BDL	14

The data was measured by AQUA CHECK kit (HiMedia, Mumbai, India and Multi Water Quality Checker U-50 Series Instrument (Horiba, Japan)) where BDL refers to "Below Detection Level"; ppm= parts per million.

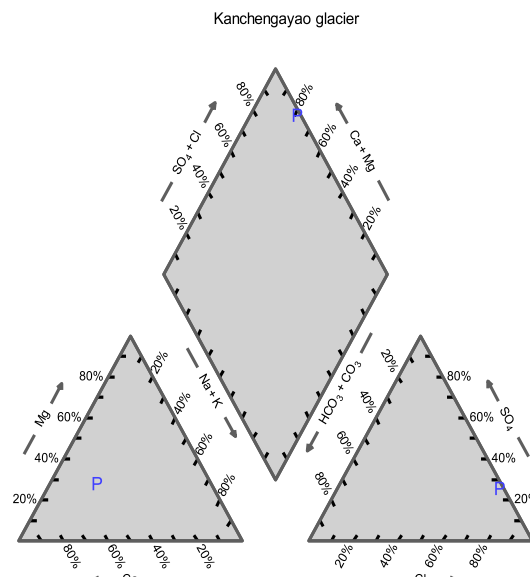


Fig. 1. Piper diagram of Kanchengayao glacier ice physicochemical analysis

Isolation and biochemical characterization of psychrotolerant *Pseudomonas* sp.

Incubation in *Pseudomonas* Isolation Agar at 15°C for 3 weeks at 15°C helped us to isolate psychrotolerant *Pseudomonas* sp. from glacier ice core samples. Twenty two pure bacterial isolates, isolated from enrichment method and streak plate method, were preserved in 40% glycerol at -80°C. Based on morphological characteristics, these bacterial isolates were selected for further analysis, i.e., Gram-staining, spore staining, colony morphology, growth temperature, NaCl and pH tolerance (Table 2). The bacterial isolates were all rod-shaped, non-spore formers and mostly produced white cream color colonies as shown in (Table 2). The Gram-staining results showed that all the isolates were Gram-negative. Among 22 bacterial isolates, during the initial screening for enzymatic activity of the isolates, it was observed that four isolates (KGG15, KGG22, KGG60, KGG62) were positive for amylase production, four isolates

Table 2. Morphological characterization of the bacterial isolates

Isolates	Growth on agar plates				Morphology on the basis of staining		
	Colony color	Margin	Elevation	Form	Simple Staining	Gram Staining	Spore Staining
KGG2	Yellow	Erose	Flat	Irregular	Rod	-ve	-ve
KGG6	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG13	White	Entire	Flat	Circular	Rod	-ve	-ve
KGG14	White	Entire	Flat	Circular	Rod	-ve	-ve
KGG15	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG16	White	Erose	Flat	Circular	Rod	-ve	-ve
KGG17	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG20	Yellow	Entire	Flat	Irregular	Rod	-ve	-ve
KGG22	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG25	White	Entire	Flat	Circular	Rod	-ve	-ve
KGG28	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG29	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG35	Brown	Erose	Flat	Circular	Rod	-ve	-ve
KGG38	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG44	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG50	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG45	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG51	White	Entire	Flat	Circular	Rod	-ve	-ve
KGG53	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG59	White	Undulate	Flat	Circular	Rod	-ve	-ve
KGG61	White	Entire	Convex	Circular	Rod	-ve	-ve
KGG62	White	Undulate	Flat	Circular	Rod	-ve	-ve

where (-ve) indicates in the case of Gram staining that the isolates were Gram Negative bacteria and in case of spore staining the isolates showed negative response i.e. they did not form any spores.

(KGG17, KGG25, KGG28, KGG59) were positive for protease production and all the isolates were catalase positive as shown in Fig. 2. Carbohydrate fermentation test showed that majority of the isolates were able to ferment simple sugars like dextrose, whereas few isolates were able to ferment sugars like dulcitol, lactose, mannose, arabinose, raffinose, and ribose, however, most

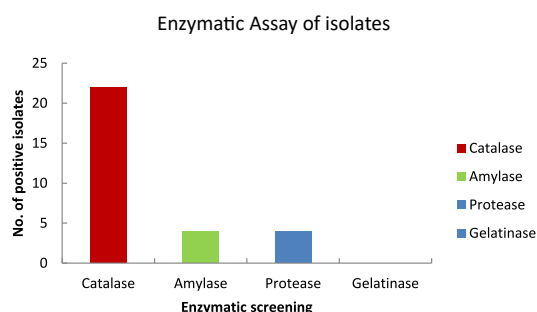


Fig. 2. Enzymatic activity test of Kanchengayao glacier isolates

of the bacterial isolates were unable to ferment fructose, sucrose, and xylose as shown in Table 3.

Antibiotic resistance

Recent studies have detected antibiotic resistant bacteria and their resistance genes in the natural environment which are geo-graphically isolated from such area³⁰⁻³². Interestingly from Kanchengayao glacier ice core samples we also found three class of antibiotic resistant bacteria against penicillin (Ampicillin 100%), glycopeptides (Vancomycin 86.36%) and Tetracycline 27% respectively (Table 4). This paves for an interesting study for future as these glaciers are never exposed to antibiotics and also are usually free of any anthropogenic activities.

Tolerance to different; Temperature, NaCl and pH

The temperature dependent growth profile of the isolates showed that the majority of the isolates had optimum growth at 20°C but all could tolerate temperature >25°C≤ 40°C (Fig.

Table 3. Carbohydrate Fermentation Test of the bacterial isolates

Isolates	Carbohydrate									
	(D-) Arabinose	(D+) Dextrose	(D+) Dulcitol	(D-) Fructose	(D+) Lactose	(D-) Mannose	(D+) Raffinose	(D-) Ribose	(D+) Sucrose	(D+) Xylose
KGG2	+	+	-	-	+	+	-	-	-	-
KGG6	-	+	-	-	-	-	-	-	-	-
KGG13	-	-	-	-	-	-	-	-	-	-
KGG14	-	-	-	-	-	-	-	-	-	-
KGG15	-	+	-	-	-	-	-	-	-	-
KGG16	-	+	-	-	-	-	-	-	-	-
KGG17	-	+	-	-	-	-	-	-	-	-
KGG20	+	+	-	-	+	+	-	-	-	-
KGG22	-	+	-	-	-	+	-	-	-	-
KGG25	-	-	-	-	-	+	-	-	-	-
KGG28	-	+	-	-	-	-	-	-	-	-
KGG29	-	+	-	-	-	-	-	-	-	-
KGG35	-	-	-	-	-	-	-	-	-	-
KGG38	-	+	-	-	-	-	-	-	-	-
KGG44	-	+	-	-	-	-	-	+	-	-
KGG50	-	+	-	-	-	-	-	-	-	-
KGG45	-	+	-	-	-	-	-	-	-	-
KGG51	-	-	+	-	-	-	+	+	-	-
KGG53	-	-	+	-	-	-	+	+	-	-
KGG59	-	+	+	-	-	-	-	-	-	-
KGG61	-	-	-	-	-	-	-	-	-	-
KGG62	-	+	+	-	-	-	-	-	-	-

The isolates were tested against various carbohydrates and the (+) sign indicates that the isolates were able to ferment that particular carbohydrate and (-) sign indicates that the isolates were unable to ferment that particular carbohydrate.

3). It suggested that these isolates are psychrotolerant, rather than psychrophilic in nature. The salinity tolerance was also tested and it was found that majority of the isolates grew well at minimum concentrations of NaCl at 15°C (Fig. 4). pH dependent growth profile suggested that nine isolates showed good growth at pH5 and pH6, followed by three isolates at pH8 and two isolates at pH2 (Fig. 5). From our study we found that 11 bacterial isolates were acidophilic in nature, while others preferred neutral pH or slightly alkaline pH.

Phylogenetic analysis of 16S rDNA sequencing of isolates

Based on the similarity criterion of at least 99% of the 16S rRNA gene sequence, it was found that all 22 bacterial isolates belonged to genus *Pseudomonas* and was from seven diversified *Pseudomonas* species. 13 isolates showed 99% homology to *Pseudomonas azotoformans*, two isolates had 99% homology to *Pseudomonas poae*, three isolates had 99% homology to *Stenotrophomonas maltophilia*

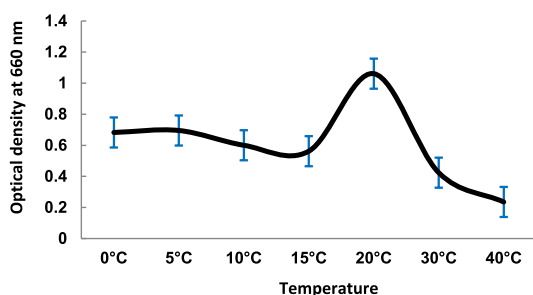


Fig. 3. Temperature Dependent growth of Kanchengayao glacier isolates

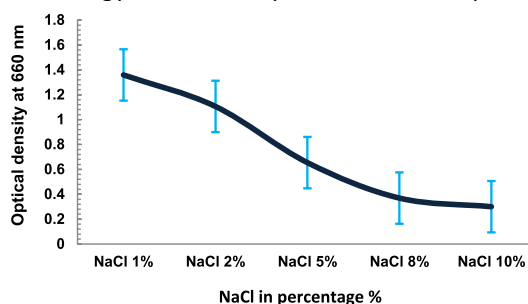
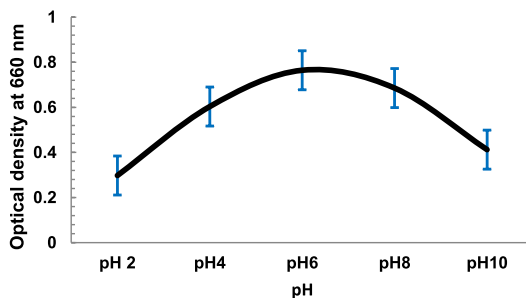


Fig. 4. NaCl Dependent growth of Kanchengayao glacier isolates

Table 4. Antibiotic Assay of the bacterial isolates

Isolates	Antibiotic disc with their Concentration			
	Zone of inhibition (in mm)			
	Van30	TE30	Amp10	S10
KGG2	R	R	R	S
KGG6	R	R	R	S
KGG13	R	R	R	S
KGG14	R	S	R	S
KGG15	R	I	R	S
KGG16	R	I	R	S
KGG17	R	I	R	S
KGG20	S	R	R	S
KGG22	R	I	R	S
KGG25	S	R	R	S
KGG28	R	I	R	S
KGG29	R	I	R	S
KGG35	S	R	R	S
KGG38	R	I	R	S
KGG44	R	I	R	S
KGG50	R	I	R	S
KGG45	R	I	R	S
KGG51	R	S	R	S
KGG53	R	S	R	S
KGG59	R	I	R	S
KGG61	R	I	R	S
KGG62	R	I	R	S

The isolates were tested against various antibiotics and the (R) sign indicates that the isolates were resistant, (I) sign indicates that the isolates intermediate and (S) sign indicates that the isolates sensitive.

**Fig. 5.** pH Dependent growth of Kanchengayao glacier isolates

and others were *Pseudomonas fluorescens*, *Pseudomonas reactants*, *Pseudomonas hibiscicola* and *Pseudomonas synxantha*. The accession number and identity of all the isolates are given in Table 5. The phylogenetic tree was made using MEGA7 using the Neighbor-Joining method Fig. 6.

DISCUSSION

Diverse form of microorganisms, including algae and fungi, has been reported on the surface of many glaciers around the world. These microorganisms make up the primary colonizer of the snow and ice, which also include cold-tolerant animals such as insects and copepods. In North East Himalayas, Kanchen-gayao glacier is located at North Sikkim, India and it is a debris-free (clear glacier) transverse valley glacier (Fig 7). This glacier originated from the southern slope of Mount Kanchengayao, treading north-south face having latitude 27°59'57.872'N and longitude 88°37'8.785'E at an altitude of 1393m. The melt water of this glacier feeds into river Thangu which is a tributary of river Lachen. Kanchengayao glacier is surrounded by a cold desert and in its lower valley small Rhododendron trees are present along with some other vegetation. Possibly, nutrient and bacteria from nearby or distantly located places gets transferred to accumulation zone of glacier through air currents³³. Also, the soil bacteria might affect the distribution of *Pseudomonas* species on the surface of the glacier accumulation zone.

Our result showed that the most dominant *Pseudomonas* species on Kanchen-gayao glacier were *Pseudomonas azotoformans* followed by *Pseudomonas poae*, *Stenotrophomonas maltophilia*, *Pseudomonas fluorescens*, *Pseudomonas reactants*, *Pseudomonas hibiscicola* and *Pseudomonas synxantha*. Diversity of *Pseudomonas* sp. in glaciers has been reported in various parts of the world¹²⁻¹⁴. These gram negative bacteria are abundant in environmental niches and are clinically important also. The most abundant *Pseudomonas* sp. in our glacier ice core samples was *Pseudomonas azotoformans*. This gram negative bacterium has been reported as an effective biocontrol agent against plant pathogens³⁴⁻³⁶ and it also was reported as a good biotechnological candidate which has biomineralization property to fix the concrete cracks by producing calcite³⁷. So, it will be interesting to study this psychrotolerant bacterium isolated from glacier in future. *Pseudomonas poae* is also reported to have biocontrol activity^{38,39}. *Pseudomonas fluorescens* is an aerobic plant growth promoting rhizobacterium (PGPR) and has biocontrol activity^{40, 41}. Some bacteria have the ability to synthesize nanoparticles metabolically and this has amazed the scientific

Table 5. Identification of the bacterial isolates through GenBank 16S rRNA gene sequence accession numbers

Isolates	Closest Relative Species	% of Identify	Gene Bank Accession No.
KGG35	<i>Pseudomonas fluorescens</i>	99.42	KY129832
KGG59	<i>Pseudomonas reactants</i>	99.3	KY129833
KGG13	<i>Pseudomonas hibiscicola</i>	99.4	KY129834
KGG2	<i>Stenotrophomonas maltophilia</i>	99	KY129838
KGG14	<i>Pseudomonas synxantha</i>	99	MH079449
KGG15	<i>Pseudomonas azotoformans</i>	99	MH079450
KGG51	<i>Pseudomonas poae</i>	99.4	MH079451
KGG62	<i>Pseudomonas azotoformans</i>	99	MH079452
KGG6	<i>Pseudomonas azotoformans</i>	99	MH157226
KGG20	<i>Stenotrophomonas maltophilia</i>	99	MH157227
KGG25	<i>Stenotrophomonas maltophilia</i>	99	MH157228
KGG28	<i>Pseudomonas azotoformans</i>	99	MH157229
KGG38	<i>Pseudomonas azotoformans</i>	99	MH157230
KGG44	<i>Pseudomonas azotoformans</i>	99	MH157231
KGG45	<i>Pseudomonas azotoformans</i>	99	MH157232
KGG53	<i>Pseudomonas poae</i>	99.33	MH157233
KGG50	<i>Pseudomonas azotoformans</i>	99	MH157234
KGG61	<i>Pseudomonas azotoformans</i>	99	MH157235
KGG16	<i>Pseudomonas azotoformans</i>	99	MH157236
KGG17	<i>Pseudomonas azotoformans</i>	99	MH157237
KGG22	<i>Pseudomonas azotoformans</i>	99	MH157238
KGG29	<i>Pseudomonas azotoformans</i>	99	MH157239

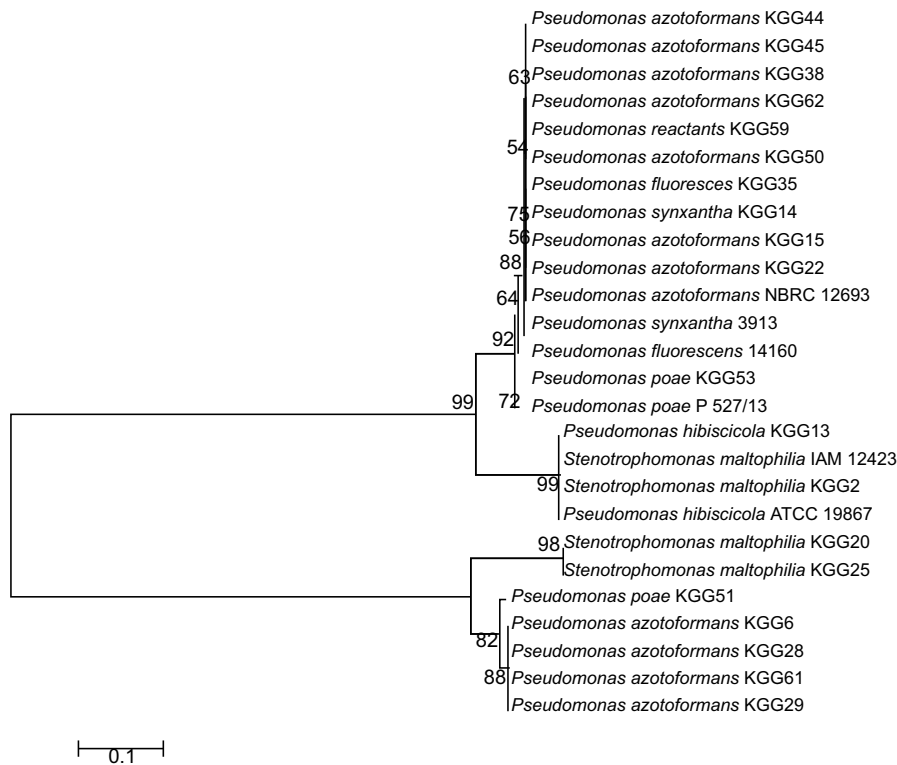


Fig. 6. Phylogenetic tree of Kanchengayao glacier isolates



Fig. 7. Kanchengayao glacier

community and industries at large for harnessing this unique capability to produce nanoparticles in cost effective method^{42,43}. One of our isolates *Pseudomonas hibiscicola* have been reported in many literatures as a potential candidate showing this property^{44,45}. So, it will be another interesting study in future. *Stenotrophomonas maltophilia* is an environmental bacterium found in various ecological niches like river, plant rhizospheres, soil etc. It is an emerging multi-drug-resistant global opportunistic pathogen and it was also present in our glacier sample.

Glacier study shall reveal in future many such diversified microorganisms and their biotechnological applications render them as suitable candidates for future industrial strains. Sikkim glacier houses plethora of microbes and it's an important one in terms of monitoring climatic changes in the Himalayas.

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CONFLICT OF INTEREST

The author declares that there are no conflict of interest.

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