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Cultivable bacterial diversity along the altitudinal zonation and vegetation range of tropical Eastern Himalaya

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Abstract: The Northeastern part of India sprawls over an area of 262 379km^2 in the Eastern Himalayan range. This constitutes a biodiversity hotspot with high levels of biodiversity and endemism; unfortunately, is also a poorly known area, especially on its microbial diversity. In this study, we assessed cultivable soil bacterial diversity and distribution from lowlands to highlands (34 to 3 990m.a.s.l.). Soil physico-chemical parameters and forest types across the different altitudes were characterized and correlated with bacterial distribution and diversity. Microbes from the soil samples were grown in Nutrient, Muller Hinton and Luria-Bertani agar plates and were initially characterized using biochemical methods. Parameters like dehydrogenase and urease activities, temperature, moisture content, pH, carbon content, bulk density of the sampled soil were measured for each site. Representative isolates were also subjected to 16S rDNA sequence analysis. A total of 155 cultivable bacterial isolates were characterized which were analyzed for richness, evenness and diversity indices. The tropical and sub-tropical forests supported higher bacterial diversity compared to temperate pine, temperate conifer, and sub-alpine rhododendron forests. The 16S rRNA phylogenetic analysis revealed that *Firmicutes* was the most common group followed by *Proteobacteria* and *Bacteroidetes*. Species belonging to the genera *Bacillus* and *Pseudomonas* were the most abundant. Bacterial CFU showed positive but insignificant correlation with soil parameters like pH (r=0.208), soil temperature (r=0.303), ambient temperature (r=0.443), soil carbon content (r=0.525), soil bulk density (r=0.268), soil urease (r=0.549) and soil dehydrogenase (r=0.492). Altitude (r=-0.561) and soil moisture content (r=-0.051) showed negative correlation. Altitudinal gradient along with the vegetation and soil physico-chemical parameters were found to influence bacterial diversity and distribution. This study points out that this is a biome with a vast reservoir of bacteria which decrease with increasing altitudes, and highlights the microbiological importance of the poorly studied Eastern Himalayan range, justifying efforts to explore the prevalence of novel species in the biome. Rev. Biol. Trop. 61 (1): 467-490. Epub 2013 March 01.

Key words: cultivable bacteria, Eastern Himalyan range, tropical region, altitude, 16S rRNA, diversity indices.

The Eastern Himalayan range, one of the biodiversity hotspots (Myers *et al.* 2000), is facing imminent threat due to anthropogenic interventions through tourism, agriculture, industrial activities and housing. This hotspot known to harbor plants and animals with high levels of biodiversity and endemism, is poorly understood as far as microbial diversity is concerned. Despite its high potential for biodiversity conservation, Eastern Himalayan range has attracted little attention from scientists and conservationists. As strategies for conserving species and communities focuses as much on species richness and endemism (Lovett *et al*. 2000, Myers *et al*. 2000, Kier & Barthlott 2001), information about the microbiologically unexplored Eastern Himalayan range would greatly increase the potential of finding novel species (Kapur & Jain 2004).

Soil microbiota plays important roles in soil aggregation and cycling of nutrients through cellulose and lignin breakdown, and

nitrogen fixation (Donnelly *et al*. 1990, Hu & van Bruggen 1997, Atlas & Bartha 2002). The microbial diversity of soil is huge (Alexander 1977, Rondon *et al*. 1999, Ward 2002) and hence, a representative estimate of microbial diversity is a prerequisite for understanding the functional activity of microorganisms in ecosystems (Garland & Mills 1994, Zak *et al*. 1994). The role of soil enzymes is well established by way of relationships among soil enzymes, the environmental factors and biological transformations important to soil fertility (Moore & Russell 1972, Paul & Mclaren 1975, Tabatabai 1977, Bremner & Mulvaney 1978, Brzezinska *et al*. 1998, Baum *et al.* 2003, Makoi & Ndakidemi 2008, Gao *et al*. 2010, Sumathi *et al.* 2012). Our knowledge of soil microbial diversity is limited in part by our inability to culture them and other by the lack of survey in certain areas of the Earth. However, surveying of 16S rRNA genes targeted by primers in soils, as well as other techniques like metagenomics, has permitted a more direct census of studying soil microbial diversity (Kirk *et al*. 2004, Palmer *et al*. 2006).

The immense variety of the climatic, edaphic and altitudinal variations have resulted in a great range of diverse and extreme ecological habitats in the Eastern Himalayan range. Lying between 22º11'190'' - 28º23'261'' N and 89º86'821'' - 97º42'683'' E, and sprawling over 262 379km^2, Northeastern region of India represents the transition zone between the Indian, Indo-Malayan and Indo-Chinese biogeographic regions and is a meeting place of the Himalayan mountains with peninsular India. The region occupies 7.7% of India's total geographical area and supports a rich biodiversity spanning from tropical evergreen forests, temperate conifer, to sub-alpine rhododendron forests. The faunal and floral diversity of this region (Chatterjee *et al*. 2006) including the mountain forests of Asia (Culmsee *et al*. 2011) have been well studied. However, information on microbial diversity from this range is relatively sparse and no comprehensive study has so far been made to explore and document the bacterial diversity of the region as a whole.

Most studies of bacterial diversity in soils have focused on inland desert or coastal locations (Vishniac 1993). Investigations of bacteria in extreme environments such as the Antarctic region soils have focused on abundance and diversity and the influence of climatic conditions. These bacteria were assigned to known genera including *Arthrobacter, Micrococcus, Bacillus* and *Pseudomonas* (Vishniac 1993). This study is the first attempt to assess the bacterial diversity along the high altitudes of the Eastern Himalayan range under different forests types and soil physico-chemical conditions. This aimed at generating a baseline data on the cultivable bacterial diversity from this unique place of the world.

MATERIALS AND METHODS

Research area: Northeast India in the Eastern Himalayan range is lying between 22º11'190'' - 28º23'261''N and 89º86'821'' - 97º42'683''E, and sprawling over 262 379km^2; this represents the transition zone between the Indian, Indo-Malayan and Indo-Chinese biogeographic regions, and a meeting place of the Himalayan mountains and peninsular India, characterized by a great range of ecological habitats under different forests types, that were selected for the present study. Soils from 40 different locations covering different altitudes and having different vegetation types (Champion & Seth 1968) were sampled and analyzed during 2008 to 2011. Based on the altitude having specific vegetation and forest types, the sites were grouped into four altitudinal ranges *viz*., AR-I (34m.a.s.l to 1 000m.a.s.l.), AR-II (1 001m.a.s.l. to 2 000m.a.s.l.), AR-III (2 001m.a.s.l. to 3 000m.a.s.l.) and AR-IV (3 001m.a.s.l. to 3 990m.a.s.l.).

Sample collection and sampling site parameters: Soil samples were collected using a sterilized soil corer (inner diameter 5.5cm) from a depth of 10-30cm. In order to document maximum bacterial population and diversity, five soil samples were selected from each location and the samples were pooled before

analysis. The soil samples were kept in sterile containers and stored at 4ºC until processing within 24h (Joshi *et al*. 1991). The geographical location of each sample collection site was recorded using digital GPS (Garmin 7.6).

Soil parameters: The soil temperature was measured on site by using a soil thermometer. Soil pH was measured using a soil-water mixture 1:5 (w/v) with a pH meter. Soil moisture content was determined gravimetrically by oven drying 10g of fresh sieved soil for 24h at 105ºC (Anderson & Ingram 1993). Furthermore**,** bulk density was determined by Blake & Hartge (1986) method using soil corer, while soil organic carbon was determined by using Walkley & Black's rapid titration method as described by Anderson & Ingram (1993).

Soil Dehydrogenase activity: The dehydrogenase is used as a general criterion to determine soil microbial activity and is considered a good measure of soil microbial oxidation activity; soil bacterial dehydrogenase activity was estimated following the method as described by Kumar (2011). After incubating at 37ºC for 24h, the formazan formed was extracted with 10mL ethanol and estimated spectrophotometrically at 485nm. The formazan concentration was calculated from its standard curve. The dehydrogenase activity is expressed as gram of formazan formed per gram of dry weight of soil.

Soil Urease activity: Soil enzymes activities including urease activities are sensors of soil degradation since they integrate information about microbial status and soil physicochemical conditions (Wick *et al.* 1998, Aon & Colaneri 2011, Baum *et al.* 2003); the urease test has also been used to detect bacteria from soil (Zaved *et al*. 2008). Urease activity in soils was assayed by the buffer method (Tabatabai 1994) which involves the determination of the NH_4^+ released when a soil sample is incubated with THAM (Tris hydroxymethyl aminomethane) buffer at the optimal pH (pH 9.0) with or without toluene, and urea (0.2M) at 37ºC for 2h.

Isolation, enumeration and preservation of bacteria: The total colony forming units (CFU) of cultivable soil bacteria were determined using a soil dilution plate-count technique. A total of 10g of soil samples were suspended in 100mL sterile 0.85% NaCl solution and mixed by shaking in a shaker incubator at 120rpm for 4-5min to establish a dilution series; these were used as inoculum for plate count of colony forming units (CFU). Aliquots of 100µL from different dilutions were transferred and spread onto Nutrient agar, Muller Hinton agar and Luria-Bertani agar plates in triplicates separately. Three different culture media were used in order to record maximum diversity of cultivable bacteria; however, nutrient agar was considered as the standard media based on the isolation efficiency. The agar plates were incubated at 35-37ºC for 24-48h and colonies formed on the plates were counted for enumeration. Well-isolated colonies with different morphologies were randomly chosen from each plate and streaked on fresh plates. Isolates were checked for purity by re-streaking and then inoculated into nutrient agar slants and stored at 4ºC. Simultaneously, the pure cultures of isolates were preserved in 20% glycerol water at -20ºC (Kumar *et al*. 2011).

Growth and preliminary characterization: Colony morphology was determined after two to seven days growth on agar plates incubated at 35-37ºC. Each isolate was subjected to Gram staining and was examined for cellular morphology and arrangement. Various tests for biochemical characterization were performed following standard protocols given by Holt *et al*. (1994).

Molecular characterization: A total of 155 selected isolates were subjected to 16S rDNA sequence analysis following the methods as described by Kumar *et al*. (2011). Briefly, Genomic DNA was extracted using Genomic DNA isolation kit (HiPurA Bacterial and Yeast

DNA Purification Spin Kit, HiMedia, India). The 16S rRNA gene sequences were amplified by Gene AMP 9700 (Applied Biosystems, USA) PCR using two universal bacterial 16S rRNA gene primers (i.e., 27F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'-TACGGYTACCTTGTTACGACTT-3'). PCR mixtures (25µL) contained approximately 30ng of DNA, 2µM each forward primer 27F and reverse primer 1492R, 1.5mM of $MgCl_2$ (Taq Buffer), deoxynucleoside triphosphates (250µM each of dATP, dCTP, dGTP and dTTP) and 0.6U of Taq polymerase. PCR cycle used for the reaction comprised an initial denaturation for 5min at 94ºC, and this was followed by 30 cycles each of denaturation at 94ºC for 1min, annealing at 55ºC for 1min, and extension at 72ºC for 2min, and the final extension for 5min at 72ºC. Approximately, 1 500 nucleotides were amplified. For negative controls sterile distilled water was used in place of DNA template. Amplified products were purified using QIAquick Gel Extraction Spin Kit (QIAGEN, Germany).

The purified PCR products were bi-directionally sequenced by Genetic Analyzer ABI 3130XL (Applied Biosystems, California, USA) with Big Dye (3.1) Terminator protocol using the forward, reverse and internal primers corresponding to *Escherichia coli* positions 357F, 926F, 685R and 1100R. Sequencing reaction was performed with 20µL reaction mixture containing approximately 50ng of template DNA and 1pmol of sequencing primers. Post reaction cleanup and resuspension were performed for removal of unincorporated dye terminators from the sequencing reaction using 125mM EDTA, 3M sodium acetate and 70% ethanol. Basic Local Alignment Search Tool (BLAST) was used to determine the phylogenetic neighbors from the nucleotide database of National Centre for Biotechnology Information (NCBI) (Altschul *et al*. 1997) and EzTaxon (the database of type strains with validly published prokaryotic names available online at http://www.eztaxon.org/) (Chun *et al*. 2007).

The correlation between the different environmental factors, soil physico-chemical parameters and bacterial counts was determined by calculating Pearson product moment correlation coefficients (Zar 1984). The correlations were considered significant if $p<0.05$. Karl Pearson Correlation Coefficient (Parametric test) was done using Minitab 11.5 Statistical Software to study the influence of soil physico-chemical and environmental parameters on soil bacterial distribution/diversity. The diversity of the bacterial community was calculated using the Shannon-Wiener's (H') method (Jost 2006). However, the entropy value (H') of the Shannon index is not itself a measure of diversity. Conversion of this value to effective number of species, or true Diversity (D), is the key to a unified and intuitive interpretation of diversity (Jost 2006). The complement of Simpson's index, (d') (Krebs 1978) was also estimated. Bacterial alpha diversity was estimated with Fisher's α (Magurran 2004). Evenness (J) of species was evaluated using the formula as given by Pielou (1966). The number of species divided by the square root of the number of individuals results in Species richness, ***S***. Species accumulation curve (Colwell *et al*. 2004), show the rate at which new species are found within a community and can be extrapolated to provide an estimate of species richness. It was plotted using PAST 2.10 software (Hammer *et al*. 2001).

RESULTS

Research area and soil physico-chemical properties: Tropical and sub-tropical forests were prevalent at lower altitudes whereas temperate pine to temperate conifer and sub-alpine rhododendron forests were abundant at higher altitudes (Table 1). The prevalent vegetations, ambient temperature, soil pH, soil temperature, soil moisture content, soil carbon content and bulk density were found to vary along the altitudes (Table 1). Soil physico-chemical factors, ambient temperature and/or substrate availability were found to influence soil bacterial growth and population density at various level of significance ($p<0.05$) (Fig. 1). Soil carbon was found to have significant positive

TABLE 1
Physico-chemical properties of soil, CFU counts and microbial enzymatic activities in the soil collected from different survey sites situated at different altitudes of the eastern Himalayan range along with forest types

Altitudinal Range	Survey site with altitude (m.a.s.l.)	Soil pH	Soil Temp.(^{0}C)	Ambient Temp. (^{0}C)	Moisture Content (%)	Carbon Content (%)	Bulk Density (g/cm^3)	Urease (mg) NH_4 + -N/g dry soil	Dehydrogenase (mg) TPF/g dry soil	Colony Forming Unit (CFU) /g dry soil	Forests types with dominant vegetation
AR-I	West Chandrapur (34)	6.2	26	27.5	17.2	5.01	1.2	0.28	0.14	$6 \times 10^3 \pm 1.0 \times 10^3$	Tropical evergreen, Tropical semi- evergreen, Tropical pine to Sub-tropical evergreen, Mixed moist deciduous forests (*Myrica* spp., *Altingia excela*, *Pyrus* sp., *Shorea robusta*, *Mesua ferra*, *Clerodendron* sp., *Dipterocarpus retusus*, *Exbucklandia* sp., *Castanopsis* sp., *Quercus grifithi*, *Duabanga grandiflora*, *Terminalia indica*, *Mesua ferra*, *Bombax ceiba*, *Elaeocarpus* spp., *Quercus* spp., *Terminalia myriocarpa*, *Gmelia arborea*, *Magnolia* spp., *Michelia oblonga*).
	Naharlagun (134)	6.4	21	31	21	4.89	1.1	0.26	0.16	$2.4 \times 10^6 \pm 0.8 \times 10^6$	
	Fifth Mile (210)	4.3	24	25.8	10.8	4.92	1.2	0.22	0.14	$1.0 \times 10^6 \pm 0.5 \times 10^6$	
	Ganga lake (336)	4.9	19	26.1	19	4.91	2.1	0.22	0.12	$6.4 \times 10^5 \pm 0.9 \times 10^5$	
	Medziphema (412)	4.8	24	29.1	13.2	4.88	1.0	0.21	0.11	$1.1 \times 10^6 \pm 0.6 \times 10^6$	
	Rimassar (562)	5.0	20.5	23.7	6.5	4.68	1.2	0.23	0.12	$2.2 \times 10^4 \pm 0.4 \times 10^4$	
	Mawshun (688)	4.9	19.5	25.9	3.4	4.76	1.1	0.24	0.14	$5.1 \times 10^5 \pm 0.9 \times 10^5$	
	Keibul Lamjao (785)	4.7	26	34.7	17.6	4.42	1.1	0.19	0.14	$6.0 \times 10^3 \pm 0.8 \times 10^3$	
	Lawngthlai (829)	5.7	28	30.4	16	4.3	1.0	0.20	0.12	$1.8 \times 10^4 \pm 0.4 \times 10^4$	
	Lalmati (930)	4.6	23	24.3	18.5	4.28	0.9	0.24	0.10	$1.1 \times 10^6 \pm 0.3 \times 10^6$	
AR-II	R. Vanhne village (1010)	4.7	18.2	24.8	9.8	4.3	1.2	0.22	0.10	$2 \times 10^6 \pm 0.3 \times 10^6$	Tropical semi-evergreen to Sub-tropical evergreen, Sub-tropical Pine, Mixed moist deciduous forests, Temperate broadleaved, Temperate pine forests (*Croton* sp., *Exbucklandia* spp., *Manglietia caveana*, *Myrica esculenta*, *Salix* spp., *Camelia* sp., *Schima* sp., *Bombax ceiba*, *Elaeocarpus* spp., *Quercus* spp., *Terminalia myriocarpa*, *Gmelia arborea*, *Castanopsis* spp., *Magnolia* spp., *Michelia oblonga*, *Pinus kesiya*, *P. roxburghii*, *P. wallichiana*, *Taxus* sp., *Castanopsis* spp., *Magnolia* spp., *Quercus* spp., *Acer* spp., *Alnus nepalensis*, *Rhododendro* spp.).
	Mawsmai (1192)	5.3	22	27.2	18.6	4.12	1.1	0.20	0.11	$3.8 \times 10^6 \pm 0.7 \times 10^6$	
	Tyrshi (1319)	5.3	22	31.2	15.9	3.96	1.0	0.19	0.12	$1.4 \times 10^6 \pm 0.5 \times 10^6$	
	NEHU (1393)	5.7	27	33.1	12	4.80	1.1	0.18	0.11	$1.0 \times 10^6 \pm 0.5 \times 10^6$	
	Sum suh (1452)	5.4	12.5	17	11.4	3.78	1.2	0.22	0.11	$3.3 \times 10^7 \pm 0.5 \times 10^7$	
	Mawkadong (1575)	4.5	20	22.5	16.2	3.80	1.1	0.16	0.10	$2.7 \times 10^5 \pm 0.3 \times 10^5$	
	Mao (1696)	6.5	25	27.8	33.7	3.93	0.9	0.16	0.11	$7.8 \times 10^6 \pm 1.1 \times 10^6$	
	Sacred Forest (1796)	4.5	20	23.5	33.6	3.95	0.7	0.14	0.12	$5.3 \times 10^6 \pm 0.6 \times 10^6$	
	Damthang (1852)	4.7	15	19	12.8	2.78	0.9	0.14	0.10	$2.1 \times 10^4 \pm 0.5 \times 10^4$	
	Shillong Peak (1950)	5.7	19.5	23.8	9.6	3.96	0.9	0.12	0.12	$3.2 \times 10^8 \pm 0.4 \times 10^8$	

TABLE 1 (Continued)
Physico-chemical properties of soil, CFU counts and microbial enzymatic activities in the soil collected from different survey sites situated at different altitudes of the eastern Himalayan range along with forest types

Altitudinal Range	Survey site with altitude (m.a.s.l.)	Soil pH	Soil Temp.(^{0}C)	Ambient Temp. (^{0}C)	Moisture Content (%)	Carbon Content (%)	Bulk Density (g/cm^3)	Urease (mg) NH_4 + -N/g dry soil	Dehydrogenase (mg) TPF/g dry soil	Colony Forming Unit (CFU) /g dry soil	Forests types with dominant vegetation
AR-III	Pelling (2008)	4.9	20.0	22.6	13.2	3.8	0.9	0.12	0.11	$3.1 \times 10^7 \pm 0.2 \times 10^7$	Temperate broadleaved, Temperate pine, Temperate conifer (*Acer pectinatum*, *A. oblongum*, *Alnus nepalensis*, *Rhododendron* spp., *Castanopsis indica*, *Pinus merkusii*, *P. roxburghii*, *P. wallichiana*, *P.kesiya*, *Quercus* spp., *Taxus* sp., *Cupressus torulosa*, *Abies spectabillis*, *A. delavayi*, *Taxus* sp.).
	Pfutsero (2120)	5.2	21.0	23.0	14.0	3.81	0.9	0.12	0.11	$2 \times 10^7 \pm 0.5 \times 10^7$	
	Uttarey (2205)	5.2	18.0	22.8	15.0	3.9	1.0	0.11	0.11	$2.1 \times 10^6 \pm 0.2 \times 10^6$	
	Bomdila (2320)	4.8	17.5	22.4	10.4	3.78	0.9	0.12	0.10	$1.8 \times 10^6 \pm 0.5 \times 10^6$	
	Dzukou (2435)	4.7	18.2	23.0	11.2	3.76	1.1	0.11	0.09	$1.7 \times 10^6 \pm 0.3 \times 10^6$	
	Broksher (2599)	5.1	14	18	18.2	2.77	1.0	0.12	0.09	$4.3 \times 10^5 \pm 0.2 \times 10^5$	
	Riga (2672)	4.8	16.0	19.0	19.0	2.9	0.9	0.13	0.09	$3 \times 10^5 \pm 0.5 \times 10^5$	
	Bakhim (2744)	5.2	14.9	18.9	20.2	3.1	1.0	0.12	0.09	$3.2 \times 10^4 \pm 0.3 \times 10^4$	
	Namdapha (2880)	5.1	15.3	18.7	21.8	2.72	1.0	0.12	0.09	$3.2 \times 10^4 \pm 0.3 \times 10^4$	
	Tado (2964)	5.0	16.0	18.4	22.4	2.68	0.9	0.11	0.08	$4 \times 10^4 \pm 0.4 \times 10^4$	
AR-IV	Thingbu (3010)	4.9	15.0	15.7	22.8	2.84	0.8	0.12	0.09	$4.1 \times 10^4 \pm 0.5 \times 10^4$	Temperate conifer, Sub-alpine conifer, Rhododendron forests (*Pinus wallichiana*, *Cupressus torusola*, *Abies spectbilis*, *A. Delavayi*, *Castanopsis indica*, *Alnus nepalensis*, *Taxus* sp., *Alnus* spp., *Rhododendron nivale*, *R. anthopogon*, *R. thomsonii*, *Sedum* sp., *Festuca* sp., *Rhodiolia* sp.).
	Chiwabhanjang (3127)	5.0	16.0	16.4	22.4	2.81	0.9	0.12	0.08	$4.6 \times 10^4 \pm 0.6 \times 10^4$	
	Varsey (3220)	4.9	13	17	19.1	2.76	0.9	0.14	0.08	$1.8 \times 10^3 \pm 0.2 \times 10^3$	
	Chira (3389)	4.8	14.6	16.8	21.8	2.7	0.9	0.13	0.08	$2 \times 10^3 \pm 0.2 \times 10^3$	
	Mandala (3435)	5.1	14.2	15.9	22.7	2.71	1.0	0.12	0.09	$1.9 \times 10^3 \pm 0.2 \times 10^3$	
	Tawang (3528)	5.0	15.0	17.1	21.2	2.6	0.9	0.12	0.08	$1.6 \times 10^4 \pm 0.2 \times 10^4$	
	Thangsing (3642)	4.9	14.9	16.9	21.9	2.58	1.0	0.12	0.08	$2.6 \times 10^4 \pm 0.3 \times 10^4$	
	Tsomgo lake (3755)	4.9	14	16	23.2	2.4	1.1	0.12	0.09	$2 \times 10^6 \pm 0.3 \times 10^6$	
	Baba Mandir (3840)	5.8	12	15	12.8	2.28	0.9	0.12	0.07	$3 \times 10^5 \pm 0.4 \times 10^5$	
	Thegu (3990)	5.2	12	14	28.7	1.7	0.8	0.09	0.07	$5 \times 10^6 \pm 0.6 \times 10^6$	

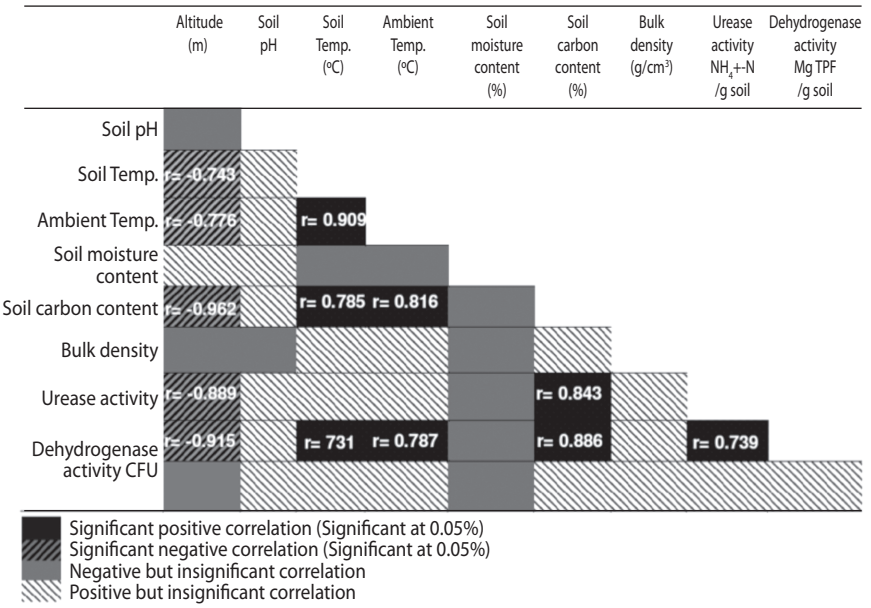

Fig. 1. Pearson Correlation analysis showing associations amongst various environmental factors, soil physico-chemical parameters and bacterial counts.

correlation with soil temperature (r=0.785, p<0.05) (Fig. 1).

Enzymes activity: The enzymes activities showed negative correlation with altitude, as highest activities for both urease and dehydrogenase enzymes were recorded at lower altitudes and vice versa (Table 1). Urease activity ranged from 0.09 to 0.28 (SD=±0.0508) (mg) NH_4^+/g of dry soil, whereas that of dehydrogenase varied from 0.07 to 0.16 (SD=±0.04788) (mg) TPF/g of dry soil. A significant positive correlation was observed between soil dehydrogenase and urease activity. Soil carbon was found to have significant positive correlation with urease and dehydrogenase activity (Fig. 1).

Cultivable bacteria: The bacterial colonies observed on agar plates ranged from $1.8x10^3$ to $3.2x10^8$ (SD=±$6.3x10^7$) cfu/g of dry soil. There was significant variation in the soil physico-chemical parameters and bacterial CFU counts along the altitudinal gradient (Table 1). The CFU counts of bacteria decreased with increasing altitude, as highest CFU counts were recorded at lower altitudes and lowest CFU counts were recorded at higher altitudes (Table 1, Fig. 2). Soil bacterial distribution showed positive but insignificant

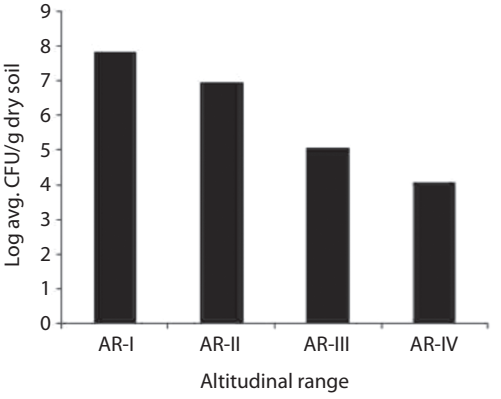

Fig. 2. Variations in Log average CFU of bacteria at different altitudinal ranges (AR-I: 34-1 000m.a.s.l.; AR-II: 1 001-2 000m.a.s.l.; AR-III: 2 001-3 000m.a.s.l. and AR-IV: 3 001-3 990m.a.s.l.).

correlation with soil pH, soil temperature, ambient temperature, soil carbon content, soil bulk density, urease and dehydrogenase activities whereas altitude and soil moisture content showed negative correlation (Fig. 1).

Phylogenetic diversity of bacterial isolates: A total of 155 bacterial isolates (Table 2) were obtained using cultivation based method from the entire altitude (34-3 990m.a.s.l) under the study area. Characterized isolates belonged to *Proteobacteria*, *Firmicutes* and *Bacteroidetes*, which comprised of *Alpha-*, *Beta-* and *Gamma-Proteobacteria*, *Bacilli* and *Flavobacteria* (Table 3), distributed to 27 genera and 77 species. From among the 155 isolates; 103 belonged to *Firmicutes*, 47 to *Proteobacteria* (two to α-*Proteobacteria*, 34 to γ-*Proteobacteria* and 11 to β-*Proteobacteria*) and five to *Bacteroidetes*. The phylogentic trees for the respective groups (*Beta-* and *Gamma-Proteobacteria*, *Firmicutes* and *Flavobacteria*) are constructed using rooted Neighbor-joining tree based on 16S rRNA (more than 1,400 bases) gene sequences of the isolates and the related species obtained from the database of type strains with validly published prokaryotic names at the EzTaxon server. The scale bars given in the phylogenetic trees correspond to the expected number of changes per nucleotide position (Figs. 3-6C).

The predominant phyla characterized in the entire stretch of altitudes were *Firmicutes* followed by *Proteobacteria* and *Bacteroidetes* (Fig. 7). These three phyla were found to occur in all the four altitudinal ranges with varying density. Under *Proteobacteria*, β-*Proteobacteria* and *γ-Proteobacteria* were isolated from all the four altitudinal ranges whereas α-*Proteobacteria* was isolated only from AR-III and AR-IV. *Bacillus* and *Lysinibacillus* were the dominant genus under the class

TABLE 2
Taxonomic affiliations of bacteria isolated from soils determined by 16S rRNA genes sequences showing closest identified match in the EzTaxon database

Altitudinal Range	Sequenced Isolate	Colony Colour	Closest match	% Similarity	Nucleotide Bases	Accession number
AR-I	GP1	Yellowish	*Bacillus aryabhattai*	99.9	1516	JF825995
	IT2	Yellowish	*Bacillus aryabhattai*	99.8	1475	JF825997
	NH1	Offwhite	*Bacillus aryabhattai*	97.8	1476	JN208177
	DG2	Creamish	*Bacillus aryabhattai*	97.1	1415	JN208193
	LT2	Yellowish	*Bacillus aryabhattai*	99.7	1457	JQ433931
	TZ1	Creamish	*Bacillus cereus*	97.7	1482	JN208187
	DG4	Offwhite	*Bacillus cereus*	98.2	1449	JN208195
	MK8	Whitish	*Bacillus cereus*	99.1	1479	HM769816
	BK-4	Offwhite	*Bacillus cereus*	99.9	1434	JN695698
	SI1	Creamish	*Bacillus cereus*	100	1476	JQ433945
	LM6	Whitish	*Bacillus thuringiensis*	99.9	1487	JN208185
	I7	Whitish	*Bacillus thuringiensis*	99.8	1476	HQ600994
	MB1	Offwhite	*Bacillus thuringiensis*	99.6	1489	HQ600985
	KL10	Creamish	*Bacillus thuringiensis*	99.9	1474	JN695703
	ZR1	Offwhite	*Bacillus thuringiensis*	100	1490	JQ433944
	AM3	Offwhite	*Bacillus mycoides*	98.5	1473	JF825987
	TZ2	Red	*Serratia marcescens*	99.8	1440	JN653468
	NN6	Yellowish	*Bacillus marisflavi*	99.6	1425	HQ600999
	BH1	Yellowish	*Bacillus marisflavi*	98.3	1484	JN208191
	M11	Creamish	*Aeromonas salmonicida*	100	1478	HQ600986
	LM4	Creamish	*Acinetobacter baumannii*	97.9	1530	JF825999

TABLE 2 (Continued)
Taxonomic affiliations of bacteria isolated from soils determined by 16S rRNA genes sequences showing closest identified match in the EzTaxon database

Altitudinal Range	Sequenced Isolate	Colony Colour	Closest match	% Similarity	Nucleotide Bases	Accession number
	AM10	Offwhite	*Lysinibacillus xylanilyticus*	99.7	1413	HQ600997
	ZR6	Light brown	*Lysinibacillus xylanilyticus*	100	1477	JQ433935
	GP6	Whitish	*Bacillus safensis*	97.5	1469	JN208186
	DG12	Offwhite	*Bacillus pseudomycoides*	98.8	1481	JN208202
	DP6	Offwhite	*Bacillus pseudomycoides*	99.9	1446	JN975935
	WC1	Purple	*Chromobacterium piscinae*	98.8	1434	HQ601002
	MZ5	Purple	*Chromobacterium piscinae*	99.5	1477	JN653466
	UB5	Offwhite	*Bacillus tequilensis*	99.7	1466	HQ601004
	DT10	Yellow	*Burkholderia anthina*	99.7	1436	HQ601008
	IT1	Offwhite	*Burkholderia anthina*	99.9	1436	JF825996
	IT4	Brownish	*Lysinibacillus fusiformis*	99.9	1490	JN695724
	FM3	Brownish	*Pseudomonas monteilii*	99.5	1520	JF825993
	SJ6	Offwhite	*Paenibacillus terrigena*	97	1477	HQ600989
	PRI2	Creamish	*Aquitalea denitrificans*	98.1	1454	JN208179
	LM1	Whitish	*Bacillus methylotrophicus*	99.5	1431	JF825998
	SJ7	Offwhite	*Bacillus thioparans*	99.2	1439	JF825989
	HU9	Yellowish	*Staphylococcus cohnii*	99.9	1441	JF825988
	AM6	Whitish	*Staphylococcus equorum*	100	1468	JN975939
	PRI1	Creamish	*Bacillus soli*	97.6	1362	JN208178
	PRI3	Brownish	*Bacillus drentensis*	99.1	1494	JN208180
	DG1	Yellowish	*Bacillus megaterium*	99.8	1394	JN208192
	HU1	Brownish	*Bacillus vallismortis*	99.7	1487	JN975946
	GL1	Offwhite	*Bacillus vallismortis*	99.6	1481	JN975949
	MZ1	Whitish	*Bacillus novalis*	99.5	1489	JN975931
	HU2	Offwhite	*Bacillus weihenstephanensis*	99.4	1473	JN975948
	NH6	Orange	*Chryseobacterium defluvi*	98.1	1458	JN975947
	TX4	Whitish	*Brevibacillus agri*	99.3	1449	JN975944
	HW1	Orange	*Exiguobacterium profundum*	99.9	1477	JQ433938
	LT3	Yellowish	*Ralstonia insidiosa*	97.4	1407	JQ433928
	CT1	Offwhite	*Acinetobacter nosocomialis*	99.4	1474	JQ433922
	KC1	Light orange	*Staphylococcus saprophyticus*	99.9	1481	JQ433926
	KV2	White	*Bacillus amyloliquefaciens*	99.8	1464	JQ433942
AR-II	IT6	Offwhite	*Bacillus aryabhattai*	99.9	1484	JN695723
	GO5	Yellowish	*Bacillus aryabhattai*	100	1485	JN695716
	HL3	Offwhite	*Bacillus aryabhattai*	100	1476	JQ433947
	HW2	Offwhite	*Bacillus aryabhattai*	99.9	1474	JQ433939
	KM8	Offwhite	*Bacillus cereus*	100	1344	JN695715
	MP4	Whitish	*Bacillus cereus*	99.9	1454	JN695726
	BH3	Offwhite	*Bacillus cereus*	100	1490	JN695704
	WC2	Creamish	*Bacillus thuringiensis*	99.9	1488	JN695705
	NP3	Offwhite	*Bacillus thuringiensis*	100	1472	JN695706
	SA1	Whitish	*Bacillus thuringiensis*	100	1358	JN695708
	T1	Offwhite	*Bacillus mycoides*	99.5	1490	HQ601014
	MB2	Reddish	*Serratia marcescens*	99.5	1436	HQ601000
	MS10	Reddish	*Serratia marcescens*	99.5	1452	JN653470

TABLE 2 (Continued)
Taxonomic affiliations of bacteria isolated from soils determined by 16S rRNA genes sequences showing closest identified match in the EzTaxon database

Altitudinal Range	Sequenced Isolate	Colony Colour	Closest match	% Similarity	Nucleotide Bases	Accession number
	FM2	Creamish	*Bacillus amyloliquefaciens*	99.7	1458	JN695707
	M4	Creamish	*Aeromonas salmonicida*	100	1475	HQ600987
	TH5	White	*Staphylococcus warneri*	99.9	1452	JN208183
	DT12	Offwhite	*Lysinibacillus xylanilyticus*	99.3	1344	JN208190
	SF10	Yellowish	*Lysinibacillus xylanilyticus*	99.9	1407	HQ600993
	GO6	Brownish	*Lysinibacillus xylanilyticus*	100	1480	JF825994
	TZ6	Offwhite	*Bacillus safensis*	99.9	1468	JN975945
	MZ4	Offwhite	*Bacillus pseudomycoides*	99.1	1489	JN695727
	MA9	Whitish	*Bacillus subtilis*	99	1480	HM769817
	MS9	Whitish	*Bacillus subtilis*	100	1407	JN653469
	GL3	Offwhite	*Bacillus tequilensis*	99.5	1473	JN975956
	N14	White	*Bacillus tequilensis*	99.9	1442	HQ601003
	M8	Creamish	*Pseudomonas mohnii*	99.8	1452	HQ600990
	I15	Offwhite	*Viridibacillus arenosi*	98.9	1466	HQ601001
	MA10	Brownish	*Viridibacillus arenosi*	99.3	1449	HQ601010
	TS1	Offwhite	*Pseudomonas jessenii*	98.1	1302	JN208188
	T4	Offwhite	*Paenibacillus taichungensis*	99.7	1463	HQ600992
	MB4	Pink	*Serratia nematodiphila*	98.9	1477	HQ600995
	RH6	Light orange	*Chryseobacterium defluvii*	98	1436	JN975952
	N4	Offwhite	*Enterococcus durans*	99.5	1486	HQ600998
	KL8	White	*Staphylococcus cohnii*	100	1452	JN695710
	MP1	Brownish	*Sporosarcina ureae*	99.4	1422	JN695711
	T7	Offwhite	*Citrobacter farmeri*	99	1467	HQ600991
	DG6	Creamish	*Lysinibacillus sphaericus*	97.2	1443	JN208197
	I8	Offwhite	*Brevibacillus laterosporus*	97	1397	HQ600996
	PRI4	Purple	*Chromobacterium piscinae*	99.8	1431	JN653467
	CT2	Whitish	*Acinetobacter nosocomialis*	99.5	1462	JQ433924
	HL4	Offwhite	*Bacillus ginsengi*	97.5	1474	JQ433930
	TN1	Pale	*Ralstonia mannitolilytica*	99.4	1459	JQ433936
AR-III	FM5	Offwhite	*Bacillus aryabhattai*	99.8	1472	JN695721
	RH2	Offwhite	*Bacillus aryabhattai*	99.9	1471	JN975942
	DG5	Whitish	*Bacillus cereus*	99.8	1483	JN208196
	AM1	Offwhite	*Bacillus cereus*	100	1464	JN975936
	TN3	White	*Bacillus cereus*	100	1453	JQ433946
	KV1	White	*Bacillus cereus*	100	1482	JQ433949
	MZ2	Offwhite	*Bacillus thuringiensis*	99.9	1487	JN695718
	TX3	Yellowish	*Bacillus thuringiensis*	99.9	1425	JN975933
	SF1	Offwhite	*Bacillus mycoides*	100	1484	JN975958
	SF3	Brownish	*Bacillus mycoides*	99.9	1482	JN975959
	MA3	Reddish	*Serratia marcescens*	99.4	1421	HQ601009
	N6	Offwhite	*Bacillus marisflavi*	99.7	1452	HQ601012
	I4	Brownish	*Lysinibacillus parviboronicapiens*	99.1	1471	HQ601013
	DT3	Creamish	*Lysinibacillus parviboronicapiens*	99.1	1484	JN208189
	WZ2	Creamish	*Acinetobacter baumannii*	98.5	1461	HQ601006
	DG10	Yellowish	*Enterobacter cloacae*	97.4	1488	JN208201

TABLE 2 (Continued)
Taxonomic affiliations of bacteria isolated from soils determined by 16S rRNA genes sequences showing closest identified match in the EzTaxon database

Altitudinal Range	Sequenced Isolate	Colony Colour	Closest match	% Similarity	Nucleotide Bases	Accession number
	CM5	Creamish	*Ponticoccus gilvus*	99.9	1429	JN695725
	N5	Offwhite	*Pantoea ananatis*	99.5	1395	HQ601011
	WC5	Creamish	*Bacillus subtilis*	99.9	1475	JN975953
	MP2	Brownish	*Bacillus methylotrophicus*	99.7	1469	JN975950
	SP8	Creamish	*Kluyvera ascorbata*	98.7	1466	JF825990
	CM2	Brownish	*Pseudomonas plecoglossicida*	99.9	1475	JN695722
	AM2	Offwhite	*Pseudomonas moorei*	99.1	1472	HQ600988
	BM3	Yellowish	*Pseudomonas arsenicoxydans*	98.8	1455	JN208184
	KL3	Offwhite	*Pseudomonas azotoformans*	99.7	1428	JN695709
	CK5	Offwhite	*Pseudomonas gessardii*	99.6	1480	JN695712
	WC4	Whitish	*Staphylococcus cohnii*	100	1450	JN695701
	DP1	Whitish	*Staphylococcus arlettae*	99.9	1442	JN695717
	GO1	Creamish	*Chryseobacterium taiwanense*	99.3	1448	JN975957
	VT3	Offwhite	*Comamonas thiooxydans*	100	1470	JQ433933
	HL1	Creamish	*Pseudomonas nitroreducens*	99.4	1460	JQ433932
	SH1	Whitish	*Bacillus pseudomycoides*	99.2	1497	JQ433948
AR-IV	DG7	Offwhite	*Bacillus aryabhattai*	97.3	1464	JN208198
	GP2	Offwhite	*Bacillus aryabhattai*	99.8	1467	JN975951
	GL5	Brownish	*Bacillus aryabhattai*	100	1490	JN975940
	BM3	Offwhite	*Bacillus cereus*	98.7	1469	JF825991
	SJ3	Offwhite	*Bacillus cereus*	99.8	1440	JN975941
	GO2	Whitish	*Bacillus cereus*	99.6	1474	JN975954
	CK1	Whitish	*Bacillus thuringiensis*	99.7	1454	JN975943
	CM1	Creamish	*Bacillus thuringiensis*	99.9	1432	JN695713
	SJ4	Brownish	*Psychrobacillus psychrodurans*	98.7	1465	JN975955
	DG8	Offwhite	*Cupriavidus metallidurans*	98.8	1359	JN208199
	CM4	Offwhite	*Lysinibacillus parviboronicapiens*	99.1	1466	JN975932
	DG9	White	*Staphylococcus warneri*	100	1464	JN208200
	TX2	Brownish	*Enterobacter cloacae*	99.1	1468	JN695719
	BM-1	Whitish	*Pseudomonas koreensis*	99.9	1371	JN695699
	TH1	Orange	*Chryseobacterium culicis*	99.2	1439	JN208181
	BM-2	Creamish	*Pseudomonas mohnii*	99.9	1447	JN695700
	TS3	Brownish	*Pseudomonas vancouverensis*	99.8	1459	JN975934
	BM1	Offwhite	*Viridibacillus arvi*	98.9	1475	JN695714
	TS2	Whitish	*Pseudomonas baetica*	99.4	1446	JN975937
	TS4	Brownish	*Pseudomonas taiwanensis*	99.1	1447	JN975938
	TH4	Offwhite	*Acinetobacter pittii*	99.8	1451	JN695720
	TH6	Brownish	*Pseudomonas extremaustralis*	100	1466	JN695702
	TH2	Offwhite	*Pseudomonas extremaustralis*	98.8	1432	JN208182
	VT1	Orange	*Chryseobacterium ureilyticum*	98.9	1457	JQ433943
	RV4	Whitish	*Mitsuaria chitosanitabida*	100	1419	JQ433927
	ZR3	Creamish	*Pseudomonas geniculata*	99.6	1469	JQ433923
	KR5	Yellowish	*Sphingobium yanoikuyae*	99.5	1433	JQ433940
	SS1	White	*Bacillus pseudomycoides*	99.6	1487	JQ433941

TABLE 3
Isolated bacterial phyla and class for the identified bacteria

Phylum	Class	Genus*
Proteobacteria	Alpha	*Ponticoccus* (1), *Sphingobium* (1)
	Beta	*Burkholderia* (2), *Chromobacterium* (3), *Aquitalea* (1), *Cupriavidus* (1), *Commamonas* (1), *Mitsuaria* (1), *Ralstonia* (2)
	Gamma	*Aeromonas* (2), *Acinetobacter* (5), *Citrobacter* (1), *Kluyvera* (1), *Pantoea* (1), *Pseudomonas* (17), *Serratia* (5), *Enterobacter* (2)
Firmicutes	Bacilli	*Bacillus* (74), *Brevibacillus* (2), *Enterococcus* (1), *Lysinibacillus* (10), *Paenibacillus* (2), *Staphylococcus* (8), *Viridibacillus* (3), *Psychrobacillus* (1), *Sporosarcina* (1), *Exiguobacterium* (1)
Bacteroidetes	Flavobacteria	*Chryseobacterium* (5)

*Figures in the parentheses indicate total number of representative isolate(s) for a particular genus.

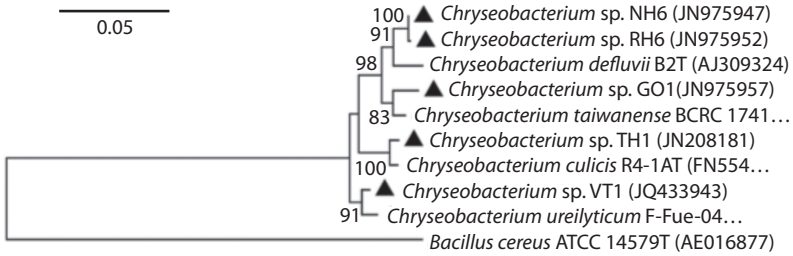

Fig. 3. Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) gene sequences for the group *Flavobacteria*.

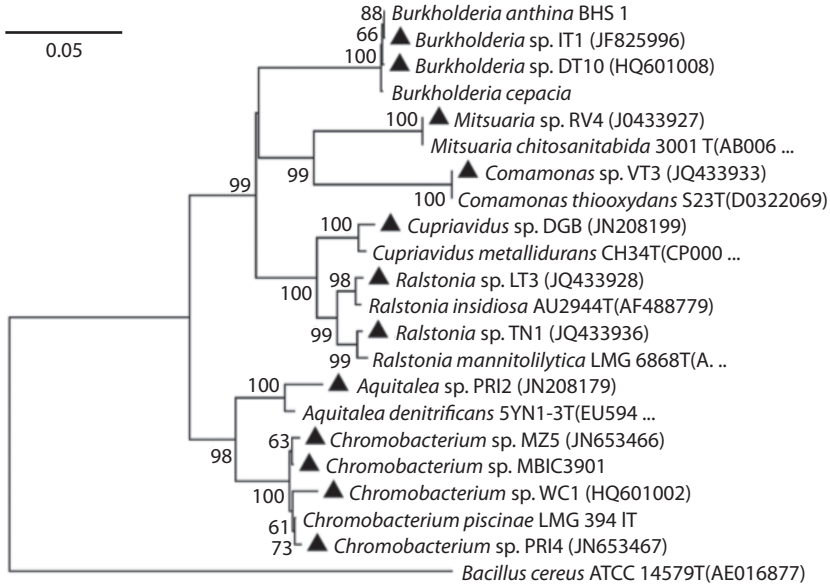

Fig. 4. Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) gene sequences for the group β-*Proteobacteria.*

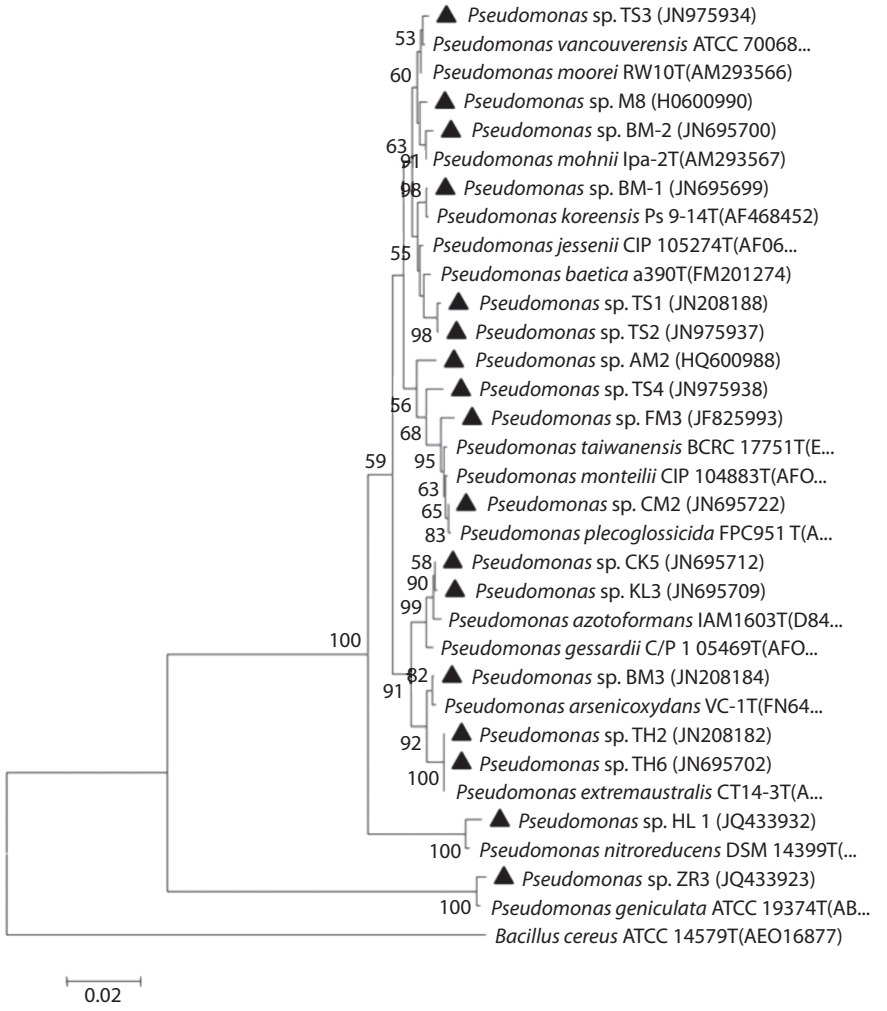

Fig. 5. (A). Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) for the group γ-*Proteobacteria*.

Bacilli. Similarly, *Serratia* and *Pseudomonas* belonging to class γ-*Proteobacteria* were frequently characterized from all the altitudinal ranges. Under β-*Proteobacteria*, *Burkholderia* and *Chromobacteria* were dominant. *Sphingobium* and *Ponticoccus* were the isolated genera under α-*Proteobacteria*. *Chryseobacterium* was the only genus identified under *Flavobacteria* (Table 3). The genus *Bacillus* was the most dominant among all the isolated genera followed by *Pseudomonas* and *Lysinibacillus*. Similarly, diversity index of genus *Bacillus* (0.353) was found to be the highest followed by *Pseudomonas* (0.242) and *Lysinibacillus* (0.176) (Fig. 8).

Diversity indices and data analyses: Shannon-Wiener's index (H'), Diversity (D), Complement of Simpson's index (d'), Fisher's alpha index, richness and evenness index for the studied range was found to be 3.92, 50.40, 0.966, 60.77, 6.18 and 0.903, respectively (Table 4). The Shannon-Wiener's index (H') for the entire range varied from 2.99 to 3.35. The calculated values for Shannon-Wiener's, Diversity, complement of Simpson's indices

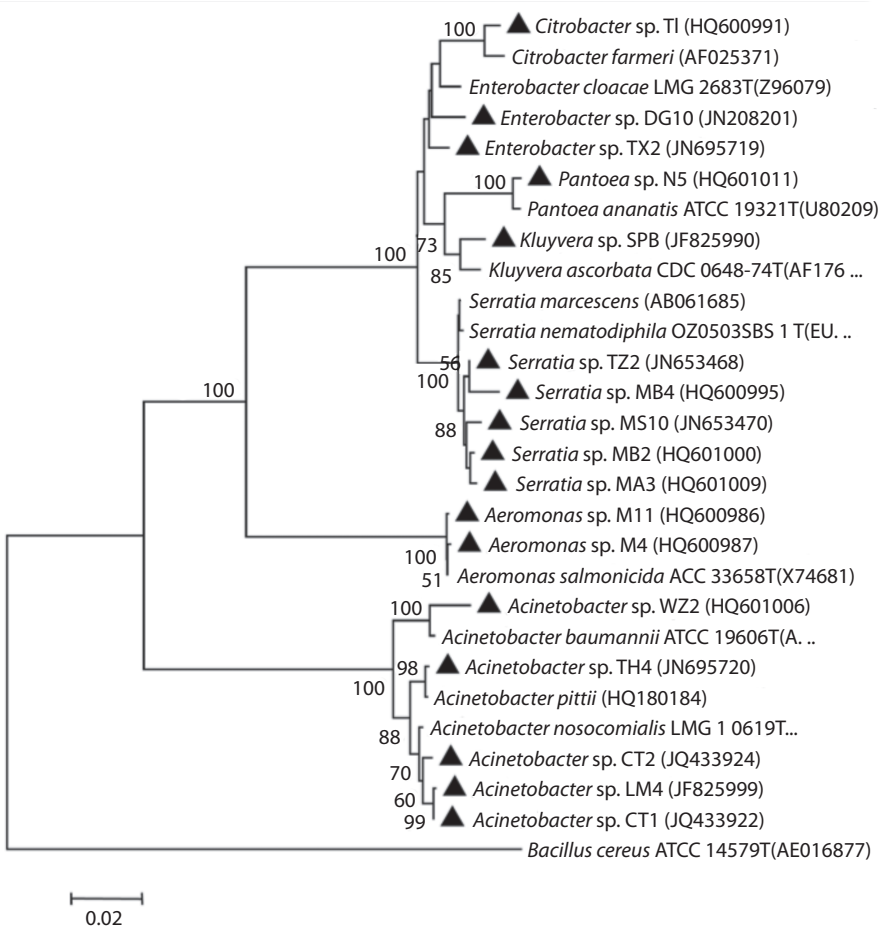

Fig. 5. (**B**) Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) for the group γ-*Proteobacteria*.

TABLE 4
Statistical analysis of bacterial diversity, evenness and species richness at various altitudinal ranges

	Total	Altitudinal range			
		AR-I	AR-II	AR-III	AR-IV
Number of isolates	155	53	42	32	28
Number of different species	77	35	29	25	22
Species richness (*S*)	6.18	4.81	4.47	4.42	4.16
Evenness (*J*)	0.903	0.944	0.961	0.968	0.970
Shannon Diversity Index (*H'*)	3.92	3.35	3.23	3.12	2.99
Diversity (*D*)	50.40	28.50	25.27	22.65	19.88
Complementary of Simpson Diversity Index (*d'*)	0.966	0.955	0.953	0.949	0.943
Fisher alpha	60.77	44.9	41.42	52.64	47.37

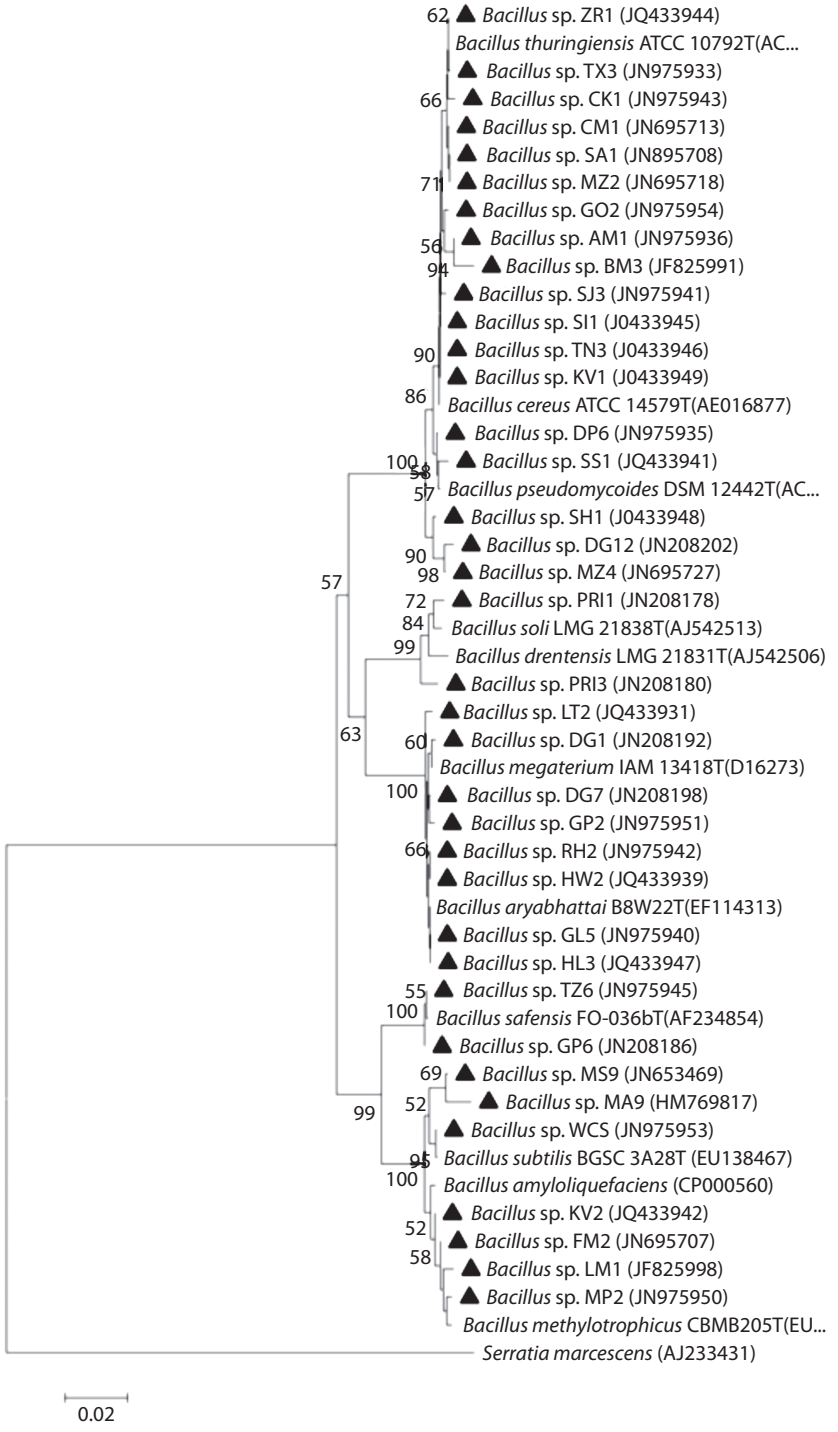

Fig. 6. (**A**) Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) for the group *Firmicutes.*

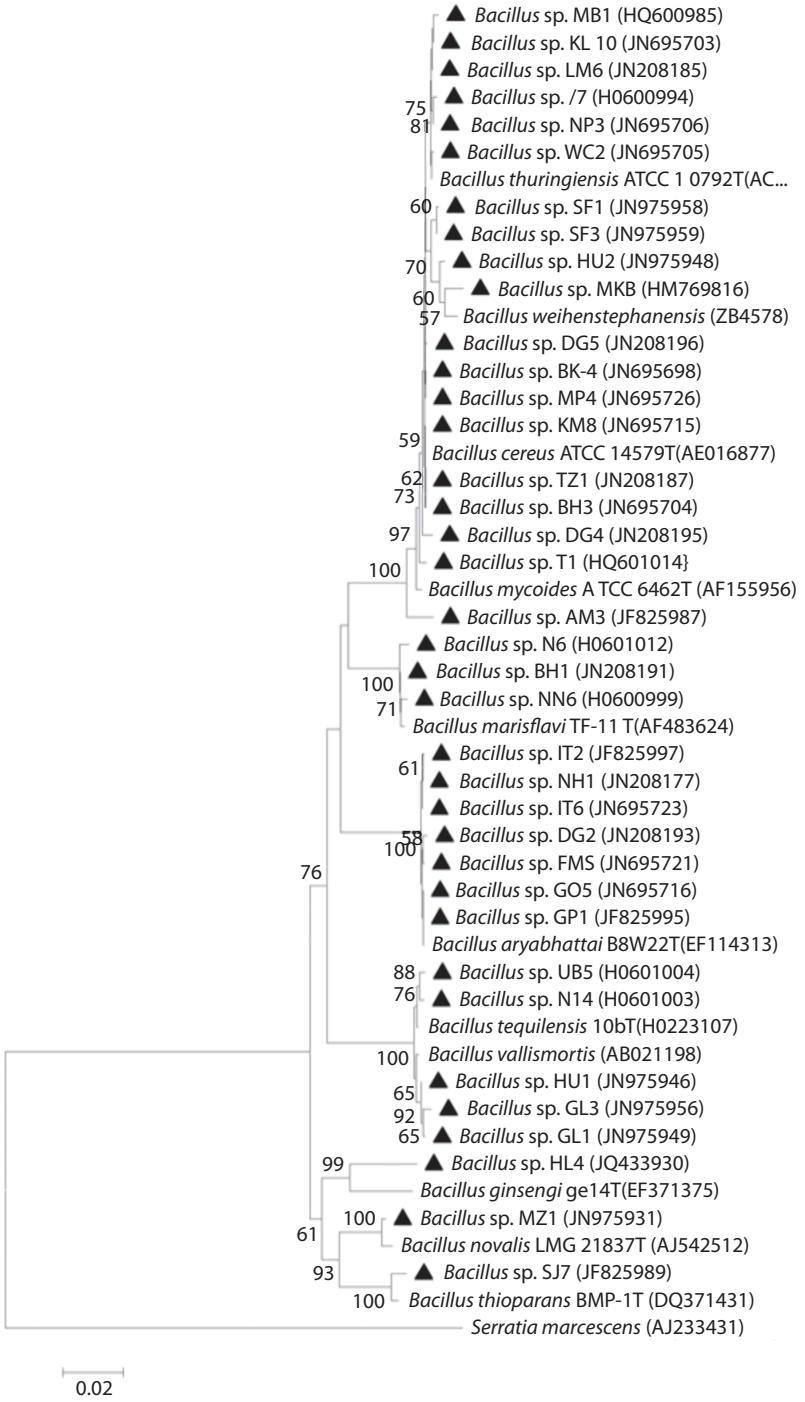

Fig. 6. (**B**) Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) for the group *Firmicutes*.

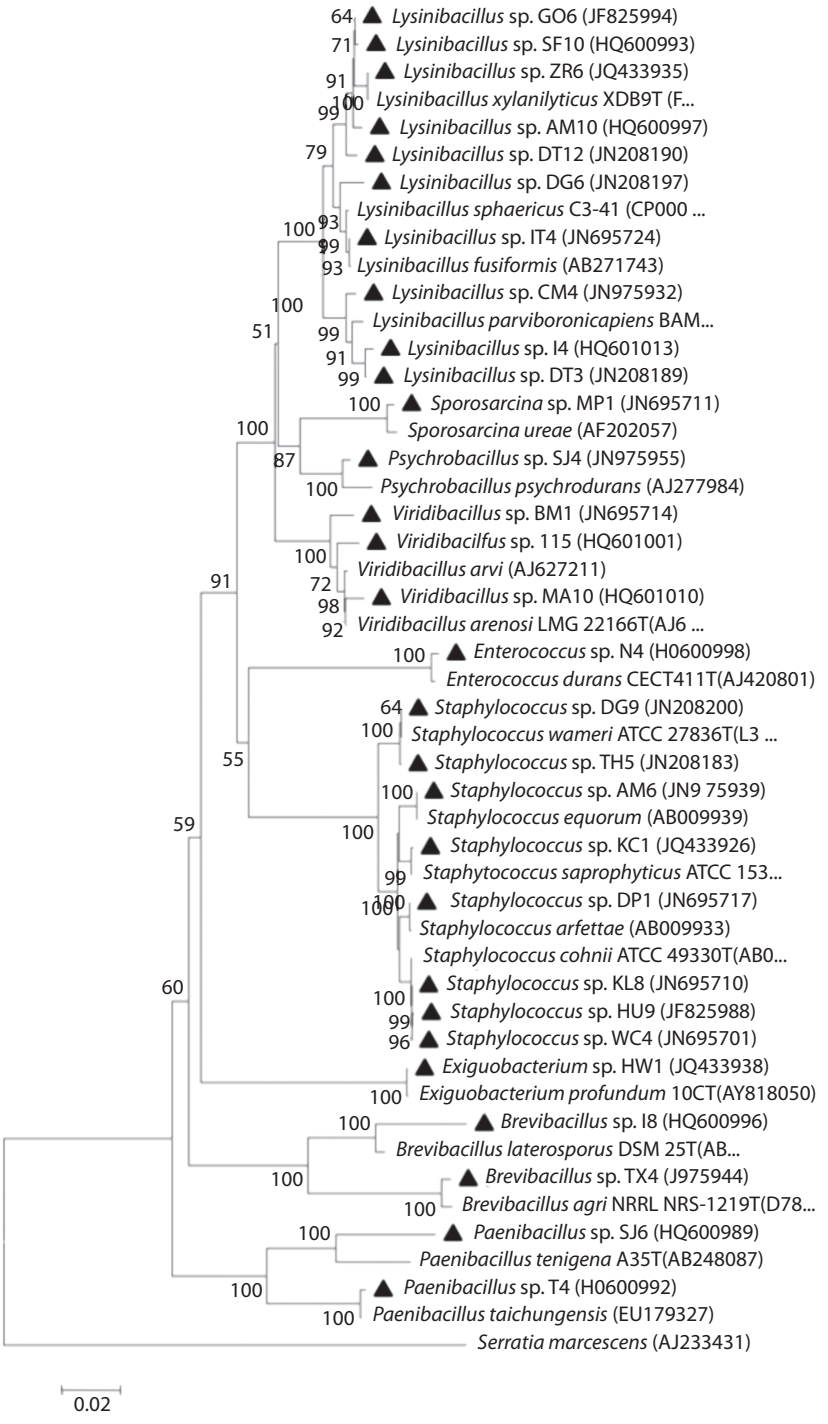

Fig. 6. (**C**) Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) for the group *Firmicutes.*

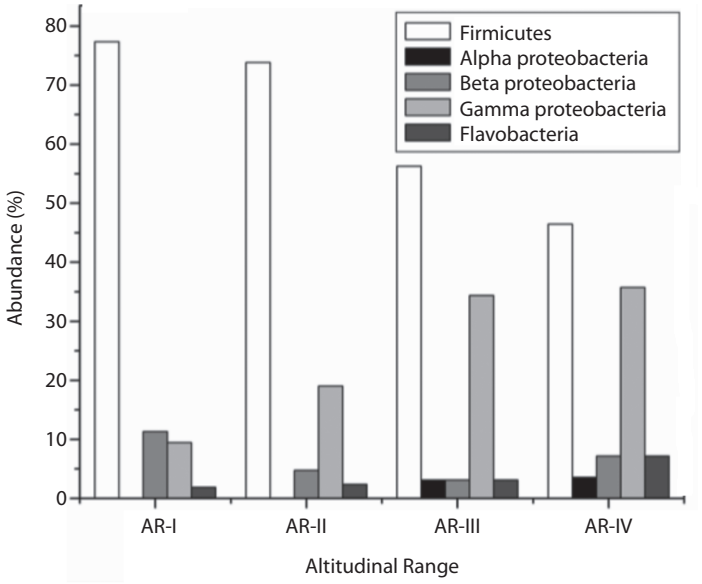

Fig. 7. Relative abundance of bacterial groups at different altitudinal range.

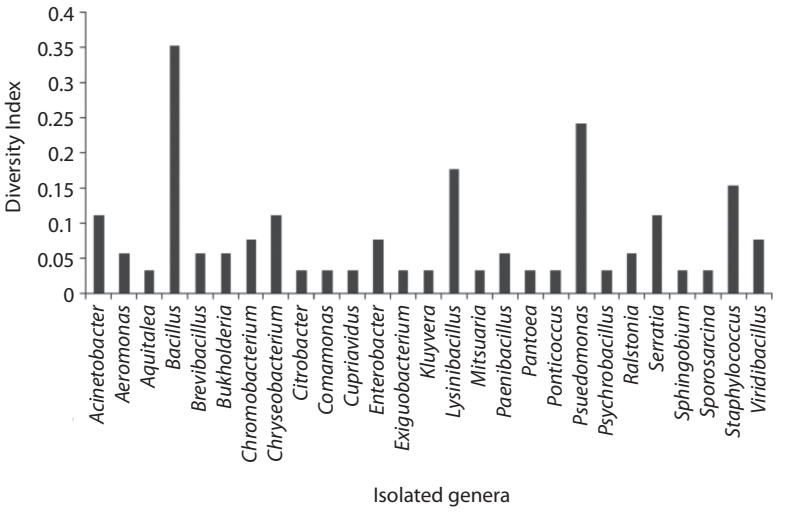

Fig. 8. Diversity index of individual genus for the entire study area.

and species richness decreased with increasing altitude. However, Fisher's alpha did not follow the same trend of diversity (Table 4). The highest value for most of the diversity indices as well as species richness was recorded for the lowest altitudinal range AR-I (H'=3.35, D=28.50, d'=0.955, S=4.81). Genus wise, the individual diversity index varied from 0.033 to 0.353 with *Bacillus* (0.353) having the highest diversity index, followed by *Pseudomonas*

(0.242) and *Lysinibacillus* (0.177) (Fig. 8). The species accumulation curve displayed an increasing trend that did not level off (Fig. 9).

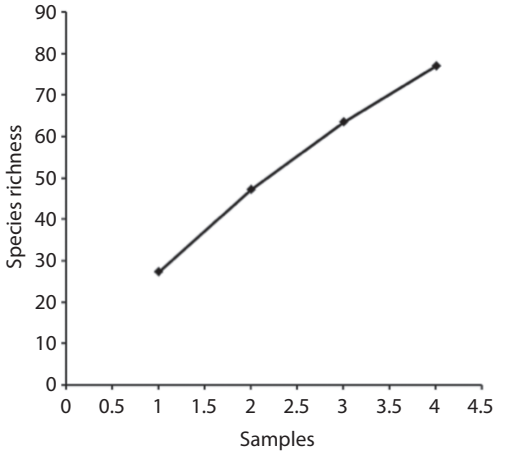

Fig. 9. Species accumulation curve plotted using PAST 2.10 for the entire study area.

Accession numbers of nucleotide sequences: The 16S rDNA nucleotide partial sequences were submitted to GenBank and accessions were obtained (Table 2).

DISCUSSION

Ecological factors and altitudinal gradient are known to influence distribution of soil micro-organisms (Lomolino 2001). The cultivable bacterial diversity across the altitude showed variation in the region revealing spatial trend and correlation with soil parameters and forest types. The variability in population densities of cultivable soil bacteria can be attributed to soil properties, physico-chemical conditions and vegetations which are among the most important factors that influence soil microbial growth, population density and diversity (Atlas 1984, Dimitriu & Grayston 2010). Alpine and sub-alpine regions situated at higher altitude with sparse vegetation represent different soil physico-chemical factors when compared to tropical and sub-tropical evergreen or deciduous forests at lower altitudes. Tropical and sub-tropical forest prevalent at lower altitudes supported higher bacterial diversity compared to temperate pine, temperate conifer and sub-alpine rhododendron forests at higher altitudes as measured by Shannon-Wiener's (H'), Complement of Simpson's (d') indices and Diversity (D). This variation in bacterial diversity may be attributed to the types of forests as vegetations are known to affect soil microbial diversity and community structures (Kowalchuk *et al*. 2002, Grayston & Prescott 2005, Han *et al*. 2007, Thomson *et al*. 2010). The value of the Shannon-Wiener's Index (H') usually lies between 1.5 and 3.5 for ecological data and rarely exceeds 4.0 (May 1975). In this study, we found that the entire study area has high value of H' (H'>3.5; Table 4) and of Fisher's α suggesting prevalence of diversity in the region.

Richness and evenness of bacterial communities reflect selective pressure that shape diversity within communities. Measuring these parameters is most useful when assessing the relationships of soil physico-chemical and environmental parameters on bacterial diversity in soil (Kapur & Jain 2004). However, in the present study correlation was found to be insignificant. Similar findings have been reported by Bryant *et al*. (2008) and Fierer *et al.* (2011). The insignificant decrease in diversity with altitude is attributed to higher bacterial diversity at sub-tropical and temperate soil. One of the possible reasons for higher bacterial diversity at intermediate elevation could be due to the fact that the trees of sub-tropical and temperate regions including *Cedrus*, *Pinus* and *Taxus* in Himalayan range are known to exert slightly stimulatory effect on the microorganisms and therefore support relatively higher microbial population (Pandey *et al.* 2006). Similar effects of vegetations on soil microbial diversity and community structures has also been reported by several other workers (Kowalchuk *et al*. 2002, Grayston & Prescott 2005, Han *et al*. 2007, Thomson *et al*. 2010). The present study corroborates with the findings of Collins & Cavigelli (2003) and Fierer

et al (2011) who had reported decrease in soil pH with increase in altitude. Insignificant correlation between soil temperature and bacterial diversity could be due to the variation in sampling time and season.

Members of the phyla *Proteobacteria* and *Firmicutes* are the most abundant soil bacteria, as revealed by analysis of 16S rRNA gene (Janssen 2006, Bruce *et al*. 2010, Lin *et al*. 2010). In the present study, γ-*Proteobacteria* was found to be more common as compared to β-*Proteobacteria*, which could be due to the soil physico-chemical factors as pH is found to influence the distribution of *Proteobacteria* (Lin *et al*. 2010). The abundance of *Burkholderia* species among β-*Proteobacteria* is due to its nutritional versatility as saprophyte and also being a common resident of rhizosphere soil (Estrada-De los Santos *et al*. 2001, Coenye & Vandamme 2003). Within the *Firmicutes*, 75.19% of the isolates belonged to *Bacillus*. The high number and high diversity index value of *Bacillus* as compared to the other isolated genera in the study suggests that aerobic or facultative anaerobic, spore-forming bacteria are abundant in the forest soils of this Himalayan range which supports the patterns observed in temperate and tropical soils (Teixeira *et al*. 2010). Abundance of *Serratia* and *Bacillus* species is attributed to their capability of adapting to a wide range of environmental conditions. Both are distributed widely in nature and are commonly found in soil as saprophytic organisms (Vilain *et al*. 2006). Plants benefit from the presence of *B. cereus* since it is capable of inhibiting plant diseases and also enhances plant growth (Jensen *et al*. 2003). *Serratia marcescens*, an enterobacteria, on the other hand has a unique ability to produce extracellular enzymes. Several such enzymes have been shown to have the ability to degrade chitin, a substance which mainly comprises fungal cell walls (Hejazi & Falkiner 1997). *Lysinibacillus xylanilyticus* is a xylan-degrading soil bacteria widely found in forest soil (Lee *et al*. 2010). *B. aryabhattai* is recorded in large number in the present study and its abundance in soil indicate good soil health as it performs wide range of enzymatic activities and play active role in soil nutrient decomposition and mineralization (Shivaji *et al*. 2009, Yadav *et al*. 2011). *Bacillus* from high altitudes of Eastern Himalayan range has been reported to produce thermostable enzyme (Devi *et al*. 2010). The cultivation approach showed that certain isolates belonging to *Bacillus*, *Brevibacillus*, *Lysinibacillus* and *Enterobacter* spp. may represent novel species as they have relatively low 16S rRNA sequence similarity≤97% (Table 2) to the known species.

Determination of dehydrogenase and urease activity in general is a criterion used to determine soil microbial activity (Burns 1978, Makoi & Ndakidemi 2008). Dehydrogenase activity is an indicator of potential non-specific intracellular enzyme activity of the total microbial biomass and may be considered a good measure of microbial oxidative activities in soils (Subhani *et al*. 2001).

The present study is the first broad survey on the bacterial community structure in the Eastern Himalayan soils under different vegetations and soil physico-chemical conditions. This study clearly points out that this biome represents a vast reservoir for bacterial discovery. Moreover, the species accumulation curve showed that the rate of species increase with sampling effort had not yet reached an asymptote, indicating that the diversity of the area had not yet been fully captured. The bacterial diversity data may become a baseline to compare how anthropogenic and climatic interventions in the coming years change the composition and diversity of bacteria across the altitudes of this anthropogenically exposed range. Further functional analyses are required for understanding the functional diversity (Hollister *et al*. 2010) and its possible exploration for bioprospection potentials and conservation value of the microbiota. Unless the area is protected, human generated disturbances such as tourism, agriculture, industrial activities, transient farming and housing may rapidly reduce the forest cover and with it, the associated microbial biodiversity value of the region in the near future, by exerting severe impacts

on its rich but poorly known microflora. Future efforts focused on deep sequencing of soil microbial diversity and novel characterization methodologies to recover and describe taxonomically diverse novel species of this range are required to confirm these first conclusions, and thereby add data needed to support decisions on conservation and sustainable utilization of this biodiversity hotspot.

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RESUMEN

La parte noreste de la India se extiende sobre una superficie de 262 379km^2 en la cordillera oriental del Himalaya. Es un punto de acceso con altos niveles de biodiversidad y endemismo; desafortunadamente, también es una zona poco conocida, sobre todo su diversidad microbiana. En este estudio se evaluó la diversidad de bacterias cultivables del suelo, su diversidad y distribución de las tierras bajas a las altas (34 a 3 990m.s.n.m). Se caracterizaron los parámetros físico-químicos del suelo y tipos de bosques a lo largo del gradiente altitudinal y se correlacionaron con la distribución y diversidad bacteriana. Los microbios del suelo se cultivaron en placas de agar enriquecido Muller Hinton y Luria-Bertani, e inicialmente se caracterizaron mediante métodos bioquímicos. Parámetros tales como actividad de la deshidrogenasa y ureasa, temperatura, contenido de humedad y de carbono, pH y densidad aparente del suelo se midieron en cada sitio. Aislamientos representativos también se sometieron al análisis secuencial de 16S rADN. Un total de 155 aislamientos bacterianos cultivables se caracterizaron para estimar los índices de riqueza, equidad y diversidad. Los bosques tropicales y subtropicales albergan una mayor diversidad bacteriana en comparación con los bosques templados de pino y coníferas, y los bosques subalpinos de rododendro. El análisis filogenético de 16S rARN reveló que *Firmicutes* fue el grupo más común, seguido de *Proteobacteria* y *Bacteroidetes*. Especies pertenecientes a los géneros *Bacillus* y *Pseudomonas* fueron las más abundantes. Las UFC bacterianas mostraron una positiva pero insignificante correlación con los parámetros del suelo, tales como pH (r=0.208), temperatura (r=0.303), temperatura ambiente (r=0.443), contenido de carbón (r=0.525), densidad aparente (r=0.268), ureasa (r=0.549) y deshidrogenasa (r=0.492). La altitud (r=-0.561) y el contenido de humedad del suelo (r=-0.051) mostraron una correlación negativa. Se encontró que el gradiente altitudinal, junto con la vegetación y los par**ámetros físico-químicos influyeron** en la diversidad bacteriana y la distribución. Este estudio señala que este es un bioma con un vasto reservorio de bacterias que disminuyen con la altitud y pone en relieve la importancia microbiológica de la pobremente estudiada zona del este del Himalaya, lo que justifica los esfuerzos para explorar la prevalencia de nuevas especies en el bioma.

Palabras clave: bacterias cultivables, zona del este del Himalaya, región tropical, altitud, 16S rARN, índices de diversidad.

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