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 262 379 km² 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 3 990 m.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 Himalayan 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 379km², Northeastern region of India 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. 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 (Vishnic 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 (Vishnic 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 379km²; 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 1000m.a.s.l.), AR-II (1001m.a.s.l. to 2000m.a.s.l.), AR-III (2001m.a.s.l. to 3000m.a.s.l.) and AR-IV (3001m.a.s.l. to 3990m.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
The soil samples were kept in sterile containers and stored at 4°C 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 105°C (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 37°C for 24h, the formazan formed was extracted with 10mL ethanol and estimated spectrophotometrically at 485nm. 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 37°C 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 35-37°C 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 4°C. Simultaneously, the pure cultures of isolates were preserved in 20% glycerol water at -20°C (Kumar et al. 2011).

**Growth and preliminary characterization:** Colony morphology was determined after two to seven days growth on agar plates incubated at 35-37°C. 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 MgCl₂ (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 94°C, this was followed by 30 cycles each of denaturation at 94°C for 1min, annealing at 55°C for 1min, and extension at 72°C for 2min, and the final extension for 5min at 72°C. 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. (°C) | Ambient Temp. (°C) | Moisture Content (%) | Carbon Content (%) | Bulk Density (g/cm³) | Urease (mg NH₄⁺-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 x 10⁷±1.0 x 10⁸                 | Tropical evergreen, Tropical semi-evergreen, Tropical pine to Sub-tropical evergreen, Mixed moist deciduous forests (Myrica spp., Altingia excelsa, Pyrus sp., Shorea robusta, Mesua ferra, Clerodendron spp., Dipterocarpus retusus, Exbucklandia sp., Castanopsis sp., Quercus griffithii, 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 x 10⁶±0.8 x 10⁶              |                                                        |
|                   | Fifth Mile (210)                   | 4.3     | 24              | 25.8              | 10.8               | 4.92              | 1.2                 | 0.22                     | 0.14                          | 1.0 x 10⁶±0.5 x 10⁶              |                                                        |
|                   | Ganga lake (336)                   | 4.9     | 19              | 26.1              | 4.91               | 2.1               | 0.22                | 0.12                     | 6.4 x 10⁵±0.9 x 10⁶            |                                                        |
|                   | Medziphema (412)                   | 4.8     | 24              | 29.1              | 13.2               | 4.88              | 1.0                 | 0.21                     | 0.11                          | 1.1 x 10⁶±0.6 x 10⁶              |                                                        |
|                   | Rimassar (562)                     | 5.0     | 20.5            | 23.7              | 6.5                | 4.68              | 1.2                 | 0.23                     | 0.12                          | 2.2 x 10⁴±0.4 x 10⁴              |                                                        |
|                   | Mawshun (688)                      | 4.9     | 19.5            | 25.9              | 3.4                | 4.76              | 1.1                 | 0.24                     | 0.14                          | 5.1 x 10⁵±0.9 x 10⁵              |                                                        |
|                   | Keibul Lamjao (785)                | 4.7     | 26              | 34.7              | 17.6               | 4.42              | 1.1                 | 0.19                     | 0.14                          | 6.0 x 10⁵±0.8 x 10⁵              |                                                        |
|                   | Lawngthlai (829)                   | 5.7     | 28              | 30.4              | 16                 | 4.3               | 1.0                 | 0.20                     | 0.12                          | 1.8 x 10⁶±0.4 x 10⁶              |                                                        |
|                   | Lalmati (930)                      | 4.6     | 23              | 24.3              | 18.5               | 4.28              | 0.9                 | 0.24                     | 0.10                          | 1.1 x 10⁶±0.3 x 10⁶              |                                                        |
|                   | R. Vanhne village (1010)           | 4.7     | 18.2            | 24.8              | 9.8                | 4.3               | 1.2                 | 0.22                     | 0.10                          | 2 x 10⁶±0.3 x 10⁶               |                                                        |
|                   | Mawsmai (1192)                     | 5.3     | 22              | 27.2              | 18.6               | 4.12              | 1.1                 | 0.20                     | 0.11                          | 3.8 x 10⁶±0.7 x 10⁶              |                                                        |
|                   | Tyrshi (1319)                      | 5.3     | 22              | 31.2              | 15.9               | 3.96              | 1.0                 | 0.19                     | 0.12                          | 1.4 x 10⁶±0.5 x 10⁶              |                                                        |
|                   | NEHU (1393)                        | 5.7     | 27              | 33.1              | 12                 | 4.80              | 1.1                 | 0.18                     | 0.11                          | 1.0 x 10⁵±0.5 x 10⁵              |                                                        |
|                   | Sum suh (1452)                     | 5.4     | 12.5            | 17.1              | 11.4               | 3.78              | 1.2                 | 0.22                     | 0.11                          | 3.3 x 10⁵±0.5 x 10⁵              |                                                        |
|                   | Mawkadong (1575)                   | 4.5     | 20              | 22.5              | 16.2               | 3.80              | 1.1                 | 0.16                     | 0.10                          | 2.7 x 10⁵±0.3 x 10⁵              |                                                        |
|                   | Mao (1696)                         | 6.5     | 25              | 27.8              | 33.7               | 3.93              | 0.9                 | 0.16                     | 0.11                          | 7.8 x 10⁶±1.1 x 10⁶              |                                                        |
|                   | Sacred Forest (1796)               | 4.5     | 20              | 23.5              | 33.6               | 3.95              | 0.7                 | 0.14                     | 0.12                          | 5.3 x 10⁵±0.6 x 10⁵              |                                                        |
|                   | Damthang (1852)                    | 4.7     | 15              | 19.5              | 12.8               | 2.78              | 0.9                 | 0.14                     | 0.10                          | 2.1 x 10⁴±0.5 x 10⁴              |                                                        |
|                   | Shillong Peak (1950)               | 5.7     | 19.5            | 23.8              | 9.6                | 3.96              | 0.9                 | 0.12                     | 0.12                          | 3.2 x 10⁶±0.4 x 10⁶              |                                                        |

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**Altitudinal Range**: AR-I, AR-II

**Survey site with altitude (m.a.s.l.)**: West Chandrapur (34), Naharlagun (134), Fifth Mile (210), Ganga lake (336), Medziphema (412), Rimassar (562), Mawshun (688), Keibul Lamjao (785), Lawngthlai (829), Lalmati (930), R. Vanhne village (1010), Mawsmai (1192), Tyrshi (1319), NEHU (1393), Sum suh (1452), Mawkadong (1575), Mao (1696), Sacred Forest (1796), Damthang (1852), Shillong Peak (1950).
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. (°C) | Ambient Temp. (°C) | Moisture Content (%) | Carbon Content (%) | Bulk Density (g/cm³) | Urease (mg NH₄⁺-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 x 10⁷ ±0.2 x 10⁷               | Temperate broadleaved, Temperate pine, Temperate conifer (*Acer pectinatum*, *A. oblongum*, *Alnus nepalensis*, *Rhododendron spp.*, *Castanopsis indica*, *Pinus merkusii*, *P. roxburghii*, *P. wallichiana*, *P. kesya*, *Quercus spp.*, *Taxus sp.*, *Cupressus torulosa*, *Abies spectabilis*, *A. delavayi*, *Taxus sp.*). |
|                   | Plutsero (2120)                      | 5.2     | 21.0            | 23.0              | 14.0                | 3.81               | 0.9                 | 0.12                          | 0.11                              | 2 x 10⁷ ±0.5 x 10⁷               |                                        |
|                   | Uttarey (2205)                       | 5.2     | 18.0            | 22.8              | 15.0                | 3.9                | 1.0                 | 0.11                          | 0.11                              | 2.1 x 10⁶ ±0.2 x 10⁶              |                                        |
|                   | Bomdila (2320)                       | 4.8     | 17.5            | 22.4              | 10.4                | 3.78               | 0.9                 | 0.12                          | 0.10                              | 1.8 x 10⁶ ±0.5 x 10⁶              |                                        |
|                   | Dzukou (2435)                        | 4.7     | 18.2            | 23.0              | 11.2                | 3.76               | 1.1                 | 0.11                          | 0.09                              | 1.7 x 10⁶ ±0.3 x 10⁶              |                                        |
|                   | Broksher (2599)                      | 5.1     | 14              | 18                | 18.2                | 2.77               | 1.0                 | 0.12                          | 0.09                              | 4.3 x 10⁶ ±0.2 x 10⁶              |                                        |
|                   | Riga (2672)                          | 4.8     | 16.0            | 19.0              | 2.9                 | 0.9                | 0.13                 | 0.09                          | 3 x 10⁵ ±0.5 x 10⁵               |                                        |
|                   | Bakhim (2744)                        | 5.2     | 14.9            | 18.9              | 2.02                | 3.1                | 1.0                 | 0.12                          | 0.09                              | 3.2 x 10⁴ ±0.3 x 10⁴              |                                        |
|                   | Namdapha (2880)                      | 5.1     | 15.3            | 18.7              | 2.18                | 2.72               | 1.0                 | 0.12                          | 0.09                              | 3.2 x 10⁶ ±0.3 x 10⁶              |                                        |
|                   | Tado (2964)                          | 5.0     | 16.0            | 18.4              | 22.4                | 2.68               | 0.9                 | 0.11                          | 0.08                              | 4 x 10⁴ ±0.4 x 10⁴                |                                        |
|                   | Tingbu (3010)                        | 4.9     | 15.0            | 15.7              | 2.28                | 2.84               | 0.8                 | 0.12                          | 0.09                              | 4.1 x 10⁴ ±0.5 x 10⁴              |                                        |
|                   | Ghinwanghanjang (3127)               | 5.0     | 16.0            | 16.4              | 2.24                | 2.81               | 0.9                 | 0.12                          | 0.08                              | 4.6 x 10⁴ ±0.6 x 10⁴              |                                        |
|                   | Varsey (3220)                        | 4.9     | 13              | 17                | 1.91                | 2.76               | 0.9                 | 0.14                          | 0.08                              | 1.8 x 10⁵ ±0.2 x 10⁵              |                                        |
|                   | Chira (3389)                         | 4.8     | 14.6            | 16.8              | 2.18                | 2.7                | 0.9                 | 0.13                          | 0.08                              | 2 x 10⁴ ±0.2 x 10⁴                |                                        |
|                   | Mandala (3435)                       | 5.1     | 14.2            | 15.9              | 2.27                | 2.71               | 1.0                 | 0.12                          | 0.09                              | 1.9 x 10⁵ ±0.2 x 10⁵              |                                        |
|                   | Tawang (3528)                        | 5.0     | 15.0            | 17.1              | 2.12                | 2.6                | 0.9                 | 0.12                          | 0.08                              | 1.6 x 10⁵ ±0.2 x 10⁵              |                                        |
|                   | Thangsing (3642)                     | 4.9     | 14.9            | 16.9              | 2.19                | 2.58               | 1.0                 | 0.12                          | 0.08                              | 2.6 x 10⁴ ±0.3 x 10⁴              |                                        |
|                   | Thongsong lake (3755)                | 4.9     | 14              | 16                | 2.32                | 2.4                | 1.1                 | 0.12                          | 0.09                              | 2 x 10⁶ ±0.3 x 10⁶                |                                        |
|                   | Bopa Mandir (3840)                   | 5.8     | 12              | 15                | 12.8                | 2.28               | 0.9                 | 0.12                          | 0.07                              | 3 x 10⁵ ±0.4 x 10⁵                |                                        |
|                   | Thugu (3990)                         | 5.2     | 12              | 14                | 2.87                | 1.7                | 0.8                 | 0.09                          | 0.07                              | 5 x 10⁶ ±0.6 x 10⁶                |                                        |
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$^3$) 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

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**Table 1**

| Altitude (m) | Soil pH | Soil Temp. (°C) | Ambient Temp. (°C) | Soil moisture content (%) | Soil carbon content (%) | Bulk density (g/cm$^3$) | Urease activity NH$_4^+$-N /g soil | Dehydrogenase activity TPF /g soil |
|--------------|---------|-----------------|-------------------|--------------------------|------------------------|-------------------------|-----------------------------------|----------------------------------|
|              |         |                 |                   |                          |                        |                         |                                   |                                  |

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

**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 phylogenetic 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          | Bacillus pseudomycoides      | 99.9  | 1490         | JN695724         |
| FM3              | Brownish          | Pseudomonas monteillii       | 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 amylophilusaciens   | 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 thuringensis        | 99.9  | 1488         | JN695705         |
| NP3              | Offwhite          | Bacillus thuringensis        | 100   | 1472         | JN695706         |
| SA1              | Whitish           | Bacillus thuringensis        | 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 amylo liquefaciens | 99.7 | 1458 | JN95707         |
| 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 | JF25994        |
| 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 taichangensis | 99.7 | 1463 | HQ600992        |
| MB4               | Pink              | Serratia nematodiphila      | 98.9 | 1477 | HQ600995        |
| RH6               | Light orange      | Chryseobacterium deflavii   | 98   | 1436 | JN975952        |
| N4                | Offwhite          | Enterococcus durans         | 99.5 | 1486 | HQ600998        |
| GL8               | White             | Staphylococcus cohnii       | 100  | 1452 | JN975910        |
| MP1               | Brownish          | Sporosarcina ureae          | 99.4 | 1422 | JN975711        |
| 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               | Whitis            | Acinetobacter nosocomialis  | 99.5 | 1462 | JQ433924        |
| HL4               | Offwhite          | Bacillus ginsengi           | 97.5 | 1474 | JQ433930        |
| TN1               | Pale              | Ralstonia mannitolylitica   | 99.4 | 1459 | JQ433936        |

**AR-III**

| FM5               | Offwhite          | Bacillus aryabhattai        | 99.8 | 1472 | JN95721         |
| 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 | JN95718         |
| 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        |
| Altitudinal Range | Sequenced Isolate | Colony Colour | Closest match          | % Similarity | Nucleotide Bases | Accession number |
|------------------|-------------------|---------------|-------------------------|-------------|-----------------|-----------------|
| CM5              | Creamish          | Offwhite      | *Ponticoccus gilvus*    | 99.9        | 1429            | JN695725        |
| N5               | Offwhite          | Pantoena ananatis | *Bacillus subtilis*    | 99.5        | 1395            | HQ601011        |
| WC5              | Creamish          | Brownish      | *Bacillus methylobacter* | 99.7        | 1469            | JN975953        |
| MP2              | Brownish          | Yellowish     | *Bacillus methylobacter* | 99.7        | 1469            | JN975950        |
| SP8              | Creamish          | Orange        | *Kluyvera ascorbata*    | 98.7        | 1466            | JF825990        |
| CM2              | Brownish          | Offwhite      | *Pseudomonas plecoglossicida* | 99.9        | 1475            | JN965722        |
| AM2              | Offwhite          | Orange        | *Pseudomonas moorei*    | 99.1        | 1472            | HQ600988        |
| BM3              | Yellowish         | Creamish      | *Pseudomonas arsenicoidans* | 98.8        | 1455            | JN208184        |
| KL3              | Offwhite          | Yellowish     | *Pseudomonas azotoformans* | 99.7        | 1428            | JN975909        |
| CK5              | Offwhite          | Yellowish     | *Pseudomonas gessardii* | 99.6        | 1480            | JN965712        |
| WC4              | Whitish           | Yellowish     | *Staphylococcus cohnii* | 100         | 1450            | JN965701        |
| DP1              | Whitish           | Brownish      | *Staphylococcus arlettae* | 99.9        | 1442            | JN965717        |
| GO1              | Creamish          | Whitish       | *Chryseobacterium taiwanense* | 99.3        | 1448            | JN975957        |
| VT3              | Offwhite          | Yellowish     | *Comamonas thiocyanicans* | 100         | 1470            | JQ433933        |
| HL1              | Creamish          | Orange        | *Pseudomonas nitroreducens* | 99.4        | 1460            | JQ433932        |
| SH1              | Whitish           | Orange        | *Bacillus pseudomycoides* | 99.2        | 1497            | JQ433948        |
| AR-IV            |                    |               |                         |             |                 |                 |
| DG7              | Offwhite          | Offwhite      | *Bacillus aryabhattai*  | 97.3        | 1464            | JN208198        |
| GP2              | Offwhite          | Offwhite      | *Bacillus aryabhattai*  | 99.9        | 1467            | JN975951        |
| GL5              | Brownish          | Offwhite      | *Bacillus aryabhattai*  | 100         | 1490            | JN975940        |
| BM3              | Offwhite          | Yellowish     | *Bacillus cereus*       | 98.7        | 1469            | JF825991        |
| SJ3              | Offwhite          | Offwhite      | *Bacillus cereus*       | 99.8        | 1440            | JN975941        |
| GO2              | Whitish           | Yellowish     | *Bacillus cereus*       | 99.6        | 1474            | JN975954        |
| CK1              | Whitish           | Yellowish     | *Bacillus thuringiensis* | 99.7        | 1454            | JN975943        |
| CM1              | Creamish          | Yellowish     | *Bacillus thuringiensis* | 99.9        | 1432            | JN965713        |
| SJ4              | Brownish          | Yellowish     | *Psychrobacillus psychrophilus* | 98.7        | 1465            | JN975955        |
| DG8              | Offwhite          | Yellowish     | *Cupriavidus metallidurans* | 98.8        | 1359            | JN208199        |
| CM4              | Offwhite          | Yellowish     | *Lysinibacillus parviboronicapensi* | 99.1        | 1466            | JN975932        |
| DG9              | White             | Yellowish     | *Staphylococcus warneri* | 100         | 1464            | JN208200        |
| TX2              | Brownish          | Orange        | *Enterobacter cloacae*  | 99.1        | 1468            | JN965719        |
| BM-1             | Whitish           | Yellowish     | *Pseudomonas koreensis* | 99.9        | 1371            | JN965999        |
| TH1              | Orange            | Yellowish     | *Chryseobacterium culicis* | 99.2        | 1439            | JN208181        |
| BM-2             | Creamish          | Yellowish     | *Pseudomonas nohii*     | 99.9        | 1447            | JN965700        |
| TS3              | Brownish          | Yellowish     | *Pseudomonas vancouverensis* | 99.8        | 1459            | JN975934        |
| BM1              | Offwhite          | Orange        | *Viridibacillus arvi*   | 98.9        | 1475            | JN965714        |
| TS2              | Whitish           | Yellowish     | *Pseudomonas baetica*   | 99.4        | 1446            | JN975937        |
| TS4              | Brownish          | Yellowish     | *Pseudomonas taiwanensis* | 99.1        | 1447            | JN975938        |
| TH4              | Offwhite          | Orange        | *Acinetobacter pittii*  | 99.8        | 1451            | JN965720        |
| TH6              | Brownish          | Yellowish     | *Pseudomonas extremaustralis* | 100         | 1466            | JN965702        |
| TH2              | Offwhite          | Yellowish     | *Pseudomonas extremaustralis* | 98.8        | 1432            | JN208182        |
| VT1              | Orange            | Yellowish     | *Chryseobacterium ureilyticum* | 99.8        | 1457            | JQ433943        |
| RV4              | Whitish           | Orange        | *Mitsuaria chitosanitabida* | 100         | 1419            | JQ433927        |
| ZR3              | Creamish          | Orange        | *Pseudomonas genticulata* | 99.6        | 1469            | JQ433923        |
| KR5              | Yellowish         | Orange        | *Sphingobium yanoikuyae* | 99.5        | 1433            | JQ433940        |
| SS1              | White             | Yellowish     | *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), Comamonas (1), Mitsuaria (1), Ralstonia (2) |
|                 | Gamma          | Aeromonas (2), Acinetobacter (5), Citrobacter (1), Klyvera (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.

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

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**Fig. 4.** Rooted Neighbor-joining tree based on 16S rRNA (more than 1 400 bases) gene sequences for the group β-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.*
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.
and \textit{Lysinibacillus} (0.177) (Fig. 8). The species accumulation curve displayed an increasing trend that did not level off (Fig. 9).

The species accumulation curve plotted using PAST 2.10 for the entire study area.

\textbf{Fig. 9.} Species accumulation curve plotted using PAST 2.10 for the entire study area.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{species_accumulation_curve.png}
\caption{Species accumulation curve plotted using PAST 2.10 for the entire study area.}
\end{figure}

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

\section*{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 vegetation 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 vegetation are known to affect soil microbial diversity and community structures (Kowalchuk \textit{et al.} 2002, Grayston & Prescott 2005, Han \textit{et al.} 2007, Thomson \textit{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 $\alpha$ 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 \textit{et al.} (2008) and Fierer \textit{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 \textit{Cedrus}, \textit{Pinus} and \textit{Taxus} in Himalayan range are known to exert slightly stimulatory effect on the microorganisms and therefore support relatively higher microbial population (Pandey \textit{et al}. 2006). Similar effects of vegetation on soil microbial diversity and community structures has also been reported by several other workers (Kowalchuk \textit{et al} 2002, Grayston & Prescott 2005, Han \textit{et al} 2007, Thomson \textit{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 (Holister 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 379km² 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 diversidad 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 rARN. 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 parámetros 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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