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Diversity of Culturable Soil Micro-fungi along Altitudinal Gradients of Eastern Himalayas

Lamabam Sophiya Devi, Polashree Khaund, Fenella M. W. Nongkhlaw and S. R. Joshi*

Microbiology Laboratory, Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong 793022, Meghalaya, India

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Very few studies have addressed the phylogenetic diversity of fungi from Northeast India under the Eastern Himalayan range. In the present study, an attempt has been made to study the phylogenetic diversity of culturable soil fungi along the altitudinal gradients of eastern Himalayas. Soil samples from 24 m above sea level to 2,000 m above sea level altitudes of North-East India were collected to investigate soil micro-fungal community structure and diversity. Molecular characterization of the isolates was done by PCR amplification of 18S rDNA using universal primers. Phylogenetic analysis using BLAST revealed variation in the distribution and richness of different fungal biodiversity over a wide range of altitudes. A total of 107 isolates were characterized belonging to the phyla Ascomycota and Zygomycota, corresponding to seven orders (Eurotiales, Hypocreales, Calosphaeriales, Capnodiales, Pleosporales, Mucorales, and Mortierellales) and Incertae sedis. The characterized isolates were analysed for richness, evenness and diversity indices. Fungal diversity had significant correlation with soil physico-chemical parameters and the altitude. Eurotiales and Hypocreales were most diverse and abundant group of fungi along the entire altitudinal stretch. Species of *Penicillium* (D = 1.44) and *Aspergillus* (D = 1.288) were found to have highest diversity index followed by *Talaromyces* (D = 1.26) and *Fusarium* (D = 1.26). Fungal distribution showed negative correlation with altitude and soil moisture content. Soil temperature, pH, humidity and ambient temperature showed positive correlation with fungal distribution.

KEYWORDS : Altitude, Diversity indices, Microfungal diversity, Richness, 18S rDNA

Introduction

The relationship between biodiversity of soil fungi and ecosystem function is an issue of paramount importance, particularly in the face of global climate change and human alteration of ecosystem processes. Soil micro-flora performs a variety of ecosystem functions that are crucial to maintaining ecosystem stability [1-3]. Fungi are an important component of the soil micro-biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. The saprobic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition of plant structural polymers, such as, cellulose, hemicellulose and lignin, thus contributing to the maintenance of global carbon cycle [4]. Researches on distribution of fungi in soil have focused in agricultural soils and less is known about the occurrence of fungi under natural soil conditions [5, 6]. Fungal flora of the soil is attributed to native soils [7]. Some fungi are widely distributed in soil while others are limited to certain habitats. The distribution of fungi is influenced by the abundance and nature of the organic content of the soil, as well as by other soil and climatic conditions, surface vegetation and soil texture. Soil micro-fungal diversity has important implications in ecosystem stability and productivity. They are a well recognized source for variety of chemicals, several of which are valuable pharmaceuticals, agrochemicals and industrial products [8].

Fungi have historically been identified and classified using morphological characteristics such as sexual structure. However, fungi, especially microscopic ones, often have few useful morphological features and show pronounced morphological variability [9]. Many fungi are anamorphic without sexual production, yet possess a surprisingly high level of genetic variation [10]. Furthermore, an increasing number of morphologically indistinguishable (cryptic) species have recently been described [11]. Accordingly, the use of morphology for fungal identification and classification can be severely biased. In the past decade, the ability to

*Corresponding author <E-mail : srjoshi2006@yahoo.co.in>

identify species at the molecular level has changed our understanding of the species concept for different groups of fungi. Particularly, molecular phylogenetic approaches avoid subjectivity in determining the limits of a species by relying on the concordance of more than one gene genealogy and have been approved to well suit teleomorphic and anamorphic fungi [12].

Northeast (NE) India is regarded as one of the biodiversity hotspots of the globe [13] and blessed with a wide range of physiography and ecoclimatic conditions. It forms one of the major regions of tropical forests in India, especially the species-rich tropical rain forests [14]. The tropical semi-evergreen and moist deciduous forests in the lowlands of this region extend south and west into the subcontinent, and east into Southern China and Southeast Asia. The subtropical forests of the region follow the foothills of the Himalaya to the west and extend into Southeast China in the east. Himalayan temperate and subalpine zone forests extend from northern Pakistan and adjacent Afghanistan through NE India to Southwest China. Northeast region of India sprawling over 262,379 sq.km, 22~30° N and 89~97° E comprising of the states of Arunachal Pradesh, Assam, Meghalaya, Manipur, Tripura, Mizoram, Nagaland and Sikkim can be physiographically categorized into the Eastern Himalayas, Northeast hills (Patkai-Naga Hills and Lushai Hills) and the Brahmaputra and Barak Valley plains. NE 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. At the confluence of the Indo-Malayan, Indo-Chinese and Indian biogeographical realms, this region is unique in providing a profusion of habitats, which features diverse biota with a high level of endemism. Highly undulating topography leads to marked variation in edapho-climatic conditions, and has resulted in great variation in altitude, irrespective of distance due to which it is rich in diverse groups of flora and fauna which have been documented but the microbial groups have not attracted any attention as yet. In the present study, an attempt has been made to investigate soil micro-fungal community structure and phylogenetic diversity from soil from different altitudes of NE India under Eastern Himalayan range and to assess if altitude has any impact on the diversity of fungi in the range.

Materials and Methods

Study area. The study area comprises of an area sprawling over 262,379 sq.km in NE India spread from 22~30° N and 89~97° E at altitude from 24 m above sea level to 2,000 m above sea level (Fig. 1).

Collection of soil samples. Soil samples were collected from various vegetational and climatic zones spread over different altitudes of NE India under Eastern Himalayan range. Surface and sub-surface soil samples were collected aseptically from different microhabitats. Every sample was a mixture of soils from 5 to 10 holes at a depth from 0 to 30 cm. The soil samples were transported in sterile containers to the laboratory where it was stored at 4°C until processing.

Fig. 1. Map showing sampling area under Eastern Himalayan range.

Physico-chemical characteristics of soil. Different physical parameters of the sampling site were recorded. The ambient temperature and humidity was recorded using a Hygrometer. The altitudes of the sampling sites were determined by using GPS (Garmin 76 CSX, USA). Soil pH was determined using a soil-water mixture (1 : 5, w/v) with a pH meter (Chemiline, India).

Isolation and enumeration of fungi. The colony forming units (CFU) of the fungi were determined by serial dilution method. 10 g of the soil sample was suspended in 90 mL of sterile 0.86% NaCl solution and shaken vigorously on a magnetic stirrer for 30 min to obtain uniform suspension. A serial dilution up to 10^{-5} was made and 0.1 mL aliquot from each dilution was inoculated on potato dextrose agar (PDA; HiMedia, India) plates supplemented with Streptomycin (20 ppm) and incubated at 25°C for 48~72 hr. All fungal colonies which appeared on plates were counted. Morphologically different strains were isolated and purified. Purified fungal isolates were maintained on PDA slant (stored at 4°C) and also in sterile distilled water (stored at −80°C).

Characterization of fungal isolates. Fungal isolates were examined and identified to genus level using light microscopy. Classical morphological features of the fungal isolates were used for these preliminary identifications of the fungal isolates. Selected fungal isolates were characterized using molecular approach. Genomic DNA of the isolates

was extracted using genomic DNA miniprep purification spin kit (Qiagen, Germany). The universal 18S rRNA gene primers; forward nu-SSU-0817-5, 5'-TTAGCATGGAAT-AATRRAATA-3' and reverse nu-SSU-1536-3, 5'-ATTGC-AATGCYCTATCCCCA-3' [15] were used for amplification of the 18S rRNA gene. The PCR reaction mixture comprised of 10 μL fungal DNA, 5 μL 10 × PCR buffer, 1.5 μL of 50 mM $MgCl_2$, 1 μL of 10 mM dNTP, 0.25 μL Taq polymerase, 40 pM each of the forward and the reverse primers in a total reaction volume of 50 μL [16]. Amplification of DNA was carried out in 9700 Gold thermal cycler (Applied Biosystems, Warrington, UK) under the following conditions: initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 10 sec, extension at 72°C for 30 sec, and a final extension at 72°C for 2 min. Approximately 740 nucleotides were amplified. Amplified PCR products were purified using QIA quick gel extraction kit (Qiagen) and were bi-directionally sequenced (BigDye terminator; Applied Biosystems).

Sequence similarity searches were performed for each of the representative fungal sequences against the non-redundant database maintained by the National Center for Biotechnology Information (NCBI) using the BLAST algorithm (http://www.ncbi.nlm.nih.gov).

Nucleotide sequence accession number. The 18S rDNA nucleotide partial sequences were submitted to GenBank and accessions were obtained.

Statistical analysis. The diversity of the culturable fungal diversity in our study was estimated using the Shannon index (H') [17]. However 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 [18]. Simpson's index of diversity was also estimated [19]. The reciprocal form of Simpson's index (1/D) usually is presented, ensuring that the index increases with increasing diversity. Evenness (J) of species was evaluated using the formula as given by Pielou [20]. The number of species divided by the square root of the number of individuals results in species richness, S.

The correlation between the different environmental factors, soil physico-chemical parameters and fungal counts was determined by calculating Pearson product moment correlation coefficients [21]. The correlations were considered significant if $p < 0.05$. Karl Pearson correlation coefficient was done using SPSS ver. 20 (IBM, NY, USA).

Results and Discussion

There was significant variation in the soil physico-chemical parameters and fungal CFU counts along the altitudinal gradient (Table 1).

Soil temperature, pH and moisture are some of the major factors affecting the fungal population and diversity [22]. Altitude is also considered important factor acting on fungal diversity. Generally, an increase in altitude is accompanied by a decrease in temperature, affecting negatively fungal diversity. In our study, the range of soil pH along the altitudinal stretch did not show much variation. The results obtained in our study clearly indicate that there is a marked decrease in the number of isolates with increasing altitude which is in agreement with other studies [23, 24]. The CFU counts of fungi decreased with increasing altitude, as highest CFU counts were recorded at lower altitudes and lowest CFU counts were recorded at higher altitudes (Table 1). However, there was a sharp increase in CFU count at 1,000~1,500 altitudinal range which can be attributed to the tropical semi-evergreen to sub-tropical evergreen forest prevalent in the range. Bissett and Parkinson [25] have demonstrated that moisture, soil pH and temperature were the most important abiotic variables influencing the distribution and community composition of alpine soil fungi. Microbial community structure and diversity are sensitive to any small change in their microhabitat especially due to anthropogenic activities [26-28]. Soil physico-chemical factors, ambient temperature and humidity were found to influence soil fungal distribution and population density at various level of significance ($p < 0.05$, $p < 0.01$). Fungal distribution showed negative but insignificant correlation with altitude ($r = –0.269$) and soil moisture content ($r = –0.107$). Soil parameters like pH ($r = 0.065$), soil temperature ($r = 0.340$), ambient temperature ($r = 0.214$) showed positive but insignificant correlation whereas humidity ($r = 0.410$) showed positive significant correlation with the fungal distribution (Table 2).

Cultivation of fungi from the four different altitudinal ranges yielded a total of 107 isolates (Table 3). Sequence and phylogenetic analysis revealed the soils to be rich in fungal diversity belonging to the phyla Ascomycota and Zygomycota, corresponding to seven orders (Eurotiales, Hypocreales, Calosphaeriales, Capnodiales, Pleosporales, Mucorales, and Mortierellales) and Incertae sedis which were distributed in 22 genera and 44 species (Table 4). Ascomycota was the dominant phyla in all the four altitudinal ranges. Eurotiomycetes presented the highest relative abundance in the three altitudinal ranges (1~500, 1,000~1,500, and 1,500~2,000) followed by Sordariomycetes whereas in the altitudinal range 500~1,000 Sordariomycetes had the highest relative abundance followed by Eurotiomycetes (Fig. 2). Dothideomycetes was not characterized from the altitudinal range 500~1,000 which could be attributed to the vegetation types. In the present study, under Euritoiales, *Penicillium* and *Aspergillus* were observed to be the most diverse genus and were evenly distributed in the entire stretch. Among Ascomycota,

Table 1. Soil physico-chemical parameters and fungal colony forming unit (CFU) counts along the altitudinal gradient

Altitudinal range in meter above sea level (masl)	Sampling site with altitude (masl)	Soil temperature (°C)	Ambient temperature (°C)	Humidity (%)	Soil pH	Soil moisture content (%)	CFU	Average CFU/g soil
1~500	Baruakandi, 24	29.0	30.0	98	6.1	19.0	98×10^3	20.08×10^3
	Monabari, 83	19.5	24.2	87	5.6	15.2	1.3×10^3	
	Hurua, 101	26.5	28.2	98	5.8	19.8	37×10^3	
	Midpu area, 184	22.0	26.2	87	5.8	15.4	2.13×10^2	
	Kolcho vill, 234	26.5	35.7	77	5.8	17.0	0.84×10^3	
	Ganga Lake, 336	19.0	26.1	80	4.9	19.0	1.9×10^3	
	Megdziphema, 412	24.0	29.1	78	4.8	13.2	9.2×10^3	
	Khurongkulai, 476	25.0	32.1	88	6.1	16.9	10.3×10^3	
500~1,000	Nongpoh, 522	22.5	29.6	60	6.0	14.6	2.3×10^3	13.98×10^3
	Rimassar, 562	20.5	23.7	67	5.0	6.5	17×10^3	
	Saiha, 575	27.5	34.2	75	5.1	18.5	28×10^3	
	Thenzawl, 759	25.5	32.7	68	5.8	17.9	2.9×10^3	
	Keibul Lamjao, 785	26.0	34.7	71	4.8	17.6	6.06×10^3	
	Khongjom, 802	30.0	33.0	75	5.7	15.1	9.04×10^3	
	Ngariyan, 881	28.0	30.4	86	5.7	16.0	18×10^3	
	Haulawng district, 941	24.0	30.8	67	5.7	27.6	9.6×10^3	
	Lalmati, 931	25.0	24.1	93	4.6	18.5	1.0×10^3	
	Kolasib, 900	25.0	31.5	78	6.5	22.0	17.9×10^3	
1,000~1,500	R.Vanhne village, 1010	25.5	32.4	91	4.7	9.8	91.8×10^3	19.86×10^3
	Rum Saitbakon, 1015	18.2	24.8	60	4.8	9.8	22×10^3	
	Mawsmai, 1195	22.0	28.7	71	5.3	18.6	6×10^3	
	Ialong, 1359	27.0	29.2	62	5.2	28.3	13×10^3	
	Thadlaskein, 1345	26.0	30.7	66	5.4	15.9	13×10^3	
	NEHU Campus, 1393	27.0	33.1	43	5.7	12.0	4×10^3	
	Sumsuh, 1427	23.5	30.6	88	5.4	11.4	6.7×10^3	
	Jakrem, 1493	23.0	26.0	71	4.9	17.4	2.4×10^3	
1,500~2,000	Cherrapunjee, 1574	18.5	25.3	76	5.2	22.0	19.8×10^3	5.48×10^3
	Mawkadong, 1575	20.0	22.5	81	4.5	16.2	3×10^3	
	Mawsynram23, 1578	20.0	25.7	73	5.0	4.0	6×10^3	
	Mao, Senapati, 1696	25.0	27.8	53	6.5	33.7	2.4×10^3	
	Sacred forest, 1796	20.0	23.5	77	4.5	33.6	4×10^3	
	Shillong Peak, 1950	19.5	23.8	84	5.7	9.6	1.5×10^5	
	Mawsynram1, 1634	21.0	23.9	39	5.9	13.0	2.6×10^3	
	3rd Mile, Upper Shillong, 1597	23.0	26.7	78	4.6	21.0	4.5×10^3	

Table 2. Pearson correlation coefficients analysis of different parameters

Parameters	Altitude (masl)	Soil temperature (°C)	Ambient temperature (°C)	Humidity (%)	Soil pH	Soil moisture content (%)
Soil temperature (°C)	$p = -0.246$					
	$r = 0.199$					
Ambient temperature (°C)	$p = -0.240$	$p = 0.764$**				
	$r = 0.209$	$r = 0.000$				
Humidity (%)	$p = -0.369$*	$p = 0.049$	$p = -0.167$			
	$r = 0.049$	$r = 0.800$	$r = 0.385$			
Soil pH	$p = -0.252$	$p = 0.399$*	$p = 0.378$*	$p = -0.108$		
	$r = 0.187$	$r = 0.032$	$r = 0.043$	$r = 0.578$		
Soil moisture contant (%)	$p = 0.168$	$p = 0.165$	$p = 0.013$	$p = -0.118$	$p = 0.185$	
	$r = 0.383$	$r = 0.393$	$r = 0.946$	$r = 0.543$	$r = 0.336$	
CFU count/g soil	$p = -0.269$	$p = 0.340$	$p = 0.214$	$p = 0.410$*	$p = 0.065$	$p = -0.107$
	$r = 0.158$	$r = 0.071$	$r = 0.264$	$r = 0.027$	$r = 0.737$	$r = 0.581$

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 3. Phylogenetic affiliations of fungi isolated from soils on the basis of 18S rRNA genes sequences showing closest match and their NCBI accession No.

Altitudinal ranges in meter above sea level (masl)	Isolate name	Taxon	Closest match with accession No.	Similarity value (%)	Accession No.
1~500	MR1	Eurotiales	*Aspergillus* sp. (HM773225.1)	100	JN040731
	CB-6	Eurotiales	*Aspergillus versicolor* (JN546128.1)	100	JQ256469
	NG2-1	Eurotiales	*Penicillium purpurogenum* (JX022616.1)	99	JF968421
	NG5-3	Eurotiales	*Penicillium charlesii* (FJ430768.1)	99	JF968422
	AM-2	Eurotiales	*Penicillium* sp. (HM773243.1)	99	JQ256464
	MP-2	Eurotiales	*Penicillium purpurogenum* (FJ941868.1)	100	JQ256465
	DI-1	Eurotiales	*Penicillium* sp. (HQ317210.1)	99	JN247750
	MR-2	Eurotiales	*Penicillium decumbens* (HQ871900.1)	98	JN040743
	MR4	Eurotiales	*Penicillium purpurogenum* (FJ941868.1)	98	JN040746
	NL4	Eurotiales	*Penicillium piceum* (GU477623.1)	86	JN040747
	SL6	Eurotiales	*Penicillium* sp. griseofulvum (JN886803.1)	99	JN247760
	BK-1	Eurotiales	*Penicillium* sp. (HM773243.1)	99	JQ256466
	SJ3	Eurotiales	*Penicillium chrysogenum* (EU203859.1)	99	JQ256467
	MZ4-2	Eurotiales	*Penicillium purpurogenum* (FJ941868.1)	98	JQ256473
	HU6	Eurotiales	*Penicillium purpurogenum* (GU573849.1)	96	JN040728
	SJ2	Eurotiales	*Penicillium* sp. (HQ317210.1)	99	JN040733
	SL2	Eurotiale	*Chromocleista malachitea* (FJ358346.1)	99	JN040744
	SL9	Eurotiale	*Chromocleista malachitea* (FJ358346.1)	99	JN040737
	RH5	Eurotiales	*Thysanophora longispora* (AB075440.1)	73	JN040748
	GL3	Eurotiales	*Talaromyces leycettanus* (AY526487.2)	74	JN040735
	MZ4-8	Eurotiales	*Talaromyces flavus* (GU733356.1)	100	JQ256470
	HU5	Eurotiales	*Penicillium piceum* (GU477623.1)	87	JN040739
	CB-4	Eurotiales	*Aspergillus* sp. (HM773226.1)	99	JQ281523
	AM-1	Eurotiales	*Talaromyces leycettanus* (AY526487.2)	73	JN040738
	MZ4-1	Eurotiales	*Talaromyces byssochlamydoides* (HQ600966.1)	99	JQ074020
	MZ4-4	Eurotiales	*Penicillium purpurogenum* (JQ074027.1)	99	JQ074021
	NG5-2	Eurotiales	*Penicillium purpurogenum* (JX022616.1)	98	JF968433
	MZ4-5	Eurotiales	*Talaromyces flavus* (GU733356.1)	99	JQ074022
	MZ7-3	Eurotiales	*Penicillium purpurogenum* (FJ941868.1)	99	JQ074027
	MZ7-4	Eurotiales	*Talaromyces flavus* (GU733356.1)	99	JQ074028
	MZ4-3	Eurotiales	*Penicillium decumbens* (FJ458446.1)	99	JQ256475
	MZ4-12	Eurotiales	*Penicillium* sp. (DQ184697.1)	99	JQ074031
	MZ4-8a	Hypocreales	*Fusarium* sp. (JF807402.1)	99	JQ074023
	SL3	Hypocreales	*Nectria lugdunensis* (AY357278.1)	98	JN247757
	SL13	Hypocreales	*Hypocrea koningii* (JQ278021.1)	99	JN040745
	MZ2-3	Hypocreales	*Fusarium oxysporum* (JQ926985.1)	99	JQ281526
	DTEA-3	Hypocreales	*Fusarium oxysporum* (JQ926985.1)	99	JN040741
	IN2	Hypocreales	*Fusarium oxysporum* (JQ926985.1)	100	JQ256468
	WC6	Hypocreales	*Cladosporium* sp. (EU167574.1)	99	JN040749
	WS9	Hypocreales	*Trichoderma viride* (FJ598872.1)	94	JF927993
	SL5	Calosphaeriales	*Pleurostomophora richardsiae* (AY761066.1)	93	JN247755
	SL8	Mortierellales	*Mortierella wolfii* (AF113425.1)	98	JN247749
500~1,000	MZ6-3	Eurotiales	*Aspergillus nomius* (JF416646.1)	99	JQ074026
	RM4	Eurotiales	*Thysanophora penicillioides* (AB075434.1)	98	JF968434
	NN8	Eurotiales	*Penicillium griseofulvum* (JN886803.1)	99	JF968426
	MZ10-6	Eurotiales	*Penicillium decumbens* (HQ871900.1)	99	JQ074033
	MZ9-1	Eurotiales	*Penicillium* sp. (FJ430775.1)	99	JQ074034
	MZ1-3	Eurotiales	*Talaromyces byssochlamydoides* (HQ600966.1)	100	JQ281525
	MZ14-1	Eurotiales	*Talaromyces flavus* (GU733356.1)	100	JQ281530
	MZ5-9	Eurotiales	*Talaromyces flavus* (GU733356.1)	99	JQ074036
	MZ5-3	Eurotiales	*Chromocleista malachitea* (FJ358346.1)	100	JQ074030
	MZ5-8	Hypocreales	*Chamaeleomyces granulomatis* (HM635076.1)	98	JQ256474
	MZ5-4	Hypocreales	*Hypocrea rufa* (JF922009.1)	100	JQ074032
	MZ5-2	Hypocreales	*Fusarium* sp. (JF922007.1)	98	JQ074024
	MP5	Hypocreales	*Fusarium* sp. (EU710822.1)	99	JN040730
	MZ6-2	Hypocreales	*Fusarium oxysporum* (JF922007.1)	99	JQ074025

Table 3. Continued

Altitudinal ranges in meter above sea level (masl)	Isolate name	Taxon	Closest match with accession No.	Similarity value (%)	Accession No.
	NH2	Hypocreales	*Fusarium oxysporum* (EU710822.1)	99	JF968424
	NN1	Hypocreales	*Fusarium oxysporum* (JQ926985.1)	99	JN040732
	MZ1-4	Hypocreales	*Hypocrea rufa* (JF922009.1)	100	JQ281528
	MZ10-7	Hypocreales	*Fusarium oxysporum* (JQ926985.1)	100	JQ281529
	R-1	Hypocreales	*Hypocrea rufa* (JF922009.1)	100	JQ281531
	MZ1-1	Hypocreales	*Tricoderma* sp. (HM439095.1)	98	JQ281524
	KL-1	Incertae sedis	*Apiospora montagnei* (JN546134.1)	99	JN040736
	MZ3-5	Mucorales	*Absidia glauca* (JQ004925.1)	98	JQ074019
	NG7-5	Mucorales	*Absidia gluaca* (JQ004925.1)	95	JF968423
1,000~1,500	OT1	Eurotiales	*Aspergillus niger* (JX112703.1)	99	HQ600982
	N6	Eurotiales	*Penicillium purpurogenum* (GU573849.1)	99	HQ600973
	N9	Eurotiales	*Aspergillis* sp. (JX112703.1)	99	HQ600974
	N10	Eurotiales	*Penicillium charlesii* (FJ430769.1)	99	HQ600975
	N12	Eurotiales	*Penicillium charlesii* (FJ430769.1)	99	HQ600976
	N13	Eurotiales	*Penicillium janthinellum* (AB293968.1)	99	HQ600977
	MZ8-2	Eurotiales	*Penicillium* sp. (FJ430775.1)	99	JQ074029
	MZ13-2	Eurotiales	*Penicillium decumbens* (HQ871900.1)	100	JQ281528
	T1	Incertae sedis	*Apiospora montagnei* (FJ941864.1)	99	HQ600984
	N5	Capnodiales	*Davidiella tassiana* (FJ941874.1)	99	HQ600972
	N2	Mucorales	*Umbilopses* sp. (HM161751.1)	99	HQ600971
	ZP7	Mucorales	*Absidia gluaca* (AF157118.1)	97	JF968429
	UB7	Mucorales	*Mucor genevensis* (HM623319.1)	99	JN247758
1,500~2,000	CHE2-1	Eurotiales	*Aspergillus fumigates* (FJ941867.1)	99	JN247753
	MI5	Eurotiales	*Penicillium griseofulvum* (FJ717697.1)	99	HQ600965
	MI6	Eurotiales	*Penicillium charlesii* (HQ600976.1)	99	HQ600969
	SF1	Eurotiales	*Paecilomyces lilacinus* (JF824691.1)	99	HQ600978
	SF3	Eurotiales	*Penicillium charlesii* (FJ430769.1)	99	HQ600979
	SF7	Eurotiales	*Penicillium olsonii* (FJ717701.1)	99	HQ600980
	SF8	Eurotiales	*Penicillium glabrum* (GU733357.1)	93	HQ600981
	SP5	Eurotiales	*Aspergillus terreus* (HM773229.1)	99	JN860210
	MAU-1	Eurotiales	*Aspergillus flavus* (JF824683.1)	100	JQ824836
	MAU-2	Eurotiales	*Talaromyces flavus* (GU733356.1)	100	JQ824837
	MAU-3	Eurotiales	*Aspergillus awamori* (JQ012801.1)	100	JQ824838
	MAU-4	Eurotiales	*Penicillium* sp. (HQ317210.1)	99	JQ824839
	MAU-5	Eurotiales	*Aspergillus fumigates* (HQ871892.1)	99	JQ824840
	CHE2-3	Hypocreales	*Hypocrea koningii* (JQ278021.1)	99	JN247752
	MD1	Pleosporales	*Alternaria alternata* (HQ691422.1)	98	JF968425
	CHE2-4	Mucorales	*Absidia glauca* (AF113409.1)	97	JN247751
	MD2	Mucorales	*Umbelopsis* sp. (JF895927.1)	99	HQ600968

Table 4. Taxonomic grouping of fungi isolated from different altitudes

Phylum	Class	Order/Group	Genus with the number of species (given within parentheses)
Ascomycota	Eurotiomycetes	Eurotiales	*Chromocleista* (1), *Paecilomyces* (2), *Aspergillus* (8), *Penicillium* (9), *Talaromyces* (3), *Thysanophora* (2)
	Sordariomycetes	Hypocreales	*Fusarium* (2), *Trichoderma* (1), *Hypocrea* (2), *Emericellopces* (1), *Nectria* (1), *Chamaeleomyces* (1), *Niesslia* (1)
		Incertae sedis	*Apiospora* (1)
		Calosphaeriales	*Pleurostomophora* (1)
	Dothideomycetes	Capnodiales	*Davidiella* (1), *Cladosporium* (1)
		Pleosporales	*Alternaria* (1)
Zygomycota	Zygomycetes	Mucorales	*Umbelopsis* (2), *Absidia* (1), *Mucor* (1)
		Mortierellales	*Mortierella* (1)

diversity index of genus *Penicillium* (1.44) was found to be highest followed by *Aspergillus* (1.29) and *Talaromyces* (1.26). *Fusarium* and *Hypocrea* belonging to the class Hypocreales were dominant genus among the phyla

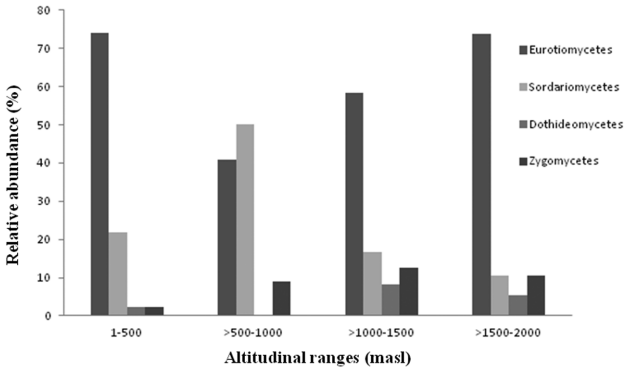

Fig. 2. Relative abundance (%) of fungal order along the altitudinal gradient of the studied Himalayan range.

Ascomycota with diversity index of 1.16 and 1.15 respectively.

Shannon-Wiener's index, Diversity index, reciprocal of Simpson's index, species richness and evenness index for the studied range was found to be 3.473, 32.233, 0.9585, 4.291, and 0.732 respectively (Table 5). The Shannon-Wiener's index (H') for the entire range varied from 2.99 to 3.35. The highest value for most of the diversity indices as well as species richness was recorded for the altitudinal range 1,000~1,500 (H' = 3.473, D = 32.233, 1/D = 0.9585, S = 4.291).

These findings provide important insights that aid our understanding of the diversity of fungi in natural ecosystems since fungi comprise important component of microbial diversity in high altitudes and are considered key organisms in inland ecosystems. Information on fungal diversity and functions might provide scope for bioprospecting of new source of drugs and other industrially important biomolecules and enzymes. New technologies, particularly in nucleic acid analysis, bioinformatics, analytical chemistry, and habitat sampling and characterization place the study of microbial diversity on the cutting edge of science. *Bacillus* from high altitudes of eastern Himalayan range has been reported to produce thermostable enzyme [29]. Previous study also demonstrated that the fungi isolated from soil systems of high altitudinal cold climatic zones in eastern Himalayan range were capable of synthesizing stable silver nanoparticles [30]. Despite the acknowledged value of microorganisms especially fungi, our knowledge of their diversity in NE India under Eastern Himalayan range is still very scarce. This calls for continued research to inventorize and protect the unexplored resources not only for the conservation of natural ecosystems but also for the future benefits through bioprospection.

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Table 5. Statistical analysis of fungal diversity at different altitudinal ranges

Diversity indices	Altitudinal ranges in meter above sea level (masl)				Total
	1~500	500~1,000	1,000~1,500	1,500~2,000	
Species richness	3.097	2.772	4.083	3.671	4.291
Reciprocal of Simpson's index	0.913	0.9008	0.9444	0.9252	0.9585
The Shannon Index	2.739	2.437	2.947	2.698	3.473
Diversity	15.472	11.439	19.049	14.85	32.233
Evenness	0.7368	0.88	0.9524	0.9281	0.7325

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