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Study on Air Bacteria at Different Altitudinal Locations in Tezpur to Tawang Axis

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ABSTRACT

Microflora plays an important role in modulating environmental quality. Among microflora, bacteria are omnipresent in the environment. Pathogenic bacteria, present in air, are known to affect significantly the health and well-being of human, animal or plant populations. Air bacteria monitoring is thus essential for surveillance of pathogenic microorganisms from public health perspective besides its significant implications in detection and mitigation of biothreat related issues. Despite the geo-politically strategic importance of northeast India, there is scarcity of data on human health and disease surveillance. Considering these facts, we, for the first time studied the bacterial diversity of air at six important sites adjacent to the international border in the northeast region of India, having an altitude range of 73 m (Tezpur) to 4170 m (Sela Pass) above sea level. Standard microbiological techniques, such as Tryptone Soya Agar, Mannitol salt and McConkey agar strips and plates were used for air bacterial load assessment and culture for subsequent analysis using biochemical and molecular techniques. Following RFLP study, twenty six different bacterial colonies were isolated. Subsequently, bacteria identification was carried out by examining the substrate utilisation patterns, sequencing 16S rRNA gene and phylogenetic analysis. Results of the study reveal that the isolates mostly belong to two genera *Bacillus* and *Staphylococcus* (eleven in each genus), along with *Micrococcus, Pseduomonas* and *Acinetobacter*. Based on significant match of our sequences with that of medically important bacterial 16S rRNA sequences available at 16SpathDB 2.0 and review of available literature, we found that a number of these bacterial species have the pathogenic potential. In this manuscript we report our results and discuss the importance of air bacterial surveillance from the perspective of human health, hygiene and biothreat mitigation.

Keywords: Air microflora; Altitudinal variations; Bacteria; High altitude region; Bio-security

1. INTRODUCTION

Bacteria, a major group of microorganisms, are ubiquitous in the atmosphere[1-4]. They are easily transported by wind over long distances due to their minute size, and extended duration of residence in the atmosphere. The concentration of bacterial particles is higher on the land than the sea (approx. 100-1000 fold less)[4]. Bacteria may enter into the atmosphere from varied sources including soil, water, human/animals and plants and even from clouds[5-6]. Bacteria play an important role in the health and wellbeing of human, animals and plant populations. In addition, aeroallergens related to bacteria also have important implications in public health and hygiene[4,7]. It is well established that anthropogenic activities affect the concentration and characteristics of bacterial population present in a given region[8]. Increase in population and related activities, and degradation of air quality leads to atmospheric pollution by increasing bacterial concentration[9]. According to a recent report, degradation of air quality with consequences for health and hygiene is becoming serious problem in India[10]. Moreover, airborne bacteria have been implicated in the causation of several diseases including allergies and serious infections.

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There are locations in the north-eastern parts of India that attracts many visitors. The route to Tawang (Arunachal Pradesh state) from Tezpur (Assam state) of India is famous for its remarkable natural splendour of Himalayan ranges, in addition to its socio-political and military importance. This route takes visitors from Tezpur (Assam) to Tawang via towns such as Bhalukpong, Bomdila, and Dirang through the folds & furrows of the mighty mountain. Study of the diversity and abundance of airborne bacterial populations have several implications and a subject of concern related to health, hygiene and bio-security issues. Although, these regions are having enormous strategic importance, however, terrains and geo-climatic conditions are quite difficult, leading to paucity of data on health and communicable diseases. Nevertheless, significant incidences related to Acute Respiratory Infection (ARI) are reported with a rapidly increasing trend. Among the various communicable diseases reported by the Indian States/UTs during the year 2017, the acute respiratory infection (ARI) accounted for the maximum percentage of morbidity (69 %) and mortality (23 %) as reported in the National Health Profile 2018 (Central Bureau of Health Intelligence, Ministry of Health & Family Welfare, Government of India, http://cbhidghs.nic.in/). This increment of ARI might have etiologic association with air borne pathogens.

Keeping these facts in mind, a surveillance program was undertaken to study the air bacterial diversity of five selected locations of Arunachal Pradesh, nearby international border having an altitude up to 4170 m above sea level, and one site in Assam (where the laboratory is located).

2. MATERIALS AND METHODS

2.1 Sampling Sites and Collection

Samples were collected from six selected sites of different altitudes of northeastern part of India namely Tezpur of Assam and Bhalukpong, Bomdila, Dirang, Sela pass (a high altitude mountain pass) and Tawang of Arunachal Pradesh (Fig. 1). Road distance from Tezpur to Tawang is approximately 360 km. Coordinates, weather and demographic data of the study sites are presented in Table 1. Sample collection for the study was done as stated below from the selected sites which are commonly commutable places.

Sampling was done using HiMedia Air Sampler system (model: LA002 of HiMedia Laboratories Pvt. Limited, India with centrifugal impaction mechanism for capturing particle, having a flow rate of 280 l/min and agar volume of 15 ml). Three different readymade agar strips (TSA-Agar for total count, Mannitol-Salt-Agar for Staphylococci and MacConkey-Agar for coliform bacteria, obtained from HiMedia Laboratories Pvt. Limited, India) were used consecutively to collect the air microflora of a given sampling site. Samples were collected during the first week of April of 2011 and 2012 during visits through these locations. Seven days of weather data were also collected from local weather stations for temperature (max/min) and precipitation as shown in Table 1. Air sampler was placed vertically upward at an approximate height of one meter from ground level and kept on for 9 min duration for the collection of samples at each site for each agar strip. Strips were immediately placed inside sterile containers, incubated for 24 h and colonies developed on the agar strips were counted, noted and stored in cool boxes for transportation to laboratory for further analysis. Twenty six different colonies collected from all six locations on the TSA agar strip were streaked on nutrient agar for pure colonies and subsequently cultured in the nutrient broth for further investigations.

Figure 1. Figure showing the sites for collection of samples (downloaded from: https://maps.google.co.in/).

2.2 PCR amplification, RFLP and Sequencing

Bacterial cells were pelleted from culture media by centrifugation, followed by washing once with PBS (pH 7.4) and resuspended in lysis buffer. DNA was extracted according to manufacturer's protocol using a commercially available bacterial genomic DNA isolation kit (Sigma Aldrich, India). Subsequently, extracted DNA quality and quantity was checked by agarose gel electrophoresis and micro-volume UV-spectrophotometry (PicoDrop, UK) respectively. Ten fold diluted purified DNA extracts were then subjected to PCR amplification of partial 16S gene region using universal primers 16S1: 5'-gag ttt gat cct ggc tca-3' and 16S2: 5'-cgg cta cct tgt tac gac tt-3' [11], using commercially available PCR reagents (Fermentas, Thermo Scientific) and a thermal cycler (Genetix Asia Pvt Ltd, India). All the PCR reaction were set up inside

Table 1. Showing the Coordinates, weather and demographic data of selected study sites

			Tezpur	Bhalukpong	Bomdila	Dirang	Sela Pass (top)	Tawang
Coordinates	Lat	N	26°42'	27°01'	27°18'	27°19'	27°31'	27°34'
	Long	E	92°47'	92°64'	92°22'	92°16'	92°30'	91°52'
Elevation		m; ASL	73	213	2217	1929	4170	3048
Temperature	Max	°C	31±2	27±2	19±1	18±1	10 ± 1	12±1
	Min	°C	20±2	19±2	6±2	7±1	0 ±1	1±1
Precipitation		mm	4-5	1-2	1-3	2-3	2-4	0-1
Population		x1000	60	5.5	7.12	4.8	No permanent settlement	4.8
Cloud cover			Partial	Partial	Clear	Partial	Partial	Partial
Wind speed		km/h	8-9	8-10	10-12	11-12	20-21	17-18

PCR Work bench (Esco, Singapore), appropriate controls were run along with samples and precautions to avoid PCR carry-over contamination were followed strictly[12]. Amplified PCR products were run on agarose gel, sliced and purified using agarose gel extraction kit (Sigma Aldrich, India). An aliquot of the purified amplicons were subjected to endonuclease digestion using each of the three restriction endonucleases (Fermentas or Sigma Aldrich, India) *Alu I*, *Msp I* and *Hae III*, separately in reaction mix containing matching buffers. Tubes were then incubated at recommended temperature for 3 h. Fragments of restriction endonuclease digested amplicons were separated on agarose gels, visualised and images captured for comparison of restriction fragment length polymorphism (RFLP) patterns Based on the similarity/dissimilarity of the banding patterns, a total of 26 cultures were randomly selected for sequencing and further analysis. Another aliquot of purified amplicons were rechecked on agarose gels, quantified (PicoDrop, UK) and sent for sequencing (BioLinkk, New Delhi, India). Both the strands of the amplicons were directly sequenced using the same forward and reverse primers used in PCR amplification (16S1 and 16S2).

2.3 Analysis of Sequenced Data

All the 16S sequences generated in this study were visually inspected; ends trimmed, manually edited using Bioedit version 7.0.5.3[13] and were subsequently subjected to nucleotide search using BLAST[14], EzTaxon[15] and the 16SPathDB servers[16]. The 16SPathDB database was used for its utility in efficient and accurate identification of listed medically important bacteria. Following the algorithm of bacterial identification, suggested by Woo and colleagues[16], first three best matches were tabulated. Reference GenBank/EzTaxon sequences of different bacterial species, that showed high match with each individual sequences were retrieved, multiply aligned with all the query sequences using ClustalW program incorporated in Bioedit. Finally, Kimura's two-parameter method was employed to estimate genetic distances and the phylogenetic tree was constructed by Neighbor-Joining (NJ) method. The consistency of the pairwise comparison and phylogenetic tree analysis was assessed by bootstrap resampling (1000 replicates). Phylogenetic and molecular evolutionary analyses were performed using MEGA version 5.0[17]. All the 16S sequences generated in this study have been submitted in the GenBank (KC898299 through KC898324). Principal Coordinate Analysis (PCOORD) using default parameters was also performed on the sequences, using analysis tools available at the HFV Database Project, maintained by LANL (http://hfv.lanl.gov/content/sequence/PCOORD/PCOORD.html). The PCOORD program[18] searches for meaningful patterns in sequence data with no *a priori* knowledge and also attempts to summarize the difference in the sequences in a limited number of axes or dimensions (a combination of positions in a sequence that behave similarly).

2.4 Carbon Source Utilisation Study

The above mentioned 26 selected bacterial cultures were examined for carbon source utilisation based identification using Biolog GENIII micro plate on a MicroStation ID System (Biolog Inc., USA) installed at Sophisticated Instrumentation facility (SIF), Delhi University. Results obtained were compared with BIOLOG database (Microlog System TM, Release 4.0) and isolates were identified. This system reports bacterial identity based on probability and similarity of "metabolic fingerprint" with "metabolic fingerprint" of reference bacterial strains available in the Biolog databases, and a 50 % similarity is considered as a cut-off for species identification. Further statistical analyses were done using SPSS 14.0 (SPSS, Inc).

3. RESULTS AND DISCUSSION

After incubation, diverse colonies were found in three different agar strips with maximum colonies developed on TSA-Agar for total count followed by Mannitol-Salt-Agar for Staphylococci and MacConkey-Agar for coliform bacteria as shown in Fig. 2. Areas like Tezpur were found with highest diversity and number of bacterial colonies in all three different agar strips, followed by Bhalukpong, whereas, Sela pass had the least diversity and CFUs on the same strips. This finding of total bacterial count and diversity directly corroborates with the population density of the sampling sites, strongly supporting previous findings that population and anthropogenic activities directly influence the quality of air[2].

RFLP pattern of the generated amplicons were studied (data not shown) and based on the similarity/dissimilarity of patterns, representative 26 samples were processed for PCR amplification and sequencing. These edited sequences were then subjected to database search for identification based on

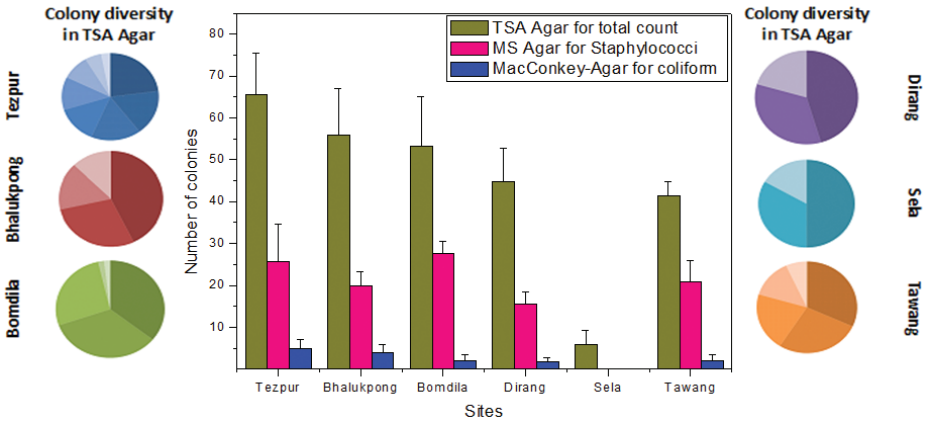

Figure 2. Bacterial colony diversity (CFU) in different agar strips and colony diversity on TSA agar.

Table 2. Comparison table of the identification of isolates through 16s rRNA gene analysis (using EzTaxon and GenBank databases) and Biolog data

Isolate	Gen Bank Accn no	EzTaxon				GenBank (NCBI)				Biolog		
		Hit	Accession	Country	%	Hit	Accession no.	%	Country	*Hit*	Probability %	Similarity %
A1TW	KC898299	*Acinetobacter schindleri LUH5832(T)*	AJ278311	Belgium	99.67	*Acinetobacter schindleri*	JX315564	99	China	*Not presented*		
A2TW	KC898300	*Bacillus tequilensis 10b(T)*	HQ223107	USA	100.00	*Bacillus subtilis*	KC534274	100	India	*B. pumilus*	88.4	54.6
A4TW	KC898301	*Staphylococcus equorum subsp. equorum ATCC 43958(T)*	AF527483	Switzerland	99.84	*Staphylococcus equorum*	JQ680456	99	Denmark	*S. hymolyticus*	94	64.8
A5TW	KC898302	*Bacillus tequilensis 10b(T)*	HQ223107	USA	99.68	*Bacillus subtilis*	EU870498	99	S Africa	*B subtilis*	n/a	15.8
A6TW	KC898303	*Bacillus tequilensis 10b(T)*	HQ223107	USA	99.68	*Bacillus subtilis*	EU870498	99	S Africa	*B subtilis*	n/a	22.8
A7TW	KC898304	*Bacillus safensis FO-036b(T)*	AF234854	USA	100.00	*Bacillus pumilus*	KC675187	100	China	*B pumilus*	75.5	60.6
A8DI	KC898305	*Staphylococcus hominis subsp. novobiosepticus GTC 1228(T)*	AB233326	Japan	100.00	*Staphylococcus hominis*	KC429620	100	China	*S kloosii*	80.8	55.7
A9DI	KC898306	*Staphylococcus arlettae ATCC 43957(T)*	AB009933	Japan	100.00	*Staphylococcus arlettae*	JX646766	100	India	*S aureus*	87.6	57.2
A12DI	KC898307	*Staphylococcus arlettae ATCC 43957(T)*	AB009933	Japan	100.00	*Staphylococcus arlettae*	JX646766	100	India	*S aureus*	87.6	57.2
A14BO	KC898308	*Staphylococcus xylosus ATCC 29971(T)*	D83374	Japan	100.00	*Staphylococcus xylosus*	KC456590	100	Chiina	*S xylosus*	90.1	72.3
A15BO	KC898309	*Bacillus tequilensis 10b(T)*	HQ223107	USA	100.00	*Bacillus subtilis*	KC534274	100	India	*B pumilus*	99.7	68.7
A16BO	KC898310	*Acinetobacter lwoffii DSM 2403(T)*	X81665	Germany	100.00	*Acinetobacter lwoffii*	JQ815204	100	China	*Not presented*		
A17TZ	KC898311	*Bacillus vallismortis DSM 11031(T)*	AB021198	Japan	99.84	*Bacillus amyloliquefaciens*	JQ837275	99	China	*B valismortis*	67.6	51.7

Table 2. Comparison table of the identification of isolates through 16s rRNA gene analysis (using EzTaxon and GenBank databases) and Biolog data

Isolate	Gen Bank Accn no	EzTaxon				GenBank (NCBI)				Biolog		
		Hit	Accession	Country	%	Hit	Accession no.	%	Country	Hit	Probability %	Similarity %
A18TZ	KC898312	*Bacillus tequilensis 10b(T)*	HQ223107	USA	99.84	*Bacillus tequilensis*	KC565672	99	China	*B licheniformis*	84.9	61.7
A19TW	KC898313	*Pseudomonas psychrotolerans C36(T)*	AJ575816	Austria	100.00	*Pseudomonas oryzihabitans*	KC335294	100	India	*Not presented*		
A20TZ	KC898314	*Staphylococcus warneri ATCC 27836(T)*	L37603	ATCC	99.84	*Staphylococcus warneri*	KC213932	100	Lithuania	*S sciuri*	98.6	83
A22BP	KC898315	*Bacillus safensis FO-036b(T)*	AF234854	USA	100.00	*Bacillus pumilus*	KC675187	100	China	*B pumilus*	99.7	68.7
A23BP	KC898316	*Micrococcus yunnanensis YIM 65004(T)*	FJ214355	China	100.00	*Micrococcus yunnanensis*	KC634108	100	S Korea	*M luteus*	34.3	58.2
A24TZ	KC898317	*Staphylococcus epidermidis ATCC 14990(T)*	L37605	ATCC	98.05	*Staphylococcus epidermidis*	KC634103	98	S Korea	*S sciuri*	n/a	4.7
A25BP	KC898318	*Bacillus marisflavi TF-11(T)*	AF483624	S Korea	99.68	*Bacillus marisflavi*	KC414706	99	S Korea	*B licheniformis*	77.7	53.5
A26TZ	KC898319	*Staphylococcus saccharolyticus ATCC 14953(T)*	L37602	ATCC	99.51	*Staphylococcus haemolyticus*	JQ001918	99	India	*S. hymolyticus*	94	64.8
A27BP	KC898320	*Staphylococcus saccharolyticus ATCC 14953(T)*	L37602	ATCC	99.35	*Staphylococcus haemolyticus*	JQ001918	99	India	*S. hymolyticus*	94	64.8
A28TZ	KC898321	*Staphylococcus warneri ATCC 27836(T)*	L37603	ATCC	99.84	*Staphylococcus warneri*	KC354484	99	Czech Republic	*S sciuri*	71.1	54.3
A29TZ	KC898322	*Staphylococcus saccharolyticus ATCC 14953(T)*	L37602	ATCC	99.84	*Staphylococcus saccharolyticus*	AB646616	99	Japan	*Not presented*		
A30TZ	KC898323	*Bacillus vallismortis DSM 11031(T)*	AB021198	Japan	99.84	*Bacillus amyloliquefaciens/ vallismortis*	JQ837275/ KC565644	99	China	*B amyloliquefaciens*	83.1	63.5
A31TW	KC898324	*Bacillus stratosphericus 41KF2a(T)*	AJ831841	India	100.00	*Bacillus pumilus*	KC492092	100	India	*B valismortis*	n/a	31.2

homology of nucleotide sequences with reference sequences available in different databases. Initially we submitted the sequence data as FASTA files to EzTaxon (EzTaxon server 2.1, http://147.47.212.35:8080/index.jsp) and 16SpathDB (147.8.74.24/16SpathDB/) servers. Database search results showed similar number (eleven each) of *Streptococci* and *Bacilli*. Although the sequences were found to be concordant upto the genus levels, but, for some sequences, variation in the identification at species level was evident. Hence, the sequences were subsequently searched individually using BLASTn server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the comparison of search results of all three search servers are presented in Table 2. Interestingly, 16SPathDB search has resulted (Table 3) in matching of the all but one sequence data with medically important bacterial database (>98% best match criteria). This match has clinical importance, since the 16SPathDB comprises of 16SrRNA GenBank sequences of well characterised (genotypic/phenotypic) bacterial strains, specifically isolated from human subjects, and are listed as potential or probable human pathogen in the Manual of Clinical Microbiology[19]. To resolve this ambiguity of species identification, all closely related sequences, as reported by three different servers were retrieved and phylogenetic analysis of the samples were performed as shown in Fig. 3. The results of phylogenetic analysis were also strongly supported by the PCOORD analysis results showing well defined clustering of the sequences at genus and species levels as shown in Fig. 4.

Table 3. Comparison table of the identification of isolates through 16PathDB analysis

Isolate	Genus identification	Species identification					
		Best match		2nd best match		3rd best match	
		Hit	%	Hit	%	Hit	%
A1TW	*Acinetobacter sp.*	*A. schindleri*	99.68	*A. guillouiae*	98.38	*A. johnsonii*	98.22
A2TW	*Bacillus sp.*	*B. amyloliquefaciens*	99.51	*B. atrophaeus*	99.19	*B. subtilis*	99.03
A4TW	*Staphylococcus sp.*	*S. equorum*	99.68	*S. succinus*	99.19	*S. xylosus*	99.19
A5TW	*Bacillus sp.*	*B. amyloliquefaciens*	99.51	*B. atrophaeus*	99.19	*B. subtilis*	99.03
A6TW	*Bacillus sp.*	*B. amyloliquefaciens*	99.51	*B. atrophaeus*	99.19	*B. subtilis*	99.03
A7TW	*Bacillus sp.*	*B. pumilus*	100.00	*B. pumilus*	100.00	*B. idriensis*	98.21
A8DI	*Staphylococcus sp.*	*S. hominis*	100.00	*S. lugdunensis*	99.35	*S. epidermidis*	99.35
A9DI	*Staphylococcus sp.*	*S. arlettae*	100.00	*S. saprophyticus*	99.51	*S. gallinarum*	99.35
A12DI	*Staphylococcus sp.*	*S. arlettae*	100.00	*S. saprophyticus*	99.51	*S. gallinarum*	99.35
A14BO	*Staphylococcus sp.*	*S. xylosus*	100.00	*S. saprophyticus*	99.68	*S. gallinarum*	99.51
A15BO	*Bacillus sp.*	*B. amyloliquefaciens*	99.51	*B. atrophaeus*	99.19	*B. subtilis*	99.03
A16BO	*Acinetobacter sp.*	*A. lwoffii*	100.00	*A. radioresistens*	97.89	*A. calcoaceticus*	97.57
A17TZ	*Bacillus sp.*	*B. amyloliquefaciens*	99.84	*B. atrophaeus*	99.51	*B. subtilis*	99.35
A18TZ	*Bacillus sp.*	*B. amyloliquefaciens*	99.35	*B. atrophaeus*	99.51	*B. subtilis*	99.35
A19TW	*Pseudomonas sp.*	*P. oryzihabitans*	100.00	*P. pseudoalcaligenes*	95.62	*P. mendocina*	95.3
A20TZ	*Staphylococcus sp.*	*S. warneri*	99.84	*S. pasteuri*	99.51	*S. aureus*	99.19
A22BP	*Bacillus sp.*	*B. pumilus*	100.00	*B. pumilus*	100.00	*B. idriensis*	98.21
A23BP	*Micrococcus sp.*	*M. luteus*	99.83	*M. antarcticus*	99.33	*M. lylae*	99.16
A24TZ	*Staphylococcus sp.*	*S. caprae*	98.05	*S. epidermidis*	98.05	*S. saccharolyticus*	97.89
A25BP	*Bacillus sp.*	*B. licheniformis*	97.41	*B. anthracis*	96.6	*B. amyloliquefaciens*	96.6
A26TZ	*Staphylococcus sp.*	*S. haemolyticus*	99.51	*S. saccharolyticus*	99.51	*S. aureus*	99.35
A27BP	*Staphylococcus sp.*	*S. haemolyticus*	99.35	*S. saccharolyticus*	99.35	*S. aureus*	99.19
A28TZ	*Staphylococcus sp.*	*S. warneri*	99.84	*S. pasteuri*	99.51	*S. aureus*	99.03
A29TZ	*Staphylococcus sp.*	*S. saccharolyticus*	99.68	*S. caprae*	99.51	*S. epidermidis*	99.51
A30TZ	*Bacillus sp.*	*B. amyloliquefaciens*	99.84	*B. atrophaeus*	99.51	*B. subtilis*	99.35
A31TW	*Bacillus sp.*	*B. pumilus*	99.68	*B. pumilus*	99.68	*B. idriensis*	97.89

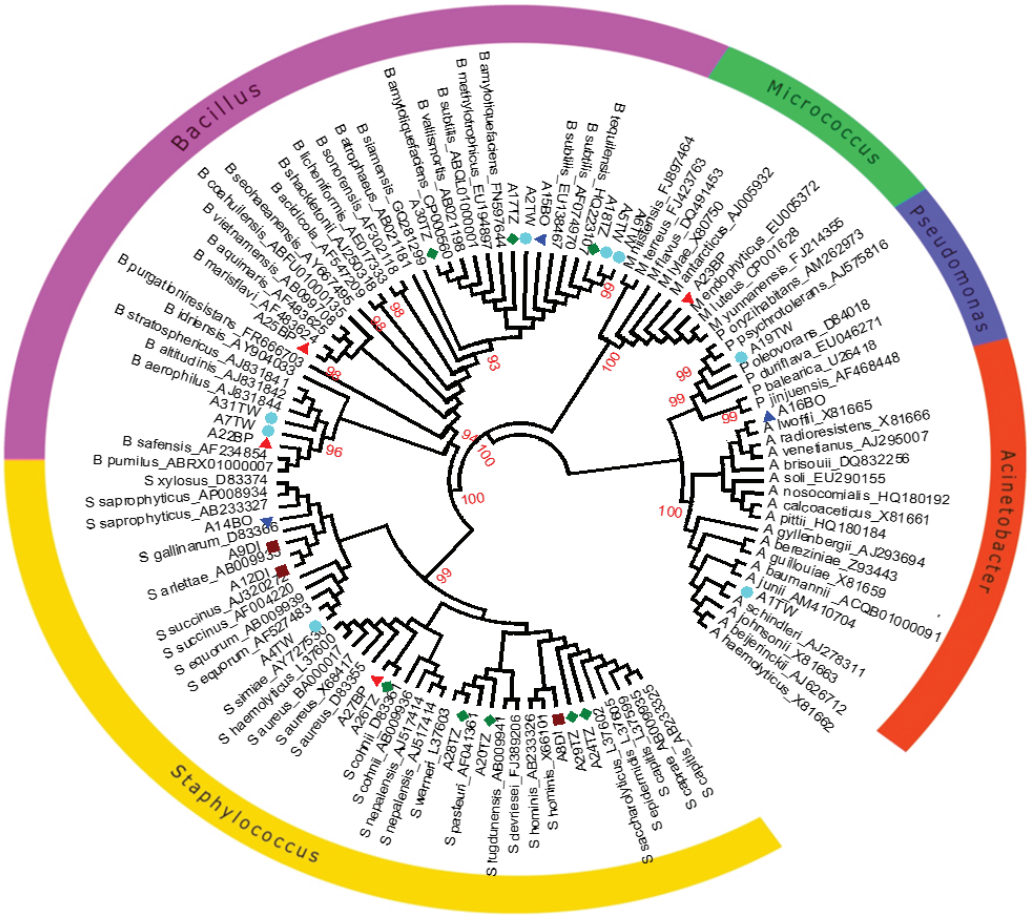

Figure 3. Phylogenetic analysis of 26 bacteria on the basis of 16s rDNA sequence analysis.

Apart from identification using 16s rRNA genetic region, we also attempted identification using "metabolic fingerprint" based on unique pattern of carbon sources utilisation for all 26 samples. Hierarchical cluster using Pearson correlation based on carbon utilisation pattern of 22 samples yielded similar grouping of isolates as shown in Fig. 5. Again, the results were found to be in agreement with genetic data up to the genus levels. In some of the isolates, the genetic identification did not match the metabolic identification at the species level. Similar discordance between genetic and metabolic identification has also been observed and reported elsewhere[20,21]. Nevertheless, on review of available literature, we found that a number of the bacterial species that we sampled have the potential to act as human pathogens as shown in Table 4.

Although our understanding of microbial community profile in the atmosphere is presently inadequate, research interests have gained momentum recently due to its significance in human/animal/plant health and hygiene[35]. The advent of genetic techniques like 16S rRNA amplification, sequencing and

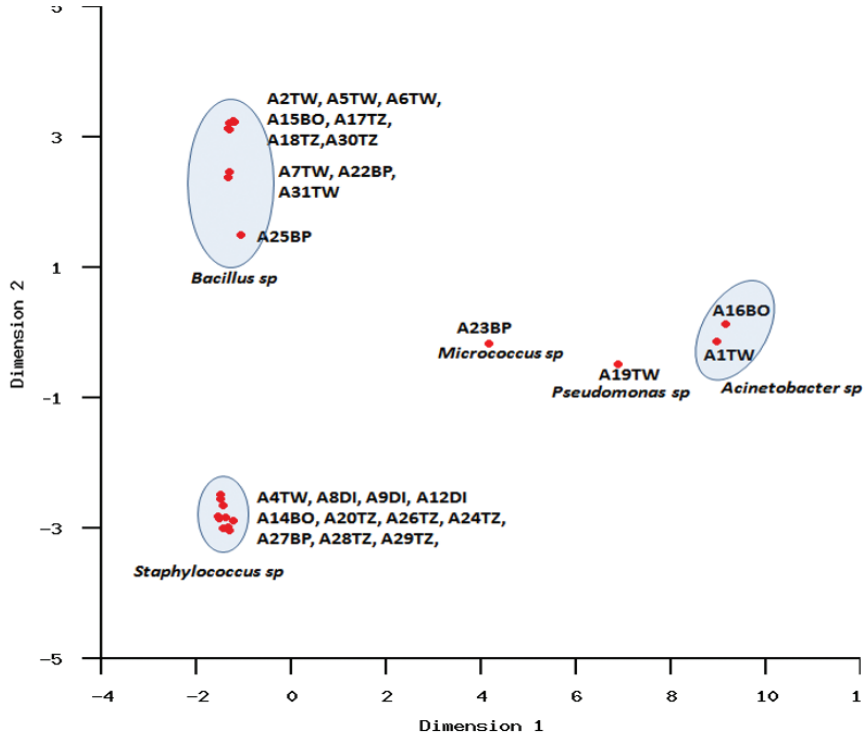

Figure 4. Results of Principle Coordinate analysis showing clustering of sequences based on similarity among them.

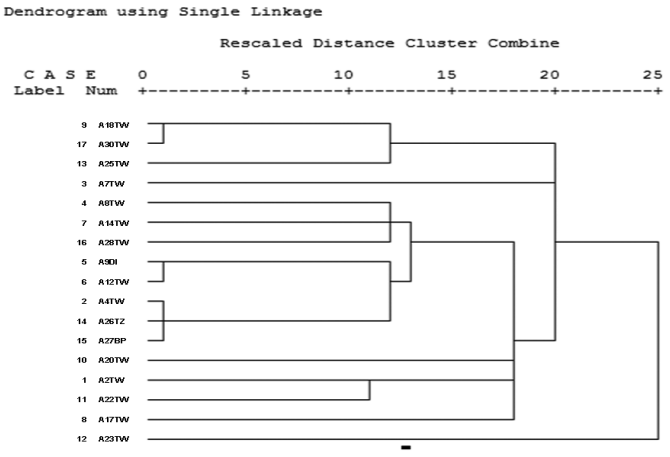

Figure 5. Pearson correlation (nearest neighbour) based on unique pattern of carbon sources utilisation (metabolic fingerprint) of bacteria.

Table 4. Table showing human diseases associated (based on available literature) with some of the bacterial species found in this study

Bacteria	Diseases reported	Ref.
Bacillus pumilus	Cutaneous infection, food poisoning in human	22, 23
Staphylococcus saccharolyticus	Infective endocarditis and bacteremia	24
Staphylococcus warneri	Vertebral osteomyelitis and native valve endocarditis, bacteremia	25, 26
Staphylococcus haemolyticus	Erythroderma	24, 27
Staphylococcus epidermidis	Nosocomial infections	28, 29
Micrococcus yunnanensis	Opportunistic pathogen	30
Pseudomonas oryzihabitans	Peritonitis	31
Acinetobacter lwoffii	Gastritis	32, 33
Staphylococcus xylosus	Acute Pyelonephritis	34

phylogenetic analysis have greatly enhanced our capabilities in exploring and identification of the airflora[4]. it has been proposed that microorganisms may remain attached to particles or get dispersed and deposited through clouds, fog, etc to earth's surfaces, impacting environment quality. Since human/ animals mainly depend upon the air they breathe, microbiological monitoring of air is essential to evaluate the hygienic conditions of an environment. It has been shown that exposure to pathogenic air borne bacteria are the main cause of adverse health effects including unexplained infections, allergies, and toxic reactions[36-38]. It has also been well established that one of the most important source of airborne bacteria is the presence of human or related anthropogenic activities[39-42].

Lastly, with the enormous advancement of scientific understanding of microbial manipulation and the changing conflict strategies, the threat of unscrupulous and deliberate misuse of micro-flora is increasing substantially. Therefore, regular surveillance and monitoring of air micro-flora is imperative to record the natural bacterial communities as well as any changes (natural or deliberate) that may have serious implication in health, hygiene and biosecurity of the population concerned.

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