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A Review of Studies on Bacterial and Fungal Diversity in Wetland Ecosystems

Jemi K. Gandhi*, Ketan Tatu, R.D. Kamboj
Junior Research Fellow, Microbiology, Gujarat Ecological Education and Research (GEER) Foundation, Gandhinagar, Gujarat, India

Abstract

Wetlands provide habitat to plethora of bacteria and fungi. Though a large number of ecological studies are carried out on various wetlands all over the world, the aspects of bacterial and fungal diversity are often ignored. This is despite the fact that these micro-organisms play vital role in decomposition of dead organic matter and in biogeochemical cycles. The scenario of microbial studies in India is not encouraging. Moreover in Gujarat state, wetland based bacterial and fungal diversity is almost unexplored. Nonetheless, in the countries like USA, Canada, the Netherlands and China, relatively more work has been done in this domain. As Gujarat is a wetland-rich state, there is a critical need of making a beginning in this direction so that the bacterial and fungal research for this wetland-rich region reaches at par with the above-mentioned countries in near future. The time has come when wetland managers and researchers of Gujarat State and India, should go beyond charismatic groups of animals; especially birds; and look into the diversity and ecology of bacteria and fungi of the wetland ecosystems. GEER Foundation, as a part of its ecological monitoring scheme for Nal Sarovar (a Ramsar Site), has started pioneering efforts in this direction. As a first step, a review has been carried out on such studies conducted in the State, other parts of India and abroad. The present paper summarizes this review.

Keywords: *bacteria, diversity, fungi, Gujarat, India, microbes, review, wetland, world*

****Author for Correspondence*** E-mail: gandhi.jemi95@gmail.com

INTRODUCTION

Wetlands are defined as "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed 6 m" [1].

Wetlands are amongst the most productive ecosystems on the earth [2]. The estimated wetland area in the world is about 5 to 8% of the land surface of the earth [3, 4]. Owing to the variation in genesis, geographical location, water regime and chemistry, dominant species and, soil and sediment characteristics, considerable wetland diversity exists on the earth [5]. Considering the importance of wetlands ecologically and socio-economically, efforts are being made at global level for conservation and wise use of wetlands. Ramsar convention, 1971 is one such effort. Ramsar convention on wetlands of international importance is an international treaty signed in 1971 for international cooperation for the conservation and wise use of wetlands and their resources.

Wetlands have an immense diversity of micro-flora and micro-fauna, but wetland studies often ignore microbes, especially bacteria. Microbial richness of a region, which often includes multiple wetlands, is its unseen asset that needs to be explored for the benefit of human society [6]. Wetlands in a region may have a diverse microbial flora/fauna which play significant roles in those ecosystems. Particularly, soil microbial communities in wetland ecosystems play an important role in biogeochemical cycles (including carbon cycle, nitrogen, phosphorus, sulfur, and iron cycle) and are crucial to the functions of wetland systems [7].

The permanent or periodic flooding of wetland soils and the presence of wetland plant roots create dynamic oxic-anoxic interfaces that provide habitats for a wide variety of aerobic

and anaerobic microbes. The bacteria that inhabit the anoxic hydric soils of wetlands are often responsible for the formation of an oxic surface layer and a redox stratification of the oxygen-depleted zone, which is a typical characteristic of wetland soil [8]. Nutrient inputs and fast recycling due to activities of aerobes and anaerobes make these systems highly productive and in turn, attractive for humans and many other organisms [9]. The ability to characterize the bacterial community structure in wetland soils is fundamental to the understanding of wetland functions such as regulating the cycling, retention, and release of nutrients and soil carbon. These wetland functions have demonstrated significant effects on water quality and global carbon cycling [10]. However, the study of microbial diversity in wetlands is vastly unexplored relative to other ecosystems [11, 12]. Some wetland microbes release potent greenhouse gas methane, which may nullify some of the benefits of wetlands as carbon sinks. Tringe, an environmental microbiologist at the US Department of Energy's Joint Genome Institute, has been trying to determine just how much wetlands actually help offset climate change [13].

MICROBIAL RESEARCH SCENARIO FOR THE WETLANDS IN THE WORLD

Microbes in the wetland soils play a vital role in carbon, nitrogen and sulfur cycling. They also catalyze chemical transformation under alternating anoxic/anaerobic conditions found within the wetland soils [14].

Sphagnum-dominated acidic peat bogs represent one of the most extensive wetland types in North America and Eurasia [15]. Researchers led by Svetlana Dedysh at Winogradsky Institute of Microbiology have attempted to elucidate the overall bacterial community composition in the soils of an acidic *Sphagnum* peatland (a type of wetland) by a combination of 16S rRNA gene clone libraries, *fluorescence in situ hybridization* (FISH) and cultivation [16]. Molecular techniques facilitate more comprehensive investigations through cultivation-independent analyses [17]. Till date, *Sphagnum* bog has remained an exception where besides using molecular techniques, modifications of traditional culturing techniques have also been employed to obtain several isolates in the domains of Acidobacteria (24 clones), Alphaproteobacteria (20 clones), Verrucomicrobia (13 clones), Actinobacteria (8 clones), Deltaproteobacteria (4 clones), Chloroflexi (3 clones), and Planctomycetes (3 clones) [18].

Lamers *et al.* have given the most general overview of microbial conversions in wetland ecosystems by analyzing the effect of microbial activities on growth and performance of plants as many biogeochemical conversions catalyzed by microbes can ultimately control vegetation composition in wetlands [19]. Iron and methane cycling are important processes in wetlands pertaining to plant growth and to greenhouse gas emission, respectively. There is scarce information on the ecology of microbes oxidizing ferrous ion at neutral pH. In order to mitigate it, a team of researchers headed by Wang *et al.* in the Netherlands investigated spatial distribution of *Gallionella* related neutrophilic iron-oxidizers (Ga-FeOB) in co-occurrence with methane-oxidizing bacteria (MOB) in Ewijkse Waard, a riparin wetland, in Netherland [20].

Preston *et al.* applied multiple approaches to characterize depth-dependent microbial community structure and function within the James Bay Lowlands, a large peatland complex of northern Ontario, Canada [21]. Archaeal, bacterial, and fungal community structures in this peatland were characterized using community level physiological profiling, extracellular enzyme activities, and the carbon mineralization rates of various natural and synthetic substrates. Interestingly, despite the differences in nutrient content, similar dominant microbial taxa were observed at different sites peatlands.

Wu *et al*. examined diversity and composition of fungi in 10 wetlands along the Changjiang River (the third longest river in the world) and 10 other independent wetlands around China by culture-dependent and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) methods [22]. A

total of 883 isolates were obtained and identified and they belonged to 81 genera and 177 species, based on morphological characteristics and phylogenetic analysis. Among the 177 species, 169 species belonged to the *Ascomycota* and the remaining species belonged to the *Basidiomycota* and the *Zygomycota*. The most frequently occurring genera were *Penicillium* (relative frequency =16.8%), followed by *Fusarium* (15.4%), *Aspergillus* (7.6%), *Trichoderma* (5.8%) and *Talaromyces* (4.2%); other genera like *Mortierella, Acremonium, Verticillium, Cladosporium, Chaetomium, Leptosphaeria, Pycnidiophora, Stachybotrys, Paecilomyces, Alternaria, Dimorphospora* accounted for 49.8% of the total fungal diversity, wherein each genera had relative frequency ranging from 4 to 0.4%.

So far, only a few anaerobic prokaryotes have been cultivated from acidic wetlands. One of the factors that may have hampered isolation of anaerobes is the proper choice of a reducing agent for anaerobic media preparation [23]. However, modification in culturing technique by using titanium (III) citrate as a reducing agent has yielded success in isolation of several methanogens from acidic peatlands [24, 25] as well as for isolation and cultivation of some facultative anaerobic peat-inhabiting bacteria [26].

Baik *et al*. investigated strains of bacteria isolated using standard dilution plating technique and culture independent 16S rRNA gene clones from DNA extracts of water of Woopo wetland, the largest undisturbed wetland in Republic of Korea. Amplified rDNA restriction analysis (ARDRA) was applied onto single isolates and 16S rRNA gene clones and data was analyzed phylogenetically [27]. From isolated 203 bacteria, *Firmicutes* (49.8%) and *Actinobacteria* (32.0%) were predominant. Likewise, from 235 cultures, independent 16S rRNA gene clones, *Proteobacteria* (62.2%), *Actinobacteria* (15.5%), and *Bacteroidetes* (13.7%) were predominant.

Arbuscular mycorrhizal (AM) fungi are an important group of soil microorganisms, accounting for 30% of the soil microbial biomass [28]. They form a mutualistic symbiosis with 80% of vascular plants [29]. Xu *et al.* has reviewed 'Arbuscular Mycorrhizal Fungi in Wetland Habitats and their Application in Constructed Wetland' which summarizes mycorrhizal status in wetlands and the effect of flooding on AM fungal colonization [30]. *Acaulospora; Glomus; Archaeospora; Claroideoglomus claroideum; Racocetra verrucosa; Rhizophagus intraradices; Rhizophagus fasciculatum; Scutellospora; Paraglomus; Archaeospor trappei; Acaulospora koskei; Acaulospor laevis; Enterophospora columbiana; Glomus clarum; Glomus etunicatum; Glomus gerdmannii; Ambispora leptotichum; Dentiscutata heterogama; Diversisporaceae* are common AM fungi associated with plants of 99 families living in 31 different habitats.

Apart from this, microbial work has been done in several other wetlands which have been summarized in Table 1.

Table 1: *Summary of Microbial Work Done in Various Other Wetlands.*

Site	Focus Group	Work	Key Findings	Reference
Mangrove National Nature Reserves, China	Microbial communities	A high-throughput functional gene array (GeoChip 4.0) was used to analyze the functional diversity, composition, structure, and metabolic potential of microbial communities	Different microbial communities were found including carbon fixation, Carbon degradation, methane generation, nitrogen fixation, nitrification, denitrification, ammonification, nitrogen reduction, sulfur metabolism, metal resistance, antibiotic resistance, and organic contaminant degradation. Canonical correspondence analysis (CCA) results indicated the microbial community structure was largely shaped by surrounding environmental variables, such as total nitrogen, total	[31]

			carbon, pH, Carbon/Nitrogen ratio, and especially salinity.	
Delaware Estuary, USA	Bacteria	*Fluorescence in situ hybridization* (FISH) was used to examine the spatial and temporal variation in the abundance of major bacterial types in the Delaware Estuary. Basic environmental parameters were used to explore relationship between bacterial community and biogeochemical processes.	The abundance of *alpha*- and *beta*-proteobacteria and *Actinobacteria* varied systematically in the estuary and mirrored the pattern seen in lakes and oceans. Alpha-proteobacteria, were most abundant in the Bay and rare in the Delaware River. In contrast, Beta-proteobacteria and *Actinobacteria* were abundant in the Delaware River but were less in the marine waters of the Delaware Bay. Only salinity, among the several biogeochemical parameters, accounted for a substantial portion of the variation in abundance of these bacterial groups.	[32]
Four nitrogen-rich wetlands in China were chosen as field experimental sites. 1) Red Beach, Liaoning Province. 2) Freshwater marsh in Liaoning Province. 3) Baiyangdian Lake in Hebei Province. 4) Paddy field in Zhejiang Province.	Ammonia oxidizing bacteria (AOB) and Ammonia oxidizing Archea (AOA)	The composition and abundance of Archaea and Bacteria in wetlands sediments having different nitrogen concentration were investigated by quantitative real-time polymerase chain reaction, cloning, and sequencing approaches based on *amo*A genes. Moreover, the factors influencing abundance of AOA and AOB with environmental indicator were also analyzed.	AOA were distributed widely in wetland sediments, and it was found from the phylogenetic tree that archaeal *amo*A functional gene sequences from wetlands sediments cluster as two major evolutionary branches: soil/sediment and sediment/water. In different wetlands sediments, the bacteria functionally dominated microbial ammonia oxidation on the basis of molecule analysis, potential nitrification rate, and soil chemistry. The pH had great negative impact on the abundance of AOA and AOB; however ammonia concentration showed positive impact on AOB abundance only.	[33]
Two permafrost wetland in China - Lake Namco (littoral wetland) in southeast of Tibetan Plateau and Sanjiang Plain (alluvial wetland) in Northeast China.	Bacteria	16S rRNA-based quantitative PCR (qPCR) and 454 pyrosequencing were used to identify bacterial communities in soils sample	The two permafrost wetlands showed similar bacterial community compositions, which differed from those reported in other cold environments. *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* were the most abundant phyla in both wetlands, whereas *Acidobacteria* was prevalent in Sanjiang plain only. These four phyla constituted more than 80% of total bacterial community diversity in permafrost wetland soils, and Methylobacter of type I methanotrophs was dominant in soils of lake Namco.	[34]
A high water content site and a low water content site of Controlled Wetlands on the Qinghai-Tibetan Plateau,	Methanogens and Methanotrophs	The diversity and abundance of methanogens and methanotrophs were studied by using phylogenetic analysis and quantitative polymerase chain reaction (qPCR) based on *mcr*A	A total of 16 methanogenic operational taxonomic units (OTUs) and 9 methanotrophic OTUs were obtained. For methanogens, Fen cluster (58.0%) and *Methanosaetaceae* (20.3%) were	[35]

China		gene and *pmo*A gene.	the dominant groups in high moisture samples, whereas *Methanosaetaceae* (32.4%), *Methanosarcinaceae* (29.4%), and *Methanobacteriaceae* (22.1%) were prevalent in low moisture samples. *Methylobacter* (90.0%) of type I methanotrophs were overwhelmingly dominant in high moisture samples, while *Methylocystis* (53.3%) and *Methylomonas* (42.2%) belonging to types II and I methanotrophs were predominant groups in low moisture samples. Furthermore, qPCR analysis revealed abundance of methanogens and methanotrophs were higher in high moisture samples than that in low moisture samples.	
Salar de Huasco, Chilean Altiplano	Cyanobacteria	The diversity of Cyanobacteria in water and sediment samples from four representative sites of the Salar de Huasco was examined using denaturing gradient gel electrophoresis and analysis of clone libraries of 16S rRNA gene PCR products	78 phylotypes were identified in a total of 268 clonal sequences deriving from seven clone libraries of water and sediment samples. *Oscillatoriales*, *Pleurocapsales*, *Chroococcales* and *Nostocales* were reported to be dominant.	[36]
Two high altitude wetlands, Lirima and Caya, in Chilean Altiplano	Bacteria	Microbial diversity in the water column was characterized by the presence of five bacterial phyla and related genera by using DAPI. This descriptive paper highlights the unusual limnological and biological characteristics of high altitude wetlands and highlights the importance of describing their biological communities as well as their functional role, interactions and sensitivity to changes in water availability.	An average concentration of 3×10^4 cell/ml was recorded and the presence of highly pigmented microbial mats was common. In total, 30 16S rRNA gene sequences were analyzed from 61 bacterial isolates. The isolates were members of *Gammaproteobacteria* (43%), *Alphaproteobacteria* (13%), *Firmicutes* (37%), *Actinobacteria* (3.3%) and *Bacteroidetes* (3.3%). Most of the isolates exhibited high sequence identity (>98%) with the following genera: *Psychrobacter*, *Halomonas*, *Aeromonas*, *Pseudomonas*, *Paracoccus*, *Brevundimonas*, *Rhizobium*, *Exiguobacterium*, *Bacillus*, *Planococcus*, *Dietzia* and *Chryseobacterium*. Most of these taxa use different organic carbon sources and also exhibit adaptations to thrive under extreme environmental conditions such as low temperature (*Psychrobacter*, *Chryseobacterium*), high salt concentration (*Halomonas*) and desiccation (*Bacillus*)	[37]
The acidic dystrophic Lake Dubrovskoe and the acidic oligotrophic Lake Motykino in the swampy Mologa–Sheksna catchment area of upper Volga,	Bacteria and Archea	Identification of filterable and non-culturable bacteria and archea was performed by analysis of clone libraries obtained by PCR amplification of archaeal and bacterial 16S rRNA genes from water filtrates of acidic lakes.	Most of the obtained bacterial 16S rRNA gene sequences represented the class *Betaproteobacteria* and exhibited the highest homology of (94–99%) with 16S rRNA genes of representatives of the genera *Herbaspirillum*, *Herminiimonas*, *Curvibacter*, and *Burkholderia*.	[38]

Darwin State Nature Reserve, Nothern Russia			The archaeal 16S rRNA gene clone library comprised genes of Euryarchaeota representatives. One third of these genes exhibited 97–99% homology to the 16S rRNA genes of taxonomically described organisms of the orders *Methanobacteriales* and *Methanosarcinales*.	
Bakchar bog at the watershed between the Bakchar and Iksa Rivers in the western zone of the Tomsk region.	Yeasts	The abundance and the taxonomic composition of yeasts were determined by plating peaty soil samples onto a suitable media. Colonial morphotypes were differentiated and identified by examining morphological and physiological characteristics of yeast colonies with a binocular lens.	The microbiological analysis of 78 samples showed the presence of 23 yeast species belonging to genera *Bullera, Candida, Cryptococcus, Debaryomyces, Hanseniaspora, Metschnikowia, Mrakia, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces, Torulaspora* and *Trichosporon.* Peat samples from the high bog were dominated by *Rhodotorula mucilaginosa* and *Sporobolomyces roseus*, and by the ascomycetous yeasts *Candida Sporobolomyces roseus*, and by the ascomycetous yeasts spp. and *Debaryomyces hansenii.* The samples also contained two rare ascomycetous species (*Candida paludigena* and *Schizoblastosporion starkeyi-henricii*) and a dominant one yeast species (*Cryptococcus gilvescen*)	[39]
Jinshan Lake, China	Ammonia oxidizing bacteria and Ammonia oxidizing Archea	Community structures of AOB and AOA were investigated using PCR primers designed to specifically target the ammonia monooxygenase a-subunit (*amo*A) gene. Relationships between the abundance and diversity of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) and physicochemical parameters were also explored.	No significant correlations were observed between the AOB abundance and environmental variables. AOB had a higher diversity and richness of *amo*A genes than AOA. Among the 76 archaeal *amo*A sequences retrieved, 57.89, 38.16, and 3.95% fell within the *Nitrosopumilus, Nitrososphaera, and Nitrososphaera*, respectively. The 130 bacterial *amo*A gene sequences obtained in this study were grouped with known AOB sequences in the *Nitrosomonas* and *Nitrosospira* genera, which occupied 72.31 and 27.69% of the AOB group, respectively.	[40]
Mangrove wetland of the Zhangjiang Estuary, China	Nitrite dependent anaerobic methane oxidizing bacteria	Occurrence of nitrite-dependent anaerobic methane-oxidizing (n-damo) bacteria was investigated by real-time quantitative polymerase chain reaction (qPCR) assay. Phylogenetic analysis was undertaken to understand diversity of n-damo bacterial 16S rRNA and *pmo*A genes	The abundance of *Methylomirabilis oxyfera*-like bacterial 16SrRNA and pmoA genes ranged from 2.43×10^6 to 2.09×10^7 and 2.07×10^6 to 3.38×10^7 copies per gram of dry soil. The highest amount of targeting genes was all detected in the upper layer (0–20 cm). Phylogenetic analyses illustrated the depth-specific distribution and high diversity of n-damo bacteria in the	[41]

			mangrove wetland. Stable isotope experiments further confirmed the occurrence of n-damo and the potential n-damo rates ranged from 25.93 to 704.08 nmol CO2 per gram of dry soil per day at different depths of the sediment cores, with the n-damo being more active in the upper layer of the mangrove sediments.	
Sphagnum and lichen-dominated discontinuous permafrost wetland, Russia	Methane oxidizing community	Microbial diversity, activity, and composition of methane-oxidizing communities was assessed using techniques like high-throughput sequencing of the 16S rRNA genes and molecular analysis of the *pmo*A gene	Major bacterial groups identified were the *Acidobacteria* (35.4–41.2% of total 16S rRNA gene reads), *Alphaproteobacteria* (19.1–24.2%), *Gammaproteobacteria* (7.9–11.1%), *Actinobacteria* (5.5–13.2%), *Planctomycetes* (7.2–9.5%), and *Verrucomicrobia* (5.1–9.5%). Most (~80%) of all *pmo*A gene fragments revealed in peat from lichen-dominated sites belonged to "*Candidatus Methylospira mobilis".* Members of the genus *Methylocystis*, which are typical inhabitants of boreal Sphagnum peat bogs, represented only a minor group of indigenous methanotrophs.	[42]
Watershed of the Luoshijiang Wetland and adjacent agricultural soils	Ammonia oxidizing bacteria and Ammonia oxidizing Archea	The abundances and community structures of ammonia oxidizing archea (AOA), ammonia oxidizing bacteria (AOB), and methane oxidizing bacteria (MOB) was investigated by applying statistical analyzing of results of real time PCR and clone library.	AOB community size was higher than AOA in agricultural soils and lily-vegetated sediment, but lower in *A. calamus*-vegetated sediment. MOB showed a much higher abundance than AOA and AOB. Flooded rice soil had the largest AOA, AOB, and MOB community sizes. *Nitrososphaera*-like microorganisms were the predominant AOA species in garlic soil but were present with a low abundance in unflooded rice soil and cabbage soil. *Nitrosospira*-like AOB were dominant in wetland sediments and agricultural soils. Type I MOB *Methylocaldum* and type II MOB *Methylocystis* were dominant in wetland sediments and agricultural soils.	[43]
Three calcareous fens located near Ithaca, New York, USA	Arbuscular mycorrhizal fungi	Root colonization by arbuscular mycorrhizal fungi (AMF) and dark septate endophytic fungi (DSE) in 67 plant species was assessed to acertain whether mycorrhizas influence composition and diversity in calcareous fen plant communities.	AMF colonization was higher in dicots (58±3%, mean±SE) than in monocots (13±4%) but DSE colonization followed the opposite trend (24±3% in monocots and 9±1% in dicots). Dicots appear to be responding better to AMF than abundant monocots. In contrast, these monocots are more likely to respond to DSE.	[44]
The Baguazhou wetland is located in Nanjing City of Jiangsu Province (China)	Anammox bacteria	The distribution and activity of anammox bacteria in a natural freshwater wetland, located in southeastern China, by using 15N stable isotope measurements, quantitative	The potential anammox rates measured in this wetland system ranged between 2.5 and 25.5 nmol N_2 g^{-1} soil day^{-1}, and up to 20% soil dinitrogen gas production could be attributed to the anammox process.	[45]

		PCR assays and 16S rRNA gene clone library analysis	Phylogenetic analysis of 16S rRNA genes showed that anammox bacteria related to *Candidatus brocadia, Candidatus kuenenia, Candidatus anammoxoglobus* and two novel anammox clusters coexisted in the collected soil cores, with *Candidatus brocadia* and *Candidatus kuenenia* being the dominant anammox genera. Quantitative PCR of hydrazine synthase genes showed that the abundance of anammox bacteria varied from 2.3×10^5 to 2.2×10^6 copies g^{-1} soil.	
Andean wetlands, Northwest of Argentina	Arbuscular fungi	454-amplicon pyrosequencing and morphological (based on spore traits) approaches were used to assess fungal diversity in high altitude and UV radiance, hypersalinie, alkaline soil with high concentrations of toxic elements in the soil.	A total of 23 molecular operational taxonomic units and 14 distinct morphological species of AM fungi were identified. The morphological characterization of AM fungal communities revealed that *Glomeraceae* and *Claroideoglomeraceae* were the dominant families, confirming the predominance of generalist and ruderal AM fungal taxa but with stress tolerant life history traits.	[46]

Internationally, we have come a long way as far as study of wetland microbiology is concerned. Thus, starting from 11 bacterial phyla described by Woese [47], the number of divisions of bacteria has grown to at least 85, the majority of which have no cultured representatives [48, 49]. Candidate phyla radiation, labeled on the new tree of life represents unculturable bacteria which are detected using genomic methods. Not so surprisingly, bacteria make up two-thirds of all earth's biodiversity, of which, one-third is unculturable [50]. This implies that the culturing efforts of the last two centuries had managed to replicate permissive growth conditions for only a small subset of the total bacterial diversity and also a large portion of these bacteria probably belong to less exploited vastness of aquatic system [51]. Similarly, mycologists have identified approximately 74,000 species of fungi accounting for approximately 5% of the widely accepted estimate of 1.5 million fungal species [52]. This lack of species discovery is due, in part, to the microscopic nature of fungi and their frequently imbedded association of somatic body into food source by forming extensive mycelia networks. These problems, along with the lack of trained mycologists, have made identification of fungi extremely difficult, causing many mycologists to not include species identification into their research [53].

Microbial Research Scenario for the Wetlands in India

India, with its varying topography and climatic regimes, supports diverse and unique wetland habitats [54]. The available estimates about the areal extent of wetlands in India vary widely from a lowest of 1% to a highest of 5% of geographical area, but do support nearly fifth of the known biodiversity. It is known that nearly 72% of India's bio-wealth is constituted by fungi (~18%), insects (~40%) and angiosperms (~13%). But apart from this, there is no significant recognized data bank of microbial resources (especially on bacteria and fungi) and therefore, it might be worthwhile to work in this field [6].

Molecular analysis of microbial diversity, especially bacterial diversity, was undertaken for Lonar soda lake (Indian Soda Lake) located in Buldhana district of Maharashtra, by Joshi *et al.* [55]. 196 strains of aerobic and

alkaliphilic bacteria were isolated using different enrichment media. Phylogenetic analysis indicated that most of the Lonar lake isolates were related to the phylum Firmicutes, containing low G+C, Gram-positive bacteria, with different genera: *Bacillus, Paenibacillus, Alkalibacillus, Exiguobacterium, Planococcus*, *Enterococcus* and *Vagococcus*. Seven strains constituted a Gram-negative bacterial group, with different genera: *Halomonas*, *Stenotrophomonas* and *Providencia* affiliated to γ-Proteobacteria, *Alcaligenes* to β-Proteobacteria and *Paracoccus* to α-Proteobacteria. Only five isolates were High G+C, Gram-positive bacteria associated with phylum Actinobacteria, with various genera: *Cellulosimicrobium*, *Dietzia*, *Arthrobacter* and *Micrococcus*.

Choudhury *et al.* studied the distribution of Arbuscular mycorrhizal fungal (AMF) in the marshy and shoreline vegetation of Deepar Beel Ramsar site of Assam, India [56]. The study reveals the percentage of mycorrhizal colonization in the roots of different plant species. The *Vetiveria zizanioides* L. from the family Cyperaceae showed the highest (86.47%) percentage of root colonization, however, only one plant species viz. *Scirpus lateriflorus* Gmel. from the same family was found to be nonmycorrhizal. In all, total 18 AMF morphotypes were recorded which comprise four genera viz. *Glomus* (66.67%), *Acaulospora* (16.66%), *Gigaspora* (11.11%) and *Scutellospora* (5.56%).

Bacterial diversity of East Kolkata wetlands was explored by Ghosh *et al.* [57]. Isolation of 38 strains, their phenotypic and biochemical characterization and molecular identification (by direct sequencing of polymerase chain reaction (PCR)-amplified 16S rRNA gene products) was carried out by them. Das *et al.* had reported about the marine microbial diversity and its importance [58]. Marine microbes constitute a source of commercially important bioactive compounds. They also possess remarkable bioremediation capability.

Diversity of fungi in mangrove along the East coast in India was studied in terms of species diversity, frequency of occurrence and influence of physico chemical parameters on fungi by Thamizhmani and Senthilkumaran [59]. Among the 80 species of fungi isolated, totally 43 species were isolated from sediment samples followed by water with 41 species. Among the fungal members, *Aspergillus* was common genus represented with 17 species followed by *Cladosporium* with nine species; *Alternaria* and *Penicillium* with seven species respectively.

Behera *et al.* isolated sulphur oxidising bacteria from mangrove soil of Mahanadi river delta, Odisha, India, using conventional culturing techniques and evaluated their sulphur oxidation ability [60]. Results showed that out of total 30, sulphur oxidising bacteria were isolated from six different locations of mangrove soil. From the qualitative screening, it was found that out of the 30 bacterial isolates, 12 isolates could efficiently reduce the pH of the medium up to 4.2 from the initial pH 8.0. Their sulphate ion production abilities were in the range of 125–245 mg/ml. Their sulphur oxidase activities were in the range of 11.6 to 126.83 U/ml/min. From morphological and biochemical characterization, most of the isolates were identified as *Micrococcus spp.*, *Bacillus spp.*, *Pseudomonas spp.* and *Klebsiella spp.*

Nath and Kalam collected more than hundreds of soil samples from different localities of East Kolkata wetland and were able to isolate about three hundreds of visually different fungi by dilution method in Czapex Dox Agar media [6]. Out of which, probable identification of *Aspergillus* sp., *Penicillium* sp., *Sphaeropsis* sp., *Pericouic* sp., *Fusarium* sp., *Xylophpha* sp., *Umbelopsis* sp., *Cylindrocladium* sp., *Llelicocephalum* sp. were made by cultural and microscopic studies and with the help of pictorial representations available in "*Pictorial Atlas of Soil and Seed Fungi*" [61].

Arbuscular Mycorrhizal (AM) fungi belonging to the phylum Glomeromycota are widely explored. However, information on the occurrence of AM in plants growing in aquatic and wetland habitats is limited compared to that of terrestrial habitats [62]. To mitigate this, D'Souza and Rodrigues worked on seasonal diversity of AM fungi in Mangroves of Goa [63]. A total of 11 AM fungal species

representing five genera were recorded. *Glomus* was the dominant genus followed by *Acaulospora, Rhizophagus, Funneliformis*, and *Racocetra.* Within AM species, the highest relative abundance was recorded for *R. intraradices*, followed by *A. scrobiculata*, *A. laevis*, and *A. bireticulata*, and the lowest relative abundance was recorded for *R. gregaria*. *Acaulospora laevis* was recovered in all three seasons, *R. intraradices*, and *R. gregaria* were recorded in two seasons, while *G. nanolumen*, *R. fasciculatus*, and *G. multicaule* were recorded in only one season.

Despite the above mentioned microbial research for the wetlands of the country, the fact remains that, at large, in-depth studies have been done for various wetlands for the aspects of flora, fauna and water and soil quality parameters, but not for micro-organisms. This is because microbial ecologists face unique challenges as follows:

1. In order to study micro-organisms, an entire laboratory set-up with several instruments and consumables is required making such studies an expensive affair.
2. Experiments in microbiology are never one-off attempt and require constant fine tuning and trials so as to standardize protocol.
3. There occurs large number of isolates per sample and often several samples have to be collected for evaluation resulting into a problem of differentiating between various microbial populations.

The major challenge faced by microbial ecologists wanting to quantify microbial diversity from natural environment is that such diversity is subjected to seasonal changes leading to large number of samples and in turn, considerable expenses and time requirement. Moreover, to understand the microbial role in an ecosystem, it is essential to know the structure of microbial community in the ecosystem. But, gaining this knowledge is often not easy due to extraordinary complexity of microbial communities. Difficulty also arises due to the fact that limited microbial groups present *in situ* can be cultured for laboratory based microbial identification [64].

Though we have good network of R&D laboratories in India, plenty of natural and human resource wealth along with advancements in modern technology (especially biotechnology and bioinformatics), our achievements in microbial biodiversity and related areas of research are not very promising. Apparently, lack of coordination and integrated efforts for tackling complex research and development issues are some of the inherent weaknesses of systematic microbiology research [65].

Microbial Research Scenario for the Wetlands in Gujarat

Gujarat State having 17.56% of geographical area under wetlands is one of the top ranking states in India with respect to extent of wetlands. Total 14,183 wetlands have been mapped at 1:50,000 scale in the state. In addition, 9,708 small wetlands (<2.25 ha) have also been identified. Total wetland area estimated is 3474950 ha that contributes 23% of total wetlands area of the country which is the top-most among all the states [5].

Despite the abundance of wetlands in Gujarat, no wetland in the state has been explored hitherto from the view-points of bacterial or fungal diversity. GEER Foundation had surveyed micro-zooplankton in Gujarat's only Ramsar Site-Nal Sarovar during 1997–98, the findings of which have been documented as a part of a technical report titled "Environment Impact Assessment of Sardar Sarovar Project on Nal Sarovar Bird Sanctuary" [66]. Moreover, Gujarat Institute of Desert Ecology GUIDE had surveyed these microscopic animals in many wetlands of Kachchh district in late 2000s, the findings of which are documented as a part of the technical report titled "Study of Wetland Habitats in Kachchh District and Suggesting Stakeholder Driven Management Strategies [67]. Both these reports indicate that though planktons were explored (which might include some protists too), bacterial and fungal diversity studies were not carried out.

Hence, it is very essential at this stage to start at least preliminary research for exploring bacterial and fungal diversity of the wetlands of Gujarat. This is because Gujarat is a

wetland-rich State and bacteria and fungi play vital role in functioning of wetland ecosystems. It might be very appropriate if the beginning is made in this direction from Gujarat's only Ramsar Site-Nal Sarovar.

Nal Sarovar is one of the most eminent bird sanctuaries in India. It is globally reckoned, due to its rich biodiversity which includes flora and fauna, specifically birds. Being the only Ramsar Site in Gujarat (since 2012) and an Important Bird and Biodiversity Area (IBA), considerable research and survey work has been carried out at this shallow water natural wetland having an area of 147 km2 [5, 66, 68–69]. Unfortunately, microbial aspect has not been touched upon till date despite the fact that they play a vital role in photosynthesis and decomposition.

It is interesting to note that unlike conventional wetlands, which have either freshwater or saline water quality, Nal Sarovar exhibits change/transition in water quality over the seasons ranging from freshwater quality from monsoon to winter and brackish-saline water quality during summer.

Tringe marks that saltier wetland areas tend to attract microbial communities that give off less methane [13]. This implies that Nal Sarovar might have an interesting microbial dynamics with less presence of methane producing bacteria from post-monsoon to winter and higher occurrence of such microbes during summer. Microbes might be playing important role in food-web of Nal Sarovar as they serve as food for some bottom-living organisms, which might be further consumed by higher organisms like birds, for which the wetland has acquired global fame [58].

Gujarat Ecological Education and Research (GEER) Foundation has been actively involved in ecological monitoring of Nal Sarovar since 2016–17. Extensive work is being carried out to collect data pertaining to flora, fauna and their correlation with environmental parameters. Now, at this stage, it is critically required to do pioneering work to study microbial diversity. This work is of prime importance because extensive microbial ecological study of an entire wetland is rarely being done in India.

However, the complexity of wetlands and the microbial ecosystem makes such systems unfeasible for a straight forward approach. Hence, persistent efforts to culture bacteria and fungi along with interdisciplinary understanding are required to achieve a more profound understanding of microbes and microbial communities in wetlands.

CONCLUSION

The present review has attempted to have a thorough understanding about magnitude of wetland microbial studies carried out at international, national and state(Gujarat) levels. The review has indicated that wetland microbiology is inadequately studied in India and specifically more so in Gujarat. Very limited baseline studies have been undertaken in India and almost none in Gujarat. There is no comprehensive inventory of micro-organisms including bacteria and fungi) occurring even in internationally important wetlands (i.e. Ramsar Sites) in India and Gujarat. Because of this knowledge-gap, it becomes very difficult to carry out holistic ecological monitoring (i.e., monitoring inclusive of microbes) of wetlands. It is an undesirable scenario considering critical functions are played by these microbes in wetland ecosystems. Newer techniques have been emerging in the field of microbiology and by using them we can get systematic microbial research accomplished. It is recommended that microbial studies using these newer techniques should become a part of any wetland ecological monitoring and such task can be achieved by collaboration between niche organizations at both state and national levels.

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