

Original Research Article

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Prokaryotic Community Profile in a Wetland Ecosystem

T. Kavitha, J. K. Arjun, B. Aneesh and K. Harikrishnan*

Environmental Biology Laboratory, Rajiv Gandhi Centre for Biotechnology, Poojappura, Thiruvananthapuram-695 014, Kerala, India

*Corresponding author.

Abstract

The wetlands are natural ecosystems and essential part of the biosphere, through their beneficial functions helps in the mitigation of global climate change. The complexity of such ecosystems is contributed by the resident microbiome along with other flora, fauna and parameters like soil and water characteristics. Since microbes play the key role in biogeochemical cycles, the knowledge on their diversity is very essential. The culture dependent methods reveal only a minor fraction of the community since many of the microbes are recalcitrant to cultivation. In the study we have adopted the culture independent metagenomic approach to determine the prokaryote diversity, which encompasses 16S rRNA gene library construction, library sequencing and phylogenetic analysis. Out of the 600 clones generated 20 clones (bacteria) and 9 clones (archaea) were selected by Restriction Fragment length Polymorphism (RFLP) analysis and sequenced. The sequence analysis revealed that majority of the bacterial community belonged to the phylum *Proteobacteria* (45%), followed by *Acidobacteria* (25%), *Firmicutes* (15%), *Verrucomicrobia* (5%), *Chloroflexi* (5%) and *Planctomycetes* (5%). The archaeal sequences are distributed among the two phyla *Euryarchaeota* and *Crenarchaeota*.

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Introduction

Wetlands constitute about 45% of the total natural ecosystem and are distributed all around the world. They are indispensable part of the biosphere as they have unique role in recycling of nutrients, global greenhouse gas emission and high productivity (Bodelier and Dedysh, 2013; Lv et al., 2014). They are considered as the only known biological sink and source for the greenhouse gas methane and they can contribute up to 21% reduction to the total methane emission (Hanson and Hanson, 1996). Plant-microbe interactions existed in the system aids in the regulation of biogeochemical cycles of elements such as carbon, nitrogen, sulphur, iron, etc. (Lamers et al., 2012). However, the anthropogenic activities and climate change are the two

important threats to the existence of wetlands which influence microbial communities and in turn the natural functioning of wetlands. Even though various studies have reported on the microbial communities in soil and aquatic systems, how the diverse microbial communities in the wetland involve in ecosystem functioning is yet to be elucidated in detail.

The exploration of the microbial diversity in the wetland system is required to unfold the novel microbial resources and to understand their individual part in maintaining the quality of the wetland (Lv et al., 2014). The conventional culture dependent methods reveal only a small portion of the prokaryotes as 99% of them cannot be cultured under laboratory environments (Pham and Kim, 2012). Metagenomics is the fastest growing discipline in the field

of genomics which can be used as a powerful tool for the study of whole microbial communities residing in a system. Several molecular biology methods such as terminal restriction length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), quantitative PCR and cloning of 16S rRNA gene are available to decipher diversity of microbial communities in the soil (Zhen et al., 2010; Kato et al., 2011). Among these, the cloning and sequencing of 16S rRNA gene libraries have been widely used to elucidate the unknown microbial communities (Lenk et al., 2011, Bodelier and Dedysh, 2013). The present study aimed to assess the composition and diversity of prokaryotes in the Kuttanad wetland, a part of Vembanad-Kol wetland ecosystem, Kerala, designated as one of the Ramsar Sites in India through the culture independent metagenomic approach.

Materials and methods

DNA extraction and PCR amplification of 16S rRNA genes

The soil samples were collected from the paddy fields in Kuttanad wetland, transported to the laboratory at 4°C and stored at -20°C. The metagenomic DNA from the soil samples was isolated with Nucleospin soil DNA isolation kit (Macherey Nagel, USA). The purity of the extracted DNA was checked with Nanodrop 1000 spectrophotometer (Thermo scientific, USA) and stored at -20°C. The 16S rRNA gene of archaea and bacteria were amplified from the metagenomic DNA using universal primers (Achenbach and Woese, 1995; Polz et al., 1999). The amplicons were checked by agarose gel electrophoresis and purified using gel band purification kit (GE Healthcare, UK). These products were then ligated into a T/A cloning vector and transformed into chemically competent cells of *Escherichia coli* JM 109. Positive clones were screened by colony PCR using vector specific primers. The 16S rRNA genes of positive clones were digested with the restriction enzyme Csp61 (Fermentas) and checked by agarose gel electrophoresis. Based on the digestion pattern observed in the Restriction Fragment Length Polymorphism (RFLP) analysis, the clones were grouped and one clone from each group was sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing kit v. 3.1 and ABI model 3100 automated sequencer (Applied Biosystems, USA).

Phylogenetic analysis

The partial 16S rRNA gene sequences were assembled using BioEdit v. 7.1.11 software (Hall, 1999) and subjected

to homology searches in the public database, NCBI using Basic Local Alignment Search Tool (BLAST). A neighbour-joining phylogenetic tree was constructed using MEGA 6 (Tamura et al., 2013) based on sequences obtained in the study and their closest relatives from the database.

Results

The amplified 16S rRNA gene yielded was approximately 1.5 kb (Fig. 1). The metagenomic library generated composed of 600 clones of which 420 clones are for bacteria and 180 for archaea. Based on the RFLP banding patterns, 20 clones from the bacterial and 9 clones from the archaeal libraries were sequenced. The sequences were subjected to homology search in NCBI database and the sequences showing 97-100% similarity index were selected for further analysis. Most of the clones exhibited homology to the uncultured bacterium clones in the database representing different phyla.

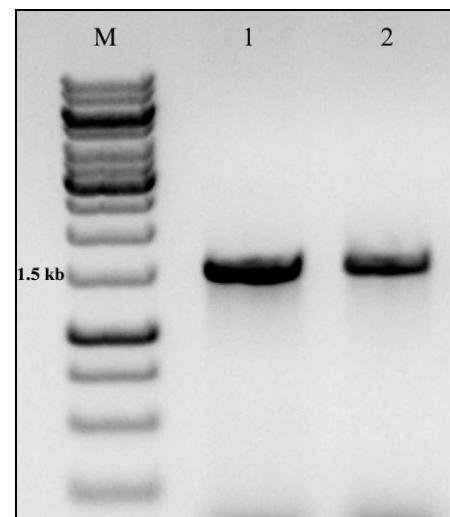


Fig. 1: 16S rRNA gene amplified from the metagenomic DNA; M-1 Kb DNA ladder, 1- Bacteria, 2- Archaea.

The distribution of the prokaryotes in the wetland is depicted in Fig. 2A and the bacterial diversity as represented in the clone library is given in Fig. 2B. The bacterial community in the wetland was dominated by the phylum *Proteobacteria* (45%). The group *Proteobacteria* was represented by the classes Alpha- (5%), Beta- (15%), Gamma- (20%) and Deltaproteobacteria (5%). Among these Gammaproteobacteria was the major class and represented by the genus *Methylobacterium*. Betaproteobacteria, represented by the genera *Thiobacillus*, *Thiomonas* and *Leeia* and the classes Alpha- and Deltaproteobacteria were represented by the genera *Sphingomonas* and *Geobacter* respectively.

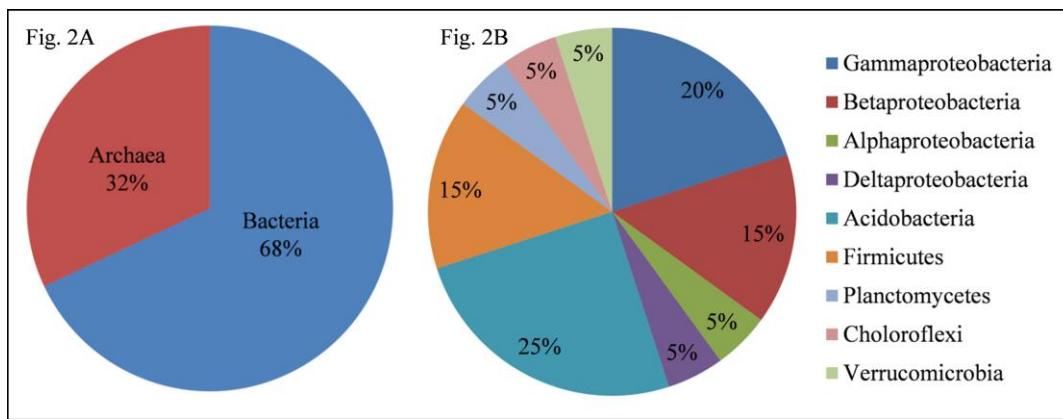


Fig. 2: Distribution of prokaryotes in the wetland: 2A- Total Prokaryotes in the wetland; 2B- Bacterial groups based on 16S rRNA clones.

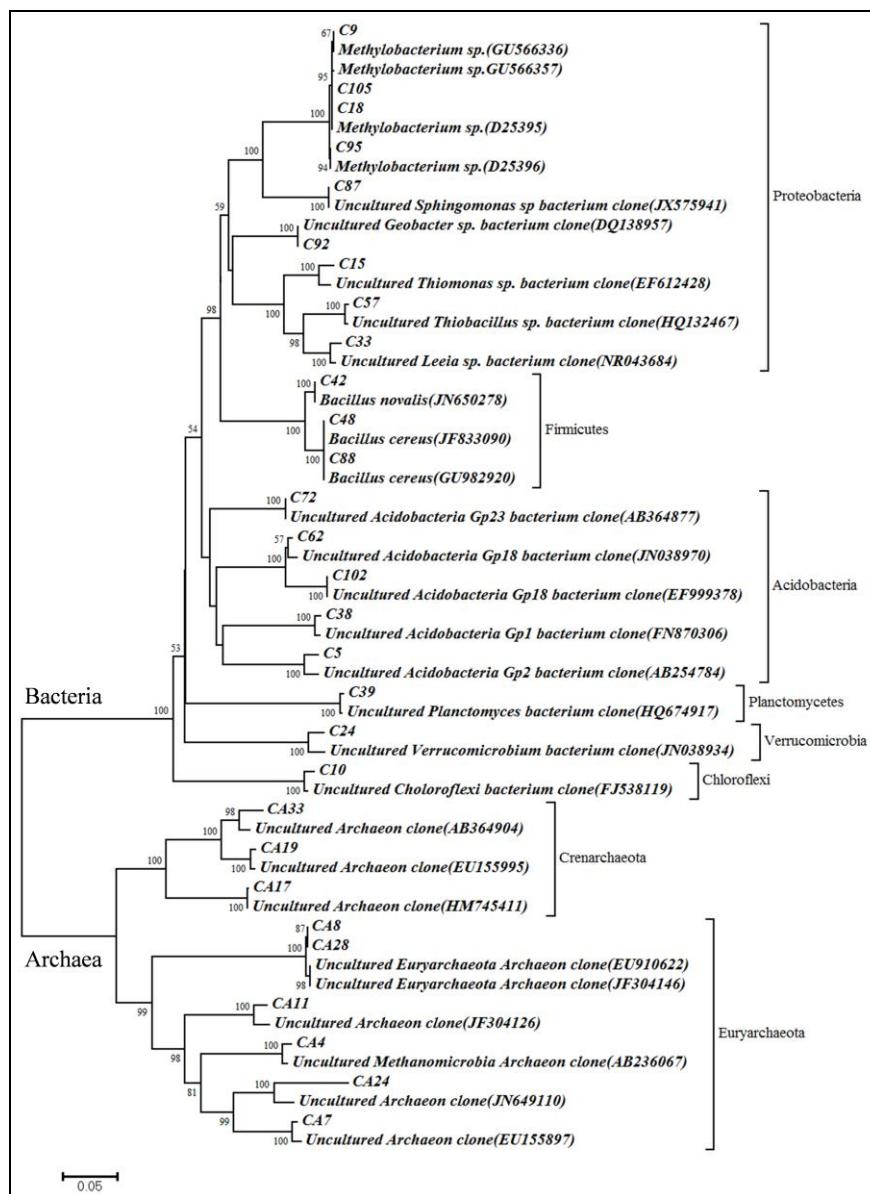


Fig. 3: Phylogenetic tree representing 16S rRNA gene sequences of the clones and their closest relatives from database.

Discussion

Wetlands are natural ecosystems endowed with rich biodiversity and high productivity, but are highly susceptible to the impacts of global climate change. Although several studies are available on the soil properties of Kuttanad wetland ecosystem, a few studies have described its microbial diversity (Thampatti and Jose, 2000; Arjun and Harkrishnan, 2011; Kabeer et al., 2014). Since microbes play the key role in biogeochemical cycles, the knowledge on their diversity is very essential. The culture dependent methods reveal only a minor fraction of the community since many of the microbes are recalcitrant to cultivation. Hence, the study applied metagenomic approaches to elucidate the prokaryote diversity in the wetland. Twenty distinct bacterial sequences were obtained from the 16S rRNA gene clone library, where majority of them represented the phylum *Proteobacteria* followed by *Acidobacteria*, *Firmicutes*, *Verrucomicrobia*, *Chloroflexi* and *Planctomycetes*.

The existence of microbial communities in different wetlands varies from one another based on their type of hydrology, soil and vegetation (Shange et al., 2013). Several studies have proved that *Proteobacteria* is the major phylotype inhabited in the soil with different classes such as Alpha-, Beta-, Gamma-, Delta- and Epsilonproteobacteria (Janssen, 2006). Our findings from the Kuttanad wetland are also in agreement with these observations where the dominant group represented is *Proteobacteria*. The members of this phylum are distributed in four classes in which Gammaproteobacteria is the predominant class followed by Beta-, Alpha- and Deltaproteobacteria. Gammaproteobacteria is represented by *Methylobacterium* sp. Wetlands are one of the significant sources of single carbon (C_1) compounds like methane, methanol, halomethanes, methylated sulphur compounds, etc (Hanson and Hanson, 1996; Jhala et al., 2014). Several species of *Methylobacterium* are reported to be facultative methylotrophs involved in the degradation of C_1 compounds such as methane, methanol and help significantly in reducing C_1 compound emission from wetlands (Balachandar et al., 2008). *Proteobacteria*, the largest group of bacteria in wetland play significant role in a number of biogeochemical cycles. *Thiomonas* sp. and *Thiobacillus* sp. represented the class Betaproteobacteria in the study, and different species of them have been reported previously involved in the nitrate-dependent anaerobic oxidation of inorganic sulphur compounds connecting the sulphur and nitrogen cycles (Haaijer et al., 2006; Katayama et al., 2006). The

class Alphaproteobacteria was represented by *Sphingomonas* sp. Availability of total organic carbon in wetlands are considerably high and the genus *Sphingomonas* was reported as the prominent component of the class in wetlands as they can metabolize a wide variety of carbon compounds and can survive even under nutrient limited conditions (Guo et al., 2011). *Geobacter* sp. representing the class Deltaproteobacteria is an anaerobe and they have been documented as an important group in wetlands involved in the regulation of sulphur and iron cycle (Imfeld et al., 2010; Lamers et al., 2012). They reduce iron and sulphur compounds to corresponding ions that can be easily absorbed by the vegetation in the wetland (Lovely and Philips, 1988; Lamers et al., 2012).

The soil pH in the wetland was found to be towards acidic and in agreement with the early findings (Thampatti and Jose, 2000). Studies have reported that *Acidobacteria* is the largest phylum in acidic wetlands (Wilhelm et al., 2011). However, in the present instance they occupied the second largest position represented as uncultured clones belonging to the groups Gp1, Gp2, Gp18 and Gp23. Since a part of the wetland is used for paddy cultivation, a variety of fertilizers and pesticides are being used, which may contaminate the ecosystem. Several species belong to the genus *Bacillus* has been reported as biodegraders of organic hydrocarbons from the polluted wetlands (John et al., 2011). In the study, two species belonged to the genus, *B. cereus* and *B. novalis* were identified from the clone library of which *B. cereus* was reported to have the capacity to degrade pesticides such as chloropyrifos, malathion and other organic compounds (Liu et al., 2012; Singh et al., 2013; John et al., 2011).

Verrucomicrobia, *Chloroflexi* and *Planctomycetes* were the minor phyla represented in the wetland. It has been reported that, in most of the soil ecosystems 73% of the total minor phyla available is comprised of these three groups. The crucial role of Verrucomicrobial strains in the regulation of methane cycle and nitrogen fixation has been reported recently (Khadem et al., 2010; Sharp et al., 2014). The members of *Planctomycetes* contributed significantly towards the slow degradation of plant derived organic matter in acidic wetlands (Ivanova and Dedysh, 2012). Even though these groups are present at minor level in the soil, their contribution towards global carbon cycle is remarkable.

Among the archaea in the wetland, *Euryarchaeota* represents the predominant phylum and the majority of

the members are methanogens. Generally phylum *Crenarchaeota* recorded a minor representation in the wetland soils (Lv et al., 2014). The anoxic zone of the wetland makes them more suitable for the survival of methanogens. *Methanomicrobia* represented from the wetland is a significant genus in the group methanogens as several members of them are involved in the assimilation of methane in addition to its production (Zhang et al., 2008). Studies have also reported that the combination of anaerobic methanotrophs along with sulphur reducing bacterial partners will enhance the oxidation of methane before reaching it into the earth's atmosphere (Boetius et al., 2000). Thus methanogens as well as methanotrophs in the wetland act as key players in the regulation of global methane cycle.

The study based on 16S rRNA metagenomic library and phylogenetic analysis has generated a basic idea on the microbial diversity in the Kuttanad wetland. However, a holistic picture on the distribution and functional diversity of microbes in the wetland can be achieved only through the sequential analysis of soil samples through different seasons and this will also bring out the microbial community succession. A considerable part of this wetland is used for agricultural practices and hence polluted through the application of various agrochemicals. Such activities in natural ecosystems can cause imbalance in microbial diversity which in turn affect the geochemical cycles. Therefore, appropriate measures have to be taken for the conservation of the wetlands for a better ecosystem functioning.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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