

8. SUMMARY

Microorganisms consist of at least 60% of earth's biomass (Singh et al., 2009). It is well known that prokaryotic cells outnumber the eukaryotic cells on Earth by several orders of magnitudes (Schleifer, 2004). Human life and activity depends on microorganisms, as they are responsible for providing basic elements of life. The prokaryotes are an indispensable part of ecosystem processes and perform some of the most important functions as highlighted earlier by Schleifer (2004) i.e. Biogeochemical cycles, existing as endosymbionts are fundamental to the survival of higher organisms, further non-cultured prokaryotes represent a huge genetical and biotechnological potential and offer an enormous source of new products and processes (Linz et al., 2017; Van Rossum et al., 2015; White III et al., 2015)

Although microbes have such a key role in sustaining basic functions for all living organisms, very little is known about their biology since only a small fraction (about 1%) can be cultured under laboratory conditions. Microbes are ubiquitous in nature, expanding their presence in all components of biosphere. One of the major ecosystems on this earth constitutes of water, either fresh or saline, covering the major portion of the earth surface. Aquatic ecosystems (any water body) account for >70% of the Earth's surface (excluding ice and groundwater ecosystems) and provide various goods and services for human populations, representing gigantic economic value (Zinger et al., 2012). Aquatic microorganisms also represent a large and diverse pool of species; According to the studies, *Bacteria* within ocean are estimated to entail more than $\sim 2 \times 10^6$ species (Curtis et al., 2002) and conduct a vast array of metabolic functions and biogeochemical processes. Among all the aquatic ecosystems, the freshwaters are among the most productive, diverse and threatened on Earth. It forms an important component of daily life, as well as an essential driving force in industrial and agricultural sector (Zinger et al., 2012). The prokaryotes are among the most important contributors involved in the transformation of complex organic compounds and minerals in freshwater sediments (Tamaki et al., 2005).

The present research aims on determining the bacterial community structure and function of one of the freshwater ecosystems i.e. the river Ganges (Ganga) of the country. According to the earlier research, majority of the studies included the assessment of physico-chemical parameters of the parts of the river including the sites of Haridwar (Joshi et al., 2009); Kanpur (Agrawal et al., 2015; Arya and Gupta, 2013; Bhatnagar et al., 2013; Praveen et al., 2013; Thareja et al., 2011); Allahabad (Kaur and Verma, 2014; Raghuvanshi et al., 2014;

Srivastava et al., 2016; Tripathi et al., 2014), Mirzapur (Khan and Nath, 2014). Further research also focused on water quality and pollution status of the river Ganges (Arora et al., 2013; Bhutiani et al., 2016; Hasan; Panwar et al., 2015; Shweta and Satyendra, 2015; Sood et al., 2008).

There are several reports on the detection and survival of coliforms in the water of river Ganga studying the presence of enteropathogenic bacteria including *Vibrio cholera*, *Vibrio fluvialis*, *Aeromonas* sp., *Plesiomonas* sp., *Salmonella* sp., *Shigella* sp., *Pseudomonas* sp., *Proteus* sp., and *E. coli*, etc in the city of Varanasi (De et al., 1993). Also, presence of multiple antimicrobial resistant *E. coli* has been reported from selected locations of the river (Hamner et al., 2007). Several other members of *Enterobacteriaceae* and potential pathogenic genera like *Staphylococcus* has been reported (Biswas et al., 2015; Indiji v, 2011; Lata et al., 2009). Several species like *Nesseria mucosa*, *Proteous mirabilis*, *Lactobacillus fermentum*, *Bacillus cereus*, *Lactobacillus acidophilus* have been reported in a study in Triveni Sangam (Kaur and Verma, 2014). Paul and Sinha have presented a research article on phosphate solubilising and total heterotrophic bacterial strains, by culture plate method and determining their phosphate solubilisation potential, from the river Ganga at Triveni, West Bengal, although the strains were not identified (Paul and Sinha, 2013).

The microbial communities present in the river water and sediments are extraordinarily diverse and complex modulated by a variety of factors that affect the local environment, therefore in order to study such an ecosystem, along with traditional culture based approach, we also applied metagenomics and sequenced the community DNA from the sites including Bijnor, Narora, Kannauj, Kanpur, Allahabad, Triveni Sangam, Mirzapur and Varanasi targeting the V3-V4 region 16S rRNA gene. The study revealed significant bacterial diversity of the water and sediment samples collected from the river Ganges. Some of the critical findings from our study include:

Culture dependent analysis

- In culture dependent analysis, some distinct phyla were observed viz. *Bacteroidetes* and *Deinococcus-Thermus* from downstream sites which have not been reported in previous research on the Ganges river.
- Twenty four strains were found to be potentially novel some of which were characterized by using a polyphasic taxonomic approach and whole genome analysis.

Cologgi et al., 2014); were recovered, which have not been reported in any previous study.

	Culture dependent	Culture independent (dominant)
Phylum level	<i>Proteobacteria</i> (46%), <i>Actinobacteria</i> (31%), <i>Firmicutes</i> (26%), <i>Bacteroidetes</i> (8%) and <i>Dienococcus-Thermus</i> (4%)	<i>Proteobacteria</i> (56%), <i>Cyanobacteria</i> (39%), <i>Bacteroidetes</i> (22%), <i>Actinobacteria</i> (14%), <i>Acidobacteria</i> (12%), <i>Firmicutes</i> (11%), <i>Nitrospirae</i> (7%), <i>Chloroflexi</i> (6%), <i>Gemmatimonadetes</i> (4%), <i>Verrucomicrobia</i> (4%) and <i>Planctomyces</i> (4%)
Genus level	<i>Pseudomonas</i> , <i>Bacillus</i> , <i>Acinetobacter</i> , <i>Rhodococcus</i> , <i>Fictibacillus</i> , <i>Pseudarthrobacter</i> , <i>Exiguobacterium</i> , <i>Alcaligenes</i> , <i>Microbacterium</i> , <i>Brevibacterium</i> , <i>Arthrobacter</i> , <i>Streptomyces</i>	<i>Stramenopiles</i> , <i>Flavobacterium</i> , <i>Synechococcus</i> , <i>Chlorophyta</i> , <i>Paulinella</i> , <i>Fusibacter</i> , <i>Nitrospira</i> , <i>Rheinheimera</i> , <i>Bacteroides</i> , <i>Rhodobacter</i> , <i>Saprospirae</i> , <i>Sinobacter</i> , <i>Pelagibacterium</i> , <i>Rhodoplanes</i> , <i>Geobacter</i> , <i>Mycoplana</i> , <i>Clostridium</i> , <i>Methylomonas</i> , <i>Sulfuricurvum</i> , <i>Trichococcus</i> , <i>Faecalibacterium</i> , <i>Microcystis</i> , <i>Syntrophus</i> , <i>Janthinobacterium</i>
Species level (dominant)	<i>Pseudomonas alcaligenes</i> , <i>Acinetobacter junii</i> , <i>Fictibacillus phosphorivorans</i> , <i>subtilis</i> , <i>Aeromonas rivuli</i> , <i>Paenarthrobacter</i> <i>nicotinovorans</i> , <i>Rhodococcus</i> <i>defluvii</i> , <i>Acinetobacter</i> <i>baumanii</i> , <i>Brevibacterium</i> <i>sediminis</i> , <i>Streptomyces</i> <i>althoticus</i> , <i>Pseudarthrobacter</i> <i>polychromogenes</i>	<i>Hydrogenophaga sp.</i> , <i>Synechococcus sp.</i> , <i>Comamonas sp.</i> , <i>Flavobacterium sp.</i> , <i>Rhodobacter sp.</i> , <i>Faecalibacterium prausnitzii</i> , <i>Sphingobacterium sp.</i> , <i>Luteolibacter sp.</i> , <i>Nitrospira sp.</i> , <i>Bacteroides sp.</i> , <i>Micrococcus</i> <i>sp.</i> , <i>Janthinobacterium lividum</i> , <i>Clostridium</i> <i>neonatal</i> , <i>Clostridium butyricum</i> , <i>Verrucomicrobium chitinophaga</i> , <i>Sulfuricurvum sp.</i> , <i>Cylindrospermopsis sp.</i> , <i>Clostridium gasigene</i>

Table 8.1. Predominant taxa (phyla, genera and species level) obtained from culture dependent and culture independent approaches.

As, in the present study, less of coliforms and faecal indicators were observed, new strategies could be devised targeting such microbial communities from Enterobacteriaceae or non-Enterobacteriaceae family, and also for screening of important clinical isolates with potential health risks. There were several unclassified groups and groups classified till family level in metagenomic study indicating prevalence of some novel taxa. Further research regarding the importance of rare species is required to fully understand their role in the ecosystem. A detailed functional metagenomics study would provide valuable information about the

metabolic potential of microbial communities present in freshwater ecosystem and its surrounding regions.

It's clearly evident from the analysis that compared with culture-independent approaches, traditionally used culture-dependent methods have a limited capacity to identify and characterize the microbes in any given environment. This is the first study covering a larger stretch from the river Ganges where water and sediment samples were collected from three stretches viz. upstream stretch (Bijnor and Narora), middle stream (Kannauj, Kanpur, Allahabad and Triveni Sangam) and downstream stretch (Mirzapur and Varanasi) including upstream and downstream of eight different sites, based on culture dependent and culture independent approaches. Overall this Gangiomics study revealed the significant bacterial diversity of the freshwater and sediment samples as we moved from a less polluted site to a more interfered state.

The study of the microbial diversity is important for understanding the link between diversity, community structure, and function. The classical microbiology and organism level genomics approach, if merged together, would help in a better understanding of overall diversity level and would provide an opportunity to obtain genomic information from major uncultivated microbial lineages. The information could be helpful in various functional aspects, as it would be possible to fish out the genes possessing important properties like antibiotic resistance, drug resistance, and secondary metabolite producing, and even bacteriophages and strategies could be designed to culture those organisms. The information could be useful and applied in clinical microbiology, virology, biotechnology, microbial forensics, and many other fields.