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Dynamics of bacterial class *Bacilli* in the deepest valley lake of Kashmir-the Manasbal Lake

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ABSTRACT

In recognition of the importance of bacteria as ecological indicators of the aquatic systems a comprehensive and systematic analysis was carried out on Manasbal Lake, the deepest spring fed valley lake of Kashmir. The main objective envisaged was to analyze bacterial community composition (BCC) and for this purpose systematic and regular sampling of waters from ten different sampling stations, predetermined in the Lake according to differences in degree of human interference and also as zones of special ecological interests were selected. The isolated species were identified according to Bergey's Manual specification by examining their micro and macro morphological characteristics and biochemical characteristics on different culture media. Further confirmation was done by sequencing the 16s rRNA gene by using universal bacterial primers 27F and 1429R. From all the sampling stations the class *Bacilli* showed a maximum relative abundance with a contribution of 16 bacterial species. The whole process resulted in the identification of *Bacillus aerius*, *Bacillus altitudinis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus ginsengisoli*, *Bacillus pumilus*, *Bacillus safensis*, *Bacillus stratosphericus*, *Bacillus subtilis*, *Bacillus tequilensis*, *Bacillus thermocopriae*, *Bacillus thuringiensis*, *Brevibacillus agri strain*, *Lysinibacillus boronitolerans*, *Lysinibacillus pakistanensis* and *Lysinibacillus sphaericus*.

1. Introduction

Genus Bacillus (Class: *Bacilli*; Phylum: Firmicutes) established in 1872 have certain features like their aerobic nature, rod shape, and catalase production that distinguishes it from other Bacillaceae (all endospore formers). It includes Gram-positive, spore-forming bacteria of diverse phenotypic characteristics, including differences with respect to nutritional requirements, growth conditions and DNA base composition [1]. The microbes belonging to genus Bacillus have been isolated from diverse environmental habitats, including alkaline [2,3], acidic [4,5], saline [6]; high temperature and low temperature [7]. It represents a heterogeneous group which included many important species [8,9]. Exact descriptions of these genera and species are based to a varying extent upon morphological, physiological, biochemical, chemo-taxonomical and on molecular traits. However, while the basic systematic structure is determined by the comparison of the sequences of the 16S rRNA [10], it is understood, in areas where discrimination through this method too is insufficient or unsatisfactory newly developed methods need to be applied. Though a large majority of *Bacillus* species are harmless saprophytes [11] some of the species of genus Bacillus like *Bacillus cereus* and *Bacillus anthracis* are reported human pathogens [12–14] with the former implicating food borne disease and the later infecting perorally. *B. anthracis* is a facultative intracellular bacterial pathogen causing cutaneous, gastrointestinal or respiratory disease in many vertebrates, including humans [15,16].

Genus Lysinibacillus (Class: *Bacilli*; Phylum: Firmicutes) was first described by Ahmed et al. [17] with *Lysinibacillus boronitolerans*, *Lysinibacillus fusiformis* and *Lysinibacillus sphaericus* as the most studied species of the genus [18]. Different species of this genus have also been isolated from diverse environs such as surface water [19], soil [17,18,20], fermented soybean food [21] and inner tissues of plants and humans [22,23]. Genus Brevibacillus (Class: *Bacilli*; Phylum: Firmicutes) was created as a new and separate genus by reclassification of the *Bacillus brevis* group of species in 1996 [24]. Some gram-positive Bacillales bacteria including Brevibacillus, are considered as good

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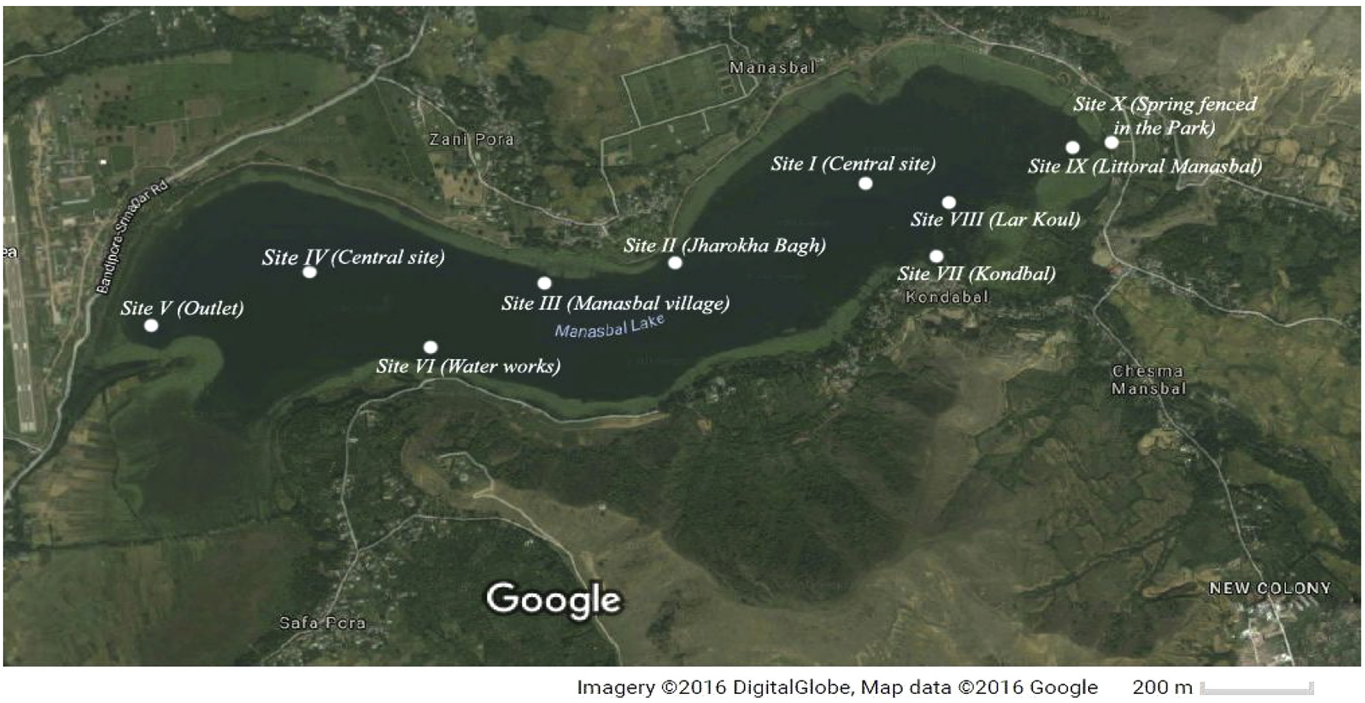

Fig. 1. Manasbal Lake and the selected sampling stations.

candidates for biocontrol approach by using antibiotic production and biofilm formation [25–27].

2. Materials and methods

2.1. Lake description and sampling sites

Manasbal lake – the deepest (about 12 m) of all freshwater lakes of Kashmir valley [28,29] is located at an altitude of 34°14′40″–34°15′20″N and 74°39′00″– 74°41′20″E. Though it is mainly fed by the springs, it is also fed seasonally by an irrigational stream (Laar-kul) on the eastern side, operational only during summer season. This lake also serves as an important natural water reservoir for the local population as water of the lake is used for various activities such as drinking, washing, bathing, recreational and agricultural purposes etc. [30]. Ten different sampling stations (Fig. 1), predetermined in the Lake according to the degree of human interference and as zones of special ecological interests were selected in the lake ecosystem for the collection of water samples to undertake the work on the community composition of the bacterial class *Bacilli*.

Table 1
Giving volume and final concentration of different reagents used in amplification process.

Reagent	Final concentration	Volume required
Taq Buffer (10X)	1 X	5.0 μl
$MgCl_2$	1.5 mM	3 μl
dNTP mix (2 mM)	0.2 Mm	5.0 μl
Forward Primer 10 pmol/μl	0.5 pmol/μl	1.0 μl
Reverse Primer 10 pmol/μl	0.5 pmol/μl	1.0 μl
Taq DNA Polymerase 5U/μl	0.03 units/μl	0.3 μl
Genomic DNA	50-100 ng	1.5 μl
MilliQ water		33.2 μl
Total volume		**50.0 μl**

2.2. Collection of water samples

Water samples from different sites of Manasbal Lake were collected seasonally for a period of two years from February 2013–February 2015 in sterilized polyethylene (PET) bottles, which were previously carefully cleaned and rinsed three to four times with autoclaved distilled water and sterilized with 70% alcohol. At the time of collection, the containers were rinsed thrice with the lake water before being used to collect the samples. During collection of samples, extreme care was exercised to avoid any contamination.

2.3. Isolation of bacteria

For the isolation of bacteria, the collected water samples were serially diluted four folds using normal saline solution followed by direct plating method [31], spreading 0.1 ml inoculum from the serial dilution tubes [32] on petri dishes containing nutrient agar medium [33]. The inoculated plates were incubated at a temperature of 37 °C for 24–48 h.

2.4. Identification of the isolated bacterial species

2.4.1. Morphological approach

For the identification of bacteria gram's staining using direct microscopy, and motility tests were performed. In order to study the micro and macro-morphological features of bacteria, they were stained and studied for size, shape, and arrangement etc. Each recognizable type of colony was streaked and re-streaked on fresh media to obtain pure cultures. Bacterial motility was ascertained by microscopic examination of cultures by using motility agar media.

2.4.2. Biochemical approach

The purified colonies of various types were identified to genus or species level using standard biochemical tests following the criteria provided in Bergey's Manual of Systematic Bacteriology. Biochemical test kits used for identification purpose utilized seven