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Biochemical characterization and identification of bacteria isolated from Mithi River in Mumbai

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***Abstract*— Mithi River is one of the rivers in the city of Mumbai which is adversely affected by the industries, illegal small scale vendors, random development of slums on the river banks and an inability to effectively mitigate the anthropogenic factors bringing down the quality of the river. Initial physicochemical characteristics and temperature were analysed on the field and they remained well in the prescribed limits. The study conducted was an attempt at understanding the biodiversity of the bacteria. Approximately 51 bacteria isolated from different samples were characterized as a pilot study. The characterization showed a satisfactory amount in the diversity of the bacterial population of the river.**

Keywords*— *Mithi River, Pollution, Bacteria, Biochemical characterization.

INTRODUCTION

Water pollution is not restricted to any particular country or actions of a set group of states, it is a global consequence. It is important to address water pollution on a larger scale as pollution and water quality degradation interfere with vital and legitimate water uses at any scale, i.e. local, regional or international (Meybeck et al., 1989). At an altitude of 246 m above sea level, the Mithi River in the east hills and gathers strength from the streams and discharges of Tulsi, Vihar and Powai Lake travelling 17.84 kms to meet the Mahim Bay (Kirtane, 2011). The area of the river is within latitude 19° 00' to 19° 15' N and longitude 72° 45' to 73° 00' E. (NEERI, 2011). The River from its origin flows through Filterpada, Bamandayapada, Marol, Sakinaka, L&T Junction, JVLR, Bail Bazaar, ChattrapatiShivaji International Airport, BKC and finally the Mahim creek and which completes its journey into the Arabian Sea. The Mithi River existed long before the developments took place. The Mithi River has been largely affected due to the land reclamations and pollution. Alarmingly, about two thirds of the streams and drainage channels in the Mithi River's catchment have also fallen prey to land reclamation and urbanization (J.Kamini, 2006). The banks of the river are occupied by slums, illegal factories, small scale industries etc. pouring untreated and unchecked amounts of wastes into the river each day. Municipal wastes too make their way from many sources along the outlets made on the walled banks of the river.

Genera of bacterial isolates have shown bioremediation capacities reported by many researchers found in their study area. Kumar et al 2005, Agarry and Solomon 2008, studied *Pseudomonads,* Kim and Oriel 1995 studied *Bacillus sps* and found degradation capacity of phenolic compounds. Aerobic degradation by a *Pseudomonas* strain of HCH, a persistent pesticide, was first reported by Matsumura et al. (1976). Klebsiella strains have been found with many metabolic properties that are interesting for bioremediation (Dworkin Martin et.al). Proteus spp. strains possessed various metabolic abilities that allowed their adaptation to different environmental conditions, such as high concentrations of heavy metals or toxic substances, which could have been exploited as sources of energy and nutrition by those bacteria increasing the possibility of employing such microorganisms in bioremediation and environmental protection (Drzewieck Dominika, 2016). The current study was taken up with an objective to study the microbial diversity of the Mithi River and identify the dominating microbial communities at the genus levelexperimentally and understand their importance of those genera in mitigation of pollution naturally, through means of existing literature for future studies.

Materials and Methods

Water sampling: Water samples were collected from six sites along the bank of the river. The locations were narrowed down on the basis of accessibility. Grab samples were collected in pre washed, dried and clean containers. The samples were transferred in an ice bath to the laboratory within four hours of collection and transferred to the broth in aseptic conditions immediately. Another sample, around 1 ml was directly inoculated on the field, in a pre-sterilized test tube containing Nutrient broth which served as a mother culture for validation in case of contamination or loss of sample.

Analysis of pH and Temperature: pH and temperature of the water sample was analysed on field during collection using a digital pH meter and thermometer respectively**.**

Isolation of organisms: The isolation of the organisms in each water sample was carried out using serial dilution. The organisms were plated on to Nutrient agar medium using spread plate technique. After 24 hours of incubation the plates were observed for well separated, clear and dominating colonies of microorganisms. These colonies were picked up individually and transferred onto a nutrient agar slant for further studies.

Biochemical characterization: Biochemical tests like Caesin Hydrolysis, IMViC (Indole test, Methyl red test, Voges-Proskauer test and Citrate utilization test), Nitrate test, Urease test were performed. (Bergey et. al 1974, Ananthanarayan et al 2005)

RESULTS AND DISCUSSION

The pH is the scale of intensity of acidity and alkalinity of water which measures the concentration of hydrogen ions. The pH of the water sample were slightly alkaline going above 7 (the neutral pH) at all stations. Table 1. pH and Temperature of water

Location	pH	Temperature
1	7.55	24.8
2	7.52	27.1
3	7.40	26.3
4	7.51	27.5
5	7.50	27.8
6	7.30	28.2

Location 1 was most alkaline followed by location 2, location 4, Location 5, Location 3 and Location 6 being least alkaline. Considering all the locations of the river the temperature ranged from 28.2°C to 24.8°C.

Biochemical Characterization: The biochemical characterization showed a varied group of activities of the microorganisms. In Location table 1 and 2, eight isolates tested positive for casein hydrolysis while in Location 3 and 4, six isolatess showed positive results and in Location 5 and 6, eight isolates tested positive for casein hydrolysis. In Indole test seven isolates in the overall locations were tested positive. In the Methyl red test eight isolates were tested positive in Location 1 and 2, twenty five isolates were tested positive at Location 3 and 4 and five were tested positive at Location 5 and 6. Voges–Proskauer test was positive for five isolates at Location 1 and 2, three organisms at Location 3 and 4 and five organisms at Location 5 and 6. Citrate utilization test was positive for all isolates at all stations except three at location 3 and 4 which showed negative results. The nitrate test showed positive results for all isolates at location 3 and 4 and six at Location 5 and 6. One isolate exhibited negative result at location 1 and 2. Overall 33 isolates showed positive results for Urease test(Table 2, 3 and 4)

Table 2. Biochemical Characterization of isolates from Location 1 and 2

Isolate no.	Caesin Hydrolysis	Indole Test	Methyl Red (MR) test	Voges–Proskauer (VP) Test	Citrate Utilization Test	Nitrate Test	Urease Test
V0	-	-	-	-	+	+	+
V1	+	-	+	-	+	+	-
V2	-	-	-	-	+	+	+
V3	+	-	+	-	+	+	-
V4	-	-	+	-	+	+	-
V5	+	-	+	-	+	+	-
V6	+	-	+	+	+	+	+
BN1	+	-	-	+	+	+	+
BN2	+	-	-	+	+	-	+
BN3	-	-	-	+	+	+	-
BN4	-	+	-	+	+	+	+
BN5	+	-	+	-	+	+	+
BN6	-	-	+	-	+	+	+
BN7	+	-	+	-	+	+	-

Table 3. Biochemical Characterization of isolates from Location 3 and 4

Isolate No.	Caesin Hydrolysis	Indole Test	Methyl Red (MR) test	Voges–Proskauer (VP) test	Citrate Utilization Test	Nitrate Test	Urease Test
P0	+	+	-	+	+	+	+
ML0	-	+	+	-	+	+	-
ML1	-	-	+	-	+	+	+
ML2	-	-	+	-	+	+	+
ML3	+	-	+	-	+	+	-
ML4	-	-	+	+	+	+	-
ML5	-	+	+	-	+	+	+
ML6	-	-	+	-	+	+	-
ML7	-	-	+	-	+	+	+
ML8	+	-	+	-	+	+	+
ML9	-	-	+	-	+	+	+
ML10	-	-	+	-	+	+	+
ML11	-	+	+	-	+	+	-
ML12	-	-	+	-	+	+	+
ML13	-	-	+	-	-	+	+
ML14	+	-	+	-	+	+	+
ML 15	-	-	+	-	-	+	-
ML 16	-	-	+	-	+	+	+
ML 17	-	-	+	-	+	+	+
ML18	+	-	-	+	+	+	+
ML19	-	-	+	-	+	+	-
ML20	-	-	+	-	+	+	-
ML21	-	-	+	-	+	+	-
ML22	+	-	+	-	+	+	+
ML23	-	-	+	-	-	+	+
ML24	-	+	+	-	+	+	-
ML25	-		+	-	+	+	+

Table 4. Biochemical Characterization of isolates from Location 5 and 6

Isolate No.	Caesin Hydrolysis	Indole Test	Methyl Red (MR) test	Voges–Proskauer (VP) test	Citrate Utilization Test	Nitrate Test	Urease Test
BK1	+	-	-	+	+	-	-
BK2	-	-	-	+	+	+	-
BK3	+	-	-	+	+	-	+
BK4	+	-	-	+	+	-	+
BK5	+	+	+	-	+	-	+
BK6	-	-	+	+	+	+	+
BK7	-	-	+	-	+	-	+
MH1	+	-	+	-	+	+	+
MH2	+	-	-	-	+	+	+
MH3	+	-	-	-	+	+	+
MH4	+	-	+	-	+	+	-

Identification of Isolates:

The dominant genera of bacteria comprised Proteus (81%), *Salmonella* (72%), *Pseudomonas* (63%), *Citrobacter* (54%) *Enterobacter* (45%) *Klebsiella* and *Staphylococcus* (36%) *Shigella*and*Bacillus (27%) Vibrio* (18%) and *Acinetobacter* (9.09%). The identification was carried out by focusing predominantly on the IMViC tests supported by the Urease tests results, as major genera belonged to the family Enterobacteriaceae. Indole negative, MR positive VP and Citrate utilization tests were characteristic to *Shigella*sps. Indole test and MR test positive while VP test and Citrate utilization test negative were characteristic to *Klebsiella, Bacillus* and *Enerobacter*sps. Indole Test and VP test

negative while MR test and Citrate utilization test were characteristic of *Salmonella*, *Proteus* and *Citrobacter*sps. Indole test negative while MR test, VP Test and Citrate utilization test positive were characteristic of *Staphylococcus*sps. These results were further narrowed down along with other tests for example Urease test to further confirm the genus. Urease tests are positive for *Klebsiella* but negative for *Salmonella.* It also helps distinguish *Proteus* since large number of species test negative for Urease test.*Pseudomonas*sps are usually glucose non fermenting bacteria which show MR and VP tests negative.*Vibrio* and *Acinetobacter* were a comparatively small group of organisms characterized by the above mentioned tests during the study period. Further tests like oxidase, catalase, H_2S (Hydrogen sulphide) and TSI (triple sugar iron agar) tests will be conducted on the available base line to identify the species.

CONCLUSION

The pH and temperature of the water were analysed as a pre requisite to understand the habitable conditions of the microbes to facilitate further in-vitro tests. Both temperature and pH have been found to be in conditions that can be easily maintained in laboratories fairly at room temperature. The dominance of the genus suggest the presence of nutrients contributed through sewage discharged through municipal sewerage lines, unauthorized outlets etc. But it need not be entirely concluded as fecal contamination because many species among the genera also normally occur in the water or soil habitat. Many bacteria in the above mentioned genera, are also reported to be efficient in carrying out bioremediation.

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