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EXPERIMENTAL ARTICLES

Exploring Bacterial Diversity from Contaminated Soil Samples from River Yamuna[1]

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Abstract—The Yamuna is the source of key water supply in the national capital region of India. Due to its immense importance, the pollution of Yamuna has become an imperative issue of study. Various initiatives have been taken by the Indian Government to decontaminate this river, but so far no possible outcome has been obtained. Therefore bioremediation may seem to be a promising approach. To assess the bioremediation potential of the microbes at river Yamuna, study of microbial diversity was carried out. *Escherichia*, *Pseudomonas*, *Bacillus*, *Thermomicrobium*, *Azoarcus*, *Nitrosomonas* and *Shigella* were the dominant genera present at the contaminated river coastal zone. The presence of *Escherichia* and *Shigella* indicated the sewage contamination in the river. On the other hand, the presence of *Pseudomonas* and *Bacillus* indicated the existence of indigenous bacterial communities capable of de-polluting the river, thus providing a promising approach to decontaminate Yamuna by natural means.

Keywords: Yamuna, bacterial diversity, T-RFLP, diversity indices

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The Yamuna is the largest tributary of the Ganges in northern India [1] and is responsible for being the key water supply in Delhi, the National Capital region.

Due to its immense importance, the pollution of Yamuna has become an important subject of study. Pollution in the river is caused mainly by the industrial and domestic sources [2]. According to Central Pollution Control Board (CPCB), India, major cause of pollution in the river is untreated sewage [3] and industrial wastes [4–6].

There are several heavy metals [5, 7], pesticides [8] and various coliform bacteria [9] that are found to be contaminating the river. Various efforts have been made by the Government of India to improve the water quality of the river. These include Common Effluent Treatment Plants (CETPs), relocation of industries, Yamuna Action Plan, Yamuna Jiye Abhiyaan, Yamuna Satyagraha etc. The CPCB constantly monitors river Yamuna at strategic locations. Majority of these plans use chemical methods for water treatment. But so far no possible outcome has been obtained and no improvement in water condition of Yamuna is reported. Hence, biodegradation seems to be a promising approach for water treatment. The river Yamuna may itself contain natural microbial population that can help in improving the quality of the river. Therefore, in the present investigation, study of bacterial diversity was carried out using both culture-dependent and culture-independent methods to explore the possibility of bioremediation.

MATERIALS AND METHODS

Bacterial community characterization. To characterize the bacterial flora present at the contaminated Yamuna river bed, five soil samples were randomly collected from Yamuna bed at Wazirabad area, Delhi, India. 1 g of each soil was pooled and homogenized in 9 mL sterile 0.9% saline (w/v) by vortexing. Dilutions were plated onto 0.1% tryptic soy agar and incubated at 28°C [10]. The number of colony forming units (CFU) was counted daily from day 1 to day 10. The percentage of fast-growing bacteria was determined by dividing the number of CFU counted on day 2 by the number of CFU on day 10. Eco-physiological index was calculated as: $-\Sigma(ni/N)(\log ni/N)$where ni is the number of visible colonies on ith day and N is the total colonies.

Terminal restriction fragment length polymorphism (T-RFLP). Soil DNA was extracted from 0.5 g of soil samples using PowerSoil™ DNA kit (MoBio, USA). The concentration (A260 nm) and purity (A260/280 nm) of DNA was checked by Nanodrop ND-1000 Spectrophotometer (Witec-AG, Switzerland). DNA samples were stored at −20°C till further use. Purified

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Table 1. Physico-chemical and geochemical properties of soil samples of river Yamuna

pH	E. C., dS/m	Organic carbon, %	Available phosphorus, kg P/ha	Available potassium, kg K/ha
8.2	0.37	0.23	22.1 M	148 M

Table 2. Statistical analyses of T–RFLP profiles of three different restriction enzymes performed using standard ecological estimates of diversity

Samples	Simpson's diversity index	Shannon entropy	Berger-Parker dominance index	Equitability index	Buzas and Gibson's index	Margalef Richness index	Berger-Parker dominance index
*Msp*I	0.8736	4.072	0.09272	0.8882	0.7009	1.431	0.09272
*Hha*I	0.87	4.047	0.1046	0.8827	0.6888	1.436	0.1046
*Rsa*I	0.8534	4.077	0.1699	0.8275	0.6061	1.396	0.1699

DNA was used as a template to amplify 16S rRNA gene using 6-carboxy-fluorescein (6-FAM)-labelled 8F and 1115R primers. Three restriction enzymes (*Hha*I, *Msp*I, *Rsa*I) were used for T-RFLP analysis. GeneMapper3.2 software (ABI) was used to analyze the electropherogram output. The sample data consisted of size in base pair (bp), peak height, peak area and data point for each terminal restriction fragment (T-RF) in each sample. T-RFLP data was analyzed using Phylogenetic Assignment Tool (https:// secure.limnology.wisc. edu/trflp/).

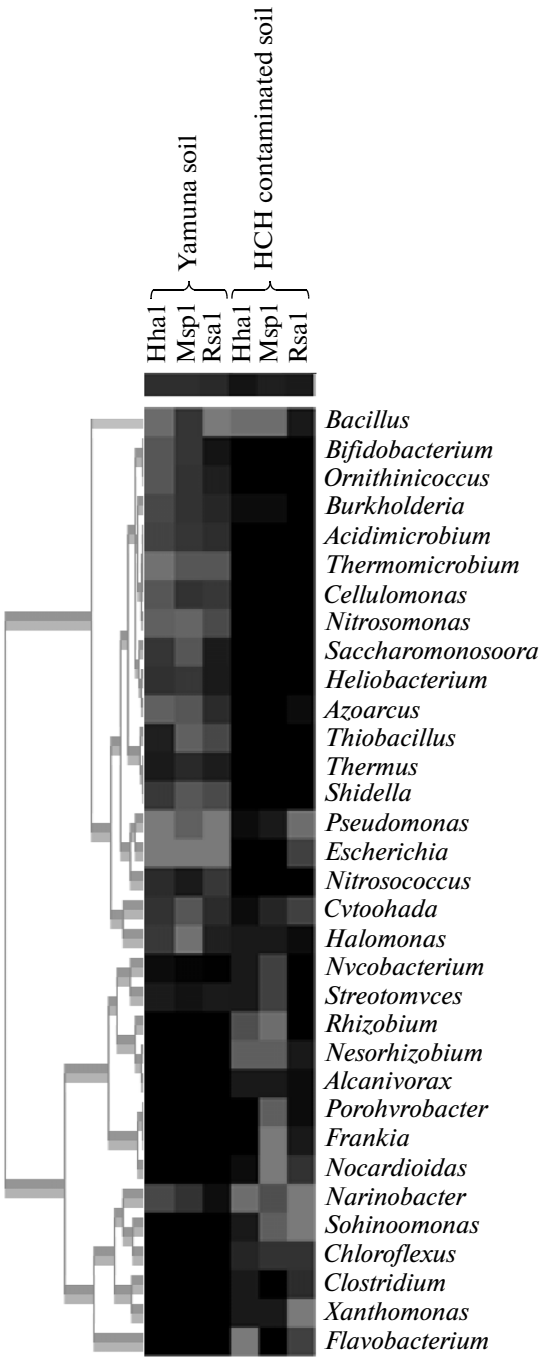
Diversity indices. To get deeper insight into the bacterial diversity and distribution of various species, diversity indices like Simpson's Diversity Index, Shannon Entropy, Berger-Parker Dominance Index, Equitability Index, Buzas and Gibson's Index, Margalef Richness Index and Berger-Parker Dominance Index were calculated using Diversity Calculator (http://alyoung.com/labs/biodiversity_calculator. html).

RESULTS AND DISCUSSION

In the present study bacterial diversity of Yamuna river soil was studied using both culture-dependent and culture-independent methods. The physico-chemical and geochemical properties were analyzed at Indian Agricultural Research Institute (IARI), New Delhi. The pH of soil was found to be 8.2, with electrical conductivity 0.37 dS/m. The soil organic matter was also checked and it was found that the Yamuna soil was poor in organic carbon (0.23%), whereas available phosphorus and potassium contents were found to be medium (Table 1).

The culture based method of De Leij [10] was used to study the bacterial community. This classical method involves the study of bacterial communities by using the growth characteristics on diluted TSA. All the collected soil samples were pooled prior to the analysis. Total CFU obtained using 0.1% TSA was $8.4 \pm 0.7 \times 10^4$ cfu/g of soil. The soil was found to contain $19.6 \pm 1.5\%$ fast growing bacteria. Evenness in the structure of microbial diversity ensures that the community has more capacity to use its varied array of metabolic pathways [11]. It has been proposed that more even the distribution, higher the Eco-physiological (EP) index [10]. EP index for the present analysis was found to be 0.716 ± 0.008, showing the evenness in the bacterial community based on the characteristic growth pattern.

To get deeper insights into the bacterial community, T-RFLP of 16S rRNA gene was carried out. T-RFLP is a method of analyzing variations in the length of terminal restriction fragments (T-RFs) of conserved molecular markers generated after restriction digestion. It is a rapid method to study community structure and dynamics [12–14] and has been used to study diversity from contaminated soils [15, 16]. Three different enzymes (*Msp*I, *Hha*I, *Rsa*I) that are frequent cutters were used in the present study. Using *Hha*I, T-RFs ranging from 56–547 bp in size were obtained. Using *Msp*I, T-RFs ranging from 74–440 bp in size were obtained, whereas T-RFs ranging from 56–762 bp were obtained using *Rsa*I. Out of these T-RFs, maximum % area was obtained for the T-RFs belonging to genera *Escherichia, Pseudomonas, Bacillus, Thermomicrobium, Azoarcus, Nitrosomonas, Shigella* (Fig. 1). The presence of genera like *Escherichia* and *Shigella* indicates the contamination of the samples from sewage [17, 18]. This is rather relevant as river Yamuna is the dumping site where all the sewage from the city is dumped. Apart from sewage contamination the residues of pesticides like HCH, DDT and endosulfan have also been reported from the river [19]. Therefore, the bacterial diversity obtained for Yamuna soil using T-RFs of three different enzymes was compared with the bacterial diversity of HCH (Hexachlorocyclohexane) contaminated soil [15]. It was found that some genera like *Pseudomonas*, Bacillus, *Marinobacter* were found in both the types of soil samples indicating that these genera may play an important role in the contaminated ecosystems. *Pseudomonas* and *Bacillus* in particular are versatile bacteria as they have the ability to degrade various xenobiotic compounds [20–23]. So, the presence of large number of



Overview of the bacterial diversity of Yamuna soil as evaluated using T-RFLP. The heatmap represents the relative percentage of each bacterial genera covered using different restriction enzymes. The bacterial diversity of Yamuna soil is being compared with the bacterial diversity from HCH contaminated soil.

T-RFs belonging to these genera indicates the existence of indigenous microbial population capable of remediating and cleaning the river naturally. Few genera that were found in Yamuna soil were found to be completely absent from HCH contaminated soil. Similarly many genera that were found in HCH contaminated soil were found to be absent from Yamuna soil (Fig. 1).

Diversity indices have been used to study the distribution of different species and in various ecological studies [15, 24, 25]. The deeper insights into the distribution and diversity covered by T-RFLP with different restriction enzymes were provided by the diversity indices (Table 2). A high value was seen for Simpson's diversity index, suggesting a high bacterial diversity present in the Yamuna soil samples. Similarly, the Equitability Index suggested evenness and Berger-Parker Dominance Index suggested that the samples were not dominated by any particular bacterial genera (Table 2). These results were found to be consistent for all three restriction enzymes used for TRFLP analysis. Together, culture-dependent and culture-independent methods support the richness and evenness at Yamuna site despite of low organic Carbon and Phosphorus content. This suggests that the bacterial population is using the xenobioic pollutant as source of C and energy. The presence of bacterial genera like *Pseudomonas* and *Bacillus* (known to degrade many xenobiotics) also contribute to the richness of the soil.

In conclusion, the present study indicated the presence of high bacterial load at the coastal zone of contaminated river. The bacterial diversity can be associated with high sewage contamination as well as with the natural agents for de-polluting the contaminated river. The microbes from the river can be screened for bioremediation potential which would eventually lead to the betterment of the water quality of the Yamuna, rescuing this dying river.

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REFERENCES

1. Dalai, T.K., Krishnaswami, S., and Sarin, M.M., Major ion chemistry in the headwaters of the Yamuna river system: Chemical weathering, its temperature dependence and CO_2 consumption in the Himalaya, *Geochim. Cosmochim. Acta*, 2002, vol. 66, pp. 3397–3416.
2. Kaur, R., Minhas, P.S., Jain, P.C., Singh, P., and Dubey, D.S., Geo-spatial analysis of land-water resource degradation in two economically contrasting agricultural regions adjoining national capital territory (Delhi), *Environ. Monit. Assess.*, 2009, vol. 154, pp. 65–83.
3. Sharma, D. and Singh, R.K., DO-BOD modeling of River Yamuna using STREAM-II for national capital territory, India using STREAM II, a 2D water quality model, *Environ. Monit. Assess.,* 2009, vol. 159, pp. 231–240.

4. Kaushik, A., Jain, S., Dawra, J., Sahu, R., and Kaushik, C.P., Heavy metal pollution of river Yamuna in the industrially developing state of Haryana, *Indian J. Environ. Health*, 2001, vol. 43, pp. 164–168.
5. Kaushik, A., Kansal, A., Santosh, Meena, Kumari, S., and Kaushik, C.P., Heavy metal contamination of river Yamuna, Haryana, India: assessment by metal enrichment factor of the sediments, *J. Hazard. Mater.*, 2009, vol. 164, pp. 265–270.
6. Khaiwal, R., Ameena, Meenakshi, Monika, Rani, and Kaushik, A., Seasonal variations in physico-chemical characteristics of River Yamuna in Haryana and its ecological best-designated use, *J. Environ. Monit.*, 2003, vol. 5, pp. 419–426.
7. Singh, M., Heavy metal pollution in freshly deposited sediments of the Yamuna River (the Ganges River tributary): a case study from Delhi and Agra urban centres, India. *Environ. Geol.*, 2001, vol. 40, pp. 664–671.
8. Pandey, P., Khillare, P.S., and Kumar, K., Assessment of organochlorine pesticide residues in the surface sediments of river Yamuna in Delhi India, *J. Environ. Protect.*, 2011, vol. 2, pp. 511–524.
9. Anand, C., Akolkar, P., and Chakrabarti, R., Bacteriological water quality status of river Yamuna in Delhi, *J. Environ. Biol.*, 2006, vol. 27, pp. 97–101.
10. De Leij, F.A.A.M., Whipps, J.M., and Lynch, J.M., The use of colony development for the characterization of bacterial communities in soil and on roots, *Microb. Ecol.*, 1993, vol. 27, pp. 81–97.
11. Werner, J.J., Knights, D., Garcia, M.L., Scalfone, N.B., Smith, S., Yarasheski, K., et al., Bacterial community structures are unique and resilient in full-scale bioenergy systems, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, vol. 108, pp. 4158–4163.
12. Kitts, C.L, Terminal restriction fragment patterns: A tool for comparing microbial communities and assessing community dynamics, *Curr. Iss. Intest. Microbiol.,* 2001, vol. 2, pp. 17–25.
13. Marsh, T.L., Saxman, P., Cole, J., and Tiedje, J.M., Terminal restriction fragment length polymorphism analysis program, a web based research tool for microbial community analysis, *Appl. Environ. Microbiol.*, 2000, vol. 66, pp. 3616–3621.
14. Dunbar, J., Ticknor, L.O., and Kuske, C.R., Assessment of microbial diversity in four southwestern United States soil by 16S rRNA gene terminal restriction fragment analysis, *Appl. Environ. Microbiol.*, 2000, vol. 66, pp. 2943–2950.
15. Lal, D., Jindal, S., Kumari, H., Nigam, A., Sharma, P., Kumari, K., and Lal, R., Bacterial diversity and RT-PCR based assessment of *linA* and *linB* gene from HCH contaminated soil, *J. Basic Microbiol.*, 2013, doi: 10.1002/jobm.201300211.
16. Gupta, S.K., Lal, D., Lata, P., Sangwan, N., et al., Changes in the bacterial community and *lin* genes diversity during biostimulation of indigenous bacterial community of hexachlorocyclohexane (HCH) dumpsite soil, *Microbiology (Moscow)*, 2013, vol. 82, pp. 234–240.
17. McMahan, L., Devine, A.A., Grunden, A.M., and Sobsey, M.D., Validation of the H_2S method to detect bacteria of fecal origin by cultured and molecular methods, *Appl. Microbiol. Biotechnol.*, 2011, vol. 92, pp. 1287–1295.
18. Rodríguez, D.C., Pino, N., and Peñuela, G., Microbiological quality indicators in waters of dairy farms: Detection of pathogens by PCR in real time, *Sci. Total Environ.,* 2012, vol. 427, pp. 314–318.
19. Kaushik, C.P., Sharma, H.R., Jain, S., Dawra, J., and Kaushik, A., Pesticide residues in river Yamuna and its canals in Haryana and Delhi, India, *Environ. Monit. Assess.,* 2008, vol. 144, pp. 329–340.
20. Konopka, A., Knight, D., and Turco, R.F., Characterization of a *Pseudomonas* sp. capable of aniline degradation in the presence of secondary carbon sources, *Appl. Environ. Microbiol.*, 1989, vol. 55, pp. 2385–2389.
21. Katz, I., Dosoretz, C.G., Mandelbaum, R.T., and Green, M., Atrazine degradation under denitrifying conditions in continuous culture of *Pseudomonas* ADP, *Water Res.*, 2001, vol. 35, pp. 3272–3275.
22. Zu, L., Li, G., An, T., and Wong, P.K., Biodegradation kinetics and mechanism of 2,4,6-tribromophenol by *Bacillus* sp. GZT: a phenomenon of xenobiotic methylation during debromination, *Biores. Technol.*, 2012, vol. 110, pp. 153–159.
23. Vyas, T.K. and Murthy, S.R., Chlorobenzene degradation by *Bacillus* sp. TAS6CB: A potential candidate to remediate chlorinated hydrocarbon contaminated sites, *J. Basic Microbiol.*, 2013, doi: 10.1002/jobm.201200758.
24. Pachiadaki, M. G., Kallionaki, A., Dählmann, A., De Lange, G.J., and Kormas, K.A., Diversity and spatial distribution of prokaryotic communities along a sediment vertical profile of a deep-sea mud volcano, *Microb. Ecol.*, 2011, vol. 62, pp. 655–668.
25. Zouache, K., Raharimalala, F.N., Raquin, V., Tran-Van, V., Raveloson, L. H., Ravelonandro, P. and Mavingui, P., Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar, *FEMS Microbiol. Ecol.*, 2011, vol. 75, pp. 377–389.