International Journal of Bio-resource and Stress Management
https://ojs.pphouse.org/index.php/IJBSM

IJBSM October 2024, 15(10): 01-08
Article AR5625a
Research Article
Natural Resource Management
DOI: HTTPS://DOI.ORG/10.23910/1.2024.5625a

Microbial Diversity of Probiotic Potential in the Sediments of Rivers Ganga and Yamuna: A Study through Metagenomic Approach

B. Patra[1], H. Purohit[1], S. Choudhary[1], P. Gaur[2], and B. K. Behera[3]

[1]Dept. of Animal Genetics and Breeding, M.B. Veterinary College, Dungarpur, Rajasthan (314 001), India
[2]Dept. of Animal Genetics and Breeding, NDRI, Karnal, Haryana (132 001), India
[3]College of Fisheries, Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh (284 003), India

Open Access
Corresponding patrabn1@gmail.com
0000-0002-5484-997X

ABSTRACT

The present study was conducted during the year January, 2016 to December, 2018 in which metagenomics approaches were utilized to identify presence of probiotics species in the river Ganga at Kanpur and Farakka stretches; and stretch of the river Yamuna. It was observed that a huge quantity of probiotic bacterial species are present in these sediments belonging to genus *Lactobacillus* including *L. brevis, L. curvatus, L. helveticus, L. fermentum*, *L. reuteri*, etc; *Bacillus* including *B. clausii*, *B. circulans, B. subtilis, B coagulans,* etc.; *Bifidobacterium species* including *B. animalis, B. bifidum, B. longum,* etc. We have found that abundance of *Pediococcus pentosaceus* was significantly higher in Farakka stretches of Ganga, whereas *Pediococcus acidilactici* was found in very higher proportion in Kanpur stretch. In this study we present the taxonomic diversity, species of different probiotics and also their relationship with different geographical locations using metagenomic approaches to provide unparalleled insight into probiotics bacteria presents in the river deposits of Ganga and Yamuna. Open Reading Frame (ORF) prediction using CLC genomics, we found one GO-term (GO:0016868) enriched in Molecular Function, 3 GO-terms (GO:0005737, GO:0044424 and GO:0005622) significantly enriched in Cellular Component and one single pathway (00500) is enriched in KEGG Pathways. The STRING functional assignment of *Lactobacillus kisonensis*-ORF1 encoded protein predicted it as a Heavy metal-associated domain (HMA) and HMA conserved site (copA, zntA). Our sediment metagenomics study able to identify important genes which can be applied for industrial use for the benefit of animal and human welfare.

KEYWORDS: *Bifidobacterium*, *bacillus* species, *lactobacillus*, *pediococcus*, *pseudomonas*

Citation* (VANCOUVER)*: Patra et al., Microbial Diversity of Probiotic Potential in the Sediments of Rivers Ganga and Yamuna: A Study Through Metagenomic Approach. *International Journal of Bio-resource and Stress Management,* 2024; 15(10), 01-08. HTTPS://DOI.ORG/10.23910/1.2024.5625a.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 19th July 2024 RECEIVED in revised form on 16th September 2024 ACCEPTED in final form on 16th October 2024 PUBLISHED on 26th October 2024

1. INTRODUCTION

The Rivers Ganga and Yamuna are getting polluted day by day from industrial swage, agricultural fertilizers, pesticides, tannery and disposal of animal and human carcasses (Behera et al., 2020). Recently Srivastava and Verma, 2023a explored microbial community from the sediments sample of the river Ganga, engaged in the degradation of xenobiotic compounds. They also reported urbanization led to the abundance of Gram-negative, chemo-organo-heterotrophs, and six antibiotic resistance genes in the downstream regions of the Ganga River water of India (Srivastava and Verma, 2023b, Srivastava and Verma, 2023c). Metagenomic landscape of river Ganga have also been studied by Rout et al., 2022, 2024. Similar metagenomics studies of bioremediation bacteria also done by Reddy et al., 2019, Das et al., 2020, Parida et al., 2022, Yadav and Dharne, 2024 on river ecosystem. Gosai et al., 2022 also explored metagenomics and bioremediation study and identified bacteria which play key role in the PAHs degradation.

Shin et al., 2019 found that *L. plantarum* JDFM LP11 increases the diversity and richness in the microbial community, and attenuates the ileal immune gene expression towards gut inflammation, promoting intestinal development in weaned piglets. Brousseau et al., 2015 studied the effects of probiotics *Pediococcus acidilactici* strain MA18/5M (PA) and *Saccharomyces cerevisiae* subsp. boulardii strain SB–CNCM I–1079 (SCB) on fecal and intestinal microbiota of nursing and weanling piglets. Results also suggest that PA and SCB have the potential as feed additives to modulate bacterial populations associated with gut health. Lin et al., 2020, Zheng et al., 2020, Valeriano et al., 2017, and Yang et al., 2018, studied the effect of dietary supplementation with *Lactobacillus* species in piglets and Hu et al., 2024 in human patients demonstrated modulating the structure of the intestinal microbiota, improving liver function, and reducing inflammatory factor levels. Peng et al., 2016, Liu et al., 2023 and Yang et al., 2024 also reported the effect of *Lactobacillus plantarum* strain on prompted growth Performance, selected antioxidant capacity, immune function indices in the serum, and cecal microbiota in broiler chicken. Milk metagenomics and cheese-making properties as affected by indoor farming and summer highland grazing was studied by Secchi et al., 2023, Bergamaschi et al., 2016, Zendri et al., 2017, Amalfitano et al., 2019, Saha et al., 2019. Probiotics, according to the currently adopted definition, are live microbes that when feed in proper quantities demonstrate a health benefit on the host (Hill et al., 2014, Sanchez et al., 2017). Not only human applications, they are also being applied in livestock industry, including fisheries, to benefits animal health by uplifting nutritional and microbial balance in intestinal tract and by improving quality of growth medium like water (Ashraf et al., 2013, Martinez-Porchas et al., 2017).

Patil et al., 2013 reported the influence of farm level interventions (like fermented juice, soil and water probiotics) on nitrogen (Ammonia oxidizing bacteria (AOB) and Nitrite oxidizing bacteria (NOB)) and sulphur [Sulphur oxidizing and reducing bacteria (SOB and SRB) recycling bacteria, opportunistic pathogens (*Vibrio* spp.) and total cultivable bacterial load and their relation with physico-chemical parameters of soil and water in three tiger shrimp *Penaeus monodon* culture ponds in Gujarat.

In the past decade, metagenomic studies (Lagkouvardos et al., 2016) have emerged as a powerful search technique for comprehensive screening of probiotic microflora present in an environment. This has also empowered a more complete calculation of the bacterial ecosystem of environmental sediment samples (Menzel et al., 2016). This study was planned with the objective of exploring metagenomics approaches to identify presence of probiotics species from the deposits of the river at Kanpur and Farakka stretches (Ganga) and at New Delhi stretches (Yamuna).

2. MATERIALS AND METHODS

2.1. *General characteristics of sediment metagenomes*

The Kanpur stretches are highly polluted by hundreds of tanneries, pesticides and heavy metals etc., whereas the Farakka stretches is very less polluted. The New Delhi regions of Yamuna are also heavily contaminated by unprocessed municipal swages, factory garbage, heavy metals etc. At the six locations of river Ganga and three locations of the river Yamuna has dissimilar physical and chemical parameters like temperature, salinity, pH, Dissolve Oxygen (DO), Total Dissolve Solid (TDS), conductivity and resistivity presented in Table 1. The Farakka stretches are non-polluted site which is compared with polluted sites of the Kanpur and New Delhi in our data analysis.

2.2. *Sample collection and DNA extraction*

The deposit samples were obtained from the river Ganga at two locations namely, Kanpur (KAN–1, KAN–2 and KAN–3) and Farakka (FAR–1, FAR–2 and FAR–3), each with three sampling sites. Whereas, from the river Yamuna, deposit sample was collected from three sampling sites at New Delhi (ND–1, ND–2 and ND–3). The nine deposit samples were obtained from six different sites of the river Ganga *viz.* Ganga Barrage, Jajmau (Jana Village near Kanpur, Uttar Pradesh, Farakka Barrage (N 24^0 $47.804^{//}$ E 87^0 $55.417^{//}$), Dhulian, Lalbagh near Farakka, West Bengal and three different sites of river Yamuna *viz.* Wazaribad, Okhla barrage, Faizupur Khaddar near New Delhi, India.

Table 1: Details of nine locations of samples collected for present metagenomics study

Sampling site	Dissolve oxygen (DO) Mg l^{-1}	Temperature (°C)	TDS (total dissolve solute) Mg l^{-1}	Conductivity µg cm^{-1}	Salinity %	Resistivity Ω. Cm	Ph
Average of Kanpur	9.52	17.87	180.24	373	0.24	2.2	8.8
Average of Farakka	6.50	30.43	95.43	207.20	0.09	5	7.8
Average of Yamuna	1.90	21.77	734.67	1447	0.74	0.9	7.56
t-test (p value)							
Kanpur vs Farakka	0.018	0.00001	0.027	0.0277	0.0224	0.066	0.0003
Yamuna vs Kanpur	0.015	0.019	0.101	0.115	0.160	0.000	0.001
Yamuna vs Farakka	0.054	0.001	0.050	0.058	0.049	0.002	0.113

2.3. *Preparation of 2x 150 NextSeq500 Shotgun Library*

The PCR augmented libraries were analysed in 4200 Tape Station system (Agilient Technologies) using D1000 Screen tape as per manufacturer instruction.

2.4. *Cluster generation and sequencing*

Afterward obtaining the Qubit concentration for the libraries and the mean peak size from Agilient Tape Station profile, the PE illumine libraries were mounted into NextSeq 500 for cluster cohort and DNA sequencing.

2.5. *Quality control and de-novo metagenome assembly*

Filtered high quality reads from each sample were then accumulated into frameworks using CLC Genomics Workbench version 9.5.2. for further analysis.

2.6. *Taxonomic arrangement of deposit metagenome*

For the identification of probiotic species from the residue metagenome samples, filtered metagenomic sequencing data were used for taxonomic ditching. A sensitive metagenome classifier-Kaiju, was used for taxonomical classification, which translate metagenomics reads into six possible ORFs and searches for maximum exact matches (MEMs) of amino acids from a given database of annotated proteins from the microbial reference genome (Menzel et al., 2016). Kaiju web interface, used Burrows–Wheeler transform algorithm to finds maximum exact matches on the protein-level and can execute millions of reads per minute that run on a standard PC.

2.7. *Evolutionary analysis of probiotics species*

To understand the phylogenetic association among the probiotic bacterium found in the residue metagenome, a manifold sequence investigation has been carried out using CLC Genomics Workbench version 9.5.2. To recognize the evolutionary association between the probiotics species, observed in the deposit tasters of the river Ganga and Yamuna, a manifold sequence analysis (MSA) was followed.

2.8. *Statistical investigation of metagenomic sequinning*

We followed standard method for statistical analysis of our data to analyse and compared our data generated from nine different locations.

2.9. *Network analysis*

After identification of the probiotic bacterial gene from sediment metagenome, the set of genes has been considering for ORF prediction using CLC genomics.

3. RESULTS AND DISCUSSION

3.1. *Phylogenetic analysis of probiotics species*

MSA revealed that probiotics bacteria are highly preserved throughout the evolution. Phylogenetic tree analysis clearly demarcated all the identified probiotics in five dissimilar groups (Figure **1**). Among the five clusters, number two

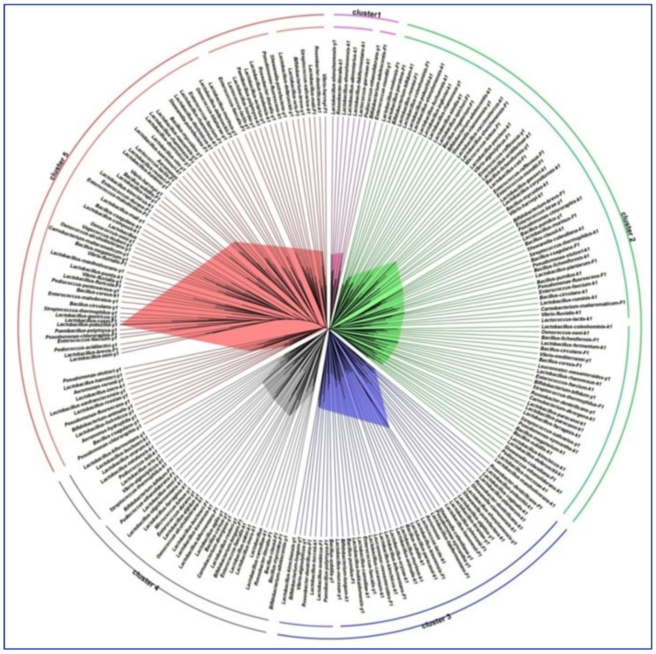

Figure 1: Phylogenetic analysis of probiotics species found in nine different location of the river Ganga along with river Yamuna where different colour represent different phylogenetic cluster. Cluster number five containing highest number of probiotics

and five contain highest number of evolutionary closed probiotics species along with different locations.

3.2. Relative abundance analysis

Statistical metagenomics investigation exposed that the genus *Lactobacillus* have relative abundance in similar proportion in all the nine specimen collection sites, however, *Lactobacillus casei*was found in comparatively high quantity in the Farakka stretches (non-polluted site) with statistical significance. *Bacillus subtilis* and *Bacillus mycoides* were observed in high quantity at the Kanpur stretch whereas *Bacillus clausii* and *Bacillus coagulans* were found in high proportion at the Farakka stretch. Our metagenomic information displayed that there are two species of *Vibrio* (*Vibrio mediterranei* and *Vibrio fluvialis*) which displayed differential in comparative occurrence among three locations and found in relatively lower proportion in Kanpur stretch as compared to Farakka stretch. Similarly, *Shewanella colwelliana* was present in different amount between three different sites and observed higher proportion at Kanpur stretch. *Enterococcus faecium* was showed in higher amount at Yamuna stretches as associated to other locations

Relative abundance of 18 genus of identified probiotics species at two distinct locations Kanpur and Farakka (Ganga) and at New Delhi sites are presented in Figure 2. It was observed that relative abundance of *Pseudomonas* was dominant in all the sampling sites of Kanpur (KAN−1, KAN−2 and KAN−3) among which KAN−3 was found highest domination with *Pseudomonas*. One interesting observation was that ND−1 sampling site of river Yamuna and KAN−2 sampling site of the river Ganga have similar bacterial abundance and rest other sites of these two river were having almost similar relative abundance of bacteria.

3.3. Functional metagenomics analysis

This analysis was done to explore the interaction networks among proteins (predicted by ORF analysis) from important genes of probiotics sp. identified from the two locations

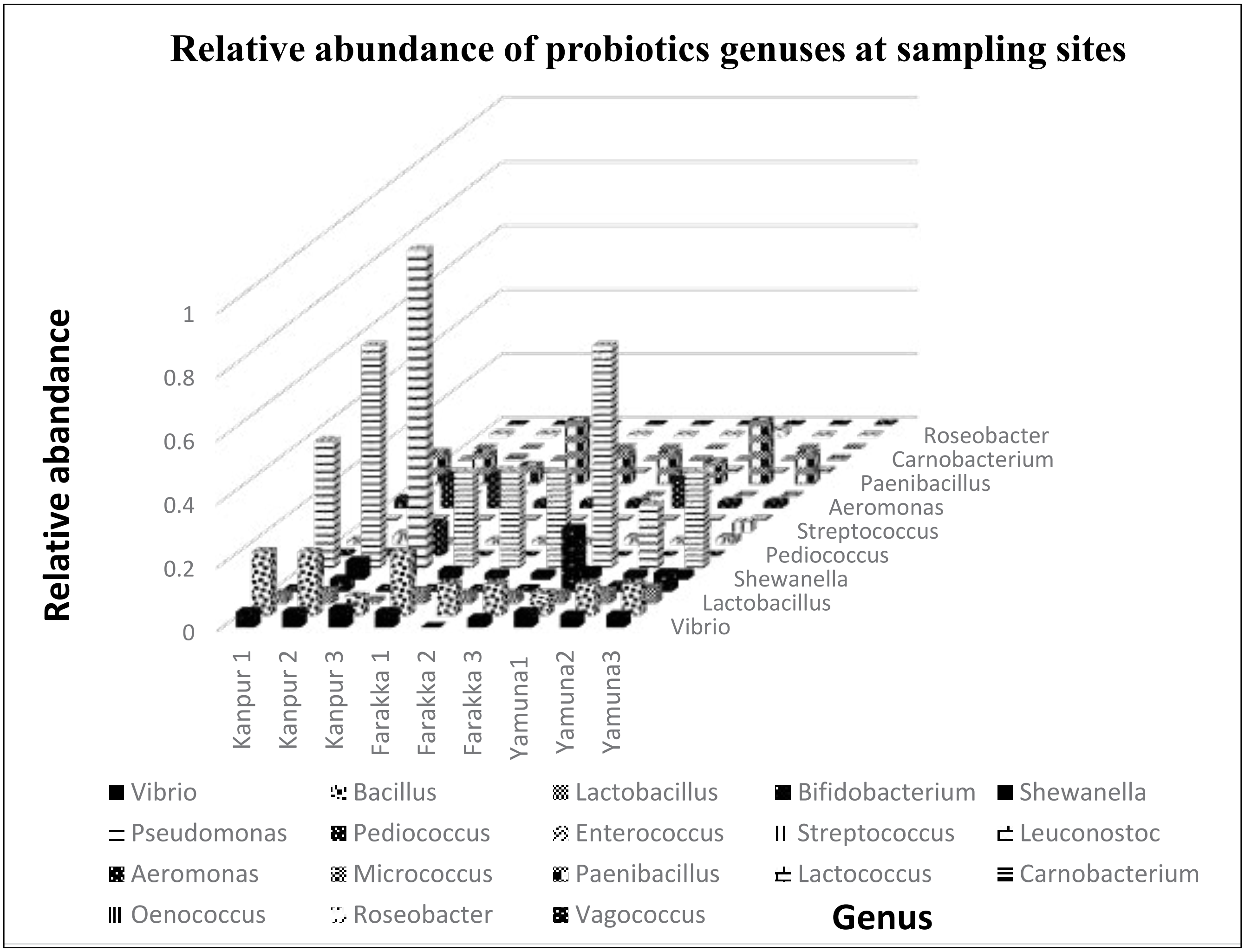

Figure 2: Relative abundance of identified probiotics at different sampling sites of river stretch Ganga and Yamuna

Kanpur and Farakka (Ganga) and one location at the river Yamuna (New Delhi). The interaction network was made using STRING web application and shown in Figure 3. It has been observed that there are 54 numbers of nodes which were involved in the protein-protein interactions (PPIs), with very low PPI enrichment p-value ($<1.0\times10^{-16}$) and average local clustering coefficient value of 0.562 (Figure 3). Figure 3 shows that all proteins in the network were integrated to each other and further functional pathways enrichment analysis of the network revealed that there are, one GO-term (GO:0016868) enriched in Molecular Function, 3 GO-terms (GO:0005737, GO:0044424 and GO:0005622) significantly enriched in Cellular Component and one single pathway (00500) is enriched in KEGG Pathways (Table 2).

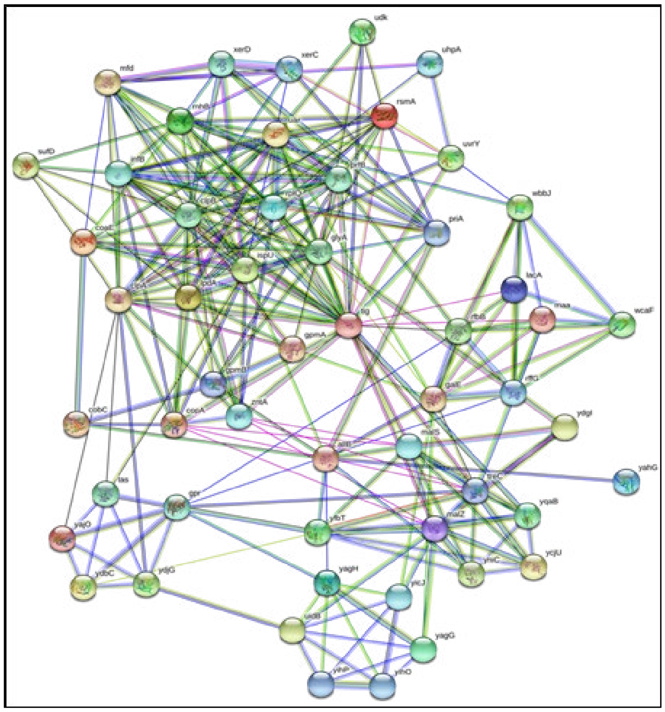

Figure 3: Protein-protein interaction network of 54 expressed protein derived from probiotics by string web application

Different locations of river Ganga have dissimilar physio-chemical parameters like temperature, salinity, pH, Dissolve Oxygen (DO), Total Dissolve Solid (TDS), conductivity, resistivity etc. Extreme contamination by unprocessed sewage in New Delhi and Kanpur locations from hundreds of tanneries, chemicals, pesticides etc. make the water unsuitable for all living beings. Due to these reasons, it creates significant differences for relative abundance of probiotics bacteria between different sites in the rivers Ganga and Yamuna. For identification of probiotics species Kaiju web application was used (Menzel et al., 2016). This Kaiju web application uses exact k–mer matches and map k–mers to the Lowest Common Ancestor (LCA) of all microbes whose genomes comprise that k-mer. The study showed that the river Gange and Yamuna harbour numerous bacterial genera of probiotics importance with mammoth taxonomic variabilities. The sensitive taxonomic classification analyses of sediment metagenome, revealed that there are 18 general and 67 probiotics species. Phylogenetic tree constructed for all the identified probiotics species, based on 16S rRNA sequence, showed very closeness among all species (Behera et al., 2020). The present finding can be supported with an earlier finding (Zheng et al., 2020) where *Lactococcus*

Table 2: Functional pathways enrichment analysis of the network

Pathway name	Pathway ID	Pathway description	Gene set name and number	False discovery rate
Molecular Function	GO:0016868	Intramolecular transferase activity, phosphotransferases	gpmB, gpmA, yqaB, ycjU(4)	0.026
Cellular Component	GO:0005737	Cytoplasm	mfd, xerD, udk, xerC, uhpA, rnhB, uar, rsmA, uvrY, prfB, rplQ, clpB, coaE, glyA, rfbB, lacA, lpdA, ispU, cobC, gpmB, gpmA, tig, allb, galE, ycjU, malZ, treC (27)	0.00222
	GO:0044424	Intracellular part	mfd, xerD, udk, xerC, uhpA, rnhB, uar, rsmA, uvrY, prfB, rplQ, clpB, coaE, glyA, rfbB, lacA, lpdA, ispU, cobC, gpmB, gpmA, tig, allb, galE, ycjU, malZ, treC, priA(28)	0.00222
	GO:0005622	Intracellular	mfd, xerD, udk, xerC, uhpA, rnhB, uar, rsmA, uvrY, prfB, rplQ, clpB, coaE, glyA, rfbB, lacA, lpdA, ispU, cobC, gpmB, gpmA, tig,allb, galE, ycjU, malZ, treC, priA(28)	0.0064
KEGG Pathways	00500	Starch and sucrose metabolism	malZ, malS, treC, ycjU, yagH(5)	0.00713

and *Streptococcus* seemed to be relatively closely related and *Lactobacillus* was observed to be phylogenetically varied. 16S rRNA sequencing data showed that *Lactobacillus* and *Pediococcus* are phylogenetically intermixed as 5 species of *Pediococcus* clustered with 32 homo and hetero fermentative *Lactobacillus* spp. (Figure 1). Recent genomics analysis explored that *Bifidobacterium* genomes display a relatively high level of conservation and also coupled to a reasonable degree of genome synteny (Papizadeh et al., 2017). The current research also explored heterogeneousness of clustering in *Lactobacillus* species and other probiotics bacteria and altogether grouped into five major clusters.

Our experiments identified several *Roseobacter* species which are beneficial to eliminate pathogenic bacteria. Grotkjær et al., 2017 reported that *Roseobacter* clade bacteria inhibit the development of fish microbe *Vibrio anguillarum*. We have detected several *Roseobacter* species which can be isolated since the deposits of river Ganga and Yamuna for therapeutic and commercial fisheries production.

With the expansion of human industrial activity, including mining, smelting, and synthetic compound creation, has led to an exponential increase in the amounts of heavy metals released into the atmosphere, water, and soil (Gaur et al., 2021). STRING functional assignment of *Lactobacillus kisonensis*–ORF1 encoded protein predicted it as a Heavy metal–associated domain (HMA) and HMA conserved site (copA, zntA). Functional metagenomics has been utilized to identify various novel genes and physiological paths, which are predominant in varied ecosystems (Kobiyama et al., 2018, Chauhan et al., 2017). To explore the interaction networks among predicted ORF derived from probiotics species of different sediment metagenome revealed that there are, one GO–term (GO:0016868) enriched in Molecular Function, 3 GO–terms (GO:0005737, GO:0044424 and GO:0005622) significantly enriched in Cellular Component and one single pathway (00500) is enriched in KEGG Pathways (Table 2).

Our metagenomic analysis from deposits tasters from diverse sites of the river Ganga and Yamuna identified enormous biodiversity among probiotic bacteria which is first reported by our research team in India. The genomic and taxonomic diversity among beneficial microbes have huge variation in cluster analysis. This sediment metagenomics will give us clue to identify them and application for industrial use for animal and human wellbeing.

4. CONCLUSION

Our metagenomic analysis from sediments samples from different locations of the river Ganga and Yamuna identified enormous biodiversity among probiotic bacteria which was first reported by our research team in India. The genomic and taxonomic diversity among beneficial microbes had huge variation in cluster analysis.

5. ACKNOWLEDGEMENT

We thank director CIFRI, Barrackpore, Dr Basanta Kumar Das for arrangement of required items for this research and Mr. Ashim Kumar Jana for sediment sample collections from nine locations across the stretches of the river Ganga and Yamuna.

10. REFERENCES

Amalfitano, N., Cipolat-Gotet, C., Cecchinato, A., Malacarne, M., Summer, A., Bittante, G., 2019. Milk protein fractions strongly affect the patterns of coagulation, curd firming, and syneresis. Journal of Dairy Science 102(4), 2903–2917. doi: 10.3168/jds.2018-15524.

Ashraf, S., Zaneb, H., Yousaf, M.S., Ijaz, A., Sohail, M.U., Muti, S., Usman, M.M., Ijaz, S., Rehman, H., 2013. Effect of dietary supplementation of prebiotics and probiotics on intestinal micro architecture in broilers reared under cyclic heat stress. Journal of Anim Physiology and Animal Nutrition (Berl) 97(1), 68–73.

Behera, B.K., Patra, B., Chakraborty, H.J., Sahu, P., Rout, A.K., Sarkar, D.J., Parida, P.K., Raman, R.K., Rao, A.R., Rai, A., Das, B.K., Jena, J., Mohapatra, T., 2020. Metagenome analysis from the sediment of river Ganga and Yamuna: In search of beneficial microbiome. PloSOne 15(10), e0239594. PMID: 33021988 DOI: 10.1371/journal.pone.0239594.

Bergamaschi, M., Cipolat-Gotet, C., Stocco, G., Valorz, C., Bazzoli, I., Sturaro, E., Ramanzin, M., Bittante, G., 2016. Cheese making in highland pastures: Milk technological properties, cream, cheese and ricotta yields, milk nutrients recovery, and products composition. Journal of Dairy Science 99(12), 9631-9646. doi: 10.3168/jds.2016–11199.

Brousseau, J.P., Talbot, G., Beaudoin, F., Lauzon, K., Roy, D., Lessard, M., 2015. Effects of probiotics *Pediococcus acidilactici* strain MA18/5M and Saccharomyces cerevisiae subsp. boulardii strain SB-CNCM I-1079 on fecal and intestinal microbiota of nursing and weanling piglets. Journal of Animal Science 93(11), 5313 –5326. doi: 10.2527/jas.2015-9190.

Chauhan, N.S., Nain, S., Sharma, R., 2017. Identification of arsenic resistance genes from marine sediment metagenome. Indian Journal of Microbiology 57(3), 299–306.

Das, B.K., Behera, B.K., Chakraborty, H.J., Paria, P., Gangopadhyay, A., Rout, A.K., Nayak, K.K., Parida, P.K., Rai, A., 2020. Metagenomic study focusing on antibiotic resistance genes from the sediments of

River Yamuna. Gene. 758, 144951. doi: 10.1016/j.gene.2020.144951.

Gaur, V.K., Sharma, P., Gaur, P., Varjani, S., Ngo, H.H., Guo, W., Chaturvedi, P., Singhania, R.R., 2021. Sustainable mitigation of heavy metals from effluents: Toxicity and fate with recent technological advancements. Bioengineered 12(1), 7297–7313. doi: 10.1080/21655979.2021.1978616.

Gosai, H.B., Panseriya, H.Z., Patel, P.G., Patel, A.C., Shankar, A., Varjani, S., Dave, B.P., 2022. Exploring bacterial communities through metagenomics during bioremediation of polycyclic aromatic hydrocarbons from contaminated sediments. The Science of The Total Environment 842, 156794. doi: 10.1016/j.scitotenv.2022.156794.

Grotkjær, T., Bentzon-Tilia, M., D'Alvise, P., Dourala, N., Nielsen, K.F., Gram, L., 2016. Isolation of TDA-producing phaeobacter strains from sea bass larval rearing units and their probiotic effect against pathogenic Vibrio spp. in Artemia cultures. Systematic and Applied Microbiology 39(3), 180–188. doi: 10.1016/j.syapm.2016.01.005.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., Sanders, M.E., 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews. Gastroenterology & Hepatology 11, 506–514. doi: 10.1038/nrgastro.2014.66.

Hu, Y.C., Ding, X.C., Liu, H.J., Ma, W.L., Feng, X.Y., Ma, L.N., 2024. Effects of *Lactobacillus paracasei* N1115 on gut microbial imbalance and liver function in patients with hepatitis B-related cirrhosis. World Journal of Gastroenterology 30(11), 1556–1571. doi: 10.3748/wjg.v30.i11.1556.

Kobiyama, A., Ikeo, K., Reza, M.S., Rashid, J., Yamada, Y., Ikeda, Y., Ikeda, D., Mizusawa, N., Sato, S., Ogata, T., Jimbo, M., Kudo, T., Kaga, S., Watanabe, S., Naiki, K., Kaga, Y., Mineta, K., Bajic, V., Gojobori, T., Watabe, S., 2018. Metagenome-based diversity analyses suggest a strong locality signal for bacterial communities associated with oyster aquaculture farms in Ofunato Bay. Gene 30(665), 149–154. doi: 10.1016/j.gene.2018.04.073.

Lagkouvardos, I., Joseph, D., Kapfhammer, M., Giritli, S., Horn, M., Haller, D., Clavel, T., 2016. IMNGS: A comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. Scientific Reports 6, 33721. doi: 10.1038/srep33721.

Lin, S., Yang, X., Long, Y., Zhong, H., Wang, P., Yuan, P., Zhang, X., Che, L., Feng, B., Li, J., Zhuo, Y., Lin, Y., Xu, S., Wu, D., Fang, Z., 2020. Dietary supplementation with Lactobacillus plantarum modified gut microbiota, bile acid profile and glucose homoeostasis in weaning piglets. The British Journal of Nutrition 124(8), 797 –808. doi: 10.1017/S0007114520001774.

Liu, X., Cao, G., Qiu, K., Dong, Y., Hu, C., 2023. *Lactobacillus plantarum* decreased ammonia emissions through modulating cecal microbiotain broilers challenged with ammonia. Animals (Basel) 13(17), 2739. doi: 10.3390/ani13172739.

Martinez-Porchas, M., Villalpando-Canchola, E., Ortiz Suarez, L.E., Vargas-Albores, F., 2017. How conserved are the conserved 16S-rRNA regions? PeerJ Life & Enviornment 5, e3036. doi: 10.7717/peerj.3036.

Menzel, P., Ng, K.L., Krogh, A., 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nature Communications 7, 11257. https://doi.org/10.1038/ncomms11257 PMID: 27071849.

Papizadeh, M., Rohani, M., Nahrevanian, H., Javadi, A., Pourshafie, M.R., 2017. Probiotic characters of Bifidobacterium and Lactobacillus are a result of the ongoing gene acquisition and genome minimization evolutionary trends. Microbial Pathogenesis 111, 118–131. doi: 10.1016/j.micpath.2017.08.021.

Parida, P.K., Behera, B.K., Dehury, B., Rout, A.K., Sarkar, D.J., Rai, A., Das, B.K., Mohapatra, T., 2022. Community structure and function of microbiomes in polluted stretches of river Yamuna in New Delhi, India, using shotgun metagenomics. Environmental Science and Pollution Research International 29(47), 71311 –71325. doi: 10.1007/s11356-022-20766-1.

Patil, P.K., Muralidhar, M., Solanki, H.G., Patel, K., Gopal, C., Pillai, S.M., 2013. Trends in beneficial and pathogenic bacterial populations and their relation with environmental parameters in tiger shrimp, Penaeus monodon culture ponds. International Journal of Bio-resource and Stress Management 4(2), 107–113. Available from: https://ojs.pphouse.org/index.php/IJBSM/article/view/337.

Peng, Q., Zeng, X.F., Zhu, J.L., Wang, S., Liu, X.T., Hou, C.L., Thacker, P.A., Qiao, S.Y., 2016. Effects of dietary Lactobacillus plantarum B1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. Poultry Science 95(4), 893 –900. doi: 10.3382/ps/pev435.

Reddy, B., Pandey, J., Dubey, S.K., 2019. Assessment of environmental gene tags linked with carbohydrate metabolism and chemolithotrophy associated microbial community in River Ganga. Gene 704, 31–41. doi: 10.1016/j.gene.2019.04.004.

Rout, A.K., Dehury, B., Parida, P.K., Sarkar, D.J., Behera, B., Das, B.K., Rai, A., Behera, B.K., 2022. Taxonomic profiling and functional gene annotation of microbial communities in sediment of river Ganga at Kanpur, India: insights from whole-genome metagenomics study. Environmental Science and Pollution Research International 29(54), 82309–82323. doi: 10.1007/s11356-022-21644-6.

Rout, A.K., Dixit, S., Tripathy, P.S., Rout, S.S., Parida, S.N., Parida, P.K., Sarkar, D.J., Kumar, Das, B., Singh, A.K., Behera, B.K., 2024. Metagenomic landscape of sediments of river Ganga reveals microbial diversity, potential plastic and xenobiotic degradation enzymes. Journal of Hazardous Materials 471, 134377. doi: 10.1016/j.jhazmat.2024.134377.

Saha, S., Amalfitano, N., Sturaro, E., Schiavon, S., Tagliapietra, F., Bittante, G., Carafa, I., Franciosi, E., Gallo, L., 2019. Effects of summer transhumance of dairy cows to alpine pastures on body condition, milk yield and composition, and cheese making efficiency. Animals (Basel) 9(4), 192. doi: 10.3390/ani9040192.

Sanchez, B., Delgado, S., Blanco-Miguez, A., Lourenco, A., Gueimonde, M., Margolles, A., 2017. Probiotics, gut microbiota, and their influence on host health and disease. Molecular Nutrition & Food Research 61(1).

Secchi, G., Amalfitano, N., Carafa, I., Franciosi, E., Gallo, L., Schiavon, S., Sturaro, E., Tagliapietra, F., Bittante, G., 2023. Milk metagenomics and cheese-making properties as affected by indoor farming and summer highland grazing. Journal of Dairy Science 106(1), 96–116. doi: 10.3168/jds.2022–22449.

Shin, D., Chang, S.Y., Bogere, P., Won, K., Choi, J.Y., Choi, Y.J., Lee, H.K., Hur, J., Park, B.Y., Kim, Y., Heo, J., 2019. Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. PLoS One 14(8), e0220843. doi: 10.1371/journal.pone.0220843.

Srivastava, A., Verma, D., 2023a. Ganga river sediments of India predominate with aerobic and chemo-heterotrophic bacteria majorly engaged in the degradation of xenobiotic compounds. Environmental Science and Pollution Research International 30(1), 752–772. doi: 10.1007/s11356-022-22198-3.

Srivastava, A., Verma, D., 2023b. Urbanization led to the abundance of gram-negative, chemo-organo-heterotrophs, and antibiotic resistance genes in the downstream regions of the Ganga river water of India. Environmental Science and Pollution Research International 30(30), 75417–75438. doi: 10.1007/s11356-023-27552-7.

Srivastava, A., Verma, D., 2023c. Comparative bacteriome and antibiotic resistome analysis of water and sediment of the Ganga River of India. World Journal of Microbiology & Biotechnology 39(11), 294. doi: 10.1007/s11274-023-03730-0.

Valeriano, V.D., Balolong, M.P., Kang, D.K., 2017. Probiotic roles of *Lactobacillus* sp. in swine: insights from gut microbiota. Journal of Applied Microbiology 122(3), 554–567. doi: 10.1111/jam.13364.

Yadav, R., Dharne, M., 2024. Utility of metagenomics for bioremediation: a comprehensive review on bioremediation mechanisms and microbial dynamics of river ecosystem. Environmental Science and Pollution Research International 31(12), 18422–18434. doi: 10.1007/s11356-024-32373-3.

Yang, C., Wang, S., Li, Q., Zhang, R., Xu, Y., Feng, J., 2024. Effects of Probiotic *Lactiplantibacillus plantarum* HJLP-1 on growth performance, selected antioxidant capacity, immune function indices in the serum, and cecal microbiota in broiler chicken. Animals (Basel) 14(5), 668. doi: 10.3390/ani14050668.

Yang, J., Qian, K., Wang, C., Wu, Y., 2018. Roles of Probiotic *Lactobacilli* inclusion in helping piglets establish healthy intestinal inter-environment for pathogen defense. Probiotics and Antimicrobial Proteins 10(2), 243–250. doi: 10.1007/s12602-017-9273-y.

Zendri, F., Ramanzin, M., Cipolat-Gotet, C., Sturaro, E., 2017. Variation of milk coagulation properties, cheese yield, and nutrients recovery in curd of cows of different breeds before, during and after transhumance to highland summer pastures. Journal of Dairy Research 84(1), 39–48. doi: 10.1017/S0022029916000583.

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., O'Toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Ganzle, M.G., Lebeer, S., 2020. A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. International Journal of Systematic and Evolutionary Microbiology 70(4), 2782–2858.

Zheng, L., Hu, Y., He, X., Zhao, Y., Xu, H., 2020. Isolation of swine-derived Lactobacillus plantarum and its synergistic antimicrobial and health-promoting properties with ZnO nanoparticles. Journal of Applied Microbiology 128(6), 1764–1775. doi: 10.1111/jam.14605.