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Factors Affecting Microbial Spatial Diversity in Aquatic Habitats of Moreh, Manipur India

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Abstract

It is believed that spatial structure has a significant role in the origin and preservation of genetic diversity. The spatial organization of most populations' habitats has the potential to have significant effects on the processes of evolution. Our goal in this work was to locate the genetic diversity of bacterial strains in various environments. In this investigation, we isolated the bacterial strains from each of the seven samples we took from water bodies from Khujailok and Lairok river, Moreh Manipur India. After gramstaining, the isolated bacteria were separated into gram-positive and gram-negative bacteria. To determine the genetic differences between the isolated bacteria, the DNA was extracted and amplified using PCR. The results showed that salinity was the most important environmental factor in explaining variance in microbial communities, surpassing TN, temperature, TP, or pH. These findings suggested that the genetic diversity and functionality of the water micro biota are influenced by environmental variables. Salinity, not TP, temperature, pH, or TN, was the most crucial environmental component.

Keywords: Manipur; Moreh; Aquatic; Bacteria; Population; Spatial diversity

Introduction

Many naturally occurring populations, like the many communities of microorganisms that create biofilms or the bacteria traversing the varied, viscous environment of the human lung, exist in complicated, spatially organized habitats (Flemming et al., 2016; Faure et al., 2018). Individual mobility restrictions make it more difficult or perhaps impossible for a population to mix over its whole geographical range. A collection of more or less autonomous subpopulations may be created at the extreme as a consequence of this mobility restriction, which drives population structure and subdivision. In these conditions, significant interactions like competition or predation tend to be local rather than global processes, occurring primarily between individuals within a constrained geographical area or "local neighborhood" (Habets et al., 2006). Therefore, a population's ecological dynamics as well as, overtime, its evolutionary dynamics, may be significantly influenced by the spatial organization (France et al., 2019).

Here, however, we concentrate on the inherent effects of spatial structure, investigating those effects that arise only as a result of mobility restrictions in an otherwise uniform external environment. Populations expanding and changing in complex natural ecosystems may also take on a structured appearance as a result of externally imposed environmental variation (e.g. resources, temperature, etc.).

One of the processes that contribute to the preservation of genetic diversity in populations and one of the key drivers of a species' genetic structure is gene flow. Mushroom-forming basidiomycete fungi exhibit geographic variation in population structure, as would be anticipated for a collection of organisms that includes species with various life histories and dispersion syndromes, according to a growing body of research (Amend et al., 2010). One of the main areas of study in evolutionary biology is the spatial organization of genetic diversity among wild populations.

Population density, breeding pattern, and environmental variability are the main determinants of structure. The capacity of plants to preserve genetic diversity within populations and broaden their global range relies on the gene flow mediated by seed and pollen dispersion (Peakall et al., 2003). Spatial genetic structure (SGS), also known as the structuring of genetic variation within and across populations, is influenced by several factors (Vekemans et al., 2004). Population genetic structure is significantly influenced by the geographical distribution of people within a population, which is influenced by dispersion mechanisms (Pometti et al., 2018).

Previous research has shown how spatial structure's provision of environmental variability causes populations to adapt and spread. Raeymaekers et al. (2017) discovered patterns of shared and unique genomic divergence across two related species of sticklebacks. To demonstrate how between-site environmental heterogeneity affects phenotypic parallel divergence, Stuart et al (2017) employed replicated pairs of lake-stream stickleback populations. The quantity of gene flow across locations accounted for a major portion of the deviation's size, although the genetic diversity throughout the genome in response to environmental change was not assessed. In response to a similar environmental setting, In the current work, we look at the effects of population fragmentation and niche creation in addition to how environmental variability affects adaptive radiation.

Materials and methodology

Sample collection

Seven water samples were taken in August 2022 from seven different neighboring water bodies from Khujailok and Lairok river, Moreh Manipur India. Each sample included 500mL of water that was extracted in sterile bottles from a depth of 1 meter below the surface. After that, the samples were put on ice before being pumped through a 0.22m polyethersulfone membrane filter (Hou et al., 2016). All water samples had their temperature, pH, DO, and salinity tested. Using an automated discrete analyzer, the concentration so froth phosphate ($PO43^{--}P$), TN, TP, and dissolved inorganic nitrogen (NO^{2-} -N, NH^{4+}-N, and NO^{3-}-N) were determined.

Bacterial Strain isolation

Individual strains were directly so lated in a lab culture at 50°C from the collected materials, as stated by Miller et al. (2006). Under 75 m mol of photon $sm^{2}s^{-1}$ of cool-white, fluorescent light with a12-h (light-dark) cycle, strains were maintained in 25ml of D medium at 50°C. Gram staining was used on the isolated isolates to determine the species of bacteria present.

DNA isolation, Polymerase Chain Reaction, and sequencing

According to the manufacturer's recommendations, DNA was extracted from each sample using a Water DNA Kit. Using a Nano Vue Plus Spectrophotometer, the content and purity of genomic DNA were assessed (GE Healthcare, USA). PCR Master Mix was used to conduct the PCR reactions, and the Polymerase Chain Reaction products were pooled at equimolar quantities. Utilizing an Illumina TruSeq DNA PCR-Free Sample Preparation Kit, the sequencing libraries were created, and index codes were added.

Statistical analysis

To ascertain the significance of diversity indexes and site characteristics, a Mantel test and multiple linear regression using the stepwise approach were carried out, and analyses were carried out using SPSS version 26.0. (SPSS, Inc.). Canonical correspondence analyses (CCA) were carried out to investigate the link between environmental conditions and microbial diversity (Dan et al., 2010).

Results

Salinity ranged from 0.42 to 32.71 percent and DO ranged from 3.45 to 19.04 mg/L in the 22 ponds' water samples, which were used to classify the samples. The pH varied from 7.31 to 9.81, while the water's temperature was between 27.70 and 32.40 C. The concentrations of NH^{+4}-N, NO2-N, NO3-N, and $PO4^{-3}$-P, respectively, ranged from 0.01-3.24 mgL-1 to 0.01-0.37 mgL-1to0.01-2.08mgL-1. Additionally, significant variations in TN (0.35-5.16mgL-and TP were found (0.01-1.32mg L-1).

Structure and Diversity in Microbial Communities

16 rRNA gene amplicons from 66 samples were sequenced using HiSeq, yielding 3,367,470 quality sequences on average (43,116-59,029) per sample. Gene fragments totalling 2,450,746 were chosen for categorization, and the pieces were then grouped into 9,988 prokaryotic operational taxonomic units. Every sample has between 659 and 1,835 OTUs found in it. Since the OTUs in each library, the diversity indices shown below were calculated. The Chao1score varied from 702 to 2,593, the Shannon index ranged from3.48 to 7.94, and the excellent coverage index ranged from 0.95 to 0.970. Classifiable sequences were divided into 58 different groups. Proteobacteria, Bacteroidetes, *Cyanobacteria, Planctomycetes, Verrucomicrobia, Firmicutes, Chlorobi, Chloroflexi, Actinobacteria*, and Chlamydiae were the most numerous and diverse groups, making up 32.12, 20.58, 12.61, 10.61, 8.35, 5.99, 2.81, 2.44, 1.94, and 0.73% of the total microbiological (Fig. 1). Even though they were less prevalent, representatives of

the phyla were present in all samples. These include the *Gemmatimonadetes* (0.1 %), *Acidobacteria* (0.33%), *Fusobacteria* (0.13%), *Tenericutes* (0.15%), *and Spirochaetes* (0.03%) *phyla. The most common phylotypes were Synechococcus* (8.76%), *Flavobacterium* (6.05%), *Pseudomonas* (1.73%), *Paenisporosarcina* (1.25%), *Rheinheimera* (1.25%), *Janthinobacterium* (1.25%), *Luteolibacter(1.25%), Shewanella(1.25%), Mycoplana* (0.93%), and *Rhodobacter* (0.93%).

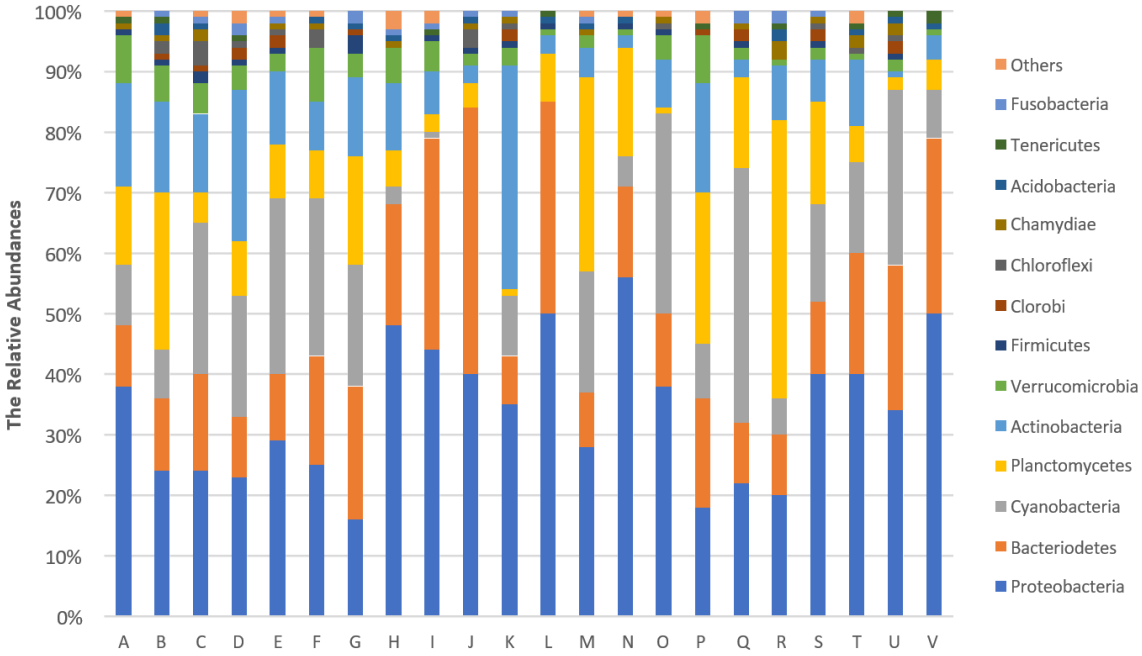

Fig. 1. Based on data from 16S rRNA gene amplicon sequencing, the relative abundance (%) of dominating phyla from all samples

Salinity as a predictor

The results of the unweighted pair group method with arithmetic mean analysis revealed that the samples were split into two groups. Group, I had three samples, whereas Group II contained four samples. Groups II and I related to samples with low and high salinities, respectively, and were primarily connected with salinity rather than sites or locations. The findings indicated that salinity may play a significant role in influencing the divergence of water microbial communities in such confined aquaculture settings.

Temperature as a predictor

To understand the significance of environmental parameters and geographical isolation of the variety of microbial communities in shrimp cultural enclosure habitats, ABT model- based analyses were performed. According to the findings, the temperature is the most important element, with a proportional effect on phylogenetic diversity and phylotypes of around 30%. The phylogenetic diversity and phylotypes of water microbial communities in shrimp cultural enclosure environments, as indicated by gradients of latitude and longitude, were not significantly influenced by geographic distance.

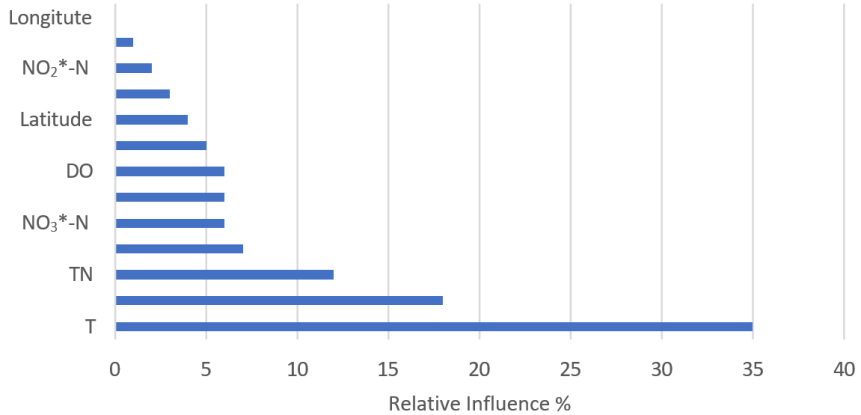

Fig. 2. Phylogenetic diversity (A) and phylotypes are affected to varying degrees by environmental factors and geographical distance (indicated by latitudinal and longitudinal).

Discussion

The primary objectives of this research were to analyze water microbial community structures and discover environmental elements that influence their composition and behavior in shrimp production enclosure environments. The function and structure of H2O microbial communities were shown to be significantly affected by salinity in shrimp culture enclosures. Temperature, pH, TP, and TN were other environmental factors that impacted the microbial populations in water. These results provide substantial support for our hypotheses. According to our first hypothesis, the key environmental elements affecting the makeup of the water microbial community may be temperature or salt. High-throughput sequencing's ability to evaluate many samples and provide in-depth sequencing has improved our understanding of the overarching tendencies in the microbial community structures of the water in shrimp-cultivation enclosures. All these factors together led to a large quantity of information gained from sequencing the 16S rRNA genes of microorganisms in pond water used in aquaculture. The microbial community variety within the samples was greater than that seen in earlier research on water microbial communities in various environments, such as tilapia ponds and estuary reservoirs (Dabadé et al., 2016).

The varied geographies and techniques used may be the cause of the variation in OTU counts in the water so confined aquaculture habitats. According to earlier studies on tilapia ponds, Chinese grasscarp ponds, and shrimp ponds, which showed an average percentage of more than 30%, Proteobacteria was the most prevalent phylum in our research. *Cyanobacteria, Actinobacteria, Bacteroidetes,* and *Planctomycetes* accounted for the remaining four most common phyla. In shrimp cultural enclosure environments, *Bacteroidetes, Planctomycetes*, and *Actinobacteria* were consistently shown to be the dominating taxa; nevertheless, thedistribution patterns of several phyla were different from those previously seen. For instance, our findings contradicted those of shrimp ponds, which suggested that *Cyanobacteria* was a prominent phylum (Biebl et al., 2005).

Furthermore, where as Chlorobi's relative abundance was low in prior studies, it was high in the current research, making it a dominating phylum. In addition, we found an oxygenic phototrophic microorganisms like *Dinoroseobacter* that use light for energy. This part of the organism lacks the necessary bacterio chlorophyll and photosynthetic reaction centers to fix CO_2, making it distinct from photosynthetic bacteria. Energy may also be produced through the oxidation of inorganic molecules by bacteria like *Rubrivivax* and *Hydrogenophaga*, which use carbon monoxide oxidation and thiosulfate reduction, respectively. Additionally, this research found many microorganisms related to the nitrogen cycle. Primary N_2-fixing *Cyanobacteria*, including *Cylindrospermopsis*, are controlled by nitrogen. Ammonia-oxidizing bacteria, such as *Nitrosococcus* and *Nitrosopumilus*, are those that convert ammonia to nitrite. The function of nitrate reduction is linked to *Glaciecola, Paenisporosarcina, Rheinheimera, Marivita, Massilia, and Flavobacterium* (Sunagawa et al, 2015).

Some microorganisms, like *Hydrogenophaga*, do both nitrification and denitrification simultaneously, acting as denitrifying bacteria in the traditional anaerobic denitrification process. Additionally, certain infections such as *Pseudomonas, Vibrio,* and *Flavobacterium* were found in our investigation and may be connected to the illnesses of cultured animals. Additionally, we discovered that several functional groups had considerably different abundances in environments with high and low salinity. High salinity water had an over representation of *Synechococcus* compared to low salinity water, whereas *Rhodobacter* and *Flavobacterium* had decreased relative abundances. These findings showed that microorganisms are important players in shrimp cultural enclosure habitats' production, nutrient cycling, and water quality. Temperature and salinity are two important environmental parameters that affect how microbial communities are organized in various habitats. But according to earlier research, salinity, not temperature, significantly contributed to the explanation of the distribution patterns of the world's microbial communities. Accordingtoour study's results, salinity acted as a more important environmental factor than temperature in influencing the divergence of H_2O microbial communities in ecosystems of shrimp cultural enclosures (Auguet et al., 2010).

Conclusion

The goal of this research is to investigate the dynamics of aquatic microbial communities in shrimp culture enclosure habitats. The salinity was the most important environmental component in explaining variance in microbial communities against temperature, Turgor Pressure, total nitrogen or pH. The salinity had a significant role in the water microbial community's ability to operate. The findings provide a place to start when predicting how microbial communities may behave in shrimp cultural enclosure habitats in response to environmental changes.

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Author Contributions

MMA, LSNB, TNB and PS conceived the concept, wrote and approved the manuscript.

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