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Molecular sequence analysis of Paenibacillus alvei isolated from Asian Sea Bass (Lates calcarifer)

Noornissabegum. M[1], Revathi. K[2*], Rekha V[3] & A.Anitha[4]

[1]PG and Research Department of Zoology, Ethiraj College for Women, University of Madras, Chennai, 606008, Tamil Nadu, India.

[2]Meenakshi Academy of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu, India.

[3,4]DKM College, Thiruvalluvar University, Vellore, Tamil Nadu, India.

*Corresponding author Email: reva63@rediffmail.com

ABSTRACT

The presence of microbial flora of edible fish *Lates calcarifer*, as candidate species which is cultured in marine, brackish as well as fresh water environment is studied, as microorganism present in aquatic ecosystem may affect its nutritional value and causes disease if consumed if proper care is not taken. This paper concentrates the study about the microbial flora present in the gut of the fish and to characterize them by applying 16S rRNA to study bacterial species effect and their roles on the bacteria from the same geographical area. A dendogram was constructed and phylogenetically analysed. Since *Lates calcarifer* is of high commercial value, the need to cultivate healthier fish is important.

Keywords: *Lates calcarifer,* 16S rRNASequence - *Paenibacillus alvei* (*Bacillus*), phylogenetic analysis – MEGA5 software.

1. INTRODUCTION

Aquatic microbial diversity is a key component of aquatic ecosystem The presence of microbial community changes with age, nutritional status, and environmental condition changes [4], environmental variables is linking towards microbial diversity with aquatic ecosystems (Singer et al., 2010).

Lates calcarifer an economically valuable food fish is due to its fast growth rate, good taste, flesh texture, high demand and high market value, microorganism found in aquatic ecosystem which may affect its nutritional value and causes disease if consumed. Microorganisms are found on the various surface organs of aquatic organisms like fish for colonization [4]. saprophytic bacteria causes the disease due to deficient in nutrients, poor water quality and overstocking. This brings economic losses in aquaculture due to mortality.

Analysis of DNA extracted from water samples made workers to investigate bacterial communities, and a range of methods targeting both rRNA and rDNA used routinely in microbial ecology. Since only 0.001 to 1% of bacteria are cultivable [7], investigators approached to modern molecular tools based on the PCR technique and phylogenetics of the 16S rRNA gene [9].

This study was carried out to provide information on bacterial flora *Paenibacillus alvei* which is isolated from the gut of marine *Lates calcarifer*, were not previously reported to be present in the

gut of this fish and are responsible for pitted keratolysis and pulmonary infection in humans. A knowledge about these micro-organisms would help one to cultivate the fish free from these microbes and to produce fishes those are reliable for human consumption.

2. MATERIALS AND METHODS

Sample collection:

The fish was collected from landing centres at Cuddalore & Parangipettai, Southeast coast of India and the collected sample immediately brought fresh to the laboratory. The fish was washed several times with sterile sea water followed by 70% ethanol to prevent contamination. The tissues in the gut were carefully removed using a sterile forceps. The gut tissue was placed in phosphate buffer saline solution and completely homogenized using a glass rod. Serial dilutions made from homogenate and 0.1 ml were spread into petriplates containing Zobell's marine agar. After 24 hours, from the incubated plates the total CFU (Colony Forming Units) was counted. The colonies were isolated, sub cultured and identified by several morphological, biochemical methods & molecular method.

Bacterial strains & Molecular characterization:

Twelve various bacterial strains were isolated, of which *Paenibacillus alvei* were found predominantly and were selected for molecular characterization following the standard method of Sambrooke *et al.,* (2001) [5].

16S rRNA gene Sequence and phylogenetic Analysis:

Sequence obtained were studied by comparisons of **16S rRNA Sequence to** the GenBank by BLAST program at NCBI (National Centre for Biotechnology Information) by the method followed by Yee *et al*., 2013 and phylogenetic tree between the bacterial strains was based on the comparative analysis of **16S rRNA Sequence** by a probable software packages MEGA version 5 [6,8].

3. RESULTS

Sample collection:

From the serial diluted samples the twelve different colonies were isolated. All the isolates were biochemically characterized and identified (Table 1).

Table 1: Bacterial identification

S.No	Culture No	Bactrial isolates
1	N1	*Micrococcus nishinomiyaensis*
2	N2	*No growth*
3	N3	*Micrococcus sedentarius*
4	N4	*Micrococcus lylae*

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5	N5	*Aeromonas hydrophila*
6	N6	*Micrococcus sedentarius*
7	N7	*Staphylococcus saprophyticus*
8	N8	*Staphylococcus saprophyticus*
9	N9	*Staphylococcus saprophyticus*
10	N10	*Bacillus cereus*
11	N11	*Micrococcus lylae*
12	N12	*Paenibacillus alvei*

Molecular characterization:

The isolate N12 was subjected to molecular characterization. The DNA from the organism was extracted and analysed both qualitatively and quantitatively. The 16s rRNA region was amplified and was visualised at 900bp. The PCR product was sequenced by Sanger's method using 3730 DNA sequencing analyzer at ABI. The sequence obtained was studied using BLAST and a dendrogram was mapped to study the phylogenetic relationship.

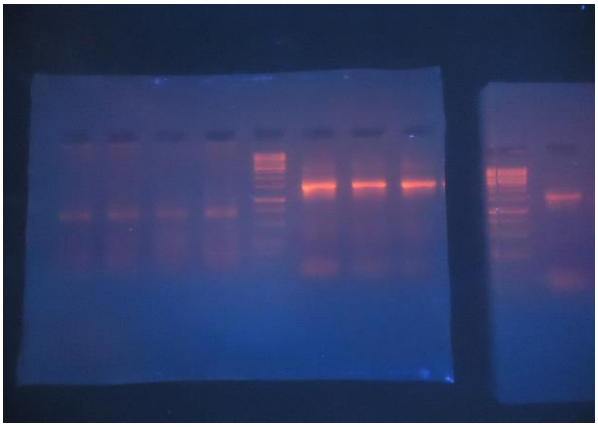

Figure 1: AMPLIFICATION OF 16s rRNA GENE (50°C)

Lane 1: 1Kb Ladder,
Lane 2: PCR product of N12,
Lane 3: PCR product of N3

1 **2** **3**

Sequence analysis:

http://annalsofrscb

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The nucleotide sequences obtained were compared with its related species using BLAST tool. Portable software Molecular Evolutionary Genetics Analysis (MEGA) version 5, was used to construct maximum likelihood tree for the obtained inter and intra species relationships. The Distance Matrix Explorer, an action menu of MEGA5 was used to calculate the pair wise difference between the obtained target sequences to its maximum aligned sequences.

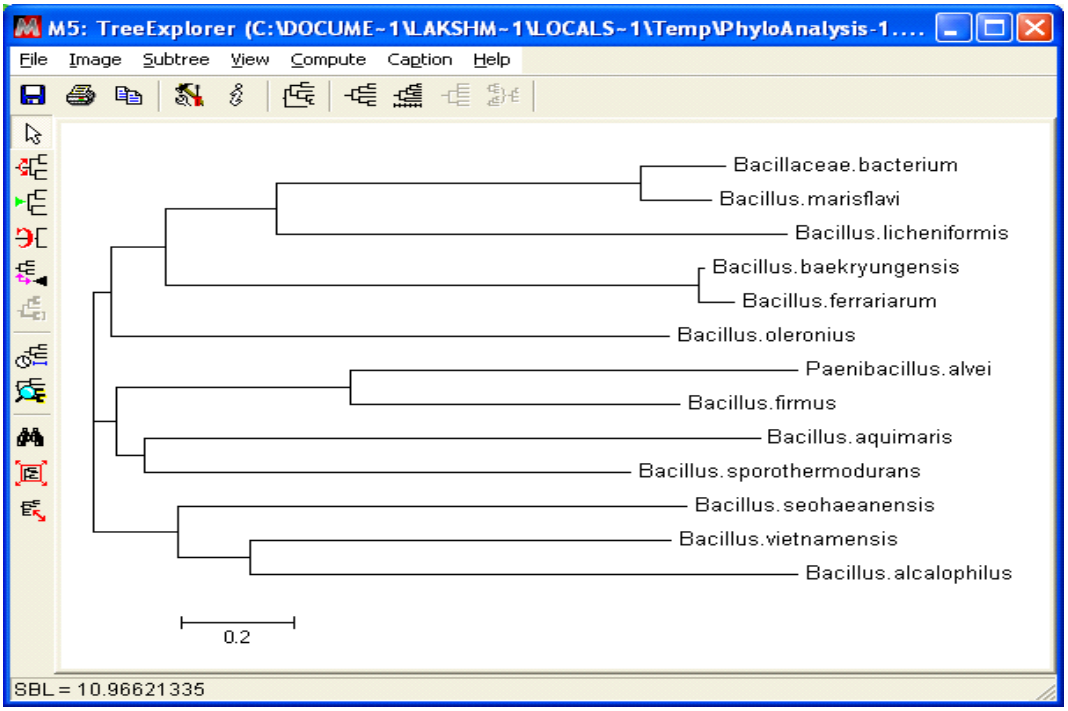

Figure 2: Dendogram for *Paenibacillus alvei* (isolated from gut of *Lates calcarifer*)

4. DISCUSSION

The *Lates calcarifer* is an anadromous fish which has a high commercial value. It is a food fish important in the tropical and subtropical regions in the Asia-Pacific. Due to relatively high market value, it is an attractive commodity of both large to small-scale aquaculture enterprises. The fish carries lot of micro-organisms from the surrounding aquatic ecosystem. The presence of these microbes in the internal tissues and other parts such as gut and intestine may affect the nutritional value of the fish. Some bacteria found in the fish may cause disease in humans if consumed.

Sequence-based microbial survey informs that cultivation methods substantially under-represent the extent of bacterial diversity. The characterization of the bacterial community in marine and freshwater fish species is done by Cultivation-independent methods using total DNA extracted from intestinal samples [1].

By this study the amount of microbial flora in the gut of the fish is studied thereby determining the genetic diversity in its living environment. Several microbes enter the gut but only those which can adapt and survive in that environment are capable of living. The species *Paenibacillus alvei* have not yet been reported to be found in the gut of the *Lates calcarifer.* This suggests a change in the existing microbial flora found in the gut of the fish.

Food poisoning (*Vibrio* sp., C. botulinum) occurs due to consumption of raw or insufficiently uncooked fish contaminated with bacteria [2]. It is a potential danger when consumed not only in causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human infecting bacteria from non-aquatic sources [3].

The strain *Paenibacillus alvei* is non-pathogenic and recently being used as a biological tool against pathogenic strains. The *Paenibacillus alvei* is found more genetically related to *bacillus firmus* than *bacillus aquimaris* and *bacillus sporothermodurans*. The *Paenibacillus alvei* is not closely related to *bacillaceae bacterium and bacillus marisflavi.* MEGA5 was used for analysis [6].

This study helps to study the ever changing aquatic environment and the invasion of new strains. This throws light to improve the cultivation techniques of the fish and prevent the presence of these types of micro-organisms in the fish which would adversely affect the shelf life of the fish and their quality. *Lates calcarifer* an edible fish would affect the sales due to the presence of pathogenic strains. This information may provide valuable information of the microbial content in the fish, their effects and help farmers to grow them devoid of pathogenic species.

The molecular phylogenetic analysis method used will be able to amplify and clone from phylogenetically diverse members of the bacteria in aquatic ecosystem for having a proved way of understanding the presence of microbial species in aquatic environment. The studies in the marine aquatic environment will tie the phylogenetic data together with data about specific microbial activities may result in long-term goal for future microbial ecology. Thus, any change in environment is a major threat to all aquatic ecosystems

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