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# BACTERIAL ISOLATES FROM AQUARICULTURE SYSTEMS: IDENTIFICATION BY 16SRDNA AMPLIFICATION AND SEQUENCE ANALYSIS

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## ABSTRACT

Aquariculture is the farming of ornamental animals and plants. As bacterial diseases cause major production and economic losses in ornamental fish culture, early identification of bacterial pathogens from such systems would help to take suitable management measures to avoid huge losses. Bacterial isolates from various aquariculture sytems were identified by PCR amplification of 16srDNA gene and nucleotide sequencing. This method is less time–consuming and more specific than conventional bacterial identification methods.

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# INTRODUCTION

Aquariculture involves farming of ornamental animals and plants[1]. Disease are the major limiting factor to the development and expansion of aquariculture. Ornamental fishes are susceptible to various diseases of bacterial, viral, fungal and parasitic origin. Bacterial diseases are the most common among the diseases affecting ornamental fishes. Majority of bacterial fish pathogens are natural inhabitants of the aquatic environment[2]. Although bacterial pathogens are conventionally identified by their cultivation and biochemical characterization, there are many bacterial species that are not cultivable by standard methods[3&4]. Biochemical identification methods also involve laborious, time-consuming processes which are at times misleading due to the presence of variants among bacterial species [5]. Molecular methods like PCR have been proved to be very useful in the identification of bacteria. PCR Amplification of 16s rDNA gene which is conserved in bacteria and sequencing enables the identification of bacterial species. This technique has been proved to be helpful to identify non-cultviable bacteria and other bacterial species in shorter time with relatively higher accuracy[6]. Rapid and accurate identification of the bacterial pathogens would help to undertake suitable management measures to avoid disease outbreaks and production losses in aquariculture. The present study was carried out with an objective to identify bacterial isolates from various aquariculture systems by 16s rDNA gene amplification systems and sequencing.

# MATERIALS AND METHODS

### *Isolation of bacteria from samples*

Water and fish samples were collected from different aquariculture systems operated with fresh water and marine water in Kolathur, Tamilnadu, India. The details of the samples are presented in Table.1. Samples of water/soil/swabs from fishes collected aseptically were used for bacterial isolation. The samples were inoculated in sterile tryptic soy broth and isolated on agar plates supplemented with or without 1% sodium chloride (NaCl) depending on the source of collection of samples (fresh water or marine). The isolates were incubated overnight at 37ºC and the individual bacterial colonies were subcultured.

### *16s rDNA gene amplification by Polymerase chain reaction (PCR)*

DNA extraction was carried out from a loopful of bacterial isolate. Briefly, the bacterial culture was washed with sterile saline and the DNA was extracted using a commercial DNA extraction kit following the manufacturer's protocol (Bangalore Genei, Bangalore). Published PCR protocol and primers were followed[7]for the amplification of 16s rDNA PCR amplification was carried out in a total volume 25 µl with 22 µl of 1x PCR master mix (Bangalore genei), 1 µl each of forward and reverse primers (30 pmoles of each) and 1 µl of DNA template from samples. PCR amplification was carried out in a PCR thermal cycler (Eppendorf, Germany). The amplified 16S rDNA products were resolved by horizontal gel electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.8 mg/ml) at 100 V. The separated PCR products were documented in a gel documentation unit (Vilber-Lourmet, France).

### *Automated sequencing and submission to GenBank*

Direct sequencing of PCR products of 16s rDNA gene was carried out using a commercial automated sequencing services (MWG, Bangalore). The sequence data were used for nucleotide sequence analysis and comparison. The bacterial isolates were identified by comparing their sequence information with the sequences available in the GenBank of National Centre for Biotechnological Information (NCBI- www.ncbi.nim.nih.gov) using Basic Local Alignment Search Tool (BLAST) program and were submitted to the GenBank for acquiring accession numbers.

## RESULTS AND DISCUSSION

PCR amplification of 16s rDNA of bacterial isolates from aquariculture systems resulted in a PCR amplicon of expected size of 1487 bp (Fig.1). Based on the homology of the nucleotide sequences, the bacteria isolated from various sources were identified and their GenBank accession numbers are presented in Table.2. In the present study, sequence analysis and homology have shown that the bacterial isolates from various aquariculture systems belong to *Aeromonas* sp, *Bacillus* sp, *Pseudomonas aeuroginosa, Vibrio sp.* and *Halomonas sp*. 16s rDNA PCR is being increasingly used for the identification of bacterial isolates that could not be identified by conventional methods as in the case of non-cultivable bacterial species[6]. It is also highly reliable as it provides unambiguous data even for rare isolates, which are difficult to identify with the phenotypic identification schemes[8]. One of the most attractive potential uses of 16S rRNA gene sequence informatics is to provide genus and species identification for isolates that do not fit any recognized biochemical profiles[9] Bacterial infections and diseases in aquariculture have been reported to cause huge production losses. *Aeromonas* sp of bacteria have been reported to be the most abundant bacteria found in fresh water aquatic environments including fish culture ponds and other aquaculture systems. *Aeromonas* are considered to be opportunistic pathogens, capable of producing disease only in weakened populations of fish or as secondary invaders in fish suffering from other diseases[10]. Mycobacterial diseases caused by bacterial pathogens of *Mycobacterium* sp. are zoonotic [11]. *Bacillus cereus* causes food borne intoxications such as nausea and vomiting and abdominal cramps, diarrhea form of disease in humans [12]. The results of this study shows that 16s rDNA amplification helps in the rapid and accurate identification of bacterial pathogens. Pseudomonas infection has been incriminated as the most common bacterial infection among fish and appear to be stress related disease of freshwater fishes especially under culture conditions [13]. *Pseudomonas* sp infections have been reported to be associated with skin infections in humans [14]. Handling of fishes infected with bacterial diseases that are zoonotic would result in the transmission of the disease to humans. Information on the bacterial species that inhabit the aquariculture systems will be helpful in the effective management of such systems so as to prevent the disease outbreak or spread of infections the humans involved in the handling activities.

**Table-1**
***SOURCE OF BACTERIAL ISOLATES***

| S.NO | SOURCE | CODE |
|---|---|---|
| 1 | Gold fish (FW) | SDDL/1/12 |
| 2 | Shrimp (MW) | SDDL/2/12 |
| 3 | Koi carp (FW) | SDDL/3/12 |
| 4 | Soil sample from rearing pond | SDDL/4/12 |
| 5 | Golden gourami (FW) | SDDL/5/12 |
| 6 | Water sample | SDDL/6/12 |
| 7 | Gourami (FW) | SDDL/7/12 |
| 8 | Pangasius (FW) | SDDL/8/12 |
| 9 | Fairy shrimp (FW) | SDDL/9/12 |
| 10 | Koi carp (FW) | SDDL/10/10 |

**Table-2**
***Identification of bacteria and accession number provided by the GenBank,***

| S.NO | CODE | BACTERIAL SPECIES | ACESSION NUMBER |
|---|---|---|---|
| 1 | SDDL/1/12 | *Aeromonas veronii* | JX861241 |
| 2 | SDDL/2/12 | *Bacillus cereus* | JX861242 |
| 3 | SDDL/3/12 | *Pseudomonas aeruginosa* | JX861243 |
| 4 | SDDL/4/12 | *Bacillus subtilis* | JX861244 |
| 5 | SDDL/5/12 | *Aeromonas* sp | JX861245 |
| 6 | SDDL/6/12 | *Aeromonas* sp | JX861246 |
| 7 | SDDL/7/12 | *Halomonas* | JX861247 |
| 8 | SDDL/8/12 | *Aeromonas* sp | JQ713335 |
| 9 | SDDL/9/12 | Uncultured *Aeromonas* sp | JX439911 |
| 10 | SDDL/10/10 | *Mycobacterium* | ---------- |

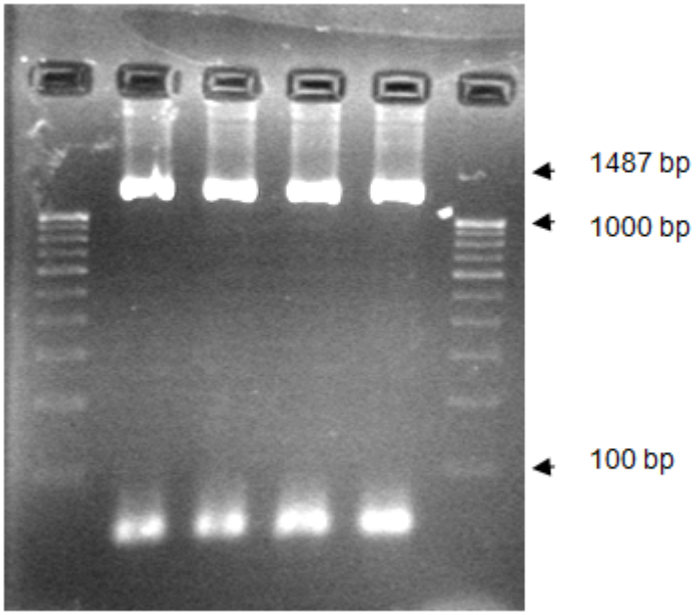


**Figure.1**
***PCR amplified 16s rDNA products of bacterial isolates from aquariculture systems***

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