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Detection of Serine Protease Gene (Ser-P) in Marine Isolates of Aeromonas Hydrophila.

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***Abstract:** Marine water samples were collected from marina beach in Chennai for screening the isolation of serine protease gene from Aeromonas hydrophila by using PCR analsis.The. Samples were collected and analysed the genomic DNA of Aeromonas hydrophila was isolated. Before analysing polymerase chain reaction with the selected isolates each primer set was tested. Polymerase chain reaction was performed to screen the ser-P genes. This study showed that PCR detection may prove to be an vital tool for the detection, identification, differentiation and distribution of serine protease genes in organisms. This polymerase chain reaction technique will give scientists an alternative way to find out the nature of pathogenicity in Aeromonas species and their distribution in isolates from different sources .Our present study clearly indicate that the PCR analysis detects serine protease genes in Aeromonas hydrophila species by using a pair of primers for gene.*

***Key words:** Aeromonas hydrophila, Environment, Gene, PCR, Serine protease.*

I. INTRODUCTION

Aeromonas hydrophila is a gram-negative, that structurally resembles members of the family fourteen species of *Aeromonas* have been discoverd, most of which have been associated with human illness (1). Major diseases are associated with *Aeromonas* species are gastro enteritis and wound infections, with or without bacterium. Gastro enteritis typically occurs after the ingestion of contaminated food materials, whereas wound infections result from exposure to contaminated water. (2,3). The pathogenesis of disease causing genes and integrons was determined in eighy one t strains of *Aeromonas species* were isolated from fresh water fishes. Polymerase chain reaction protocols was used to determine the presence of genes for cytotoxic enterotoxin such as (ser-P) (4).

II. MATERIALS AND METHODS

A. Sample Collection

Marine water samples was collected from near Marina beach in Chennai. The samples were collected in a sterile tubes.

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B. Isolation And Identification of A.hydrophila

C. Spread Plate Technique (SA Agar media)

Agar was prepared and sterilized.
At bearable warmth, Ampicillin was added and plated then, 1 ml of the samples were transferred in Starch Ampicillin agar plates and spreaded evenly by using the L-rod .The inoculated SA agar plates were incubated at room temparature for one day. The yellowish coloured organisms was scored as *Aeromonas hydrophila* and were sub cultured into the nutrient agar slants and stored at 4°C for further studies.

D. Confirmation Test

The media was prepared and sterilizezd for confirmation of . the yellow colour colonies from nutrient agar were taken and stabbed and streaked onto Kaper's multitest media tubes. The organisms isolated were sub cultured on nutrient agar and used for Gram's staining and biochemical tests.

E. Test for Indole

The isolated cultures were inoculated in peptone broth and incubated at room temparature for one day. The isolated cultures were inoculated in Glucose phosphate broth (MR-VP broth) and incubated at 37^{0}C for 48 hours. After incubation few drops of methyl red indicator was added and shake well. MR test is employed to detect the production of acid and maintenance of low pH.

F. Test for Voges Proskauer

The isolated cultures were inoculated in Glucose phosphate broth (MR-VP broth) and incubated at 37^{0}C for 48 hours. After incubation few drops of Barrit's reagent was added and shake well and give red colour.

G. Test for Citrate

The colour of the medium changes from green to blue shows the positive result.

H. Test for Urease

The isolated cultures were inoculated in the slants and incubated at 37°C for 24 hours. Change in colour of the medium to bright pink shows positive result.

I. Test for Lysine Arginine Ornithine

Isolated cultures were inoculated on LAO slants and incubated at 37^{0}C for 24 hours. Purple colour indicates positive result.

J. Activity of Protease Activity

The skimmed milk powder was dissolved by boiling it and allowed to cool. Then the skimmed milk preparation and nutrient agar were mixed and poured in petri plates. The isolated cultures were inoculated on Milk agar plate and incubated at 37ºC for 24 – 48 hours. Positive result shows clear zone formation around the bacterial colony as the organism produced protease enzyme which hydrolysed the protein casein present in the milk.

K. A.hydrophila genomic DNA isolation

The isolated cultures were inoculated in Luria Britani (LB) broth and incubate at 37° C for 24 hours and the culture was found to be turbid.

L. Analysis of PCR

The polymerase chain reaction (PCR) were performed to identify the gene

M. Serine Protease gene detection by PCR

The PCR mix was prepared in the thin walled PCR tubes in a sterile laminar hood.

***Ser-p gene*:** Forward: 5' CAGTCCCACCCACTTC 3' Reverse: 5' GCCTGAGCGAGAAGGT 3'

III. RESULTS AND DISCUSION

The enriched culture was found to be turbid showed that there was increased recovery of the organisms in the broth.

Table 1: shows the Colony on Starch Ampicillin Agar.

S.No	Sample	Colour of the sample
1	Marina beach[MB 1-7].	Yellow

The yellow colour colonies were convex, circular, regular, smooth and 2mm in size.

Fig. 1 shows that the isolation of A. hydrophila in SA agar

2.3 Isolation In Kaper's Multitest Media

After 24 hours of incubation the tubes showed yellow colour colonies

Confirming Aeromonas hydrophila.

Identification of A.hydrohila

Gram's Staining

Gram staining technique is used to identify the organisms such as bacilli to coccibacilli

Biochemical Tests

Table 2. shows the Biochemical tests for *Aeromonas hydophila.*

S.No	Tests	Results
1	Indole	+
2	Methyl Red	-
3	Voges-Proskaeur	+
4	Citrate	+
5	Urease	-
6	LOA Test	
	Lysine	+
	Arginine	-
	Ornithine	+

To detect the presence of serine protease gene in *Aeromonas hydrophila*, primers were annealed with the template DNA (isolated genomic DNA of *Aeromonas hydrophila*) and caused amplification. Totally seven isolates were subjected to PCR. In gene amplification, the isolates were confirmed of this gene with 416 bp using the 1000-100 bp marker DNA . The organism was isolated through selective media from various food and water samples and identified by Gram's staining, biochemical tests and confirmatory test. The genomic DNA was isolated and bands were observed by performing agaroes gel electrophoresis using one percent agarose gel containing EtBr.

The isolated DNA was used as template in the PCR study. From the 7 DNA isolate were selected from different sources. PCR were performed in PCR Thermocycler under the PCR conditions of the genes (5). PCR technique clearly identified ser-P gene in Aeromonas hydrophila. The PCR products were mixed with gel loading buffer and loading in one percent agarose gel containing EtBr using Tris Borate Edta buffer. Results clearly indicates that presence or absence of the ser-P gene in the isolated organisms by observing the bands at the level 416 bp for ser-P gene.

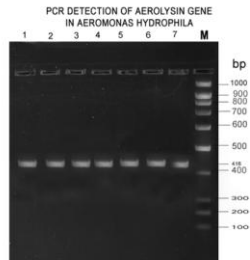

Fig.2 PCR detection of serine protease gene in aeromonas

hydrophila

A total number of 7 isolates obtained from marine samples were determined. All the isolates were identified containing *A. hydrophila* by cultural and biochemical characteristics. Overall 35% of samples examine were positive for *A.hydrophila* .The density of A . hydrophila was in the range of 2.2x10-2-9.4x10-5CFU. High level of incidence of *A.hydrophila* in marine water was recorded. On Starch Ampicillin Agar *Aeromonas hydrophila* produced yellow color colonies. On kaper's multi test medium appearance of alkaline slant and acid butt after 24 hours incubation *A. hydrophila* was identified using conventional methods. The presence of virulence gene (serine protease) was detected using PCR technique. *A.hydrophila* isolated from marine samples were studied to harvoring the virulent gene. Ser -p gene fragment(416bp) amplified from *A.hydrophila* isolates reported that 91.67% of the A. hydrophila isolates from mineral and thermal water in Italy were ser-p. Aslani and Hamzeh (2004) showed that 56% of the *A. hydrophila* isolates from diarrhoeal and healthy asymptomatic controls were positive for ser-p gene.(6).

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