PLASMID AND CELL ENVELOPE CHARACTERIZATION OF VIBRIO ALGINOLYTICUS ISOLATED FROM INFECTED TIGER PRAWN, PENAEUS MONODON

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It has been reported that indiscriminate use of antibacterial compounds in aquaculture has resulted in an increase in populations of antibiotic resistant strains of bacteria. In the present study, *Vibrio alginolyticus* isolated from infected *Penaeus monodon* has been found resistant to Furoxone (30 μg), Ampicillin (10 μg), Cloxacillin (1 μg), Bacitracin (10 units), Penicillin (10 units) and Oxytetracycline (30 μg) by antibiotic sensitivity test, suggesting the restricted prophylactic use of antibiotics in shrimp aquaculture industry. As an approach towards differentiation and identification of bacterial strain, the plasmid characterization of *V. alginolyticus* by restriction enzyme analysis was carried out which revealed the presence of 6.3 kb and 0.8 kb DNA fragments by Hind III and a 7.9 kb DNA fragment by EcoRI enzymes. The cell envelope characterization of *V. alginolyticus* by SDS-PAGE showed the presence of 12 protein bands in the range of 29 to 112.20 kD. The preliminary information on the cell envelope characterization may help in studies relating towards development of immunostimulant against vibriosis.

INTRODUCTION

Vibriosis has been considered to be the most important infectious disease of marine fishes and shrimps (Lightner, 1988). A variety of management practices including frequent disinfection of the system and use of wide range of antibiotics to reduce losses associated with vibriosis have been reported (Broch and Lea Master, 1992). This has led to the increase in population of antibiotic resistant bacterial isolates which is mostly plasmid-mediated (Toranzo et al., 1983). However, detailed information of plasmid characterisation and its correlation with antibiotic resistance of marine vibrios are lacking. As an alternative strategy against indiscriminate use of chemicals and antibiotics, recent years have witnessed to an intensive research effort directed towards the development of effective vibrio vaccines. However, the approaches towards the development of vaccine and immunostimulants against vibriosis requires detailed information on the antigenic properties of the *Vibrio* at the molecular level (Chart and Trust, 1984).
There is also a need for rapid analysis and identification of marine vibrios by molecular techniques. Studies have been initiated to differentiate and identify the marine Vibrio species based on DNA fingerprinting (Tsai et al., 1990), amplification of polymorphic DNA (Martinez et al., 1994) and by comparison of restriction enzyme patterns of plasmid DNA (Vivares et al., 1992; Tsai et al., 1993).

In the present study, the antibiotic resistance pattern, plasmid restriction profile and the cell envelope characterization of V. alginolyticus have been reported.

MATERIAL AND METHODS

Bacteria

Vibrio alginolyticus was isolated from infected shrimp (Penaeus monodon) from shrimp farm located at Nellore, Andhra Pradesh (India). The Vibrio was identified according to the taxonomic scheme of Buchanan and Gibbons (1984), and West and Colwell (1984).

Antibiotic sensitivity test

The bacterial isolate was examined for its antibiotic resistance to antimicrobial compounds. The drug sensitivity was determined by using the method of Bauer et al. (1966) on Zobells marine agar (Hi Media, Bombay). The antibiotic discs used in this study are given in Table 1.

Table 1. Sensitivity of Vibrio alginolyticus to different antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Strength per disc</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin B</td>
<td>300 µg</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30 µg</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Furoxone</td>
<td>30 µg</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 µg</td>
<td>Resistant</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>50 µg</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>1 µg</td>
<td>Resistant</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>10 units</td>
<td>Resistant</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10 units</td>
<td>Resistant</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>30 µg</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
Isolation of plasmid DNA

Plasmid DNA from *Vibrio alginolyticus* was isolated as described by Maniatis *et al.* (1982). Briefly, the bacterial cells were grown in 100 ml of 1% peptone broth supplemented with 1.5% NaCl. The bacterial pellet obtained after centrifugation (Sorvall, GSA 4 rotor) at 7000 rpm for 20 min at 4°C was resuspended in 7.5 ml of GTE buffer (50 mM glucose, 25 mM Tris (pH 8.0), 10 mM EDTA) containing lysozyme to a final concentration of 5 mg/ml. Fifteen ml of 0.2 N NaOH, 1% sodium dodecyl sulphate (SDS) solution was added and kept on ice for 10 min followed by addition of 12 ml of potassium acetate solution (5 M, pH 4.8) to the supernatant collected after centrifugation as above, RNase (22 µg/ml final concentration) was added and incubated at 37°C for 2 hour. One extraction with phenol-chloroform-isoamylalcohol was done before final ethanol precipitation.

Restriction analysis of plasmid DNA

Restriction digestion of plasmid DNA with Hind III and EcoRI restriction enzymes were performed following the specifications of the supplier (Bangalore Genei) and were subjected to electrophoresis in 0.7% agarose gel in Tris borate buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.2). Lambda DNA Hind III - Phi x 174 / Hae III (Stratagene) was used as molecular weight marker.

Isolation of cell envelope

Cell envelope from the bacteria was prepared as described by (Crosa and Hodges, 1981).

SDS-PAGE

SDS-PAGE was carried out following the method of Laemmli (1970). Cell envelope preparation, (50 µl) was mixed with equal volume of 2x electrophoresis sample buffer. Suspension was boiled for 5 min. and 50 µl was applied to 10% polyacrylamide gel. After electrophoresis (40 V, 16 hour), the gel was fixed and stained with 0.125% coomassie brilliant blue R-250 for 4 hour and destained overnight. The molecular weight (MW) of bacterial proteins were determined by comparing their migration with that of molecular weight marker (PW-1, Bangalore Genei).
RESULTS AND DISCUSSION

Our results showed that \textit{V. alginolyticus} isolate was resistant to Furoxone (30 µg), Ampicillin (10 µg), Cloxacillin (1 µg), Bacitracin (10 units), Penicillin (10 units) and Oxytetracycline (30 µg) as shown in Table 1.

The therapeutic use of oxytetracycline has been shown to enhance the production of plasmid-mediated resistance in aquatic bacteria (Shotts et al., 1976) and increase the frequency of new oxytetracycline-resistant isolates (Williams et al., 1992). In the present study, this bacterial isolate was also found to be oxytetracycline-resistant which could be plasmid-mediated. Further, curing experiments may help in finding the plasmid-mediated antibiotic resistance of the bacterial isolate as reported for fish pathogens (Toranzo et al., 1983).

Molecular analysis of pathogenic marine vibrios has revealed the carriage of a diverse assortment of extrachromosomal elements. In the present study, the plasmid DNA of the bacteria on Hind III restriction enzyme analysis showed the presence of 6.3 kb and 0.8 kb DNA fragments whereas EcoRI restriction profile showed the presence of 7.9 kb DNA fragment (Fig. 1). The preliminary information obtained on restriction enzyme analysis may help in differentiation of bacterial strain as also suggested by Vivares et al. (1992). They reported usefulness of plasmid DNA restriction patterns to differentiate bacterial strains in epidemiological studies of fish pathogenic Vibrios. Similar studies by Tsai et al. (1993) on plasmid and chromosomal fingerprints of fish pathogenic vibrios based on restriction pattern showed that \textit{V. alginolyticus} (ATCC 17749 strain) contained 16.4, 11.9, 8.3 and 6.0 kb plasmids and four restriction digestion patterns (Cla I, EcoRI, Hind III and Sac I) produced the same result for \textit{V. alginolyticus}, \textit{V. damsela} and \textit{V. vulniificus}.

Protein profile of the cell envelope of \textit{V. alginolyticus} showed the presence of 12 protein bands ranging from 29 to 112.20 kD, with total MW of 747.06 kD (Fig. 2). Further studies by immunoblotting may lead towards identification of immunogenic proteins which may be used as immunostimulant to protect shrimp against vibriosis.

ACKNOWLEDGEMENT

The authors are thankful to the Director, Central Institute of Brackishwater Aquaculture, Chennai for his kind support and assistance to carry out the present work.
Fig. 1. Restriction fragment pattern of *Vibrio alginolyticus* plasmid DNA. Lane 1, DNA MW marker lambda DNA Hind III-Phix 174/Hae III, Lane 2, Hind III restriction profile, Lane 3, EcoRI restriction profile.

Fig. 2. Electrophoretic analysis of *Vibrio alginolyticus* cell envelope in 10% SDS-PAGE. Lane 1, protein MW marker, Lane 2, cell envelope proteins.

REFERENCES


