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Isolation and identification of *Vibrio cholerae and Vibrio parahaemolyticus* from prawn (*Penaeus monodon*) seafood: Preservation strategies

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ABSTRACT

Bacterial diseases are one of the major problems which affects the production, development and expansion of aqua culture. *Vibrio* sp. are widespread in marine and estuarine environments. The several pathogenic species are commonly associated with outbreaks of *Vibrio* species and it is mainly associated with food poisonings. In this research, the occurrence of *Vibrio* sp. was studied by the isolation and it is confirmed by the biochemical methods. The growth rate was studied by changing the different operating parameters. Isolation studies were done by using enrichment and selective plating methods. The different biochemical test was carried out and inferred that the isolated organisms were *Vibrio choleraee* and *Vibrio parahaemolyticus*. The antibiotic study was also performed to find out the resistant and sensitivity of the *Vibrio* species. From the results, it was observed that this can be able to correlate the growth of *vibrio* species to a limited condition and other environmental parameters for which it will be able to find the remedial measures to prevent the growth and spreading of the diseases. Also the different preservation method was carried out to suppress the growth rate of *Vibrio* sp.

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1. Introduction

Seafood and fish was found to be an important food component for a large section of world population. Sea foods are prone to bacterial contamination, pathogens may be present at low levels when fish or shell fish are harvested, and others may be introduced during handling and processing or by unsanitary practices. Such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favorable conditions [1,5,9,24]. The World Health Organization (WHO) defines food borne illness as a disease which is caused through the consumption of contaminated food [23]. Seafood especially shells fish is a food substrate for some zoonotic *Vibrio* sp. These microorganism causes substantial number of food borne illness [4,17–19]. *Vibrio* sp are Gram-negative, facultative anaerobic motile, curved rods with a single polar flagellum [2,3,6]. *Vibrio* sp are ubiquitous bacteria and abundantly present in aquatic environments. These bacteria are particularly resistant to high salt concentration. The occurrence of *Vibrio* sp in raw sea food is common, especially seafood from region with temperate climates around the world from both natural and farm environments and all sea food types. However, most surveys are qualitative which causes difficulties in evaluating the risk relating to *Vibrio* sp in raw sea food. Different species within the genus *Vibrio* are associated with food borne infections and food spoilage. Among the members of the genus, twelve species are recognized as human pathogens such as *Vibrio Choleraee, Vibrio carchariae, Vibrio mimicus, Vibrio vulnificus, Vibrio metschnikovii, Vibrio parahaemolyticus, Vibrio cincinnatiensis, Vibrio alginolyticus, Vibrio hollisae, Vibrio furnissii, Vibrio damsel, Vibrio fluvialis* ([7,8]). Amongst that, *Vibrio Cholerae* and *V. Parahaemolyticus* are opportunistic pathogens and responsible for the most cases of food borne illness. *V. cholerae* and *V. parahaemolyticus* are recognized as serious human pathogens. *V. cholerae* is a motile, rod shaped and gram negative bacteria. These bacteria exist as natural inhabitants of aquatic ecosystems, thus making them facultative human pathogens [14,22]. *V. cholerae* is transmitted through ingestion of food or water contaminated with the bacterium, especially via feces or vomits of infected persons, directly or indirectly [10,16]. *V. cholerae* is a mesophilic organism that grows in the temperature range of 10–43 °C, with optimum

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growth at 37 °C. The pH optimum for growth is 7.6 although it can grow in the pH range of 5.0–9.6. *V. cholerae* can grow in the salt range of 0.1–4.0% NaCl, while optimum is 0.5% NaCl. *V. parahaemolyticus* is a rod shaped, slightly halophilic, gram negative and non-spore forming bacterium. *V. parahaemolyticus* is broadly distributed in marine environment and has been recognized as a major cause of food borne illness such as diarrhea and gastroenteritis resulting from the consumption of raw, undercooked or contaminated sea foods. The growth of *Vibrio* sp is majorly depending on temperature of water. The Growth of pathogenic *vibrios* occurs optimally at around 37 °C although the maximum and minimum growth temperatures are 43 °C and 5 °C respectively. The illness caused by *V. parahaemolyticus* food poisoning is gastroenteritis characterized by watery diarrhea and abdominal cramps in most cases, with nausea, vomiting, fever, and headache [11–13].

The aim of this present study was isolation of *Vibrio* sp which was present in the prawn seafood (*Penaeus monodon*) and some preservation techniques have been employed to reduce the growth of *Vibrio*. Sp such as drying, radiation, organic acid and osmotic pressure. There is a limited range of techniques currently employed to preserve food. The drying of fish is a well-understood physical process. Microbial spoilage can be reduced by drying. Radiation is effective for ensuring the microbiological safety of food naturally contaminated by *Vibrio* sp [15]. NaCl stimulates the growth of all species and is an obligate requirement for some.

2. Materials and methods

2.1. Study area and sample collection

The *Vibrio* sp were isolated from the prawn (*Penaeus monodon*). Prawn samples were collected from kovalam beach market, Chennai. It has been transported immediately within 2 h to the Environmental laboratory, SSN College of Engineering for bacteriological investigation. The collected samples were subjected to qualitative and quantitative analysis for *Vibrio* species.

2.2. Quantitative analysis

2.2.1. Enrichment method

Test samples were weighed (25 g) and transferred aseptically to a presterilized conical flask containing 225 mL of the alkaline peptone water with 3% NaCl and kept in the temperature controlled horizontal bench shaking incubator (Orbitek, India) for about 10–15 min. After 24 h duration 1 mL of inoculum was transferred to a sterile petridish and plated by using the selective media such as TCBS (Thiosulfate Citrate Bile Salt Sucrose Agar). The plates were incubated at 37 °C for 24 h. Isolated colonies with differences in morphological features were transferred into nutrient agar slants.

2.3. Staining method

2.3.1. Gram staining

Gram staining is a differential staining technique employed for studies of bacterial morphology. The smear was fixed on a clean glass slide and it was covered by using crystal violet stain for about 30–60 s. After the time interval, the slide was cleaned by placing under the tap water. Then, Grams iodine mordent was poured on slide and kept for 30–60 s. Then, the smear was decolorized by using ethanol and washed under tap water. The smear was covered with counter stain safranin for about 60 s. After the time interval, the slide was cleaned by placing under the tap water. Finally, the slide was air dried and smear was placed in the microscope under oil immersion.

2.4. Motility (hanging drop method)

Motility of the bacteria was checked by “hanging drop method”. A loop full of 24 h culture was placed on the center of the cover slip. A drop of Vaseline is placed on the four corners of cover slip. A cavity slide was kept over the drop in such a way that the drop comes with in the cavity. The Vaseline makes the cover slip to adhere to the slide. Then the whole preparation was quickly inverted so that the drop of culture was seen hanging from the cover slip.

2.5. Biochemical characterization

2.5.1. Metabolic activities

(i) Starch Hydrolysis

Starch agar medium was prepared, sterilized and poured in to petri plates. The test culture was streaked on the medium and incubated at 37 °C for 24 h. Then place 2 or 3 iodine crystals in the Petri dish cover. After inverting the plates, iodine gets vaporized. The clear zone outside the area of bacterial growth indicates extend of starch hydrolysis.

(ii) Casein Hydrolysis

Casein agar medium was prepared, sterilized and poured into petri plates. The culture was incubated at 37 °C at 24 h. The test culture was streaked on the medium and incubated. Due to the production of casienase enzyme by bacterium, medium surrounding the colony become clear since the exoenzymes has converted larger protein molecules into smaller molecules i.e. amino acid, which makes then invisible.

(iii) Gelatin Hydrolysis

The gelatin medium prepared and sterilized by tyndallization, keeping the medium in steaming for 3 consecutive days. Then the medium was inoculated heavily with the test organism by the stab inoculation and incubated for 96 h. After the incubation period the hydrolysis of the gelatin was indicated by the liquefaction of gelatin. The culture was incubated at room temperature and chilled until the control solidifies before observations of liquefactions were made.

2.6. Fermentation reactions

2.6.1. Sugar fermentation (carbohydrate fermentation test)

Carbohydrate acts as the main source of energy. The media was prepared and poured in to test tubes, introducing Durham's tube in to each tube without air bubbles. The tubes containing the media were sterilized by autoclaving at 15 lbs pressure. The culture was inoculated in to the tube and incubated in for 24 h.

2.6.2. Oxidation fermentation test (Hugh and Leifson's test)

This experiment was done to confirm the ability of the isolates to ferment glucose with the production of acid and gas. This experiment was carried out in a medium with phenol red indicator. The medium was prepared and poured in to sterilized test tubes. After cooling the medium culture was inoculated into it and inoculated. After incubation the change in color was noted. The positive reaction was show by the color change from red to yellow.

2.6.3. Indole production

The testing for indole production is important to know the

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