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Research Paper

AN EVALUATION ON THE GENETIC PROFILING AND MICROBIAL DETECTION IN PRAWNS

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The shrimps of India belong to three major families namely: Pedacidac, Palacmonidae and Sergestidae of the decapods group. They are especially rich in niacin, essential for a healthy skin and for the release of energy in the body and vitamin B complex needed for metabolic processes. The study aims at isolating the gut microorganisms such as vibriocholerae and *Staphylococcus aureus* from *P. monodon* and *A. indicus* .Vibrio were isolated by streak plate methods and *staphylococcus* were isolated by spread plate methods. There microorganisms can cause several diseases to man and including food poisoning .*Staphylococcus aureus* cause diarrhoea, increase dehydration, etc., while vibriocholerae causes cholera, renal failure, cramps, hypertension , sunken eyes, etc. CTAB extraction method was used for the isolation of genomic DNA. The isolated DNA samples were subjected to agrose gel electrophoresis .The buffer used here was 1xTAE and the DNA bands were observed in UV transilluminator. Spectrophotometric readings of the isolated DNA samples were taken at 260 and 280 nm and these readings were used to estimate the quantity and quality of DNA samples isolated. Prawns are excellent source of protein, a good source of omega 3 fatty acids and a great way to get iron, zinc and vitamin E.

Keywords: Prawn, Vibriocholerae, Staphylococcus aureus, Gut, P. monodon and A. indicus

INTRODUCTION

Prawns are an excellent source of vitamins. They are also source of including iodine, which is essential for thyroid gland function, iron for red cell formation and zinc for wound healing. Most of them are having a high commercial value. Penaceid prawns has a worldwide distribution and various species belonging to it are found both in tropical and temperate latitudes practically all of them are marine but some are known to spend a part of their life in the brackish water and even in fresh water. Significant commercial species valued for food tend to be large, and thus tend to be called prawns. Some of the marine water specious is *Panaeus japonicus, P. lastisulcatus, P. canaliculature, P. monodon, P. semisulcatus, P. inducus, P. merguiensis* and *P. pencilliatus*. All this eight species recorded from India are listened

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as prawn of economic value. *Panaeus monodon*, the giant tiger prawn or Asian tiger shrimp is a marine crustacean that is widely recorded for food. Prawns are an excellent source of vitamins. They are also source of including iodine, which is essential for thyroid gland function, iron for red cell formation and zinc for wound healing. They are especially rich in niacin, essential for a healthy skin and for the release of energy in the body and vitamin B complex needed for metabolic processes. Female can reach approximately 33 cm long, but are typically 25-30 cm long and weight 200-300 g ;males are slightly smaller at 20-25 cm long and weighing 100-170 g.

In the midgut region various types of microorganism has been found, i.e., *Vibrio cholera,* Vibrio is gram negative, rigid, curved, rods that are actively motile by means of a polar flagellum. The name 'vibrio' is derived from vibriae, meaning to vibrate. They are asporogenous, noncapsulated. Vibrios is present in marine environment, and surface waters worldwide. The *vibrio cholerae* is a short, curved, cylindrical, rod, about 1.5 µm × 0.2-0.4 µm in size, with rounded or slightly pointed ends. The cell is typically comma shaped but the curvature is often lost on subculture. S shaped or spiral forms may be seen due to two or more cells lying end to end. Pleomorphism is frequent in old cultures. The other most commonly found is *Staphylococcus aureus,* they are spherical, cocci, approximately 1 µm in diameter, arranged characteristically in grape like clusters. A few strains possess microscopically visible capsules, particularly in young cultures.

On nutrient agar, after incubation for 24 h, the colonies are large (two-four mm diameter), circular, smooth, shiny, opaque and easily emulsifiable. Most strains produce golden yellow pigment, though some may be white, orange or yellow. The pigment does not diffuse into the medium pigment production occurs optimally at 22^{o}C and only in aerobic cultures. Pigment production is enhanced when 1% glycerol mono acetate or milk medium. The pigment is believed to be a lipoprotein allied to carotene.

Many different methods and technologies are available for the isolation of genomic DNA. In general, all methods involve disruption and lysis of the starting materials followed by the removal of proteins and other contaminants and finally recovery of the DNA. Removal of protein is typically achieved by digestion with protease K followed by salting out, organic extraction or binding up of DNA to a solid phase support. DNA is usually recovered by precipitation using ethanol or isopropanol. The choice of a method depends on many factors that are required for downstream application and the time and expense.

MATERIALS AND METHODS

Collection of Samples

Two different species of fresh prawns (*Peneaus monodon, Acetes indicus)* were collected from the retail market at kollam, India and transferred to the laboratory within one hour.

Isolation of Bacteria from Prawns Intestinal Gut

The prawn samples were cleaned with distilled water and dissected at the ventral side to obtain the intestinal gut under sterile conditions. About 100 g of the gut samples from each prawn were taken and homogenized in flasks containing alkaline peptone water for Vibrio *cholerae* and isolation and phosphate buffer solution for *staphylococcus aureus* and *Vibrio cholera* [10 g + 90 mL APW].

1. 1 g samples is diluted with 10 mL APW and allow for incubation (primary enrichment).
2. The transfer of loop full culture on to TCBS plate to get isolated colonies.
3. Four loop full culture tube 10 mL alkaline PW and allow for incubation 37^0C 24 h (secondary enrichment)
4. Then it is streaked on TCBS plates and allow for incubation, followed by typical colonies identified.
5. The standard *Staphylococcus aureus* [90 mL phosphate buffer + 10 g sample]; Serial dilution up to 10^{-5} and Transfer 1 mL to BPA plate hence, typical colonies were identified

Gram Staining for Differentiation of Bacteria

This is the most important differential technique used in bacteriology. There are two groups gram-positive and gram-negative bacteria. Christian gram in 1884 developed this method. Steps of gram staining are follows:

1. Prepare the bacterial smear on a glass slide and Heat fix using a spirit lamp.
2. The bacterial cells are heat fixed on slide. Put a drop of crystal violet and wait for 2-3 min and remove excess stain with tap water.
3. The bacterial cells are stained. Stain with Gram's iodine and wait for 1-2 min, wash excess stain with tap water.
4. The bacterial cells are stained and put the slide in a beaker containing 98% ethanol to decolorize the stain.
5. Stains may or may not be decolorized. Put 1 drop of saffranin and wait for 2-4 min.
6. The bacterial cells may or may not be stained with saffranin.
7. The slide is washed with tap water, mount in glycerine and observe using oil immersion in microscope.
8. If the bacterial take gram stains and appear dark blue colored they are gram positive bacteria.
9. If the bacterial take gram stains and appear dark pink colored they are gram negative bacteria.

Isolation and Estimation of Genomic DNA from Prawn

Estimation of *Peneaus monodon* and *Acetes indicus* by CTAB extraction method.

1. Weigh 1.5 g sample, rinsed with tap water followed by distilled water, and then dried using a tissue paper.
2. Transferred the samples to pre autoclaved mortar and pestle, and ground well by adding CTAB extraction buffer.
3. Transfer the ground tissue to micro centrifuge tube. Added extraction buffer to make free flow.
4. Added 7 µL β-mercaptoethanol kept in water bath at 65^0C for 45-min. Centrifuged the tubes at 10000 rpm for 10 min at 25^0C.
5. Transferred the supernatant to fresh vials and add 200 µL phenol: chloroform: isoamylalcohol (25:24:1) mixture and mixed well. Centrifuged the tubes at 25^0C for 10 min at 10000 rpm. Repeated this step.
6. Transfer the supernatant to fresh vials and added 300 µL chloroform: isoamylalcohol (24:1) to it. Centrifuged the tube at 25^0C.
7. Collect the supernatant and added 60-µL 3M sodium acetate (pH 4.8), added double the volume-chilled isopropanol to precipitate DNA, then kept it at -20^oC for half an hour, and centrifuged at 10,000 rpm for 10 minute at 4^0C.

8. The pellet added 200 µL at 70% ethanol rinsed gently and centrifuged for 5 min.
9. Air dry the pellet and dissolved in 100 µL sterile distilled water or TE buffer.
10. Prepare 0.8% agarose gel and electrophoresis was carried out at 65 V.

Agarose Gel Electrophoresis

1. Take 100 mL 1 x TAE buffer was made from 50 x TAE buffer.
2. 50 mL of 1xTAE buffer was taken in a conical flask and added 0.4 g agarose .The agarose was melted and dissolved by heating. The solution was cooled to 40 -45^0_C and added 6 µL of ethidium bromide and mixed without forming bubbles.
3. The gel casting tray was first wiped with alcohol and was placed in the gel-casting unit. The comb was placed at the appropriate position and the gel was poured and allowed to solidify which takes about 30-45 min at room temperature.
4. When the gel is solidified, the comb is pulled out.
5. The gel was placed in buffer tank containing 1 x TAE buffer.
6. 15 µL of DNA samples mixed with 3 µL gel loading dye was loaded into the well using micropipette.
7. A DNA marker was loaded in one lane.
8. Current was switched on (65 V) and allowed DNA to run on the gel.
9. The current was switched off when the tracking dye reached two-third of the gel.
10. The gel was viewed under UV trans-illuminator.

Estimation of Genomic DNA

Quantifying of genomic DNA by using UV Spectrophotometry. The absorbance minimum at 230 nm and 260 nm. 50 µg/mL solution of DNA gives a value of 1.0 at 260 nm (A_{260}) proteins are the main contaminants in nucleic acid extract. Therefore, they have absorption maxima at 280 nm (A_{280}).

1. The spectrophotometer was switched on and allowed to warm up to 10-15 min before use.
2. The UV lamp was switched on after it was calibrated and adjusted to desired wavelength using sterile distilled water.
3. The instrument was set at zero absorbance.
4. The absorbance of the sample was noted at 260 nm and 280 nm.
5. Quantity and quality of DNA was calculated.

Quantity of DNA: 56 × A260 × Dilution factor/ 1000

Quality of DNA: A_{260} /A_{280}

RESULTS AND DISCUSSION

Isolation of *vibrio cholerae* and *staphylococcus aureus* from the gut of prawn (*P. monodon and A. indicus*). In the study, two commonly found pathogens of fish are *vibrio cholerae* was isolated by streak plate method and *staphylococcus aureus* by spread plate method. The isolated colonies of *vibrio cholerae* on TCBS agar plates were large, smooth yellow slightly flattened with opaque center and translucent peripheries. Gram staining test was done to conform the presence of *vibrio cholerae*. The isolated colonies of vibrios are gram-negative curved rods. Similarly the isolated colonies of *Staphylococcus aureus* on BPA plate were black circular smooth convex

conformed by performing gram staining test while were gram positive in pairs.

Prawn contain good amount of organic and inorganic constituents. The main constituent are proteins amino acids , carbohydrates, and lipids in addition to that prawn also contain significant proportion of minerals (Calcium, Magnesium, Manganese and Chlorine) and vitamins (A, C and D) (Sulude *et al*., 2000).

Isolation of Bacteria from Prawns Intestinal Gut

Prawn have great nutritional value but may be the cause of many food borne illness also .In our present study our major objective was to isolate gut microorganism from *P. monodon* and *A. indicus*. *V. cholerae* and *S. aureus* two potential pathogens were isolated from the gut of *P. monodon* and *A. indicus*. These microorganisms are found to cause many illnesses in human and their infections can be even fatal too so it is recommended to remove gut from prawns before consuming (Figure 1). The similar result has been reported by Anas *et al.* (2010), Abhay *et al.* (2003), and Bruce *et al.* (1991).

Isolation and Estimation of Genomic DNA from Prawn

Genomic DNA was isolated from two prawn species *P. monodon* and *A. indcus* .The isolated genomic DNA was subjected to agrose gel electrophoresis in 0.8% gel. The DNA bands were observed in UV transilluminator.

UV spectrophotometer was used to estimate the quantity and quality of isolated DNA samples. The quantity of isolated DNA samples ranged from 1.62-1.95 .Good quality DNA ranges from values 1.75-1.90. Almost all DNA samples were of good quality except *P. monodon* sample 1 (Shown in Table 1 and Figure 2). It was slightly RNA contaminated. The similar result has been reported by Chiaki (2001), David *et al.* (2008), Desmond (2000), Hopkins *et al.* (1993) and Kirchhoff *et al.* (1987).

Figure 1: Culture Plate of the Bacteria Isolated from Prawns Intestinal Gut and Identification of Microorganism

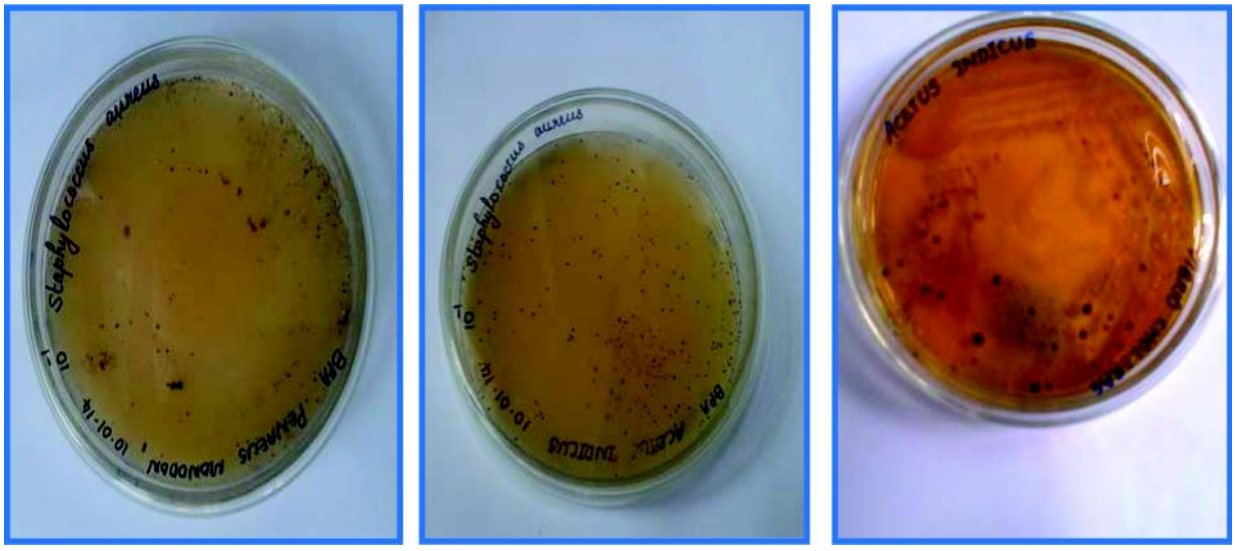
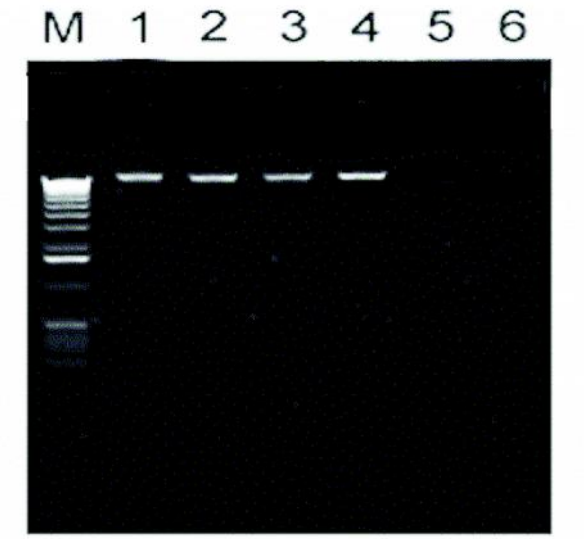


Table 1: Estimation of Quality and Quantity of Genomic DNA from P. monodon and A.indcus

Species	A_{260}	A_{280}	Quality A_{260}/A_{280}	Quantity $A_{260}\times50\times600/1000$
Peneaus monodon				
Sample 1	0.065	0.034	1.91	1.95
Sample 2	0.063	0.035	1.80	1.90
Acetes indicus				
Sample 1	0.054	0.029	1.87	1.62
Sample 2	0.059	0.032	1.84	1.77

Figure 2: Agarose Gel Electrophoresis of Various Samples of P. monodon and A.indcus



Note: Lanes are as follows; M lane – Marker, 1 lane – Peneaus monodon sample 1, 2 lane – Peneaus monodon sample 2, 3 lane – Acetus indicus sample 1, 4 lane – Acetus indicus sample 2.

CONCLUSION

Prawns are important types of seafood that are consumed worldwide. They are good source of protein. They are very low in fat and calories, hence making them a very healthy choice of food. Several microorganisms are known to inhabit the gut of prawns. The study aims at isolating the gut microorganisms such as *vibriocholerae* and *staphylococcus aureus* from *P. monodon* and *A.indicus* .Vibrio were isolated by streak plate methods and *staphylococcus* were isolated by spread plate methods. There microorganisms can cause several diseases to man and including food poisoning .*Staphylococcus aureus* cause diarrhoea , increase dehydration, etc., while *vibriocholerae* causes cholera, renal failure, cramps , hypertension, sunken eyes, etc.

CTAB extraction method was used for the isolation of genomic DNA. The isolated DNA samples were subjected to agrose gel electrophoresis .The buffer used here was 1xTAE and the DNA bands were observed in UV

transilluminator. Spectrophotometric readings of the isolated DNA samples were taken at 260 and 280 nm and these readings were used to estimate the quantity and quality of DNA samples isolated. Prawns are excellent source of protein, a good source of omega 3 fatty acids and a great way to get iron, zinc and vitamin E. They are also low in saturated fats. Inspite of all these good qualities, the gut of prawns harbor different pathogenic microorganisms .So it is better to consume prawns only after renowing their gut.

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