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PROBIOTICS FROM *ARTEMIA* AND ITS APPLICATION ON CONTROLLING THE BACTERIAL PATHOGENS IN AQUACULTURE SYSTEM

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ABSTRACT

The present work was conducted to isolate the gut bacteria of *Artemia* and to use them as probiotics to control the bacterial pathogens in aquaculture systems. In the present study, two of bacterial species were isolated from the gut of *Artemia* which was collected from Puthalam saltpan (Kanyakumari, India). Among the gut isolates of *Artemia*, best two species, *Alteromonas* sp. and *Acetobacterium* sp. were selected. These two isolates were tested for its efficiency and antagonistic activity against V*ibrio harveyi* and *Alteromonas* sp. *Artemia* gut isolates, when introduced in to the gut of adult *Artemia* along with pathogenic bacteria, they multiplied and colonized faster and seen in higher number than the pathogenic bacteria . During the present study it was found that *Acetobacterium* sp. effectively controlled the growth of *V. harveyi* and *Alteromonas* sp. than *Aeromonas* sp. Hence this study adds more towards the use of gut isolates of *Artemia* as probiotics and on controlling the bacterial pathogens in an aquaculture system to solve the bacterial Pathogenicity.

KEYWORDS: ***Artemia*, Aquaculture, Gut isolates, Probiotics and Pathogens**

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INTRODUCTION

The probiotics are defined as a live microbial adjuvant which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards diseases, or by improving the quality of its ambient environment. Although probiotics may also contribute substantially to the health and zoo technical performance in a nutritional way and although it is sometimes impossible to separate feeding of aquatic organisms from environmental control, this review is limited to the use of probiotics as a biological control agents in aquaculture[1]. A common way to select probiotics is to perform *in vitro* antagonism tests, in which pathogens are exposed to the candidate probiotics or their extracellular products in a liquid or solid medium[2]. Compounds other than bacteriocins and antibiotics have been suggested to play a major role in the amensalism that may occur between bacterial species. It was found that a large proportion of marine bacteria produced bacteriolytic enzymes against *V. Parahaemolyticus*[3]. Many studies have demonstrated the presence of bacterial strains showing *in vitro* inhibition towards pathogens known to occur in aquaculture[4]. This shows that the ability to inhibit other bacteria is not uncommon for bacteria found in aquaculture environments. However, it has not been demonstrated that production of such inhibitory compounds occurs under *in vivo* conditions and the ecological relevance of the production of inhibitory compounds toward other bacteria is still unclear. In recent years, increased interest has been focused on the search for probiotics that may improve health conditions in the intensive rearing of marine organisms[5]. In the present study, the probiotic bacteria were isolated from healthy adult *Artemia* gut and were identified. Experiments were also conducted to study the role of these bacteria on antagonistic activity against pathogenic bacteria.

MATERIALS AND METHODS

1. Sample collection

The healthy *Artemia* were collected from Puthalam saltpan of Kanyakumari District and were transported to the laboratory under aseptic condition.

2. Isolation of bacteria from Artemia gut

The gut of *Artemia* was dissected and the gut tissues were ground and the samples were plated on nutrient agar and TCBS plates by spread plate technique. The plates were kept for incubation at 37ºC for 24-48 hours. After incubation the colonies were counted and the strains were isolated and stored in nutrient agar slants at 4ºC for further use.

3. Identification of bacteria

3.1. Microscopic examination

The microscopic examination of bacteria was done by following Gram's staining and Motility Test.

3.2. Biochemical identification

Various biochemical tests were performed for the identification of bacteria present in the gut. These tests include Indole production test., Methyl red test, Vogues – Proskauer test, Citrate utilization test, Oxidase test, Urease test, Triple sugar iron test (TSI), Carbohydrate fermentation test, Starch hydrolysis and Casein hydrolysis test.

4. Pathogenicity test of Artemia

After five days of establishment with gut isolated bacteria, the control and experimental *Artemia* were challenged with already isolated aquatic pathogens *V. harveyi* ($4.75x10^{-5}$ CFU/ml) and *Aeromonas* sp. ($5.35x10^{-5}$ CFU/ml). Test pathogens from the respective dilution were added individually in the *Artemia* culture medium and reared the *Artemia* for 5 days. During the time of experiment, the survival rate of *Artemia* was noted. After pathogenic challenge test, the gut was removed from the challenged *Artemia* and enumerated the colonized pathogenic and gut bacteria.

5. Antagonistic activity test

Antagonistic activity of gut isolated bacteria against pathogens preparation was assayed by agar well diffusion method[6].

6.SDS Poly Acryl amide Gel Electrophoresis (SDS- PAGE)

The characterization of extracellular metabolites was done by SDS- PAGE[7].

RESULTS

1. Isolation and identification of Artemia gut isolated bacteria

The microorganisms were isolated from the gut of *Artemia.* The samples were streaked on 20% Nacl containing nutrient agar medium. The isolated colonies were subjected to Gram's staining, motility, Indole production, MR-VP, Catalase, Oxidase, Triple sugar ion test and carbohydrate fermentation test. Based on the results obtained, two species such as *Alteromonas* sp, *Acetobacterium* sp. were identified.

2. Colonization of gut isolated bacteria and pathogen in gut of Artemia

In *Alteromonas* sp. and *Acetobacterium* sp. the colonization rate was found to be as 5.14 and 5.62 x 10^5 CFU/ml respectively (Table-1) .

Table 1

Colonization rate of gut isolated bacteria and aquatic pathogens in the gut of Artemia

Sl. No.	Microorganisms	Colonization rate (x10^5 CFU/ml)
1	*Alteromonas* sp	5.14 ± 1.92
2	*Acetobacterium* sp	5.62 ± 2.89
3	*V. harveyi*	3.22 ± 3.12
4	*Aeramonas* sp	3.43 ± 2.92

3. Colonization of gut bacterial isolates in the gut of Artemia when inoculated along with pathogen

Among the two pathogens used for challenge study, *V. harveyi* colonized more in number than *Aeromonas* sp. From the results obtained, it was found that the gut isolates controlled the colonization of challenged pathogen in the gut of adult *Artemia*. The results are presented in Table-2.

Table 2

Colonization of gut bacterial isolates in the gut of Artemia when inoculated along with aquatic pathogens

Sl. No.	Gut isolated bacteria challenged With pathogen	Gut Isolated Bacteria (x10^5 CFU/ml)	Pathogens (x10^5 CFU/ml)
1	*Alteromonas* sp + *V. harveyi*	5.82 ± 1.92	2.24 ± 1.93
2	*Alteromonas* sp + *Aeromonas* sp	5.46 ± 1.82	1.21 ± 1.18
3	*Acetobacterium* sp + *V. harveyi*	4.92 ± 2.84	2.23 ± 2.24
4	*Acetobacterium* sp + *Aeromonas* sp	4.3 ± 3.14	1.41 ± 2.41

4. Antimicrobial activity of gut isolated bacteria against pathogens

The results of antimicrobial test showed that the gut isolated bacteria inhibited the growth of pathogenic bacteria. The gut isolated bacteria such as *Alteromonas* sp. and *Acetobacterium* sp. containing extra cellular metabolites loaded well showed zone of inhibition against the pathogens. In *Alteromonas* sp and *Acetobacterium* sp the zone of inhibition observed were found to be as 4.25 and 2.30 mm respectively. The zone of inhibition noticed against *V. harveyi* and *Aeromonas* sp. were found to be as 3.88 and 4.16 mm respectively (Table-3).

Table 3
Antagonistic activity of extracellular metabolites of gut isolated bacteria against aquatic pathogens

Sl. No	Pathogens	Gut isolated bacteria	Zone of Inhibition (mm)
1	*Aeromonas* sp	*Alteromonas* sp	4.25 ± 1.42
		Acetobacterium sp	2.30 ± 1.06
2	*V. harveyi*	*Alteromonas* sp	3.88 ± 1.36
		Acetobacterium sp	4.16 ± 1.85

5. SDS-PAGE analysis

The results of SDS – PAGE analysis showed that the molecular weight of protease was found to be as 42 KDa. This result showed that the protein response for protease activity of the two gut isolates was same.

DISCUSSION

The background behind the present study was that the *Artemia* even living in contaminated water with pathogenic bacteria, the animals are not affected by them. This phenomenon is encouraging to isolate the gut bacteria from *Artemia*. This may make the *Artemia* to live with pathogenic bacteria without any disease symptom. Among the ten species isolated from the gut, two bacterial species namely *Alteromonas* sp. and *Acetobacterium* sp. were found to be probiotic one. The use of *Lactobacillus* sp. as the probiotic bacteria in the giant shrimp was demonstrated from the earlier research conducted by Jiravanichpaisal and Chuaychuwong[8] that sates about effective treatment of *Lactobacillus* sp. against Vibriosis and white spot diseases in *Peanus monodon*. Our results also favor towards the earlier study done by Direkbusarakom[9] which reports that the *Vibrio* sp. dominate in shrimp hatchery against some fish pathogens. The mode of action of the probiotic is rarely investigated, but possibilities include in competitive exclusion, i.e. the probiotics actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and alteration of microbial metabolism by the stimulation of host immunity[10]. The two of gut isolates of *Artemia* were also found to secrete extra cellular metabolites that control other pathogenic bacteria. This is one of the unique characters of probiotics candidate, which was observed during the present study.

CONCLUSION

The major goal of this work was to isolate the gut bacteria from *Artemia* and apply them as the probiotic one to control the aquatic pathogens. These two probiotic bacteria were selected and applied on the aquatic pathogens to control the growth of the pathogens in the gut of adult *Artemia* as well as in the rearing environment. Among the two species namely,

Altermonas sp. and *Acetobacterium* sp., *Alteromonas* sp. inhibited the growth of *Aeromonas* sp. better than the *Acetobacterium* sp. One of the methods of controlling the neighbor bacteria is by the secretion of lytic enzymes, which was assayed by SDS-PAGE. This enzyme helps the bacteria to destroy the proteinacious substance present in other bacteria and thereby controlling the growth of pathogenic bacteria. In the present study, the two isolates obtained from the gut of *Artemia* secrete, namely amylolytic and proteolytic enzymes. Of these two probiotic isolates, *Acetobacterium* sp. secreted more amylase and protease enzyme than the *Alteromonas* sp. and hence the utility of *Acetobacterium* sp. in controlling the pathogenic bacteria are more than the *Alteromonas* sp. Thus, while concluding it can be stated that the gut bacterial isolates of *Artemia* can be used as a potential agent against controlling the aquatic pathogens in the aquaculture systems.

CONFLICT OF INTEREST

Conflict of interest declared none.

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