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Antibacterial Activity of Intestinal Bacteria of Gold Fish *Carassius auratus*

M.R. Rajan* and P. Akilandeswari

Department of Biology, Gandhigram Rural University, Gandhigram - 624 302, India.

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Intestinal bacteria isolated from the Gold fish *Carassius auratus* were examined for inhibitory effects against the fish pathogens *Aeromonas hydrophila* and *Pseudomonas aeruginosa* using double-layer method, disc-diffusion method and streak method. The bacteria isolated from the intestine of Gold fish were identified as *Bacillus* spp showed higher activity against *Aeromonas hydrophilla* when compared to *Pseudomonas aeruginosa*. The result suggest that *Bacillus* spp may be a suitable strain and as a biocontrol agent in fish against pathogens.

Key words: Antibacterial activity, intestinal bacteria, Gold fish.

Fishes are continuously exposed to a wide range of microorganisms present in the environment and take a large number of bacteria into their gut from water, sediment or food. Most of these bacteria are temporary residents and disappear from the intestine immediately after invading. However, some bacteria which possess the ability to tolerate the low pH in gastric juices, resist the action of bile acids, lysozyme secreted in intestines and the immune responses, and adhere to the mucus or enteric wall surface, could persist for a relatively long time and eventually make up intestinal bacteria specific to each host animal (Sera *et al.,* 1974; Sugita *et al.,*1982; Sugita *et al.,* 1988; Onarheim and Raa, 1990; Westerdhal *et al.,* 1991 and Olsson *et al*., 1992). Bacteria producing antibacterial substances were recently isolated from marine fish intestines (Onarheim and Raa, 1990; Westerdhal *et al.,* 1991; Olsson *et al.,* 1992)thereby suggesting that these bacteria may inhibit the establishment of invading bacteria in the fish intestine. It has been reported that antibacterial substances are produced by aquatic bacteria isolated from various sources, and seem to play an important role in the antagonism of bacteria in aquatic ecosystems (Gauthier and Breittmayer, 1979; Nair and Simidu, 1987 and Dopazo *et al.,* 1988). In the present study, the intestinal bacteria isolated from the Gold fish were examined for their antibacterial ability against the pathogens *Aeromonas hydrophila* and *Pseudomonas aeruginosa.*

MATERIAL AND METHODS

Gold fish (4± 0.01 g) were collected from ornamental fish farm, Kadachanenthal, Madurai, Tamilnadu, India and transported to the laboratory in polythene bags filled with aerated water. Intestinal contents from the gold fish were serially

* To whom all correspondence should be addressed.
Tel.: +91 - 0451 2452371-376
E-mail: mrrrajan1961@yahoo.co.in

diluted with sterile water and plated on nutrient agar. Plates were incubated at 37º C for 24 hrs. After incubation, bacterial colonies were invalid at random from each plate and examined for gram reaction, spore formation, cellular morphology, pigmentation, motility and production of oxidase and catalase and identified at the genus level.

The inhibition test was carried out using the double- layer method, disc- diffusion method and streak method. In double-layer method, the tested strains isolated from the fish intestine is inoculated and incubated at 37 º C for 24 hrs in liquid medium. Macro colonies of the isolates were created on agar plates by inoculating 0.1 ml droplets of the above culture .After incubation, the colonies were killed with chloroform vapors for 15-20 minutes. Target strains were inoculated and incubated at 37º C for one day in liquid medium. Then the culture is diluted with distilled water until the optical density is reached at 0.2 at 610 nm. (This means the liquid medium having 10-8 cells). The culture is further diluted 200 times and suspended in soft agar which was poured over the plates (4.5 ml/ plate). After overnight incubation the bacterial strain which produced a clear inhibitory zone indicated the antibacterial substance produced. In disc-diffusion method, an active strain was incubated in liquid medium. Then centrifuged at 9600 rpm for 15 min, and filter the supernatant fluid through a filter paper. Discs were prepared using this supernatant. Target strains were prepared by diluting with distilled water until the OD reached 0.2 at 610 nm. The culture is further diluted 200 times and 0.1 ml is spread over the agar plates and place the prepared disc on the agar plates and incubated at 37 ºC overnight. Antibacterial activity by the formation of clear zone. In streak method, the isolated and target strains were streaked side by side and incubated for 24 hrs at 37º C overnight. After incubation the isolated strain inhibits the growth of target strain. This indicates the positive result.

RESULTS AND DISCUSSION

The experimental work indicates that the isolated bacteria is identified as *Bacillus* spp. Using various biochemical tests as given in Table 1. Bacillus spp showed a very good antagonistic effect against two pathogens *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. In double – layer method, the antibacterial activity of Bacillus spp against *Aeromonas hydrophila* was more sensitive than *Pseudomonas aeruginosa*. Rosenfeld and Zobell (1947) carried out a detailed study of antibiotic-producing marine microorganisms. An average of 3.2 % of tested strains exhibited antibacterial activity against 18 different species, including 12 *Aeromonas spp* and pathogenic bacteria tested (Sugita *et al,* 1996).

Table 1. Biochemical test

Test performed	Observation
Gram reaction	Positive
Motility	Motile
Mannitol	Negative
Voges Proskauer	Positive
Oxidase	Positive
Catlase	Positive
Glucose	Positive (acid + gas)

In disc-diffusion method, the zone of inhibition was found against the pathogen *Aeromonas hydrophila*. The results reveals the increasing zone of inhibition against increasing concentration of samples. The antibacterial effect of bacteria is generally due to any of the following combination such as production of antibiotics, bacteriocins, lysozymes and proteases and alteration of pH values by organic acid production (Sugita *et al* ., 1988).The marine bacterium D2 produce antibacterial protein and was found to be kill *Vibrio anguillarum* cells at a concentration of ug/ ml (Sally G. James *et al* 1996). Finally in streak method the growth of Bacillus spp inhibit the growth of Aeromonas hydrophila and *Pseudomonas aeruginosa*. The antagonistical activity of the Lacto bacilli have been analysed on the basis of parallel streak method against 6 strains of Aeromonas and Psuedomonas genus pathogenic to fish (Jankauskiene, 2000). Lacto bacillus separated from the content of the intestinal tract of carp. Antagonistic activity has been detected in 165 strain of 168 investigated. Three strains of *Lacto bacillus* showed no antagonistic activity (Austin *et al*., 1995).

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