



Research Article

ISOLATION, ESTIMATION OF BACTERIAL LOAD, AND CHARACTERIZATION OF GUT MICROBES OF THREE GARRA SPECIES FROM BHAVANI RIVER, WESTERN GHATS, TAMIL NADU, INDIA

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ABSTRACT

Microbiomes play an important role in fish physiology, particularly in digestion, by metabolizing largely indigestible feed components for the host or synthesis of essential micronutrients. The gut flora has a dynamic effect on the host's gut and systemic immunity. This study is the preliminary exploration of gut microflora of three species *Garra mccllellandi*, *Garra stenorhynchus*, and *Garra gotyla stenorhynchus*. The total bacterial load ranges from 66×10^8 CFU/g to 81×10^8 CFU/g in the gut sample. It was found that the maximum load in *G. stenorhynchus* with 81×10^8 CFU/g followed by *Gg. stenorhynchus* with 72×10^8 CFU/ml minimum in *G. mccllellandi* with 66×10^8 CFU/ml. The isolates were subjected to various biochemical characterizations such as the Indole test, Voges Proskauer test, Methyl Red test, Carbohydrate Utilization Test, Citrate Utilization Test, Starch Hydrolysis, and Catalase Production activity. The three fish gut samples are screened for antagonistic properties for certain human pathogens which show zero antagonistic activities. The present preliminary study shows variation in the gut microflora and these microbes may be used for the development of probiotics and drug production

Keywords: Freshwater fish, Gut microflora, Bacterial load, biochemical characterization.

INTRODUCTION

The Microbial communities are called "microbiomes" which colonize in all parts like the mucosal surfaces of fish on the skin, gills, and digestive tract (Merrifield and Rodiles, 2015). The main reason for the gut microbiome variation is genetic variation, environmental factors, or dietary habits (De Bruijn *et al.*, 2018). The exogenous enzymes which are synthesized by microbiota help to increase digestion efficiency gastrointestinal tract (Ray *et al.*, 2012). The plant starches and cellulose which are largely indigestible can be metabolized by bacterial secretions. The fish microbiomes can be enhanced productivity by increasing the feed conversion efficiency (Kuebutornye *et al.*, 2020). The mucous on the skin and gills have colonies of beneficial microbes that play an important role in fish disease resistance (Zhang *et al.*,

2018). The Gut microflora plays an important role in the digestive process, growth, and disease of the host. The gut bacterial properties such as the ability to tolerate the low pH in gastric juices resist the action of bile acids, lysozyme secreted in intestines, immune responses, and adhere to the mucus or enteric wall surface, intestinal microflora specific to each host animal and providing essential nutrients such as vitamin K (Masaaki Yoshida *et al.*, 2022). The gut microbiome produces antibacterial substances which inhibit the establishment of invading bacteria in the fish intestine (Kanagasabapathy Sivasubramanian *et al.*, 2012). The feeding strategies, developmental stages, and changing environmental conditions may change the composition of the gut microbiome (Ahmad Jammal *et al.*, 2017).

The fish in the family of Cyprinidae species are slim cyprinids with flat belly and sucking mouth (Froese *et al.*,

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2017). Garra (sucker fish) is an omnivorous, eating alga, plankton, and small invertebrates that they suck off substrates like rocks. In this genus, intestinal length is remarkably constant within species and varies a lot between species, and it is useful to distinguish species and that dietary shifts have played a significant role in the evolution of Garra (Stiassny *et al.*, 2007). In the present study, an attempt has been made to investigate the variation in gut bacterial species in the gastrointestinal (GI) tract of freshwater fishes namely *Garra stenorhynchus*, *Garra maclellandi*, and *Garra gotyla stenorhynchus*. The bacterial gut flora was isolated and studied the bacterial load, morphological and biochemical characterization of isolated bacteria, and analyze the diversity of bacteria based on morphological and biochemical characteristics.

MATERIALS AND METHODS

Sample collection

The fishes *Garra stenorhynchus*, *Garra maclellandi* and *Garra gotyla stenorhynchus* were collected from the Bhavani River of western Ghats in the Coimbatore district. For each species, 15 specimens were collected and were transformed into a laboratory by using sterilized bags.

Isolation of gut microflora

The surface of the fish was sterilized with 70% alcohol and the gut was aseptically dissected from the animal's musculature under a laminar airflow chamber. The gut was weighed and placed into a 10ml of sterile double-strength phosphate-buffered saline (PBS) solution and homogenized. The bacterial suspension was streaked on nutrient agar media and the plates were incubated at 37°C for 24 h.

Spread Plate Method

A first stock solution was prepared with 9 ml distilled water in a test tube and adding 1 ml of sample to the stock solution is considered as 10^{-1} and the same procedure is repeated for 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} . The 100µl of a measured volume of each dilution is used to make a series of spread plates using an L-shape glass rod and incubated in an incubator at 37°C for 24 hours. Depending on the estimated concentration of organisms in a sample, the extent of dilution is determined. After the growth of colonies is counted using a formula

*Serial dilution = Number of colonies / Amount of plating
* Dilution factor.

Morphological Characterization

Morphological Characterization of the colony was done with the help of a compound microscope. The characteristics observed include colony size, shape, color opacity, and gram staining.

Biochemical studies

The biochemical characterization of the isolated bacteria includes the Indole test, Voges Proskauer test, Methyl Red test, Carbohydrate Utilization Test, Citrate Utilization Test, Starch Hydrolysis, and Catalase Production activity.

Antagonistic Activity of Gut Isolates

The well diffusion technique was followed to determine the antagonistic property of bacteria isolated from the gut of three fishes.

RESULTS AND DISCUSSION

The total bacterial load ranges from 66×10^8 CFU/g to 81×10^8 CFU/g in the gut sample. It was found that the maximum load in *Garra stenorhynchus* was followed by *Garra gotyla stenorhynchus* with 72×10^8 CFU/ml and minimum in *Garra maclellandi* with 66×10^8 CFU/ml (Figure 1). Morphological characterization techniques show the gram-positive, the microbes which retained the crystal violet were identified as gram-positive and the microbe which does not retain the crystal violet is identified as gram-negative. Among the isolates, sample 1 is positive another samples 2 and 3 are negative. Mortality of the sample 1 is negative and samples 2, and 3 were shown positive. Primary cultures were subcultures in the nutrient broth. For further studies, the single colony was isolated based on its morphology and subculture (Table 1). The biochemical tests for the isolate are given in (Table 2). The sample 1 results show that the isolates are positive for the Catalase, Voges Proskauer, Citrate utilization, and Urease, and negative for the Indole and Oxidase tests. These results confirm that the isolate is *Staphylococcus* species. The results of sample 2 showed that the isolates are positive for the Methyl red and Catalase test and negative for Indole, Citrate utilization test Voges Proskauer test, Urease test, and Oxidase test. These results confirm that the isolate is *Salmonella species*. The results of sample 3 showed that the isolates are positive for the Indole, Methyl red, and Catalase test and negative for the Citrate utilization test Voges Proskauer test, Urease test, and Oxidase test. These results confirm that the isolate is *E. coli*.

The antagonistic properties of a bacterial strain isolated from the gut of three fish species were (Table 3) tested for their antagonistic ability against highly virulent human bacteria such as *Listeria monocytogenes*, *Salmonella thypi*, *Salmonella aureus*, and *E. coli*. which showed negative results. The fishes have contact with the natural biota because of their aquatic environment and the various microbes are constantly ingested along with food. The transient microorganisms have a steadier and more significant collaboration with fish gastrointestinal environments (Chandni Talwar *et al.*, 2018). Fish symbioses, co-inhabitant of fish pathogens, might be a promising source of natural antimicrobial compounds (NACS) alternative to antibiotics, limiting bacterial diseases in fishes (Rafaela *et al.*, 2021). The analysis estimated the bacterial diversity in the gastrointestinal tract of three freshwater fishes. The bacterial load was from

66*10⁸ CFU/g to 81*10⁸ CFU/g in the bacterial culture. The maximum load in the sample of *Garra stenorhynchus* was followed by *Garra gotyla stenorhynchus* with 72*10⁸ CFU/ml and the minimum load in *Garra mccllelandi* with 66*10⁸ CFU/ml. A few variables, for example, bacterial host explicitness, food type, and water asset might make sense of these distinctions. The changes in the habitat and climate might affect the organization of the gastrointestinal miniature biota in fish (Verner-Jeffrey *et al.*, 2003).

Normally *Garra* benefits from the small green plants (green growth) present in the mid-water as opposed to the base mud. It doesn't uninhibitedly feed on huge plant leaves. Taking care of the environment is one of the elements that may be answerable for the presence of gut biota in these fishes. Both marine and freshwater fish have been displayed to have a particular native gut miniature biota and it might change with fish age, status, and natural circumstances (Olafsen *et al.*, 2001). Here, other than Gram-positive microbes *Staphylo coccus*, and a few Gram-negative microscopic organisms ordinarily in the gut of fish

were likewise disconnected and distinguished, like *Salmonella* and *E. Coli*. This ordinary native vegetation can act seriously, barring microorganisms, restraining colonization, and, thus, preventing disease. This work firmly proposes that powerful control of microflora in fish digestion tracts is conceivable by utilizing anti-toxin-delivering microbes. A comparable methodology might be conceivable in marine water fish utilizing gastrointestinal microorganisms with an inhibitory impact against pathogenic microscopic organisms. Subsequently, the current studies show the variation in the gut microflora of various fish species. This type of study will show the significance of business hydroponics as a supplement in formed fish feed or in the type of microscopic organisms' biofilm to accomplish colonization in the fish gut at a more significant level and might be helpful in the improvement of probiotics, drugs as well as in the modern chemical creation.

Table 1. In this table shows the colony morphological characterization of three different samples.

Test	Sample 1	Sample 2	Sample3
Gram Staining	+	-	-
Colour	Purple	Pink	Red
Shape	Cocci	Rod-shaped	Rod-shaped
Nutrient agar	Large, Opaque, Circular, Smooth	Large, Opaque, Irregular, and Transparent	Large, Opaque, Entire, and Colorless
Motility	-	+	+

Table 2. In this table show the results of the biochemical test in three different samples (+) positive result, (-) negative result

Test	Sample 1	Sample 2	Sample3
Catalase test	+	+	+
Oxidase test	-	-	-
Indole test	-	-	+
Urease	+	-	-
Methyl red test	+	+	+
Citrate utilization test	+	-	-
Voges Proskauer test	+	-	-
Strains	<i>Staphylococcus sp.</i>	<i>Salmonella sp.</i>	<i>Escherichia coli</i>

Table 3. In this table shows the result of antagonistic activity of three different samples.

Name of the sample	<i>Listeria monocytogenes</i>	<i>Salmonella thypi</i>	<i>Salmonella aureus</i>	<i>E. coli</i>
Sample 1	-	-	-	-
Sample 2	-	-	-	-
Sample 3	-	-	-	-

(+) positive result, (-) negative result

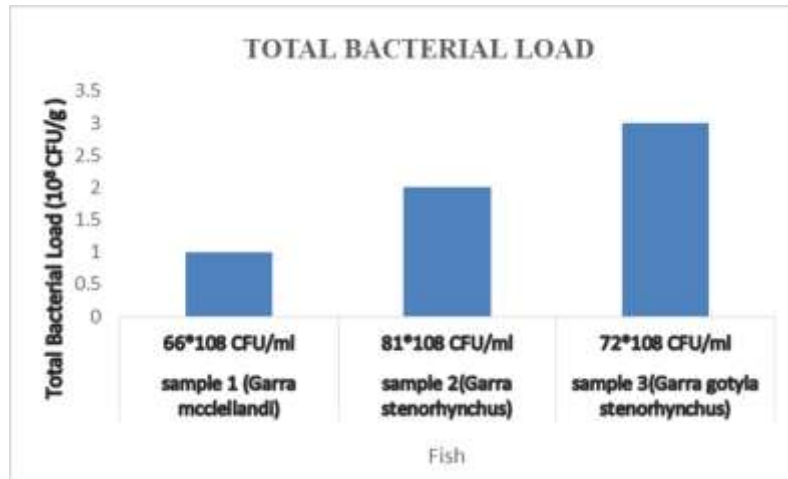


Figure 1. This graph shows the total bacterial count in the three different samples.

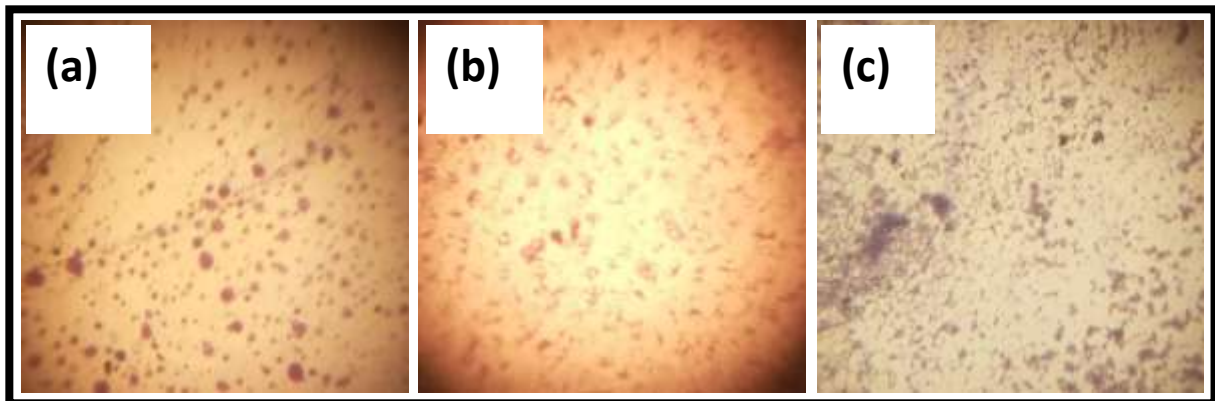


Figure 2. This image shows the gram staining method's microscopic view of the three different samples. (a). Sample 1, (b). Sample 2 and, (c). Sample 3.

CONCLUSION

In this study, the preliminary exploration of gut micro flora of three species *Garra maclellandi*, *Garra stenorhynchus* and *Garra gotyla stenorhynchus*. The total bacterial load ranges from 66×10⁸ CFU/g to 81×10⁸ CFU/g in the gut sample. It was found that the maximum load in *G. stenorhynchus* with 81×10⁸ CFU/g followed by *G. stenorhynchus* with 72×10⁸ CFU/ml minimum in *G. maclellandi* with 66×10⁸ CFU/ml. The three fish gut samples are screened for antagonistic properties for certain human pathogens which show zero antagonistic activities.

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