

MICROBIAL GUT FLORA OF A FRESHWATER FISH *GARRA MULLYA* (SYKES) FROM MUTHA RIVER, NORTHERN WESTERN GHATS, INDIA

B.S. Rudresh^{1*}, N. Dahanukar², G.M. Watve² and N.S. Renukaswamy³

¹J.C.B.M. Collage, Sringeri, Chikmagalur, Karnataka, India

²Department of Microbiology, Abasaheb Garware College, Pune, India

³Central Coffee Research Institute, Chikmagalur, India

*Email: rudresh21@gmail.com

ABSTRACT

Preliminary exploration of gut flora of *Garra mullya* (Sykes) revealed nineteen bacterial strains showing wide diversity of enzyme production, morphological and biochemical characteristics. The factors which govern the diversity assessed through principal component analysis and correspondence analysis show that isolated strain have adaptation to utilization of variety resources like citrate and sugar, and also show tolerance to variety of environmental conditions like pH, salt, and bile in *in-vitro* condition.

Key words: Gut flora, bacterial diversity, *Garra mullya*.

INTRODUCTION

Garra mullya (Sykes) is a fresh water fish commonly encountered in the Indian subcontinent and is very common throughout the Western Ghats of India. It is also a common fish in Mutha river, Pune and has a good population density (Kharat *et al.* 2000). It is commonly sold as a food fish in the local market and is known by the name "Mullya". According to Talwar and Jhingran (1991), *Garra mullya* is classified under the Order Cypriniforme, Family Cyprinidae and Sub-family Garrinae. It has a moderate commercial value. It attains a length of 10 cm and weighs about 25 g. Even though the food and feeding habits of the fish are not studied explicitly, the preliminary study of gut dissection show that the food of *Garra mullya* comprises of algae, phytoplankton and detritus matter.

For different experimental purposes, the microbial gut flora of fish has been studied by several workers. This includes, description of microbial spoilage (Joseph *et al.* 1988), relation between environment and fish micro flora (Horsely 1973), monitoring change in fish form (Allen *et al.* 1983), microbial flora as food of fish (Kamjunke *et al.* 2002), microbial flora help in production of enzymes (Bairagi *et al.* 2002) and antibiotic resistance profile of indigenous flora (Spanggaard *et al.* 1993). The micro flora of reared fish has also been studied as a source of protection against diseases (Sissons *et al.* 1989). For all these reasons, study of bacterial flora of the gut is important. In this study we have analyzed the bacterial gut flora of a freshwater fish *Garra mullya* (Sykes).

The aim of the study was to isolate the bacterial gut flora, study on the enzymes produced by the bacteria, to study morphological and biochemical characterization of isolated bacteria, and to analyze the diversity of bacteria based on morphological and biochemical characteristics.

MATERIALS AND METHODS

Collection of fish: Fresh water fish *Garra mullya* was collected from Mutha river (18°31' N and 73°51' E), Pune, northern Western Ghats. The fresh fish were bought from the local market at Verje.

Isolation of gut flora: The fish was surface sterilized with alcohol (70%) and the gut was removed by dissection. The gut was immersed in sterile saline. Presence and type of bacteria were checked by performing wet mount of the saline suspension. Bacterial suspension was streaked on nutrient agar media. The plates were incubated at 37°C for 24 h.

Morphological characterization: Morphological characterization of colony was done with the help of compound microscope. The characters observed include—colony size, shape, margin, elevation, colour, opacity, consistency, gram staining and motility.

Biochemical characterization: The biochemical characterization of the isolated bacteria include sugar utilization, respiration type, salt tolerance, pH tolerance, bile tolerance, lactose fermentation, amylase production, catalase production, oxidase production, citrate utilization, indole production, methyl red test and Voges prousker test.

RESULTS AND DISCUSSION

Nineteen bacterial strains were isolated from the gut of *Garra mullya*. The colony characters of

the isolates showed varied morphological characters. Isolate 2 was the only motile organism among the 19 isolates. Five were Gram positive and among these three were cocci, two were bacillococci and the remaining 14 were Gram negative. Among these, two were round nine were bacillococci and three bacilli (Table 1).

The biochemical characterization of the bacterial isolates is given in Table 2. It has been observed that the bacteria isolated from the gut of *Garra mullya* were aerobic and showed positive results for catalase activity. The relationship between various bacterial isolates according to their biochemical characters is shown in Fig. 1 and the Principal component analysis and correspondence analysis are given in Fig. 2.

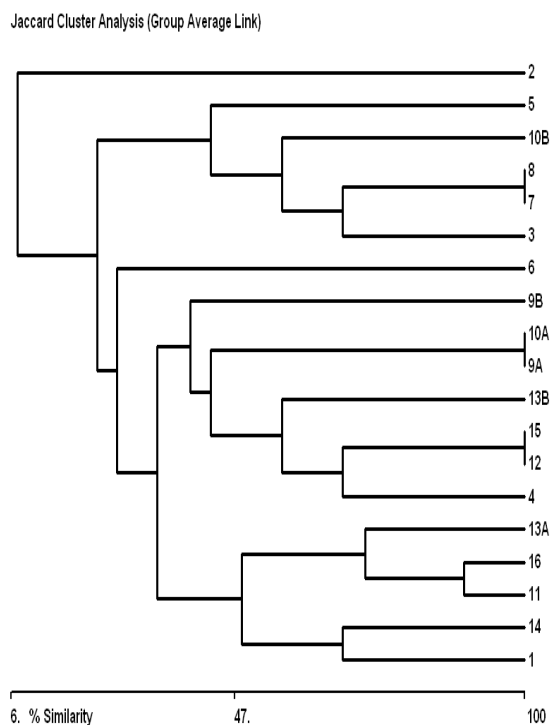


Fig. 1. Biochemical relationship between different bacterial isolates found in the gut of *Garra mullya*.

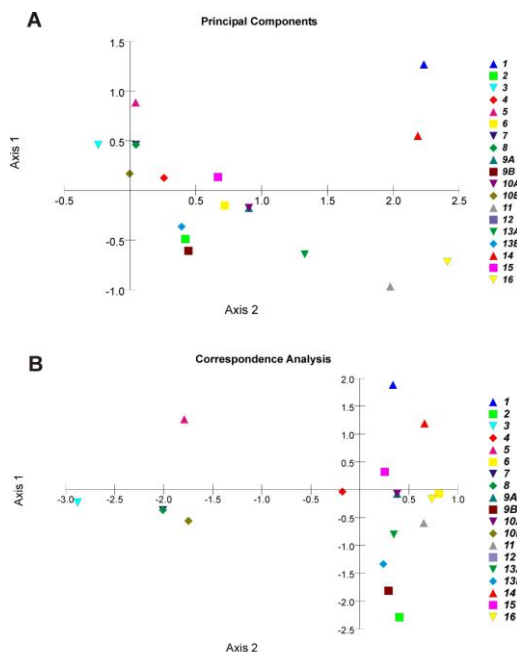


Fig. 2. Principle component analysis (A) and Correspondence analysis (B) of the bacterial isolates, based on biochemical characters.

The initial analysis suggests that the isolates belong to the order Eubacteriales as per the Bergey's Manual of Deterministic Bacteriology (Holt 2000). The isolates are suspected to be from the families Acromobacteraceae or Enterobacteriaceae, nonetheless more analysis is essential for the classification of the isolates. The isolates 2, 9B, 13A, 13B and 16 showed amylase production. It will be interesting to study these isolates and their relationship with the fish as it is known that some bacterial species complement their fish host by producing enzymes that help the fish to consume and digest food (Bairagi *et al.* 2002).

Seven bacterial isolates out of a total of 19 could grow on the McConkey agar plates, of which only three were lactose fermentative. Even though this might be an indication that these species could be from family Enterobacteriaceae it needs confirmation. As only Seven species are bile tolerant we suspect that these could be localized in the area near the gut of the fish. The remaining 11 isolates could be restricted to the distant parts of the intestine.

Out of the 19 isolates, three isolates had interesting morphology. These isolates of bacteria are normal gut flora of *Garra mullya*. If these species are indeed common in this fish and do not occur in other fish inhabiting the habitats of *Garra mullya*, there is a possibility to design a protocol to estimate the number of *Garra mullya* in the water just by looking at the count of these bacteria. The concept is hypothetical but not impossible.

The dendrogram (Fig. 2) based on the biochemical analysis suggests that there is a wide diversity in the organisms found in the *Garra mullya* gut. The factors which govern this diversity can be speculated from the analysis of PCA (Fig. 3A) and CCA (Fig. 3B) analysis. The patterns in PCA and CCA both are governed by biochemical tests involved in utilization and tolerance characterization. This indicates that adaptation to a variety of resource utilization patterns (citrate and sugar utilization in our tests) and tolerance to a variety of environmental conditions (salt, pH and bile) governs the biochemical diversity of *Garra mullya* bacterial flora.

Table1. Colony characters of the isolates.

SN	Isolates	Size (mm)	Shape	Margin	Elevation	Colour	Opacity	Consistency	Gram Staining	Motility
1	1	1.5	Round	Smooth	Flat	White	Opaque	Moist	-Ve R	Non motile
2	2	2.0	Round	Rough	Flat	White	Opaque	Moist	-Ve R	Motility
3	3	1.5	Round	Smooth	Flat	White	Opaque	Moist	+Ve Bc	Non motile
4	4	2.0	Round	Smooth	Flat	White	Opaque	Moist	-Ve Bc	Non motile
5	5	2.5	Round	Smooth	Flat	White	Opaque	Moist	+Ve Bc	Non motile
6	6	1.5	Round	Smooth	Flat	White	Opaque	Moist	-Ve Bc	Non motile
7	7	2.0	Round	Smooth	Flat	White	Opaque	Moist	+Ve C	Non motile
8	8	1.0	Round	Smooth	Low convex	Crème white	Translucent	Moist	+Ve C	Non motile
9	9A	1.0	Round	Smooth	Flat	White	Opaque	Moist	-Ve Bc	Non motile
10	9B	2.0	Oval	Rough	Flat	White	Opaque	Moist	-Ve B	Non motile
11	10A	2.0	Round	Smooth	Flat	White	Opaque	Moist	-Ve Bc	Non motile
12	10B	2.5	Round	Rough	Low convex	Yellow	Non opaque	Dry	+Ve C	Non motile
13	11	1.0	Round	Smooth	Flat	White	Opaque	Moist	-Ve B	Non motile
14	12	1.0	Round	Smooth	Flat	White	Opaque	Moist	-Ve B	Non motile
15	13A	3.0	Round	Rough	Concave	White	Nonopaque	Dry	-Ve Bc	Non motile
16	13B	1.5	Round	Rough	Flat	White	Opaque	Moist	-Ve Bc	Non motile
17	14	1.5	Round	Smooth	Flat	White	Opaque	Moist	-Ve Bc	Non motile
18	15	1.0	Round	Smooth	Flat	White	Opaque	Moist	-Ve Bc	Non motile
19	16	1.0	Round	Smooth	Flat	White	Opaque	Moist	-Ve Bc	Non motile

Note: C-cocci, BC- bacilloccoci, B- bacilli.

Table 2. Biochemical characters of the isolates.

Isolate	Oxidase	Catalase	Bile tolerance	Lactose fermentation	Citrate utilization	Indole production	Acid from glucose	VP test	Amylase	Sucrose	Maltose	Mananose	Xylose	1%Nacl	3%Nacl	5%Nacl
1	+++	+++	+	-	+	-	-	-	-	+++	++	++++	-	+++	++	+
2	-	+	-	-	-	-	-	-	++	-	-	-	-	+++	++	+
3	+++	+++	-	-	-	-	-	-	-	-	-	-	-	+++	++	-
4	+++	+++	-	-	-	-	-	-	-	-	-	-	-	+++	+	-
5	+++	+++	-	-	-	-	-	-	-	-	+	-	-	++	+	-
6	+	+++	+	+	+	-	-	-	-	-	-	-	-	++	++	-
7	+	++	-	-	-	-	-	-	-	-	-	-	-	++	+	+
8	+++	+++	-	-	-	-	-	-	-	-	-	-	-	+++	++	+
9A	++	+++	-	-	+	-	+	-	-	-	-	-	-	+++	+	-
9B	+++	++	-	-	-	-	+	-	+	-	-	-	-	++	+	-
10A	+++	++++	-	-	+	-	+	-	-	-	-	-	-	++	+	-
10B	+++	+++	-	-	-	-	+	-	-	-	-	-	-	+++	++	-
11	+++	+++	+	+	+	-	+	-	+	-	-	-	-	++	++	+
12	+++	+++	+	-	-	-	-	-	-	-	-	-	-	++	+	-
13A	+++	++	-	-	-	-	+	-	+++	-	-	-	-	++	+	+
13B	+++	+	-	-	-	-	-	-	+++	-	-	-	-	++	+	-
14	+++	+++	+	-	+	-	+	-	-	++	-	+++	-	+++	++	+
15	+++	+++	+	-	-	-	-	-	-	-	-	-	-	++	++	-
16	+++	+++	+	+	+	-	+	-	++	++	-	-	-	+++	++	+

Key: (-) Negative; (+) Positive; more positive signs indicates intensity of the results.

(VP)-Voges prousker.

REFERENCES

- Allen, D.A., B. Austin and R. Colwell. 1983. Numerical taxonomy of bacterial isolates associated with a fresh water fishery. *J. Gen. Micro. biol.* **129**:2043-2062.
- Bairagi, A., K.S. Ghosh, S.K. Kumarsen and A.K. Ray. 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. *Aqua. International* **10**(2):109-121.
- Holt, J.G. (ed.). 2000. *Bergey's Manual of Detereminative Bacteriology* (7th edition), Lippincott Willions and Wilkins, Philadelphia, USA. 259 p.
- Horsley, R.W. 1973. A review of the bacterial flora of teleosts and elasmobranchs, including methods for its analysis. *J. Fish Biology* **10**:529-553.
- Joseph, J., P.K. Surendran and P.A. Perigeen. 1988. Studies on iced storage of cultured rohu (*Labeo rohita*). *Fish. Technol.* **25**:105-109.
- Kamjunke, N., R. Mendonca, I. Hardewing and T. Mehner. 2002. Assimilation of different cyanobacteria as food and the consequences for internal energy stores of juvenile roach. *J. Fish Biology* **60**(3):731-738
- Karat, S.S., N. Dahanukar and R. Raut. 2000. Decline of freshwater fish of Pune urban area. *J. Ecological Society* **13/14**:46-51.
- Sissons, J.W. 1989. Potential of probiotic organism to prevent diarrhea and promote digestion in farm animals-A review. *J. Sci. Food Agric.* **49**:1-13.
- Spanggaard, B., I. Huber, J. Nielsen, T. Nielsen, K.F. Appel and L. Gram. 2000. The micro flora of rainbow trout intestine: a comparison of traditional and molecular identification. *Aquaculture* **182**:1-15.
- Talwar, P.K. and T. Jhingran. 1991. *Inland Fishes of India*. Oxford and IBH publications, Delhi, India. 256 p.