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The Nature of the Aerobic Gastrointestinal Bacteria of Cichlid Fish *Sarotherodon mossambicus* (Peters) and *Tilapia Nilotica* (Linneaus) Grown under Captivity

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ABSTRACT

Bacteriological examination of the gastrointestinal microflora of two fresh water cichlid fish species (*Sarotherodon mossambicus* and *Tilapia nilotica*) was performed, resulting in the becteria enumeration of total viable counts of 1.06×10^7 /g and 7.75×10^7 /g of gastro - bacteria intestinal tract plus contents (wet weight) respectively, by aerobic incubation at 30 + 1°C.

The majority (78%) of the total gut isolates from both fish species was Gram positive mesophilic which is characteristic of the higher ambient temperature in the tropics These isolates were fastidious in their nutritional requirements and together with the rest are isogenous to *bacteria autochthonous to* soil and water. The occurrence of such organisms is attributed to the feeding habits of these fish. The gastrointestinal bacteria isolated in this study are transient residents but not 'indigenous' in these cichlid fish.

Introduction

The gastrointestinal tract of animals has become a popular research area to the ecologist and microbiologist alike. In most animals it is populated by what is termed a 'normal' or 'indigenous' microbial flora. The early concept of the sterility of fish gut (Blake, 1935; Margolis, 1953) has now been replaced by the popular view that feeding fish always harbour viable bacteria in their gastrointestinal tract (Shewan and Hobbs, 1967) and that only migrating fish which undergo fasting may frequently have an empty intestinal tract or at the most a low microbial count (Bramsnaes, 1965). However the presence of a 'normal' bacterial flora in the gut of fish is a controversial issue as investigators believe that fish do not have any natural bacterial flora in their gut, and what is observed has originated from their environment (Wood, 1967) and is a function of the food injected (Liston, 1956).

The study of gastrointestinal bacteria of fish has enabled investigators to understand biological phenomena underlying spoilage of fish, microbial relationships with the host, bacterial diseases of cultivated fish and food intoxications implicated with consumption of fish. Further, gut bacteria of fish have also served as indicators of faecal pollution of waters inhabited by fish and therefore helped monitoring sanitary conditions of waters.

Sarotherodon mossambicus and *Tilapia nilotica* are two fresh water fish species of exotic origin, which were introduced into Sri Lanka as food fish. Of the two the former is reported to constitute a major fishery in itself in the North Central Province and other areas (Mendis and Fernando, 1962), and the latter probably would demonstrate locally the high potential it has exhibited in its native waters (Fryer and Iles, 1972).

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It was felt that microbiological information of the above two economically important fresh water fish would be most opportune to the developing inland fishery industry of Sri Lanka. Studies on the microbiology of fresh water fish are notably scarce, specially from the tropical region. The present report is the outcome of an investigation on the nature of aerobic gastrointestinal bacteria of the above two fish species grown under captivity.

Materials and Methods

Selection of fish

Young fish (6 – 8 months old) of the species *Sarotherodon mossambicus* and *Tilapia nilotica* grown under known culture conditions in domesticated mud ponds were obtained from fresh water fisheries station, Panapitiya, Kalutara. The selected fish which had been caught the previous day and kept overnight in a recovery pond were transported in oxygenated polythene bags containing 1/3 full of clean fresh water. They were in transit for about 1½ hrs and were in very good condition on arrival. The fish were acclimatized and transferred into aquarium tanks containing seasoned fresh water (pH 6.5 – 7.0; temperature 30±1°C). The fish were not fed and the water was neither aerated nor replenished during the course of study. The weight of *S. mossambicus* and *T. nilotica* specimen examined ranged from 32.6g to 93.2 g (average weight 62.9g) and 38.8g to 75.3g (average weight 50.9g) respectively.

Post mortem and Bacteriological Examination

The fish were caught by dip net and killed by delivering a blow to the head (Trust *et al.*, 1979); washed clean. After surface sterilization (Gibbons and Reed, 1930) the gastrointestinal tract in its entirety was removed by adopting aseptic surgery and weighed. It was homogenized in a blendor with a standard volume of ¼ strength Ringer's solution.

The resultant homogenate was serially diluted using the same diluent and suitable dilutions are plated on Nutrient agar (Difco) pH 6.8. The seeded plates were incubated aerobically at 30 ± 1°C for 24 to 48 hrs. The total viable counts per gram of intestinal tract plus contents were determined for each specimen.

Isolation of bacteria

Representatives of all the recognisable morphologically different bacterial colonies were picked off from suitable plates. They were purified and the selected pure cultures were transferred into Nutrient agar slants in Bijou bottles. After incubation at 30 ± 1° C for upto 48 hrs these were stored at 4°C as stock cultures.

Characterization and Identification of bacteria

During characterization the preserved stock cultures were recovered in Nutrient agar plates. The morphological characteristics such as pigmentation, staining, shape and arrangement of cells, motility, presence of capsules and endospores of each culture are studied.

The biochemical properties examined included oxidation and fermentation of glucose in Hugh and Leifson's (1953) normal and modified semi solid media; fermentation of and production of acid from glucose, sucrose, maltose, lactose, galactose, salicin, arabinose mannitol, inositol, starch, xylose and glycerol in broth media; production of acid from glucose, xylose, arabinose and mannitol in modified slants; presence of cytochrome 'C' oxidase, catalase, phosphatase, coagulase, arginine, ornithine and lysine decarboxylases; reduction of nitrate and nitrite; production of indole from tryptophan, H_2S from Kligler's Iron agar, ammonia from urea and production of acetyl methyl carbinol; detection of mixed acid fermentation; utilization of citrate, malate, aspartic acid, acetate and histidine

as the sole carbon source and reaction to litmus milk. Amylase activity was tested in Nutrient agar having 1% (w/v) soluble starch. Proteolytic activity was tested in Casein agar 1% (w/v) and Gelatine agar 0.4% (w/v) and lipolytic activity was tested in Tween agar having 1% (v/v) Tween 80 (Atlas). Growth in 5% (w/v) NaCl, 10% (w/v) NaCl without added NaCl, growth at pH 5 were tested in Nutrient broth, and growth at 45°C, 65°C were tested on Nutrient agar. Sensitivity to five antimicrobial agents was also tested as necessitated. All the tests were carried out in accordance with the materials and methods given in Harrigan and McCance (1976) and Buchanan and Gibbons (1974).

For the identification of Gram positive coccoid bacteria, Sub Committee Report (1965), Baird-Parker (1962, 1963, 1965 and 1966) were employed. Gram positive endospore forming bacteria were identified on the basis of Gorden and Smith (1949), Smith, Gorden and Clark (1952) and Wolf and Barker (1968). Identification of Gram negative rod shaped bacteria was based on Bain and Shewan (1968), Hendrie and Shewan (1966) and Shewan, Hobbs and Hodgkiss (1960 a & b).

Identification schemes of Cowan (1977) and Buchanan and Gibbons (1974) were adopted for the final identification of the gut isolates.

Results

The total and average viable counts obtained for the two fish species are illustrated in Table I. The average viable counts recorded for *Sarotherodon mossambicus* and *Tilapia nilotica* were 1.06×10^7 bacteria/g (range 6.11×10^4 to 5.76×10^7 bacteria/g) and 7.75×10^7 bacteria/g (range 1.25×10^6 to 3.09×10^8 bacteria/g) respectively.

TABLE 1

THE TOTAL VIABLE COUNTS OF BACTERIA ISOLATED FROM THE GASTROINTESTINAL TRACT OF *Sarotherodon mossambicus* AND *Tilapia nilotica* (after–h1 24 of incubation at 30±1°C)

Sample	*Fish*	*No. of samples*	*Number of viable bacteria g^{-1} (wet weight)*	
			Average	*Range*
Total tract plus contents	*S. mossambicus*	13	1.06×10^7	6.11×10^4
				8.12×10^5
				1.43×10^6
				1.48×10^6
				2.56×10^6
				3.38×10^6
				3.65×10^6
				4.13×10^6
				9.50×10^6
				1.03×10^7
				1.98×10^7
				2.73×10^7
				5.76×10^7
Total tract plus contents	*T. nilotica*	07	7.75×10^7	1.25×10^6
				3.48×10^6
				8.28×10^6
				5.59×10^7
				7.56×10^7
				8.90×10^7
				3.09×10^8

(Isolation medium – Nutrient Agar pH 6.8)

Out of 134 gut isolates examined 105 (about 78%) were Gram positive, of which 49 strains were rod shaped bacteria that formed endospores; 22 were coccoids and the rest being asporogenous rods. Gram negative rod shaped bacteria amounting to 29 strains (about 22%) constituted the rest of the total gut isolates (Table 2). The percentage composition of different bacteria in the gut of the two fish species is illustrated in Table 3, which shows that same types of bacteria are encountered in the gut of both fish spceies. Table 4 illustrates a summary of some selected important biochemical characters of all the gut isolates exmained and provides information on the nature of these organisms.

TABLE 2

PERCENTAGE COMPOSITION OF THE TOTAL BACTERIAL ISOLATES FROM THE GASTROINTESTINAL TRACT OF *Sarotherodon mossambicus* AND *Tilayia nilotica*

Organism	*Number of isolations*	*Number of samples from which isolated*	*Percentage*
Gram positive bacteria			
Micrococcus roseus	01	1/(15)	2.99
Micrococcus luteus	02	1/(15)	—
Micrococcus cryophilus	01	1/(15)	—
Staphylococcus saprophyticus	12	8/(15)	8.96
Planococcus sp.	04	3/(15)	2.99
Streptococcus sp.	01	1/(15)	0.74
Sarcina sp.	01	1/(15)	0.74
Bacillus sp.	49	15/(15)	36.57
Coryneform bacteria	19	7/(15)	14.16
Gram negative bacteria			
Enterobacter aerogenes	02	2/(15)	1.48
Enterobacter cloacae	06	2/(15)	4.46
Citrobacter freundii	01	1/(15)	0.74
Klebsiella pneumoniae	02	2/(15)	1.48
Aeromonas hydrophila	04	2/(15)	2.99
Vibrio sp.	01	1/(15)	0.74
Moraxella - Acenetobacter	01	1/(15)	0.75
Pseudomonas aeruginosa	03	3/(15)	6.72
Psedomonas sp.	06	4/(15)	—
Flavovacterium sp.	02	1/(15)	1.49
Unidentified Gram positive and Gram negative bacteria	16	8/(15)	11.39

* Expressed as the percentage of total number of organisms (134) isolated from 10 *S. mossambicus* and 5 *Ta nilotic* specimens.

TABLE 3

PRECENTAGE COMPOSITION* OF DIFFERENT BACTERIAL GENERA IN THE TWO FISH SPP.

Organisms	*Sarotherodon mossambious*	*Tilapia nilotica*
Bacillus sp.	40.00	28.21
'Coryneform' bacteria	12.63	17.95
Staphylococcus sp.	7.37	12.82
Micrococcus sp.	0.00	10.26
Planococcus sp.	2.11	5.13
Enterobacter sp.	8.42	5.13
Pseudomonas sp.	5.26	10.26
Aeromonas hydrophila	4.21	0.00
No. of isolates	95	39

*Expressed as the percentage of total number of organisms isolated.

DISCUSSION

Significant viable bacterial counts were obtained from the gastrointestinal tracts of all the specimen of both these cichlid fish species. Typically they assume upper limits generally accepted for free living fish (Trust *et al.*, 1978) and thus supports findings of many investigators. The magnitude of these bacterial populations suggests possible multiplication within the digestive tract of fish. In comparison, the average count obtained for *T. nilotica* spp was 7.31 times greater than that of *S. mossambicus*. Both these fish species inhabited the same waters and share common feeding habits, albeit the average weight of gut of the latter was found to be slightly higher (1.1g and 2.1g respectively).

In temperate countries it is the Gram negative psychrophilic bacteria that predominate the gut flora of fish (Shewan, 1971). In this study a high percentage (78%) of Gram positive mesophilic bacteria was isolated. The predominance of this group of organisms (*Bacillus sp.* 'Coryneform' bacteria, micrococci, staphylococci) among the gut flora is consistant with the views of Shewan and Hobbs (1967), Scholes and Shewan (1964) and Shewan (1961). The higher ambient temperature found in the tropics and the relatively high incubation temperature (30± 1°C) adopted for initial isolation appear to be conducive for more mesophilic Gram positive bacteria to propagate.

This could perhaps be the first report on the occurrence of staphylococci among the guts flora of fish. Coagulase negative non-pathogenic staphylococci are known to be ubiquitous and have been isolated also from human skin and animal carcases Baird-Parker, 1962 However the strains isolated in this study do not belong in the Staphylococcus Sub group VI of Baird-Paker (1963) which are the types reported to occur on human skin. Except staphylococci, all the other bacterial strains isolated in this study are the same strains previously reported among gut flora of marine or fresh water fish elsewhere in various parts of the world although the quantitative aspects show differences. Again this is expected becase the media and culture conditions (pH and temperature) greatly influence bacterial growth. Qualitatively the results of this study show a close resemblance to gut flora found in freshwater salmonid fish and grass carp (Trust *et al.*, 1979, 1978).

The occurrence of pigmented and non-pigmented *Bacillus* strains and 'Coryniform' bacteria in large numbers (36.57% and 14.16% respectively) together with coccoid bacteria probably reflect the feeding habits of these fish. It is well known that *T. nilotica* and *S. mossambicus* are herbivorous to omnivorous in nutrition and generally feed on detritus and soft bottom deposits as well and specially the occurrence of *Bacillus* spp. in their gut could be attributed to this feeding habit.

The bio-chemical characteristics of the gut isolates show that the majority are fastidious in nutritional requirements where the prototrophic organisms which utilize citrate as the sole carbon source amounts to less than half the population (44.79%). The fastidious group comprised of 41 *Bacillus* strains, half the coccoid bacteria and 21 Gram positive asporogenous rods. The ability to reduce nitrate which is the key step in denitrification process was observed among 66% of the isolates, where all the Gram negatives isolates, specially the pseudomonads, most *Bacillus* strains and the 'Coryneform' bacteria comprised the denitrifying group. This process is a characteristic feature encountered among microbes autochthonous to soil and water.

TABLE 4

SUMMARY OF BIOCHEMICAL CHARACTERISTICS OF THE BACTERIA ISOLATED FROM GASTROINTESTINAL TRACT OF *Sarotherodon mosambicus* AND *Tilapia nilotica*

Bio–chemical characteristics	*Number † Positive*	*Percentage Positive*
Pigmentation	60(134)	44.77
Catalase	132(134)	98.50
Kovacs's Oxidase (Cytochrome 'C')	59(134)	44.02
Utilization of Citrate as sole C source	56(134)	41.79
Reduction of Nitrate (Nitrate reductase)	90(134)	65.69
Hydrolysis of Gelatin (Gelatinase)	106(134)	80.91
Hydrolysis of Casein (Casease)	93(118)	78.81
Hydrolysis of Starch (Amylase)	46(125)	36.81
Hydrolysis of Urea (Urease)	72(134)	53.73
Production of Indole (Tryptophanase)	9 (134)	6.72
Production of Acetyl Methyl Carbinol	52(134)	38.80
Mixed acid Fermentation	27(134)	20.14
Oxidative, Hugh & Leifson's test	21(134)	15.67
Fermentative Hugh & Leifson's test	44(134)	32.84
No reaction, Hugh & Leifson's test	69(134)	51.48
Acid from Litmus Milk	22(33)	66.66
Production of Arginins Dihydrolase	29(33)	87.88
Acid from * Glucose	70(134)	52.23
Inositol	16(60)	26.66
Mannitol	34(87)	39.08
Lactose	19(84)	22.62
Acid From * * Glucose	32(38)	84.21
Arabinose	21(35)	60.0
Mannitol	28(35)	80.0
Xylose	23(36)	63.83

† Number of strains tested given in parenthesis.

* Peptone water broth.

* Modified slant medium (tested for *Bacillus* spp. only)

Further the proteolytic activity shown by a very high percentage of the gut isolates (81% hydrolysed gelatin, 79% hydrolysed casein) and the ability to split urea shown by 54% of the isolates are all characteristics of terrestrial and aquatic bacteria. This evidence strongly suggests that most of the organisms if not all, isolated in this study are similar to those found in soil and water. This is further strengthened by the fact that 52% of the isolates degnaded glucose, which is again a characteristic of terrestrial bacteria.

Thus it appears that most of the aerobic organisms isolated from the gut of these fish could have originated in the environment and are probably transient residents in the gut. Obviously, these organisms do not warrant to be placed in the status 'indigenous' organisms of the gut of these fish.

Organisms belonging in the Enterobacteriaceae (*Enterobacter, Klebsiella* and *Citrobacter* collectively 8.9%) were encountered, which cannot be considered as indicator organisms in tropical waters (Katugampola and Assim, 1958). The absence of 'faecal' coliform organisms rule out the possible existence of enteropathogenic bacteria among these gut isolates, but it is inconclusive since fish are known to purify themselves when placed in fresh water.

This study has shown that fresh water cichlid fish harbour significant bacterial populations in their gastrointestinal tract. Aerobic and facultatively anacrobic bacteria abound the gut flora which is predominantly Gram positive and mesophilic in character and these organisms reflect the environment of the fish. There is no evidence to indicate that this microflora is 'indigenous' or 'normal' to the gut of these cichild fish. These organisms are probably transient residents.

REFERENCES

BAIN, N. and SHEWAN, J. M. (1968)
Identification of *Aeromonas*, *Vibrio* and related organisms, in 'Identification methods for microbiologists, Part B' (B. M. Gibbs and D. A. Shapton Eds,), Academic Press, London and New York, 79-84 pp.

BAIRD–PARKER, A.C. (1962)
The occurrence and enumeration according to a new classification, of Micrococci and Staphylococci in Bacon and on Human and Pig skin, *J. Appl. Bact.*, **25** (3), 352-361 pp.

BAIRD PARKER, A. C. (1963)
A classification of Micrococci and Staphylococci based on physiological and Bio-chemical tests, *J. Gen. Microbiol.*, 30, 409-427 pp.

BAIRD-PARKER, A. C. (1965),
The classification of staphylococci and Micrococci from Worldwide Sources, *J. Gen. Microbiol.*, **38**, 363 - 387 pp.

BAIRD-PARKER, A. C. (1966)
Methods for classifying Staphylococci and Micrococci, in 'Identifications methods for microbiologists, Part A' (B. M. Gibbs and F. A. Skinner Eds.), Academic Press, London, 59-64 pp.

BLAKE I. (1935)
Some observations on the bacterial flora of the alimentary tract of certain salmonidae, in 'Final. Report of the Furunculosis Committee', Soctland, Edinburgh : H. M. S. O., 60-67 pp.

BRAMSNAES, F. (1965)
Handling of fresh fish, in 'Fish as Food' Vol, 4, (G. Borgstrom Eds.), Academic Press, New York, London, 1-63 pp.

BUCHANAN, R. E. and GIBBONS, N. E. (1974)–
Bergey's Manual of Determinative Bacteriology, 8th Edn., Williams and Willkins Co., Baltimore 217-632 pp.

COWAN, S. T. (1977)
Cowan and Steel's Manual for the identification of Medical Bacteria, 2nd Edn. (Revised by S. T. Cowan) Cambridge University Press, London.

FRYER, G. and ILES, T. D. (1972)
The cichlid fishes of the Great Lakes in Africa, their biology and evolution, Oliver and Boyd, Edinburgh.

GIBBONS, N. E. and REED, G. B. (1930)
The effect of autolysis in sterile tissues on subsequent bacterial decomposition *J. Bacteriol.*, **19**, 73-88 pp.

GORDEN, R. E. and Smith, N. R. (1949)
Aerobic spore forming bacteria, *J. Bact.*, **58**, 327-429 pp.

HARRIGAN, W. F. and MCCANCE, M. E. (1976)
Laboratory methods in Food and Dairy Microbiology (Revised edition prepared by W. F. Harrigan), Academic Press London, New York.

HENDRIE, M. S. and SHEWAN J. M. (1966)
The identification of certain *Pseudomonas* species, in 'Identification methods for micro-biologists, Part A' (B. M. Gibbs and F. A. Skinner Eds.), Academic Press, London, New York, 1-7 pp.

KATUGAMPOLA, D. S. and ASSIM, T. H. (1958)
Coliform organisms in domestic water supplies in Ceylon, *Cey. Journ. of Med. Scie.*, 9, 95-101 pp.

LISTON, J. (1956)
Quantitative variations in the bacterial flora of flat fish, *J. Gen. Microbiol.*, **15**, 305-314 pp.

MARGOLIS, L. (1953)
The effect of fasting on the bacterial flora of the intestines of fish, *J. Fish. Res. Board. Can.*, **10**, 62-63 pp.

MENDIS, A. S. and FERNANDO, C. H. (1962)
A guide to the fresh-water fauna of Ceylon, *Bull. Fish. Res. Stn., Ceylon*, Vol. 12.

SCHOLES, R. B. and SHEWAN, J. M. (1964)
The present status of some aspects of marine microbiology, in 'Advances in marine biology' Vol. 2, (F. S., Russell Eds.) Academic Press, London, New York, 133-169 pp.

SHEWAN, J.M. (1961)
The microbiology of sea water fish, in 'Fish as Food, Vol. 1, (G. Borgstrom Eds.), Academic Press, New York London 487-560 pp.

SHEWAN J. M. (1971)
The microbiology of fish and fishery products, *J. Appl. Bact.*, 34 (2) 299-315 pp.

SHEWAN, J.M.HOBBS, G. and (1967)
The bacteriology of fish spoilage and preservation in 'Progress in industrial microbiology,' Vol. 6 (D.J.D. Hockenhull Eds.), London Heywood Books, 169-208 pp.

SHEWAN, J. M. HOBBS, G. and HODGKISS, W. (1960)
A determinative scheme for the identification of certain genera of Gram-negative bacteria with special reference to Psuedomonadaceae *J. Appl Bact.*, 23, 379-390 pp.

SHEWAN, J.M. HOBBS, G. and HODGKISS, W. (1960)
The *Pseudomonas* and *Achromobacter* groups of bacteria in the spoilage of marine white fish, *J. Appl. Bact.*, 23. 463-468 pp.

SMITH, N.R., GORDEN, R.E. and CLARK, F.E. (1952)
Classification and identification scheme for Aerobic sporeforming bacteria, U.S. Dept. Agric. Monograph No. 16, Washington, D.C.

SUB COMMITTE: (1965)
Minutes of 1st Meeting of Sub-Committee on taxonomy of Staphylococci and Micrococci *Inten. Bull. Bacteriol Nomenclature. Taxonomy*, 15, 105-108 pp.

TRUST, T. J. BULL, L.M. CURRIE B.R. and BUCKLEY, J.T. (1979)
Obligate anaerobic bacteria in the gastrointestinal microflora of the Grass Carp (*Ctenopharyngodon idella*), Gold fish (*Carassius auratus*) and Rainbow Trout (*Salmo gairdner*), *J. Fish. Res. Board. Can.*, 36, 1174-1179 pp.

TRUST, T.J., MAC INNES, J.I. and BARTLETT, K.H. (1978)
Variations in the intestinal microflora of Salmon fishes, in'Microbiol Ecology' (M.W. Loutit and J.A.R.Miles Eds.), Springer-Verlag, Berlin, Heidelberg, New York, 250-254 pp.

WOLF, J. and BARKER, A.N. (1968)
The genus *Bacillus*-Aids to the identification of its species, in Identification Methods for Microbiologists, Part B', (B.M. Gibbs and D.A. Shapton Eds.), Aacademic Press, London, New York, 91-109 pp.

WOOD, E.J.F. (1967)
Microbiology of ocean and estuaries, Elsivier Oceanographic series, Vol. 3, Elsivier publishing company, Amsterdam, London, New York.