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Original article

Bacterial diversity on fishes and in waters from southern rivers in Mali

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ABSTRACT

Microbial diversity of fishes of river water from Bagoé, Baoulé and Sankarani in the Niger River basin in southern Mali, was investigated. Gills and intestines were sampled on forty-three (43) fishes. Also, five water samples were collected for microbial diversity analysis. Total mesophilic flora and fauna, indicator contamination were investigated in water and in the gills and intestines fish species of economic interest, freshly caught. Conventional microbiological analysis methods were used. Biochemical tests were performed for bacterial identification. The main results obtained were: (i) the bacterial density in fishes was 152.106 to 66.106 CFU/g in gills and 112±53.106 to 50±8.5.106 CFU / g in intestine, (ii) bacterial flora in fishes from rivers is composed 61% of Gram-negative and 39% Gram-positive bacilli. River waters were composed of 91% of Gram-negative bacilli and 9% of Gram-positive bacilli. In waters and fishes investigated, the different bacteria identified belong to three bacterial families: Pseudomonadaceae, Enterobacteriaceae and Bacillaceae. Bacterial flora of fishes from southern rivers was found similar to those of investigated river waters. But, the bacterial flora of the river waters had a higher proportion of Gram-negative bacilli than those in fishes.

1. Introduction

Fishes are a source of animal protein, rich in amino acids (mainly methionine), essential fatty acids and minerals (calcium and phosphorus). Therefore, fishes are a valuable contribution to the diet of malnourished populations FAO (2006). Increasingly, fishes are used in human alimentation because they present less risk to human health. However, fishes can be confronted with microbial contamination in their natural habitat and their transformation products can be a source of microbial infections and food poisoning. In the natural environment, microorganisms are usually found on the entire outer surface (skin and gills) and in the intestines of fishes. According to Shewan (1977), bacterial flora of freshly caught fishes depends on the environment in which they were captured. Also, according to the WHO / FAO (2009), the level of contamination of a fish at the time of his capture, depends on the environment and the microbiological quality of the water in which it was harvested. Indeed, the water can be extremely polluted by human and animal wastes and therefore contain several microorganisms which may contribute to it pollution. Among these microorganisms, there are dangerous groups for fish and human health such as Aeromonas hydrophila and Salmonella typhimurium.

The ichthyologic microbial flora needs special attention and should be studied not only for the preservation of fishes' health, but also for stocks management strategies in storage and for Human health preservation. The microbial flora of fishes in the rivers of the southern part in Mali supposed to be very diverse. But to our knowledge, the microbial flora of fishes in rivers in southern Mali has not yet been explored That's why the present study was performed to: (i) assess the microbial flora of fishes and rivers in southern Mali, (ii) determine the specific flora of the fishes and the river water and (iii) contribute to a better understanding of the resources of these rivers, which a long time ago, remained margin of scientific research.

2. Materials and methods

2.1. Area and sites of study

The investigations were made in pre-Sudanese and Guinean bioclimatic zones, mainly on the rivers of Baoule, Bagoé and Sankarani. The sites: Pondaga and Samorodaga on Ouassoulou, Bale on Sankarani tributary, Kabayadaga on Sankarani Babla and Farani on Baoule and Tiecounko on Bagoe, were selected for the study (Fig. 1).

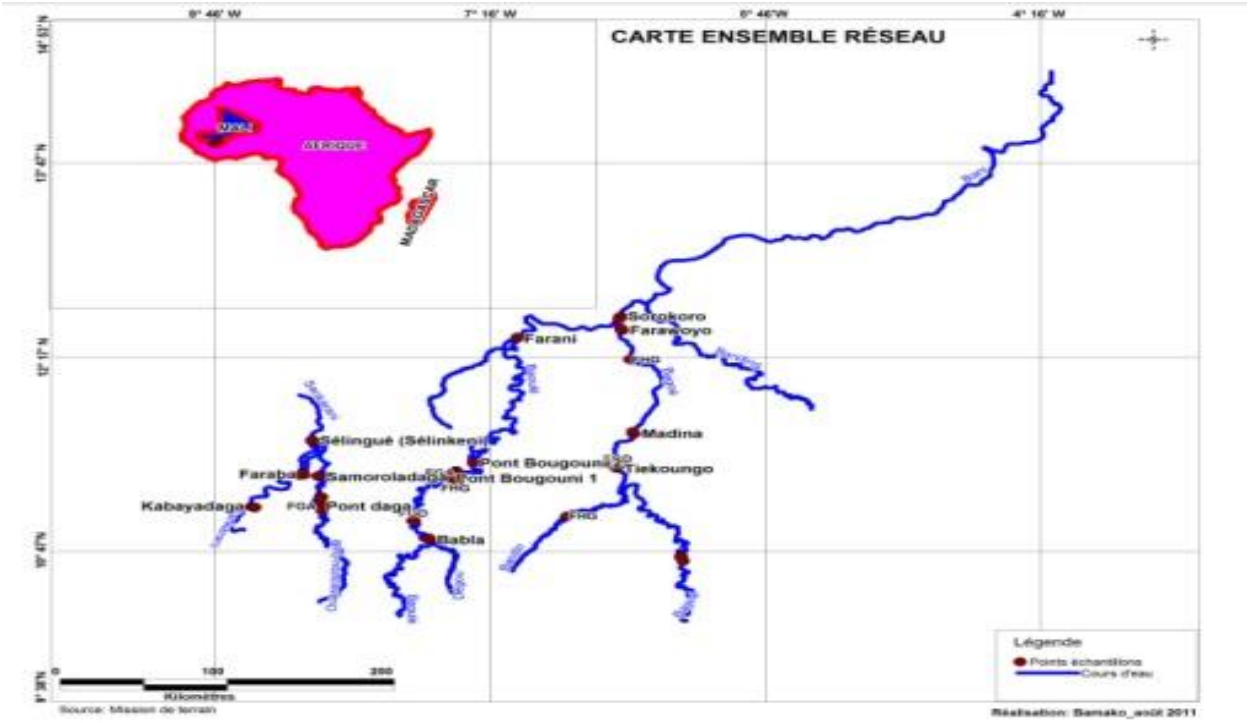

Fig. 1. Map of the study area.

2.2. Selection of fish species

Eight species of fish: Brycinus nurse, Sarotherodon galilaeus, Synodontis membranaceus, Clarias anguillaris, zillii Tilapia, Oreochromis aureus, Oreochromis niloticus Heterotis niloticus were selected based on their economic importance

2.3. Sampling

Sampling was done in three periods: October-November 2009, May 2010 and February 2011. To determine the composition and diversity of the microorganisms on the selected fish species their gills and intestines was collected. For microbiological analyzes, water samples were also collected in rivers where fish samples were collected.

3. Identification, isolation and characterization

To study bacterial diversity on fishes and river waters, dilutions of collected samples were spread on Plate Count Agar (PCA) and Nutrient Agar (NA). The inoculated plates were incubated at 37oC for 24 hours. After that, total bacteria on each plate were determined by counting. Total coliforms and Salmonella were respectively isolated on deoxycholate and Salmonella-Shigella (SS) media. Pseudomonades were isolated on King A and King B media King, (1954). The different growing colonies on each medium were purified and characterized.

3.1. Identification

To determine colony characteristics (shape, color and diameter) of bacterial isolates. Each isolate was plated on in sterile Petri dishes by the pour plate. Each inoculated plate was incubated at 37°C. For cell and colony morphology determination, negative staining was done and the morphology (form, size, mobility and presence or not of spore) of the isolates was studied under microscope as described by Reynolds et al. (1981). Gram staining of the isolates was performed as described by Bryant et al. (1983). Catalase test, using hydrogen peroxide, and glucose as carbon source was determined according to the utilization method of Hugh and Leifson (1953) were performed for the isolates identification purpose.

4. Results

In water sampled from different areas, bacterial counts vary from site to site (Table 1). The highest bacterial counts were obtained in water samples from Babla (152±52. 106 Cfu/g) and Pondaga (140±37.106 Cfu/g). Contrary to the large variation in bacterial counts among sites, we observed that water from samples can be divided in two groups according to the number of bacterial types observed: the first group (Pondaga, Babla and Farani) with five bacterial types and the second group (Samorodaga, Tiecounko and Pondaga) with four bacterial types (Table 1).

Table 1
Bacterial number and type on in water samples from different areas analyzed.

Sampling sites	Bacterial count (Cfu/g)	Bacterial types
Samorodaga	119±67. 106	4±1
Pondaga	140±37.106	5±1
Babla	152±52. 106	5±1
Farani	113±13.106	5±0
Tiecounko	70±43. 106	4±1
Pondaga	57±24.106	4±1

In general, bacterial count in water samples is largely different from that in fish organs studied (Table 2). In fact, the average bacterial count in water samples is 279.106 CFU/ml at least two times higher than those in fish intestine and grills, respectively 82.5.106 Cfu /g and 109.106 Cfu /g (Table 2).

Table 2
Bacterial number and type on fish gills, intestines and water samples analyzed.

Samples	Number of bacteria (x106 CFU / g of sample)	Bacterial types
Gills	109	4
Intestines	82,5	4
Waters	279	5

Studied fishes, the higher bacterial count (152±52. 106 Cfu/g) and the great bacterial diversity (7 bacterial types) were observed on the gills of Brycinus nurse. Our results showed that, in the same area, bacterial counts on the same organe of the same fish can vary largely. In fact, in Pondaga, bacterial count on Clarias anguillaris varies from 91±27.106 to 112±53.106 Cfu/g of intestine (Table 3).

Table 3
Population and diversity of bacteria on fishes from rivers of southern Mali.

Sample	Area S	Fish species	Bacterial count (Cfu/g)	Bacterial types
Gills	Samorodaga	Synodontis membranaceus	119±67. 106	4±1
		Sarotherodon galilaeus	128±7. 106	2±1
	Pondaga	Brycinus nurse	152±52. 106	7±1
	Babla	Brycinus nurse	113±13.106	5±1
	Farani	Brycinus nurse	124±100. 106	5±0
		Tilapia zillii	93±65. 106	3±0
	Tièkounko	Oreochromis aureus	73±25. 106	4±0
		Oreochromis niloticus	66±41. 106	3±0
		Clarias anguillaris	112±53.106	5±2
		Clarias anguillaris	91±27. 106	4±1
Intestines	Pondaga	Heterotis niloticus	77±6. 106	3±1
		Heterotis niloticus	50±8.5. 106	3±0

The less bacterial diversity (2 bacterial types) was observed on Sarotherodon galilaeus Gills (table 4). Bacterial counts on the gill of the same fish vary according to the area where the fish was caught. In fact, the bacterial count on the gills of Brycinus nurse from Pondaga is 152 Cfu/g, while the count on the same fish from Babla and Farani was respectively 113 Cfu/g and 124 Cfu/g.

Table 4
Fish species and diversity of rivers in southern Mali.

Fishes species	Bacterial types
Synodontis membranaceus	4±1
Sarotherodon galilaeus	2±1
Brycinus nurse	6±1
Tilapia zillii	3±0
Oreochromis aureus	4±0
Oreochromis niloticus	3±0
Clarias anguillaris	5±1
Heterotis niloticus	3±0

The high variation coefficient generally observed indicates a high variation on bacterial count from one fish to another.

The bacterial flora of fishes of the southern rivers in Mali consisted of 23% of Gram-positive cocci, 61% of Gram-negative bacilli, 11% Gram-positive bacilli (where 8% are non-spore forming and 3% spore-forming bacteria)

and actinomycetes 5% (Figure 2A). While the analyzed river waters were composed of 91% Gram-negative bacilli, 3% Gram-positive and 6% Gram-positive cocci (Figure 2B).

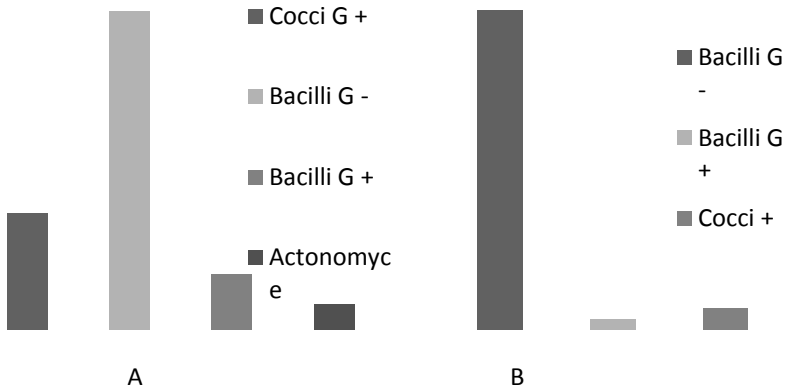

Fig. 2. Distribution of the bacterial isolates according to their Gram color and microscopic form: A) fish isolates, B) water samples' isolates.

4.1. Biochemical characterization of isolates

Simple biochemical techniques in addition to microscopy were used namely: catalase test and glucose utilization test. The results are shown in Table 3.

Table 5
Some morphological and biochemical characteristics of a few in bacteria isolated from fishes and water

Sample n°	Sample code	Form	Gram	Glucose utilisation	Catalase	Family
1	SME8 -1	rod	-	+	+	Pseudomonadaceae
2	SME8-2	rod	-	+	++	Pseudomonadaceae
3	SGA18	rod	-	+	+	Pseudomonadaceae
4	SGA19	rod	-	+	±	Pseudomonadaceae
5	BNUD	rod	-	+	+	Pseudomonadaceae
6	YSGA6	rod	-	+	+	Pseudomonadaceae
7	YCAN6	rod	-	+	+	Pseudomonadaceae
8	SME1	rod	-	-	-	Pseudomonadaceae
9	YSGA19	rod	-	-	+	Pseudomonadaceae
10	TZII	rod	-	-	+	Pseudomonadaceae
11	Sank1	rod	-	-	+	Pseudomonadaceae
12	Sank3	rod	-	-	-	Pseudomonadaceae
13	O.baléP1	rod	-	-	-	Pseudomonadaceae
14	O.balé S3	rod	-	-	-	Pseudomonadaceae
15	BNUG	rod	-	O+F+	-	Enterobacteriaceae
16	YCAN3-5	rod	-	O+/F+	+	Enterobacteriaceae
17	YCAN4-2	rod	-	O+/F+	+	Enterobacteriaceae
18	YCAN4-3	rod	-	O+/F+	+	Enterobacteriaceae
19	O.ba S2	rod	-	O+/ F+	+	Enterobacteriaceae
20	Bagoè	rod	-	F	+	Autres
21	YBNU35	rod	+	O+/F+	+	Bacillaceae
22	YCAN2	rod	+	O+/F+	+	Bacillaceae
23	Sank2	rod	+	O+/F+	+	Bacillaceae

SME: Synodontis membranaceus; YSGA: Sarotherodon galilaeus; BNUD: Brycinus nurse Dioila; O.balé P: Ouassoulou Balé Pondaga; O. Balé S: Ouassouou Balé Samorodaga;YCAN: Yanfolila Clarias anguilaris; TZI: Tilapia zilii; Sank: Sankarani; O+ F+: oxydative/ fermentative; F: fermentative.

5. Discussion

Microbial diversity of fishes and river waters in southern Mali was investigated by determining the specific microbial flora of freshly caught fishes and water samples from rivers in which fishes have been caught.

Further to laboratory tests, 106 microbial isolates were obtained from fish rivers of the South and 32 at the level within waters to which these fishes were harvested. The number of isolates obtained during this study is higher than that reported by Durand and Toumanoff (1967). These authors isolated 70 strains of twenty species of fish sick Mopti region from samples taken at different organs, blood, skin sores and intestinal contents. This discrepancy can be explained by the fact that this study focused on the bacterial flora of freshly caught fishes while studying and Toumanoff Durand (1967) concerned especially the sick fish.

The highest number of bacteria was observed respectively in Brycinus nurse, Sarotherodon galilaeus and Synodontis membranaceus. The bacterial concentration per gram of gill ranges from 152 ± 52x106 CFU/g of Brycinus nurse gill and at 66±41x106 CFU/g in Oreochromis niloticus gill. Bacterial density determined in the intestines ranged from 112±53x106 to 50±8.5x106 UFC/g of intestine. Other studies have shown that the density vary greatly, this variation depends more on the environment of the species. Trust (1975), found a density of 6x102 and 2,2 x106 bacteria / g gill. Similarly Shewan (1962), found a microbial density between 103 to 109 CFU/g of gill or intestine.

The bacterial flora of south river fishes consisted of bacilli, cocci and actinomycetes. Gram-negative bacteria accounted for 61 % against 39% for Gram-positive bacteria. Besides predominated Gram-negative bacteria, microbial flora from south river fishes contained also Gram-positive bacteria. These results are conform to those of Shewan (1977) who found that Gram-positive bacteria such as Bacillus, Micrococcus, Clostridium, Lactobacillus and coryneform in varying amounts on fishes. In the same work, Shewan found that gram-negative bacteria are the most present on fishes. Trust et al. (1979) seeking strict anaerobic found actinomycetes in fat carp. Qualitative analysis of these bacteria showed that the bacterial flora of fish from rivers is composed of 61% of Gram-negative bacilli, 11% Gram- positive, 23% of Gram-positive cocci and 5% of actinomycetes.

Our results showed that, river waters' bacterial flora is composed of 91% of Gram-negative bacilli and 9% of Gram-positive bacilli. The group of Gram- positive bacteria is composed of 3% Gram positive bacilli and 6% of Gram-positive cocci. These results are also consistent with those found by Shewan (1977) which showed that the bacterial flora of freshly caught fish depends on the environment in which he was captured, more than fish species.

On the basis of microscopic and biochemical characteristics, microbial isolates were matched to families such as Pseudomonadaceae, Enterobacteriaceae and Bacillaceae. The close Pseudomonadaceae groups were divided into two subgroups. Part of the group secreting pigment was seen as Pseudomonas and accounted for 27 % and the other part does not secrete pigments was considered as Aeromonas and accounted for 73 %. In the group of Gram- negative Enterobacteriaceae considered, total and fecal coliforms were determined and accounted for 71% and 29% respectively. The other part of this group consisted of Salmonella and Shigella. The presence of these indicators of contamination could indicate the level of pollution that can be explained by the activities of fishermen and farmers who live and work near these rivers.

6. Conclusion

This study showed that Fish Rivers in southern Mali host a rich and diverse bacterial flora. Microbial flora of fishes and river waters in southern Mali is mainly composed of Gram-negative bacilli. In general, the bacterial flora of fishes from southern rivers reflects that of waters in which we found a high proportion of Gram-negative bacteria.

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