

Biochemical Characterization of Lactic Acid Bacteria Isolated from Nile tilapia *Oreochromis niloticus*

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ABSTRACT

Lactic acid bacteria were isolated from Nile tilapia *Oreochromis niloticus* as probiotic with the use of biochemical characterization method. The investigation revealed that 10 different species of lactic acid bacteria (LAB) were isolated from the gill, skin and intestine. All the LAB naturally occurred in the three organs examined with the highest percentage of distribution of *Lactobacillus fermentum* which is 40%, and the least in three species (*L. rhamnosus*, *reuteri* and *divergence*) having 3.3% each. Nile tilapia *Oreochromis niloticus* could be a good source of Lactic Acid Bacteria (LAB) and is a promising probiotic source which could be used in aquaculture farming.

Keywords: *Oreochromis niloticus*, lactic acid bacteria, probiotic, and biochemical characterization.

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INTRODUCTION

Many different bacteria get into an organism from the environment. However, due to the natural selection, only those bacteria survive, which find favorable living conditions in that organism. Lactic Acid Bacteria (LAB) are widely distributed in various animal intestines (Devriese et al., 1987; Mitsuoka, 1980; Sakata et al., 1980) and some LAB because probiotics have played an important role in beneficial functions for industrial and aquacultural animals (Perdigon et al., 1995). There have been several reports (Perdigon et al., 1995; Salminen and Wright, 1998) of LAB occurring among the major microbial populations in animal intestines. It is well established that some LAB improve the intestinal microflora and promote the growth and health of animals (Perdigon et al., 1995). Most probiotics contain single or multiple strains of LAB and are part of the natural microflora of many animals; they are generally regarded as safe and may display antagonistic activities against pathogenic bacteria (Byun et al., 1997; Garriga et al., 1998). The intestinal microflora, especially LAB, may influence the growth and health of fish. However, few studies have reported the composition of intestinal LAB flora in fish. Lactic Acid Bacteria (LAB) are characterized as Gram-positive, usually non-motile, non-sporulation bacteria that produce lactic acid as a major or sole product of fermentative metabolism. Kandler and Weiss (1986) have classified *Lactobacillus* isolates from temperate regions according to their morphology, physiology and molecular characters. Schleifer (1987) classified LAB based on the molecular characteristics. LAB from food and their current taxonomical status have been described by Huber et al. (2004), Ringø and Gatesoupe (1998) and Salminen and von Wright (1998).

The aim of this paper is to evaluate the different species of Lactic acid bacteria present in adult Nile tilapia *Oreochromis niloticus* especially from three different sites; gill, skin and intestine, which can be used as probiotic in aqua-feed.

MATERIALS AND METHODS

Fish sample collection.

20 adult *Oreochromis niloticus* of mean weight of 52.67 ± 0.32 g were harvested from one of the ponds in the Research farm of the Department of Wildlife and Fisheries, University of Ibadan, Oyo state Nigeria and kept in a sterilized

plastic tank where it was transported to the postgraduate laboratory of the Department of Microbiology, University of Ibadan. The fish were kept in a plastic tank of dimension 75cm x 85cm x 35cm and was filled with aerated water before been taken for isolation experiment.

Lactic Acid Bacteria (LAB) isolation

The fish were starved for 24 hours before been used for the experiment. The fish were killed using sterilized dissecting knife where various organs such as the gills, skin and intestine were cut and kept into different sterile distil water. One gram of the organs (intestine, skin and gill) content was homogenized with 9 mL of sterile saline and vortexed for 1 min in stomacher. In all, ten samples were taken from each site and for the three sites, 30 samples were collected and examined for identification. Subsequently, dilution series were prepared from the homogenate in sterile saline from 10-1-10-10 and pour plated on MRS agar plates. The plates were incubated anaerobically at 37°C for 48-72 h. MRS agar and broth were used for enumeration and culture of LAB (De Man et al., 1960). Well isolated colonies with typical characteristics namely pure white, small (2-3 mm diameter) with entire margins were picked from each plate and transferred to MRS broth.

Identification of the bacterial strains: The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics (Kandler and Weiss, 1986; Sharpe et al., 1979). The used tests were: Gram reaction, cell morphology, catalase, oxidase, Indole, starch hydrolysis, motility test, MR –Methyl red, H₂S production. The sugar fermentation test includes; glucose, lactose, sucrose, salicin, fructose, xylose, raffinose, melibiose, maltose, mannitose, ramanose, galactose, sonbose, trehalose and ribose. Also, test such as reaction of the isolates to; NH₃ from arginine, growth at 4%NaOH, growth at 4°C, 15°C and 45°C were done respectively. After these entire tests were carried out, the isolates from the gill, skin and intestine were identified.

RESULTS AND DISCUSSION

The results of the test carried out are presented in Tables 1, 2 and 3. The biochemical test carried out for the isolates collected from the gill, skin and intestine of *Oreochromis niloticus* shows that all the isolates belongs to the genus lactobacillus and are all lactic acid bacteria belonging to 10 species which includes; *Lactobacillus fermentum*, *brevis*, *acidophilus*, *xylosus*, *plantarium*, *rhamnosus*, *alimentarius*, *reuteri*, *divergence* and *buchneri*.

The percentage of distribution of the 10 species of the lactic acid bacteria isolated from the gill, skin and intestine is presented in Table 4. The result showed an even distribution of the lactic acid bacteria isolated from the three sites with each having equal percentage of 33.333%. Also, considering their distribution on species basis, *L. fermentum* had the highest occurrence of 40%, followed by *L. brevis* (20%), *L. acidophilus* (10%), *L. plantarium* and *alimentarius* (6.6%), while the least was in *L. reuteri*, *buchneri* and *divergence* (3.3%) each.

The results of the biochemical characterization carried out in this experiment followed the standard methods of Kandler and Weiss, 1986; Sharpe et al., 1979 and it showed that lactic acid bacteria isolated from *Oreochromis niloticus* is not different from other laboratory isolated from other sources which are probiotic in nature as they are not different from other LAB isolated from animals and plant LAB are well distributed in the various organs of freshwater fish species such as Nile tilapia which is an indigenous fish species grown in Africa and can be used as probiotic for freshwater aquaculture and It is interesting to note that majority of the *Lactobacillus* sp. that have been isolated from adult fish were those species, which were commonly found on meat, animals and human (Kandler and Weiss, 1986). There were a few reports of isolation of LAB from fresh and seawater fish (Balcázar et al., 2007; Cone, 1982). *Lactobacillus plantarum* have been isolated from herring, Arctic krill and chilled channel catfish fillets (Hagi et al., 2004; Schroder et al., 1979; Spangguard et al., 2000). However, Maugin and Novel (1994) found that *Lactococcus* was the major flora isolated from fish. The occurrence of typical lactobacilli as described by Kandler and Weiss (1986) were rare in fish and prawn but most experiment conducted was differentiated singularly in experimental studies like what was carried out in this experiment. The presence of the LAB in these sites shows that LAB are present in the organs of fish and this may be responsible for defense mechanism in aquaculture species (Sanderson and Walker, 1993; Isolauri, 1997; Yasui et al., 1992 and Moreau et al; 1990).

Table 1: Biochemical characterization of Lactic Acid Bacteria isolated from the gill of *O. niloticus*.

Criteria	Isola 1	Isola2	Isola3	Isola4	Isola 5	Isola 6	Isola7	Isola 8	Isola9	Isola10
Gram reaction	+	+	+	+	+	+	+	+	+	+
Cellular morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Catalase	-	-	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-	-	-
Indole test	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Motility test	-	-	-	-	-	-	-	-	-	-
MR –Methyl red	+	+	+	+	+	+	+	+	+	+
H2S production	-	-	-	+	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	-	+	-	+	-	-	-	-	+	-
Sucrose	+	+	+	+	+	+	+	+	+	+
Salicin	-	-	+	+	+	+	-	+	-	+
Fructose	+	+	+	+	-	-	+	+	+	+
Xylose	+	+	+	-	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+
Melibiose	-	-	+	+	-	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+
Mannitose	+	+	+	+	+	+	+	+	+	+
Ramanose	-	-	-	+	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+	+	+
Sonbose	-	-	+	-	-	+	-	-	-	-
Trehalose	-	-	-	+	-	-	-	-	+	-
Ribose	-	-	-	+	-	-	-	-	+	-
NH3 from arginine	+	+	-	+	+	-	+	+	-	+
Growth @4%NaOH	-	-	-	-	-	-	-	-	-	-
Growth @ 4°C	+	+	-	-	+	-	+	+	+	+
Growth @ 15°C	+	+	-	-	+	-	+	+	+	+
Growth @ 45°C	+	+	-	-	+	-	+	+	+	+
Homo/ Hetero fermentation	HE	HE	HE	HE	HE	HE	HE	HE	HE	HE
Probable Isolates	<i>L. fermentum</i>	<i>L. fermentum</i>	<i>L. brevis</i>	<i>L. acidophilic</i>	<i>L. fermentum</i>	<i>L. brevis</i>	<i>L. fermentum</i>	<i>L. fermentum</i>	<i>L. xylose</i>	<i>L. fermentum</i>

Table 2: Biochemical characterization of Lactic Acid Bacteria isolated from the skin of *O. niloticus*.

Criteria	Isola 1	Isola2	Isola 3	Isola 4	Isola5	Isola6	Isola7	Isola8	Isola9	Isola10
Gram reaction	+	+	+	+	+	+	+	+	+	+
Cellular morphology	Rod	SR	Rod	Rod	Rod	Rod	Rod	SR	Rod	Rod
Catalase	-	-	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-	-	-
Indole test	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Motility test	-	-	-	-	-	-	-	-	-	-
MR –Methyl red	+	+	+	+	-	+	+	+	+	+
H2S production	-	+	-	+	+	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	+	-	-	-	-	+	-
Sucrose	+	+	+	+	+	+	+	-	-	+
Salicin	+	+	+	+	+	+	+	+	+	-
Fructose	+	-	-	+	+	-	+	-	-	+
Xylose	+	-	+	-	+	+	+	+	-	+
Raffinose	+	+	+	-	+	+	+	-	-	+
Melibiose	-	+	+	+	+	+	-	+	+	-
Maltose	+	+	+	+	+	+	+	+	+	+
Mannitose	+	+	+	-	+	+	+	-	-	+
Ramanose	-	-	-	+	+	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+	+	+
Sonbose	-	-	+	-	-	+	-	-	-	-
Trehalose	-	-	-	+	-	-	-	-	-	-
Ribose	-	-	-	+	+	-	-	+	+	-
NH3 from arginine	+	-	-	+	-	-	+	-	+	+
Growth @4%NaOH	-	-	-	-	-	-	-	-	-	-
Growth @ 4°C	+	-	-	-	-	-	+	+	+	+
Growth @ 15°C	+	+	-	-	-	-	+	+	-	+
Growth @ 45°C	+	-	-	-	-	-	+	+	-	+
Homo/ Hetero fermentation	HE	HM	HE	HE	HE	HE	HE	HM	HE	HE
Probable Isolates	<i>L. fermentum</i>	<i>L. platarium</i>	<i>L. brevis</i>	<i>L. acidophilic</i>	<i>L. alimentarius</i>	<i>L. brevis</i>	<i>L. fermentum</i>	<i>L. buchneri</i>	<i>L. divergence</i>	<i>L. fermentum</i>

Table 3: Biochemical characterization of Lactic Acid Bacteria isolated from the intestine of *O. niloticus*.

Criteria	Isola1	Isola 2	Isola 3	Isola 4	Isola5	Isola 6	Isola7	Isola 8	Isola 9	Isol10
Gram reaction	+	+	+	+		+			+	
Cell morphology	Rod	Rod	SR	Rod	SR	Rod	Rod	C	Rod	Rod
Catalase	-	-	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-	-	-
Indole test	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Motility test	-	-	-	-	-	-	-	-	-	-
MR –Methyl red	+	+	+	+	+	+	-	-	+	-
H2S production	-	-	+	-	-	-	+	+	-	-
Glucose	+	+	+	+	+	+	+	+	+	-
Lactose	-	-	-	-	-	-	+	+	-	-
Sucrose	+	+	+	+	+	+	+	-	+	-
Salicin	-	+	+	+	-	-	+	+	-	-
Fructose	+	-	-	-	+	+	+	+	+	-
Xylose	+	+	-	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	-	+	+	-
Melibiose	-	+	+	+	-	-	+	+	+	-
Maltose	+	+	+	+	+	+	+	+	+	-
Mannitose	+	+	+	+	+	+	+	+	+	-
Ramanose	-	-	-	-	+	-	-	+	-	-
Galactose	+	+	+	+	+	+	+	+	+	+
Sonbose	-	+	-	+	-	-	+	+	-	+
Trehalose	-	-	-	-	-	-	-	-	-	+
Ribose	-	-	-	-	-	-	+	-	-	+
NH3 from arginine	+	-	-	-	-	+	+	+	+	-
Growth @4%NaOH	-	-	-	-	-	-	+	-	-	-
Growth @ 4°C	+	-	-	-	-	+	-	-	+	+
Growth @ 15°C	+	-	+	-	-	+	-	-	+	+
Growth @ 45°C	+	-	-	-	-	+	-	-	+	+
Homo/ Hetero fermentation	HE	HE	HM	HE	HM	HE	HE	HE	HE	HE
Probable Isolates	<i>L. fermentum</i>	<i>L. brevis</i>	<i>L. platarium</i>	<i>L. brevis</i>	<i>L. Rhamnosus</i>	<i>L. fermentum</i>	<i>L. acidophilus</i>	<i>L. alimentarius</i>	<i>L. fermentum</i>	<i>L.reuteri</i>

Table 4: Percentage distribution of different species of Lactic Acid Bacteria isolated from *Oreochromis niloticus*

Species of LAB	Gill	Skin	Intestine	% of distribution
<i>L. fermentum</i>	6	3	3	40
<i>L. brevis</i>	2	2	2	20
<i>L. planetarium</i>	-	1	1	6.6
<i>L. buchneri</i>	-	1	-	3.3
<i>L. rhamnosus</i>	-	-	1	3.3
<i>L. xylophilus</i>	1	-	-	3.3
<i>L. acidophilus</i>	1	1	1	10
<i>L. alimentarius</i>	-	1	1	6.6
<i>L. divergence</i>	-	1	-	3.3
<i>L. reuteri</i>	-	-	1	3.3
% of distribution	33.33	33.33	33.33	100

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