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## Screening of Lactic Acid Bacteria from the Gut of *Chrysichthys nigrodigitatus* for Use as Probiotics in Aquaculture Production

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### ABSTRACT

Lactic Acid Bacteria (LAB) namely *Lactobacillus plantarum*1 and *Lactobacillus pentosus* were isolated from the gut of *Chrysichthys nigrodigitatus* and identified using Bergey's manual of determinative bacteriology (API-50 CHL, BioMérieux). They are beneficial and safe organisms that improve disturbances of the indigenous microflora and host's immune system. Gut removed from fish samples were dissected and divided into 3 regions: Fore gut, mid gut and hind gut. *Lactobacillus plantarum*1 and *Lactobacillus pentosus* were isolated from these regions using MRS (de Man, Rogosa and Sharpe) agar and broth after subsequent culture and sub culture to obtain discrete colonies. They were characterised as Gram-positive, non-motile, catalase negative and non sporulating bacteria.

**Key words:** *Lactobacillus plantarum*1, *Lactobacillus pentosus*, probiotics, *Chrysichthys nigrodigitatus*

### INTRODUCTION

Probiotics are described as preparations of living microbial cells that, when ingested in high enough concentration, beneficially affect the host's health by improving the intestinal microbial balance (Fuller, 1989). Selection of probiotic strains is achieved by screening procedures for several characteristics such as inhibitory activities against several pathogens, resistance to gastric secretions, bile tolerance and growth in faecal material. They should resist processing and storage conditions and be alive and active even after gastrointestinal passage. They should be safe and impart benefits to the host (Fuller, 1989; Havenaar and Huis in't Veld, 1992). Lactic acid bacteria are Gram-positive, non-sporulating and catalase negative rods or cocci that ferment various carbohydrates mainly to lactate and acetate. Probiotics are Lactic Acid Bacteria (LAB) characterized as Gram-positive, non-motile, non-spore forming bacteria, non pigmented (Hassan and Frank, 2001) and catalase negative. The most commonly used organisms in probiotic preparations are lactic acid producing bacteria such as lactobacilli, streptococci, bifidobacteria, bacillus spp. and fungi like *Sacharomyces cerevisiae*, *Sacharomyces boulardii* and *Aspergillus oryzae* (Fuller, 1992). However, Lactic Acid Bacteria (LAB) have attained major attention for probiotic activity and have generally been considered as good probiotic organisms (Saavedra, 2001; O'Sullivan *et al.*, 1992). The aim of the study was to screen lactic acid bacteria from the gut of *Chrysichthys nigrodigitatus* and classify them based on the morphological and biochemical characteristics.

## MATERIALS AND METHODS

**Experimental fish for analysis:** Fish samples were collected from Lagos lagoon and transferred to the Nigerian Institute for Medical Research laboratory within 1 h. Twenty individuals of different sizes were selected. The length and weight of the fish were measured before dissection. The skin was then washed with 70% ethanol before opening the ventral surface with sterile scissors. The fish were dissected to remove the gut and divided into foregut, midgut and hind gut in order to determine where there is more concentration of probiotics in these regions. One gram of intestine was taken from each fish sample and suspended in 9 mL of sterile saline diluents (0.85% NaCl). The gut samples were homogenized in a blender using saline, serially diluted and allowed to grow in MRS broth medium.

**Preparation of media:** The bacteria *Lactobacillus* spp. was isolated from fish gut by using modified MRS broth and MRS agar media. Fifty two grams of the media was suspended in one liter of distilled water each. They were mixed well, heated agitating frequently until complete dissolution of the medium. Each medium was dispensed in adequate containers and sterilized in autoclave at 121°C for 12 min. Additionally, 0.05% cysteine was added to MRS to improve the specificity of this medium for isolation of *Lactobacillus*. The pH of the media was adjusted to 6.2.

**Isolation of bacteria:** *Lactobacillus* was isolated from *Chrysichthys nigrodigitatus* by using MRS medium. One gram of each sample was dissolved into 100 mL of MRS broth at pH 6.5. After dissolving into MRS broth they were shaken homogeneously and incubated at 37°C for 24 h in aerobic condition. The cultures were subjected to five subculture at 37°C under low pH (pH 4.5) and anaerobic condition in the presence of 10% CO<sub>2</sub> to remove unwanted bacteria. After seven subcultures, the bacterial culture was streak onto MRS agar media at pH 4.8. Finally, the single colony of *Lactobacillus* was isolated observing their colony morphology and some biochemical tests. (Gram staining, catalase, endospore and motility test) and the culture were maintained in MRS broth at pH 5.5.

### Biochemical test for LAB

**Grams staining:** Heat fixed bacterial smear slide was placed on a staining rack stained with crystal violet for 1 min, washed in tap water, cover with gram's iodine for 1 min, re-washed, decolourised by washing the slide briefly in acetone (2-3 sec) and counterstained for 10 min in Safranin. The smears were washed thoroughly with water and gently air dried and observed under oil immersion.

**Methyl red test (MR):** Isolated lactic acid bacteria was inoculated with buffered glucose broth and incubated at 37°C for 48 h. After incubation, a few drops of methyl red solution were added to the culture and readings were taken immediately.

**Catalase test:** A loopful of the culture was placed on a slide and few drops of 10% hydrogen peroxide were added. The slides were observed for effervescence.

**Screening:** The lactic acid bacteria were screened for the production of *Lactobacillus plantarum* and *Lactobacillus pentosus* using Bergey's manual of determinative bacteriology (API-50 CHL, BioMérieux). The identity of the cultures was based on the characteristics of the lactobacilli as

described in Bergey's Manual of Determinative Bacteriology (Azcarate-Peril), fermentation of different carbon sources (API 50 CHL, BioMérieux). The results form a biochemical profile which, when entered in the identification software, provides the identification of *Lactobacillus plantarum*1 and *Lactobacillus pentosus*.

## RESULTS AND DISCUSSION

Bacteria isolated from the foregut, midgut and hindgut of *Chrysiethys nigrodigitatus* were identified as *Lactobacillus plantarum*1, *Lactobacillus pentosus* by observing their colony morphology, physiological and as well as some biochemical characteristics. Microscopically they were Gram-positive, rod shaped, non- motile, catalase negative and absence of endospore (Fig. 1 and 2). *Lactobacillus plantarum*1 had the highest identification value of 99.9% as the significant taxa and *Lactobacillus pentosus* as the next taxon with identification value of 0.1% as represented in Table 1. The presence of *Lactobacillus* as the main probiotic organism shows similarity with the findings of other researchers who reported maximum population of *Lactobacillus* in catfish (*Clarias orietialis*) (Dhanasekaran *et al.*, 2008). Isolation of *Lactobacillus* from the gut of brackish



Fig. 1: Non-spore forming *Lactobacillus plantarum*1



Fig. 2: Colonies of *Lactobacillus*

Table 1: Summary of the result of isolation and identification of probiotics from fish gut (foregut, midgut and hindgut)

Fish species	Probiotics identified		Concentration			ID (%)		
	Significant taxa	Next taxon	Fore gut	Mid gut	Hind gut	Significant taxa	Next taxa	Result
<i>Chrysiichthys nigrodigitatus</i>	<i>Lactobacillus plantarum</i> 1	<i>Lactobacillus pentosus</i>	++	+++	+++	99.9	0.1	Excellent ID

ID: Identification, XX: Less abundance and XXX: More abundance

catfish (*C. nigrodigitatus*) in this present study was also supported by the study of Adenike and Olalekan (2009) who reported the isolation of 44 strains of *Lactobacillus* from the gut contents of African catfish (*Clarias gariepinus*). According to a previous study which isolated and characterized *Lactobacillus* species from fish intestine obtained from 5 different fresh water and they reported that out of 120 isolates only 22 *Lactobacillus* species were selected for further studies. The result obtained in this study indicated a high accuracy of *Chrysiichthys nigrodigitatus* probiotics. The strain was isolated from fish gut and identified as *Lactobacillus plantarum*1 by both biochemical test and API 50CHL (Biomerieux). Corr *et al.* (2007) reported that bacteriocin producing LAB can effectively suppressed the growth of *Listeria monocytogenes* in fish and to eradicate its presence which was supported by Hisar *et al.* (2005) who studied the effect of *Lactobacillus sakei* lb706 on behavior of *Listeria monocytogenes* in vacuum packed rainbow trout fillets for preventing this Gram-positive food borne pathogenic bacteria. Screenings of this probiotic are required in order to develop probiotic bacteria with divers' antimicrobial potentials.

## CONCLUSION

Probiotics isolated and screened using an appropriate medium could be used as growth promoters in aquaculture production and could be administered in such a way to enter the gastrointestinal tract, to be kept alive and with the aim of improving health.

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