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Isolation and Identification of Probiotics *Pediococcus pentosaceus2 and Pediococcus pentosaceus1* from the Gut of *Tilapia Guineensis* for Use in Aquaculture Production

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Abstract: *Aquaculture industry is the fastest-growing food producing sector but fish diseases has been a major threat to the growth. Bacterial infections are one of the causes of mortality in fish and the use of antibiotics has resulted in resistant strain of bacteria in fish. Therefore role of probiotics is being considered as alternative. Studies were carried out to isolate and identify probiotics from the gut of Tilapia guineensis. Guts removed from dissected fish were suspended in 9ml of saline diluents homogenized and allowed to pour in MRS broth and agar medium. The isolated lactobacillus was re dissolved and shaken homogenously incubated at 37^0C aenarobically for 24 hrs. The culture obtained were subculture on MRS at pH 6.2 for discrete colonies. Single colony of lactobacillus was isolated using colony morphology and some biochemical tests. The isolated bacteria were identified using biochemical test and API CH in conjunction with API CHL medium for the identification of probiotics. Bacteria isolated from the gut of Tilapia guineensis were identified as pediococcus pentosaceus2 and pediococcus pentosaceus1 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*.

Keywords: *pediococcus pentosaceus2, pediococcus pentosaceus1,* probiotics and *Tilapia guineensis.*

1. INTRODUCTION

Fish diseases are major problem for the fish farming Industry and among those bacterial infections are considered to be a major cause of mortality in fish [1].

However, the excessive and inappropriate use of antibiotics has resulted in the presence of resistant strains of bacteria in fish culture [2].

In addition, there are environmental problems associated with the antibiotics [3]. Therefore, the need for alternatives is increasing and the contribution of probiotics may be considerable. With increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely accepted [4-7]. Probiotics are harmless bacteria that help in the well being of the host animal and contribute, directly or indirectly to protect the host animal against harmful bacterial pathogens. Probiotics are lactic acid bacteria (LAB) characterized as Gram- positive, non-motile, non-spore forming bacteria, non-pigmented [8], and catalyst negative.

The most commonly used organisms in probiotic preparations are lactic acid producing bacteria such as lactobacilli, streptococci, Bifidobacteria, Bacillus spp. and fungi like S*acharomyces cerevisiae, Sacharomyces boulardii* and *Aspergillus oryzae* [9, 10]. However, lactic acid bacteria (LAB) have attained major attention for probiotic activity and have generally been considered as good probiotic organisms [11, 12].

The aim of the study was to isolate and identify resident probiotics from the gut of *Tilapia guineensis* and classify them based on their morphological and biochemical characteristics.

2. MATERIALS AND METHODS

2.1 Collection of Samples and Laboratory Procedure

Freshly caught fish of *Tilapia guineensis* were procured from fish landing site at Makoko, packed in iceboxes and transferred to the Nigerian Institute for Medical Research laboratory within 1hr. 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 other to determine where there is more concentration of probiotics in these regions. 1g of intestine was taken from each fish sample and suspended in 9 ml of sterile saline diluents (0.85% NaCI). The gut samples were homogenized in a blender using saline, serially diluted and allowed to grow in MRS broth medium.

2.2 Preparation of Media

The bacteria *Lactobacillus* spp. was isolated from fish gut by using modified MRS broth and MRS agar media. 52grams of the media was suspended in one litter 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 121^0C for 12 minutes. 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.

2.3 Isolation of Probiotics

Lactobacillus was isolated from fish gut using MRS medium. 1ml of each sample was dissolved in 9 ml of MRS broth at pH 6.2. After dissolving into MRS broth they were shaken homogeneously and incubated at 37°C anaerobically for 24 hrs. The cultures were subjected to five subculture at 37°C under low pH (pH 6.2) and anaerobic condition in the presence of 10% CO to remove unwanted bacteria. After 24 hrs the broth was sub-cultured on MRS for discrete colonies, agar media at pH 6.2. Finally, the single colony of lactobacillus was isolated by 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. The isolates were prepared using a McFarland standard of 1.0 $X10^8$ by adding sterile distilled water

2.4 Some Biochemical Test for LAB

2.5 Grams Staining

Heat fixed bacterial smear slide was placed on a staining rack stained with crystal violet for 1 minute, washed in tap water, cover with grams iodine for 1 minute, re-washed, decolourised by washing the slide briefly in acetone (2-3 seconds) l and counterstained for 10 minutes in Safranin. The smears were washed thoroughly with water and gentle air dried and observed under oil immersion.

2.6 Catalyst 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.

2.7 Identification

The isolated bacteria were identified as *Lactobacillus* spp. by observing their morphological characteristics and by mean Gram staining, motility test, catalase test, 0.4% bacteriostactic phenol tolerance test and 1-10% NaCl tolerance test. 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). This is a ready- to- use medium which enables the fermentation of 49 carbohydrates on the API 50 CH strip to be studied. A suspension is made in the medium with the microorganism to be tested and each tube of the strips inoculated. Carbohydrates were fermented to acids during incubation which produced a decrease in pH. This was detected by the color change of the indicator. API analysis was used for determination of the biochemical profile of the strain and its identification.

3. RESULTS AND DISCUSSION

Bacteria isolated from the foregut, midgut and hindgut of *Tilapia guineensis* were identified as *pediococcus pentosaceus2 and pediococcus pentosaceus1* by observing their colony morphology, physiological and as well as some biochemical characteristics (Table 1). Microscopically they were Gram-positive, rod shaped, non- motile, catalase negative and absence of Endospore Plate 1and 2. Plate 3 represented the API 50 CH strip consists of wells, each one of them was rehydrated with bacterial suspension and the strips were incubated for identification of bacteria. *Pediococcus*

pentosaceus being a gram positive bacterium, non-motile with no endospore, strives anaerobically and forms a member of the industrially important lactic acid bacteria (LAB) that grows on lactobacilli MRS broth at 37^0C. Summary of the result of isolation and identification of probiotics from *Tilapia guineensis* gut (foregut, midgut and hindgut) is represented in Table 2.The result obtained indicated a high accuracy of *T. guineesis* probiotics. *Pediococcus pentosaceus* produces antimicrobial activities against many gram positive bacteria species, including the food borne pathogen *Listeria monocytogenes*. Ferguson *et al.* [13] reported that dietary supplementation of *Pediococcus acidilactici* increases the intestinal microbial load of red tilapia, *Oreochromis niloticus.* Merrifield *et al*. [14] also reported that *Pediococcus acidilactici* has a significant effect on intestinal microflora of rainbow trout, *Oncorhynchus mykiss*. Moslehi *et al.,* [15] stated that *Pediococcus pentosaceus* had positive effect on the body composition and intestinal microflora of Siberian sturgeon, *Acepenser baerri.* The author reported that *Pediococcus pentosaceus* has a proper colonization in the gut of treated fish species. Corr *et al.,* [16] reported that bacteriocin producing LAB can effectively suppressed the growth of listeria in mice and to eradicate its presence. Colony forming unit ($CFUml^{-1}$) isolated from the gut of *Tilapia guineensis* on MRS agars media is represented in Table 3. Identification and characterization of these probiotics are required in order to develop probiotic bacteria with divers' antimicrobial potentials. The probiotics may have potential use as an additive in the food industry particularly processed food in which some bacillus species are potential spoilage. Further study would be the incorporation of these probiotics into fish feed and evaluate their performance in terms of growth, nutrient utilization and lessen the diseased burden on some culturable fish species which could be of economic important in aquaculture production.

Table 1. *Colony morphology, physiological and biochemical characteristics of the isolated bacteria strain of pediococcus pentosaceus2 and pediococcus pentosaceus1*

Colony morphology/physiological features		Biochemical tests	
Characteristics	Descriptions	Characteristics	Descriptions
Cell shape	Rod	Catalase	negative (+)
Pigment	White- creamy	0.4% phenol tolerance test	Positive (+)
Gram staining	Positive	5% Nacl tolerance test	Positive (+)
spores	No endospores	Temperature	25-40 degree C
motility	Non-motile	Period of incubation	24-48hrs
Texture	Dry	Atmosphere	Anaerobic
Colony appearance	Smooth	Optimum pH	4.5-7.0

Plate 1. *Colonies of pediococcus pentosaceus2 and pediococcus pentosaceus1*

Table 3. *Summary of the result of isolation and identification of probiotics from Tilapia guineensis gut (foregut, midgut and hindgut)*

	Probiotics Identified		Concentration			%ID	%ID	Result
Fish	Sig. taxa	Next taxon	Fore	Mid	hindgut	Sig.	Next	

Specimen			gut	gut		taxa	taxa	
1	*Pediococcus pentosaceus* 2	*Pediococcus pentosaceus* 1	++	+++	+++	99.9	0.1	Excellent ID
2	*Pediococcus pentosaceus* 2	*Pediococcus pentosaceus* 1	+++	+++	+++	99.6	0.4	Excellent ID
3	*Pediococcus pentosaceus* 2	*Pediococcus pentosaceus* 1	++	+++	+++	99.6	0.4	Excellent ID
4	*Pediococcus pentosaceus* 2	*Pediococcus pentosaceus* 1	+++	+++	+++	99.1	0.9	Excellent ID
5	*Pediococcus pentosaceus* 2	*Pediococcus pentosaceus* 1	++	+++	+++	99.4	0.6	Excellent ID
6	*Pediococcus pentosaceus* 2	*Pediococcus pentosaceus* 1	++	+++	+++	99.8	0.2	Excellent ID

Keys:

ID – Identification

XX- Less abundance

XXX – More abundance.

Sig.- significance

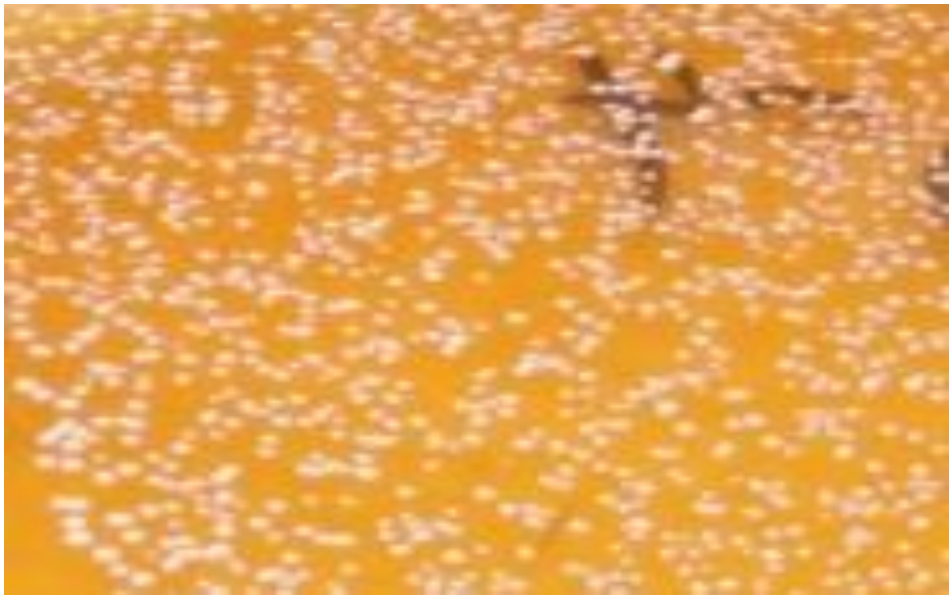

Plate 2. *Colonies of pediococcus pentosaceus2 and pediococcus pentosaceus1*

Plate 3. API kits for Identification of Probiotics.

Table 3. *Colony forming unit ($CFUml^{-1}$) isolated from the gut of Tilapia guineensis on MRS agars media.*

Fish specimens	Weight of fish (g)	Total length (cm)	Standard length (cm)	No of plates	CFUml-1
1	37.06	11.0	8.6	5	3.20×10^8
2	43.40	12.8	8.8	5	2.89×10^8
3	15.50	8.2	6.5	5	3.10×10^8
4	86.50	17.1	15.0	5	3.02×10^8
5	15.00	8.9	6.9	5	3.20×10^8

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