ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF LACTOBACILLUS SPECIES FROM FERMENTED AFRICAN LOCUST BEAN (*Parkia biglobosa*) FOR USE AS PROBIOTICS IN THE AQUACULTURE INDUSTRY.

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ABSTRACT
Probiotics are a promising feed additive to stimulate animal growth and secure a low disease response in aquaculture industry where there are high stocking densities in shrimp and fish production. Fermented locust beans (*Parkia biglobosa*) are known to be rich in protein and used as food condiment. Probiotic bacteria were isolated from this locally available food material. Culture and characterizations of isolates were carried out. Sugar fermentation patterns were determined by using an API 50 CHL system and incubation were performed anaerobically at 37°C. MRS broth culture grown at 37°C overnight was added to 9ml of MRS Agar and the bacteria were incubated at 37°C for 24 and 48 hrs. Discrete and single colony of lactobacillus was isolated using colony morphology and biochemical tests. The most significant viable taxa isolated was "*Lactobacillus fermentum*" at a pH range of (3.0 – 8.0), while the least viable taxa isolated was *Leuconostoc mesenteroides* ssp. Microscopically they were Gram-positive, rod shaped, non- motile, catalase negative and absence of Endospore.
Key words: Locust bean, probiotics, Lactobacillus.

INTRODUCTION
A growing concern about the high consumption of antibiotics in aquaculture has initiated a search for alternative methods of disease control (Gildberg *et al*., 1997) and growth promotion (Byun *et al*., 1997). Improved resistance against infectious diseases can be achieved by the use of probiotics (Gildberg, *et al*., 1997). Probiotics are living preparations of microbial cells that, when ingested in high enough concentration, beneficially affect the host's health and growth by improving the intestinal microbial balance (Fuller, 1989; Havenaar,, et al., 1992). The use of probiotics in aquaculture is proving to be highly effective in improving disease resistance, nutrition and growth of cultured organisms (Macey and Coyne, 2005). Information acquired to date shows that lactobacilli have a long history of use as probiotics without established risk to humans and remains the best proof of their safety (Naidu, *et al*., 1999; Saxelin, *et al*., 1996). No pathogenic or virulence properties have been found for lactobacilli, bifloibacteria or lactococci (Aguirre and Collins, 1993). However, lactic acid bacteria (LAB) have attained major attention for probiotic activity and have generally been considered as good probiotic organisms (Saavedra, 2001; Sullivan, *et al.,* 1992). *Parkia biglobosa* popularly known as African locust bean have found use as foods, medicinal agents and are of high commercial value. Campbell-Platt, 1980 reported *P. biglobosa has* been used for food, medicine and fodder, soil amendment, charcoal and fire wood. The fermented seeds of *P. biglobosa* are used in all parts of Nigeria and indeed the coast of Africa for seasoning traditional soups. The aim of the study was to isolate, identify and classify them based on the morphological and biochemical characteristics.

MATERIALS AND METHODS
Seed collection and Extraction
Freshly harvested African locust beans (*Parkia biglobosa*) were purchased from Mushin market in Lagos. Good quality grains were selected and the broken ones discarded. According to Odunfa (1981), selected grains were dehusked and cooked in an aluminium pot and transfer to dish, well covered and allowed to ferment for 4 days. They were ground in an ordinary grinder. The supernatant and residues were allowed to grow in MRS broth medium.
Preparation of Media:The bacteria *Lactobacillus* spp. was isolated from African locust bean 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^{0}C 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.
Isolation of LAB: One gram of each sample was dissolved into 100ml of MRS broth at pH 6.5. After dissolving into MRS broth they were shaken homogenously and incubated at 37^{0}C for 24 h in anaerobic condition. The cultures were subjected to five subculture at 37^{0}C under low pH (pH 4.5) and anaerobic condition in the presence of 10% CO_2 to remove unwanted bacteria. After five subcultures, the bacterial culture was streak into 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). Cultures were maintained in MRS broth at pH 5.5.

Identification: The isolated bacteria were identified as *Lactobacillus* spp. by observing their morphological characteristics and by 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 using 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 micro organism 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.

RESULTS AND DISCUSSION

Bacteria isolated from African locust beans (*Parkia biglobosa*) were identified as *Lactobacillus fermentum* and *Leuconostoc mesenteroides* by observing their colony morphology, physiological, some biochemical characteristics and sugar fermentation tests. Microscopically they were Gram-positive, rod shaped, non- motile, catalase negative and absence of Endospore Plate 1and 2. *Lactobacillus fermentum* had the highest identification value of 92.9% as the significant taxa and *Leuconostoc mesenteroides* as the next taxon with identification value of 7.1% as represented in Table 1. The strain was isolated from African locust bean (*Parkia biglobosa*) and was identified as *Lactobacillus fermentum* by both biochemical test and API 50CHL (Biomerieux) sugar fermentation tests (Table 2 and Plate3) . Corr *et al*., 2007 reported that bacteriocin producing LAB can effectively suppress the growth of *Listeria monocytogenes* in fish and to eradicate its presence. Uabio-Egbenni, *et al*., (2009) reported the presence of *Leuconostoc mesenteroides* as one of the LAB group in *Parkia biglobosa* after morphological, biochemical and sugar fermentation tests. Adimpong *et al* 2012 reported *Lactobacillus fermentum* and *Leuconostoc mesenteroides* among species identified and isolated from fermented food products. Screening of this probiotic is required in order to develop probiotic bacteria with diverse antimicrobial potentials. The probiotics may have potential use as an addictive in the food industry particularly processed food in which some *Bacillus* species are potential spoilage organisms.

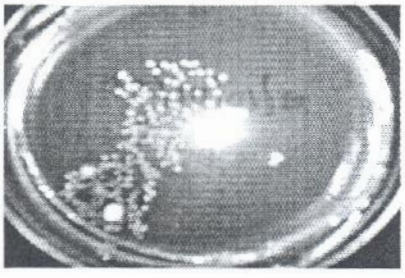


Plate 1: Non- spore forming *Lactobacillus fermentum*

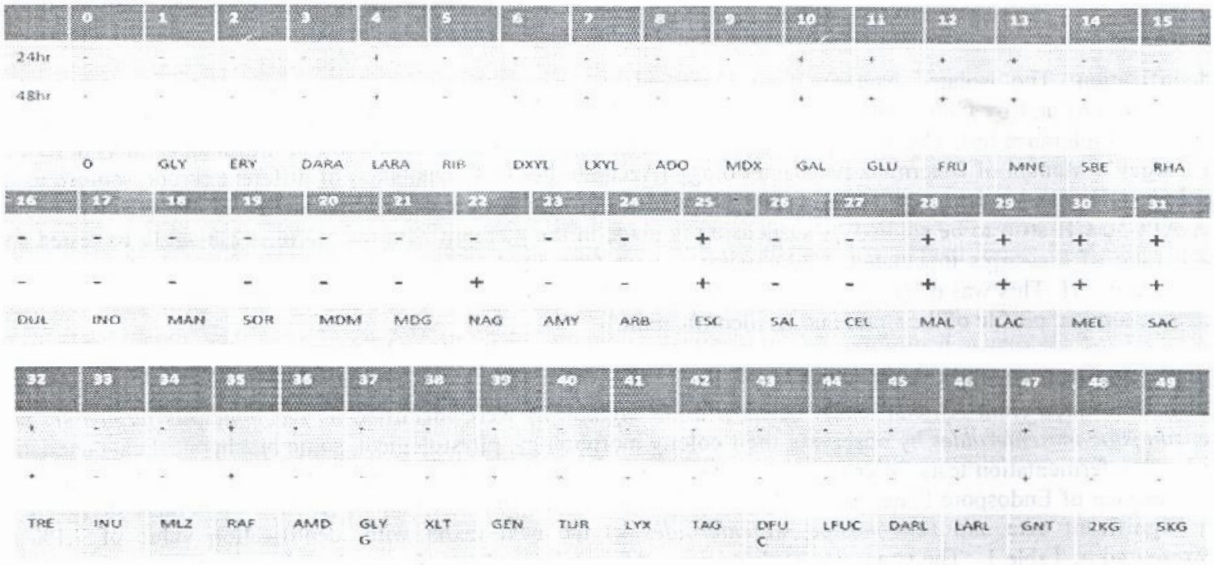
Plate 2: Colonies of Lactobacillus

Table 1: Summary of the result of isolation and identification of probiotics from African locust beans (*Parkia biglobosa*)

	Probiotics Identified		%ID	%ID	Result
Species	Sig. taxa	Next taxon	Sig. taxa	Next taxa	
Parkia biglobosa	*Lactobacillus fermentum*	*Leuconostoc mesenteroides*	92.9	7.1	Acceptable ID

Keys: ID – Identification, Sig. : significance

Table 2: Biochemical and morphological properties of Lactic acid bacteria Isolated from locust beans during fermentation.



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