

**PROBIOTIC PROPERTIES OF PEDIOCOCCUS SPECIES ISOLATED
FROM SORGHUM**

by

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SCHOOL OF POSTGRADUATE STUDIES
UNIVERSITY OF LAGOS
CERTIFICATION

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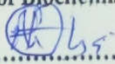
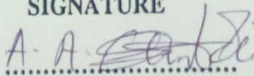
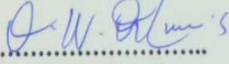
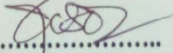
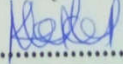
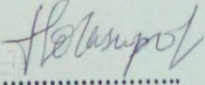

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DEDICATION

To the Almighty God who made all things possible and all who contributed one way or the other the delivery of the child of my mind, more importantly, my parents: Chief and Mrs. J. S. Otunba.

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DECLARATION

We hereby declare that this thesis titled “Probiotic Properties of *Pediococcus* species isolated from Sorghum” is a record of original research work carried out by **OTUNBA**, Ahmed Adebisi, in Biochemistry, Department of Biochemistry, University of Lagos, Nigeria.

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ABSTRACT

Probiotic microorganisms exert strain-specific health-promoting activities on humans and animals. Lactic acid bacteria (LAB) have been reported to play a major role in fermentation, enhancing nutritive values of fermented products in addition to acidifying the products which prevents the growth of some spoilage and pathogenic organisms. This study investigated the probiotic potentials of LAB isolates from indigenous fermented sorghum products (*Ogi-baba* and *Pito*). These LAB isolates were screened for potential probiotic properties by antagonistic activity against eight enteropathogenic clinical bacteria isolates (*Escherichia coli*, *Klebsiella sp.*, *Helicobacter pylori*, *Bacillus sp.*, *Staphylococcus sp.*, *Salmonella sp.*, *Pseudomonas sp.* and *Listeria monocytogenes*) as indicator organisms using the agar well diffusion technique. The organisms were also screened for acid tolerance, bile tolerance, antibiotic susceptibility, production of lactic acid, diacetyl and hydrogen peroxide. β -galactosidase assay was also carried out. Genomic DNA was extracted from two LAB isolates, which were selected as the best probiotic isolates for scoring more than 70% of potential probiotic properties. The 16S rRNA were amplified and sequenced. The sequence data were submitted to the Genbank and subjected to Basic Local Alignment Search Tool (BLAST) and molecular phylogenetic analyses to identify the isolates. The isolates were identified as strains of *Lactobacillus plantarum* and *Pediococcus pentosaceus*. The identified strains were screened for production of antibacterial agents other than organic acids and hydrogen peroxide. The proteinaceous antibacterial agent secreted was purified by ammonium sulphate precipitation and carboxy-methyl ion exchange chromatography, electrophoresed on Tricine-SDS-PAGE and characterized. Eleven lactic acid bacteria were

isolate from the fermented sorghum products and were tentatively identified as strains of *Lactobacillus* sp., *Leuconostoc* sp., *Streptococcus* sp. and *Pediococcus* sp. Two isolates were observed to exhibit highest probiotic potentials and were identified as strains of *Lactobacillus plantarum* and *Pediococcus pentosaceus*. The sequence data for the two isolates were assigned accession numbers KP883298 and KP883297 respectively. *P. pentosaceus* inhibited growth of both representative Gram negative and Gram positive microorganisms (*E. coli* and *L. monocytogenes* respectively) on bacteriocin screening medium whereas *L. plantarum* strain showed no activity. However, the *P. pentosaceus* strain PB2 showed β -galactosidase activity as well as *L. plantarum* strain OB6. Proteinase K inactivated the antibacterial activity of the *P. pentosaceus* strain (PB2) suggesting that the antibacterial agent was proteinaceous in nature. The partially purified (12.62 fold) proteinaceous substance was observed to be 4.87 KDa in size with optimal activity at 40°C and pH 5; against both *E. coli* and *L. monocytogenes*. Plasmid screening revealed the presence of two plasmids with 0.9 and 1.2 kb sizes in the *P. pentosaceus* strain. The antibacterial activity was retained when the PB2 was cured with ethidium bromide. This revealed that the antibacterial peptide is chromosome encoded. The study revealed that *Pediococcus pentosaceus* strain (PB2) isolated from fermented sorghum may be potentially used as probiotic to prevent and treat some enteropathogen induced gastrointestinal disorders and for the management of lactose intolerance.

KEYWORDS: Sorghum, Bacteriocin, *Ogi-baba*, *Pito*, *Pediococcus pentosaceus*, peptide.