

**A STUDY OF PROBIOTIC POTENTIALS OF LACTIC ACID
BACTERIA ISOLATED FROM FERMENTING CASSAVA**

ORIKE, EMMANUEL LUCKY

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B. Sc.(Hons.) Microbiology, Ife.

**A THESIS SUBMITTED TO THE DEPARTMENT OF
MICROBIOLOGY, FACULTY OF SCIENCE, OBAFEMI AWOLOWO
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AWARD OF A MASTER OF SCIENCE DEGREE IN MICROBIOLOGY.**

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AUTHOR: ORIKE, EMMANUEL LUCKY

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This is to certify that this research work was carried out by **ORIKE, Emmanuel Lucky**, in the department of Microbiology, ObafemiAwolowo University, Ile-Ife, Nigeria.

O. OmafuvbeDate

(Supervisor)

Dr. B.

Dr. D. A. Akinpelu

(Head, Department of Microbiology)

Date

DEDICATION

This work is exclusively dedicated to the creator of the universe, Almighty God for being the ultimate source of knowledge and wisdom, to my lovely mother, Mrs. J. D. Orike and finally to Barrister and Mrs. NiyiKosile.

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ABSTRACT

This study was undertaken to isolate, identify lactic acid bacteria (LAB) from fermenting cassava and investigate their probiotic properties with a view to selecting the best strain(s) that could be useful in the probiotication of pasteurized orange juice.

Matured cassava tubers were obtained from the ObafemiAwolowo University Teaching and Research Farm Ile Ife, Nigeria. The cassava tubers were peeled, cut into small sizes, washed, soaked in clean tap water and left to ferment at room temperature for up to 96 h. Aliquot (5 mL) of the soak water was aseptically obtained for isolation of LAB following standard methods. The probiotic properties such as acid and bile salt tolerance, antagonistic activities against selected pathogens, hemolytic activity and exopolysaccharide production of the isolated LAB were studied. The best LAB strain(s) were selected based on their probiotic properties for probiotication of orange juice. Fresh ripe oranges were squeezed, the juice extracted and pasteurized at 95°C for 30 seconds. The juice were inoculated (10^8 cell/mL orange juice) with the selected LAB strains singly and in combination and stored at room temperature ($30 \pm 2^\circ\text{C}$) and 4°C for 20 days. During storage, samples were obtained at 5 days interval for pH, titratable acidity and viable counts.

Twenty-four strains of LAB were isolated from fermenting cassava which showed varied tolerance to various acidic pH levels and bile salt concentrations. The isolates did not display hemolytic activity which was indicative that they were non-pathogenic. Twenty-three of the LAB strains showed antagonistic activity due to the production of organic acids against *Staphylococcus aureus* and *E. coli* NCIB 86. About 83.3% of the LAB strains produced exopolysaccharide. Of the 24 strains, *Lactobacillus plantarum* (A₂) and *Lactobacillus delbrueckii*

(C₂) were selected based on their technological properties as possible probiotic strains for probiotication of pasteurized orange juice. The viability of the selected probiotic LAB in the pasteurized orange juice were within the recommended population (10^6 - 10^8 cfu /mL) after 20 days of storage at 4°C while it dropped to 10^5 at room temperature storage. The organoleptic evaluation showed that the probiotic orange juice inoculated with *L. delbrueckii* (C₂) and the mixed culture of the probiotic strains were not significantly different from the uninoculated pasteurized orange juice in all the organoleptic attributes (taste, aroma, appearance and general acceptability) scored by the taste panelists, an indication that *L. delbrueckii*(C₂) played an important role in contributing to the development of these sensory attributes.

The study concluded that *L. plantarum* and *L. delbrueckii* might be useful as probiotic culture for the production of healthy functional drinks which might solve the problem associated with probiotic dairy products especially for lactose intolerant people.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The word 'probiotic' comes from the Greek language 'pro bios' which means 'for life' opposed to 'antibiotics' which means 'against life'. The term 'probiotic' was first used in 1965 to describe substances which stimulate the growth of other microorganisms (Lilley and Stillwell, 1965). 'Probiotic' was thereafter used in different meaning according to its mechanism and the effects on human health and was defined as live microbial supplement which affects host's health positively by improving its intestinal microbial balance. The definition was broadened to include mono or mixed culture of live microorganisms which are applied to animal and man (Sanders, 2003; Guarner *et al.*, 2005). Some modern definitions include more precisely a preventive or therapeutic action of probiotics; Charteris *et al.* (1997) for example, defined probiotics as microorganisms which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition. Since probiotics have been found to be effective in the treatment of gastrointestinal diseases (Marteau *et al.*, 2001), they can be considered to be therapeutic agents.

Probiotics can also be referred to as single or mixed cultures of live microorganisms that when applied to animals or humans in adequate amounts, confer a health benefit on the host by improving the properties of the indigenous microflora (Tabbers and Benninga, 2007). According to Boirivant and Strober (2007), probiotics are a heterogeneous group of non-pathogenic bacteria that are functionally defined by their ability to allay inflammation when introduced into the inflamed intestine. A number of health benefits such as

antimutagenic effects, anticarcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol, and immune system stimulation have been claimed for probiotic food. Because of the potential health benefits, probiotic organisms are increasingly being incorporated into a number of dairy foods. Increased commercial interest in exploiting the proposed health attributes of probiotics has contributed significantly to the rapid growth and expansion of this sector of the market (Stanton *et al.*, 2001). Food manufacturers are enthusiastic about developing such products because the added ingredients give increased value to food. Research is now being carried out to incorporate probiotic microorganisms in non-dairy food products such as chocolates and juices. The non-dairy probiotic foods are very popular in European countries and a number of companies are manufacturing probiotic juices, chocolates and other non-dairy probiotic foods (Stanton *et al.*, 2001). There are several technological factors related to probiotic microorganisms like oxygen tolerance, acid and bile tolerance, heat tolerance, and type of food carrier, which affect the manufacture of probiotic food products. A lot of researches are ongoing to overcome these impediments.

1.1 LACTIC ACID BACTERIA

Lactic acid bacteria are industrially important microorganisms recognized for their fermentative ability and their health and nutritional benefits (Schmidt *et al.*, 1994). The most studied lactic acid bacteria are those belonging to *Lactobacillus* and *Bifidobacterium* genus. These lactic acid bacteria generally can produce a number of antimicrobial substances. Besides some metabolic end products such as lactic acid and hydrogen peroxide, some lactic acid bacteria also can produce bacteriocins (Holck *et al.*, 1992). Lactic acid bacteria are the most prominent non-pathogenic bacteria that play a vital role in our

everyday life, from fermentation, preservation and production of wholesome foods, and vitamins to prevention of certain diseases and cancer due to their antimicrobial action. Lactic acid fermentation is generally inexpensive often requiring little or no heat in the process, making them fuel-efficient as well (Keith, 1991).

1.2 STATEMENT OF RESEARCH PROBLEM

Lactobacillus has been noted to have nutritional benefits, improved lactose utilization, have anti-carcinogenic activities and protection against other diseases (Englund, 1992).

In previous studies, the majority of probiotic bacteria (members of the genera *Lactobacillus*, *Bifidobacterium* and *Enterococcus*) have been isolated either from humans or animals (Annuk *et al.*, 2003; Kim *et al.*, 2010; Puniya *et al.*, 2012), milk products, sugar cane plants (Liliana, 2006), fresh water fishes (Gonzales *et al.*, 2000). However, information on isolation of lactic acid bacteria with probiotic potentials from fermenting cassava is scanty.

1.3 SPECIFIC OBJECTIVES OF THE RESEARCH

The specific objectives of the study are to:

1. isolate and identify lactic acid bacteria from fermenting cassava tuber;
2. screen the lactic acid bacteria isolates for probiotic properties such as exopolysaccharide (EPS) production, antimicrobial activity against selected pathogens, hemolytic activity, tolerance to acidic pH, bile salt; and
3. apply the lactic acid bacteria isolates with probiotic attributes in pasteurized orange juice as a possible non-dairy based carrier for delivery to humans.

For more information, please contact ir-help@oauife.edu.ng

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