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ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY OF LACTIC ACID BACTERIAL (LAB) IN AFRICA LOCUST BEANS (*Parkia biglobosa*) JACQ. BENTH

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ABSTRACT

Africa locust beans "*Parkia biglobosa*" also known as Iru or dawadawa is prepared traditionally through fermentation said to contain Lactic acid bacteria (LAB) that has health, spice and nutritional benefits for human consumption. The presence of lactic acid bacteria in edible substances qualify them to be classified as probiotics. Hence this study was carried out to isolate and test the antimicrobial susceptibility potential of LAB from processed Africa locust beans in three samples: the slurry, wet and dried samples. Samples were isolated on deMan Rogosa and Sharpe (MRS) agar at 37 ^{o}C for 24 hours. The data obtained were subjected to Complete Randomised Design (CRD). A total number of 49 LAB isolates were found with 25 from the Slurry, 13 from wet and 11 from the dried. The antimicrobial susceptibility testing was carried out on 49 isolated that were catalase negative of which almost all the isolates were sensitive to six out of eight antibiotics tested. Out of all the isolates subjected to 8 antibiotics, 96 % of the isolates from slurry and wet samples were resistant to Ceftazidime and Cloxacilline while 100% of the dried isolates were resistant to Ceftazidime and Cloxacilline. The result showed that there are significant differences in the colonies, catalase negative and antimicrobial susceptibility found between the three samples. This implies that more potential LAB were found in the slurry used for the processing of Africa locust beans..

Keywords: *Parkia biglobosa*, antibiotics, probiotics, catalase, lactic acid bacteria

Introduction

The African locust beans also known as *Parkia biglobosa* is a perennial tropical leguminous tree which occurs in the rainforest and arid zones of some African countries. The tree has the capacity to withstand drought and also the seeds are embedded in a mealy pulp that is high in energy value. (Builders *et. al*., 2014) Apart from being a good source of plant protein to man, it serves as good source of protein to animal feeds, chick and fish (Obun, 2007, 2008 and Audu *et al*., 2008). The fermentation of African locust beans is initiated by *Bacillus sp.* to produce spices called "iru" or "dawadawa" through alkaline fermentation has been described by several authors (Tamag, 1998, Ouaba *et al*., 2003; Bridget *et al*., 2004; Ademola *et al*., 2011;). The production and preparation of food by fermentation has good advantage such as the destruction of undesirable flavours and odours, production of good flavor, increase digestibility, synthesis of desirable constituents, and changes in physical state, longer shelf-life and destruction of inhibitors (Odunfa, 1985; Ademola *et al*., 2013).

Previous studies by Antai and Ibrahim (1984) and Odunfa (1985) have shown that

several microorganisms are associated with Africa locust beans fermentation and stated that the most abundant and the major dominant agent of the fermentation after 24 hours was *Bacillus subtilis*. Microbial fermentation of Africa locust beans have been found to involve only bacteria since fungi found have been regarded as incidental and does not play any notable role in its fermentation (Ikenebomah and Ingram, 1986). Most bacteria found in the process are facultative anaerobes, while approximately 10% are aerobic after 36 hours of fermentation. In food science, fermentation is the process of converting carbohydrates to alcohol and carbon dioxide or organic acids using microorganism that could be yeast or bacteria under anaerobic/aerobic condition (Williams and Dennis, 2011). The primary benefits of fermentation are the conversion of sugar and other carbohydrate to usable end products. According to Steinkraus (1996) the traditional fermentation of foods serves several functions. These include enhancement of diet through improvement of flavours, aroma, food texture, preservation and shelf-life extension. The extended shelf-life arises as due to lactic acid and alcohol generated during fermentation. Other functions of food fermentation include, enhancement of food quality with protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient availability, detoxification of anti-nutrient decrease in cooking time and fuel requirement.

Probiotics are microorganism which when administered in adequate amount confer a health benefit on the host (FAO/WHO 2010). Lactic Acid Bacteria (LAB) are widespread microorganism which can be found in any environment rich mainly in carbohydrates. Such sources include plants, fermented foods and the mucosal surfaces of humans, terrestrial and marine animals. In the human and animal bodies, LAB are part of the normal microbial or microflora, the ecosystem that naturally inhabits the gastrointestinal and genitourinary tracts, which comprised by a large number of different bacteria species with a diverse amount of strains (Aureli *et al*., 2011; Barinov *et al*., 2011). LAB have been used in food preservation and for the modification of the organoleptic characteristics of foods, for example, flavor and texture (Barinov *et al*., 2011). Nowadays, LAB play important role in the industry for the synthesis of chemical, pharmaceuticals or other useful products. Also the biotechnological production of lactic acid has recently been reported to offers a solution to the environmental pollution by the petrochemical industry (Hamdan and Sonomoto 2011).

It is a known fact that the fermentation of *P. biglobosa* is initiated by *Bacillus spp* with the assistance of some other bacteria: however, no effort has been made to study the Lactic acid bacteria present at the different stages of the production of “Iru”. This study therefore seeks to identify the LAB present in the dried and fermented Africa locust beans and compare it with those present in the slurry obtained after cooking the Africa locust beans.

Materials and Method

The African locust beans were obtained from the market and processed as briefly described herein as follows; 500g of seeds were boiled under normal atmospheric pressure for 8 hours in order to soften the cotyledon. Thereafter, the locust beans were left in the cooking chamber overnight. The seeds were then de-hulled using the fabricated de-haulling machine and washed thoroughly to remove the coat. The cotyledons were spread onside the clean and warmed calabash tray and wrapped with a thick blanket and kept in clean dark cupboard for 48 hours to allow fermentation. The slurry was collected

aseptically in sample bottle and allowed to ferment for 48 hours.

Preparation of Culture Media

Peptone water (Oxoid U.K) and Deman Rogosa and Shape (MRS) media (Oxoid, U. K) were used for serial dilution of all the samples and preparation of Lactic Acid Bacteria species. The media used were dissolved by boiling for five minutes with frequent agitation to achieve homogenization before been autoclaved. They were allowed to cool down to about 45 ^{o}C before inoculating them with the microorganisms. All media were prepared according to the manufacturers' instructions.

Isolation of Lactic Acid bacteria

1ml of the fermented slurry was transfer aseptically from the sample bottle to 9 ml MRS broth already prepared and incubated under micro-aerophilic condition at 37 ^{o}C for 24 hours. After incubation, the broth was subjected to serial dilution 10^{1} - 10^{10} using peptone water. The dilution 10^{3} - 10^{8} were plated out by transferring 0.1ml broth aseptically on a sterile Petri dish. The inoculated plates were incubated under micro-aerophilic condition at 37 $^{\circ}$C for 24 hours. The viable colony forming units were counted and the morphology was observed. The above procedure was repeated for three weeks for different batches. 1 g of the wet fermented locust bean and 1 g of unfermented dried samples were subjected to the same procedures as described above

Antimicrobial Susceptibility Test of Isolated Lactic Acid Bacteria

The antimicrobial susceptibility test of potential lactic acid bacteria isolated from Samples to antibiotics were tested by disc diffusion method. The isolates LAB from fermented samples were screen out to be pure by checking their morphology and that are catalase negative. The broth that confirmed sufficiently growth were selected out and diluted with 1ml sterile distilled water and taken into 9 ml sterile distilled water ascetically given 1 in 10-fold dilution of the LAB. 20 ml of sterilize and melted MRS agar was poured in sterile petri dish aseptically and allowed to solidify. Thereafter, a sterile swab stick was dip into the diluted LAB and streak on the surface of the solidified MRS agar aseptically to form a uniform bacteria lawn. Plates were then incubated in a micro-aerophilic condition at 37 ^{0}C for 24 hours. The antimicrobial agent studies were ceftazidime (30ug), cefuroxime (30ug) gentamicin (10ug), ceftriaxone (30ug) Erytomycin (5ug), cloxacillin (5ug) ofloxacin (5ug) and augumentin (30ug) used for all the LAB isolates from the samples. This procedure was repeated for all the isolated LAB from the slurry, wet and dried samples of Africa locust beans.

Results and Discussions

Isolation of Potential Lactic Acid Bacteria

Colonies on all the MRS Agar plate grew proportionately according to the dilution (10^{3} - 10^{8}). Howbeit, the plates inoculated with 10^{8} was selected for analysis**.** The total number of LAB colonies counted on 5 plates and the mean values obtained from different samples are presented in Table 1. Mean values of LAB colonies counted from 5 batches of the isolation from the Slurry, wet and dried samples were 228.4, 183.6 and 123.8 respectively. This result shows that the number of colonies found in water samples were more than the others and there was significant difference between the groups at 5 % level of probability.

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Table 1: Mean values for colonies and catalase negative isolates found in samples of African locust beans

Samples	Colonies		Catalase negative	
	Total	Average	Total	Average
Slurry	1142	228.4[a]	25.0[a]	5.0
Wet	918	183.6[b]	13.0[b]	2.6
Dry	619	123.8[c]	11.0[b]	2.2

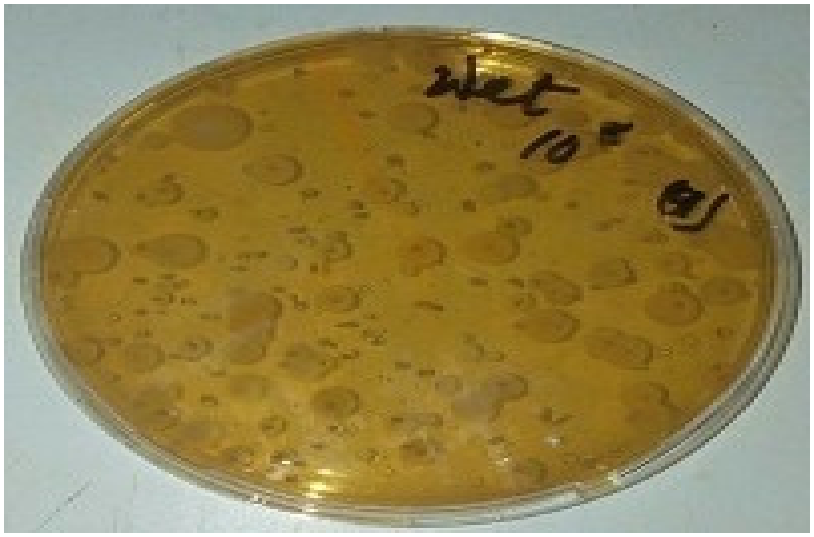

Plate 1: Catalase Negative Isolates on African Locust Beans MRS Agar Plates

The mean values for catalase negative found in processed African locust beans are also presented in Table 1. The total number of catalase negative isolate found in the slurry, wet and dried samples were 25, 13 and 11 respectively. The slurry had the highest catalase negative isolates follows by the wet and the dried samples. The results shows that there are significant differences among the groups (water, wet and dried) at 5 % level of probability. This implies that there is variation in the number of catalase negative isolates presents among the samples.

Antimicrobial susceptibility test of all potential isolated LAB

The antimicrobial susceptibility test of potential lactic acid bacteria isolated from Samples to antibiotics were tested by disc diffusion method. Out of the 25 isolates that were cultured on MRS agar for slurry that were catalase negative, all the 25 isolates were resistance to Cloxacilline (CXC) of 5ug, likewise, all the 25 Isolates were susceptible to Cefurozime (CRX), Ceftriaxone (CTR), Erythromycine (ERT),

Oxfloxacin (OFL), and Augumetin (AUG). However, 1 isolate was resistant against Gentamicin (GEN) and 1 isolate was susceptible to Ceftazidime (CAZ) of 30 ug (Table 2)

Similarly, 13 isolates obtained from the Wet samples that were catalase negative, 12 isolates were resistance to Cloxacilline (CXC) and Ceftazidime (CAZ), while all the 13 isolates were susceptible to Cefurozime (CRX), Erythromycine (ERT), Oxfloxacin (OFL), and Augumetin (AUG) while only 1 isolate was resistant against Gentamicin (GEN) and Ceftriaxone (CTR) (Table 2).

Lastly, 11 isolates that were cultured from dried African locust beans that were catalase negative, the 11 isolates were resistance to Cloxacilline (CXC) and Ceftazidime (CAZ), 11 isolates were sensitive to Cefurozime (CRX), Erythromycine (ERT), Oxfloxacin (OFL) and Augumetin (AUG) while only 1 isolate was resistant against Gentamicin (GEN) and Ceftriaxone (CTR) (Table 2).

Table 2: Antimicrobial susceptibility testing (antibiogram) of potential LAB isolated from processed African locust beans

antibiotics	CAZ	CRX	GEN	CTR	ERT	CXC	OFL	AUG
Slurry (25)	1S, 24R	25S	24S, 1R	25S	25S	25R	25S	25S
Wet (13)	1S, 12R	13S	12S, 1R	12S, 1R	13S	12R, 1S	13S	13S
Dried (11)	11R	11S	10S, 1R	10S , 1R	11S	11R	11S	11S

R = resistance, S= susceptible

As can be seen in plate 2, the cleared zones on the MRS agar plate indicate susceptibility (sensitivity) while the portions that has no clear zone indicate resistance against the antibiotics. The difference among the groups (water, wet and dried) in terms of antibiogram of the isolates were observed in Figure 3. The slurry has the highest number of isolates that were sensitive to most of the antibiotics followed by the wet and dries samples

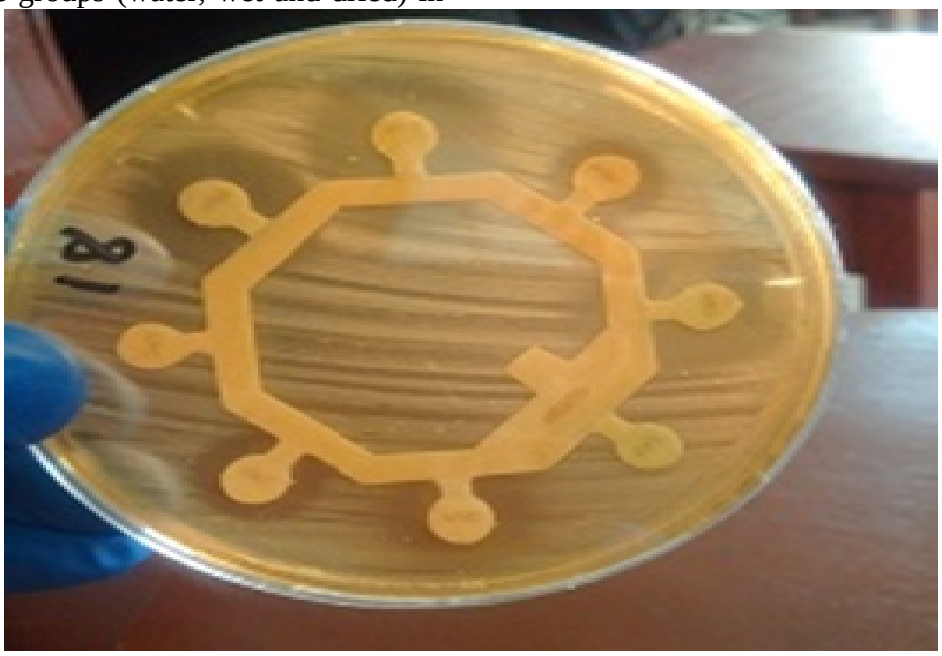

Plate .2 Antimicrobial Susceptibility Test plate

From these results, the colonies and catalase derived from processed African locust beans were higher in slurry than the others. Out of 1142 colonies, only 25 isolates were catalase negative in the slurry, 13 catalase negative from 918 colonies from wet and 11 catalase negative from 619 colonies from dried processed African locust beans. The values of colonies and catalase obtained in this study for processed African locust beans were higher than some other food items found in previous research works of Abubakr and Adiwish (2017). The total number of colonies and catalase negative found in these processed food items might be different due to the different source of sample. It has been shown that this is one of the preliminary criteria for the classification of an organism to be called LAB probiotics, and the probiotic properties of LAB isolates would be effective to dietary source of antioxidants (Abubakr and Adiwish 2017). In this study, various compounds such as di-acetyl, organic acid, hydrogen peroxide, lactic acid may be found in the samples collected from processed African locust beans because of the lactobacilli found during lactic acid fermentation of samples (Lindgren and Dobrogozy1990; Brink *et al.*, 1994).

It is possible for the isolate from processed African locust beans to have antimicrobial tendencies against some diseases because similar results were discovered in previous reports by (George and Anosike 2011). They isolated three difference LAB derived from fermented pap water also known as corn liquor and showed that they have antimicrobial effect against diarrhogenic *Escherichia. coli.*

In this study, all the isolates that were found to be catalase negative were subjected to antimicrobial susceptibility test which shows majorities of the isolates were susceptible to most of the antibiotics used. Out of all the isolates subjected to 8 antibiotics, 96 % of the isolates from the slurry and wet samples were resistant to CAZ and CXC while 100% of the dried isolates were resistant to CAZ and CXC. Previous report showed that the lactobacilli isolated from commercial products in Europe comprised strains resistant to tetracycline (29.5%), chloramphenicol (8.5%) and erythromycin (12%) (Temmerman *et al*., 2003). This shows that there will be no resistant strain or little resistance strains among the isolated potential LAB derived from the processed African locust beans. It has been reported in many previous researches that food fermented by culture of LAB has high biochemical and antioxidant activity (Kullisaar *et al*., 2003; Villani *et al*., 2005). Among LAB, species of lactobacillus have potential probiotic effects in human health. Lactobacillius spp are important members of the healthy human microbiota (Naaber *et al*., 1998).

Conclusion

This study reveals Africa locust beans to contain different strains of Lactic acid bacteria (LAB) and some other important component which has inhibitory effect on different or specific pathogenic microorganisms. The study complemented the knowledge on the use of Africa locust beans (Iru) as spices and part of condiment used by traditional herbalist for treatment of some illnesses. Combination of the therapeutic, spice and antibiotics use of *P. bioglobosa* will boost the use of this important non-wood forest product. Further research is needed on this potential LAB at the molecular level in order to ascertain the safety use of *P. biglobosa* as a probiotic which could adjust the balance of intestinal flora, reduce serum cholesterol, inhibiting and reducing the risk of tumors and revitalizing the immune system. Pharmaceutical industries could tap into this

research and produce new probiotics and antibiotics.

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