Identification of *Lactobacillus* Species Associated with Selected African Fermented Foods

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Lactobacillus, African Fermented Food

Two hundred isolates of *Lactobacillus* were obtained from seven indigenous fermented foods namely: 'fufu' (fermented cassava), 'iru' (fermented African locust bean), 'kenkey' and 'ogi' (fermented maize), 'kunu-zarki' (fermented millet), 'ugba' (fermented African oil bean) and 'wara' (fermented skimmed cow milk). *Lactobacillus* species identified were *Lactobacillus casei* (17.0%), *L. lactis* (4.5%), *L. plantarum* (41.5%), *L. brevis* (11.5%), *L. jensenii* (5.5%), *L. acidophilus* (3.5%) *L. cellobiosus* (5.0%), *L. delbrueckii* (2.5%), *L. salivarius* (3.5%), *L. leichmannii* (3.5%) and *L. fermentum* (2.0%). The commonest species isolated was *L. plantarum*. This paper highlight the importance of *Lactobacillus* in the processing of African fermented foods.

Introduction

Many African foods are fermented before consumption and the microorganisms involved in African foods fermentation are restricted to a few groups of yeasts and bacteria (Odunfa, 1985). Lactic acid bacteria particularly *Lactobacillus* are involved in the fermentation of many African stachy foods. *Lactobacillus* constitute a most important member within the lactic acid bacteria group and is probably one of the most useful microorganisms in food processing. Their activities and applications make possible the manufacture of many fermented foods, especially when used in singles or in mixed cultures with other types of bacteria (Chassy, 1985). In view of the widespread use of *Lactobacillus* in the preparation of foods and other fermentation processes (Mckay and Baldwin, 1990) and the scantyness of information, about the *Lactobacillus* species found in African fermented foods. The previous information have been geared towards gathering information on the general lactic acid bacteria, yeasts and moulds. This study attempt to provide a detailed information about the different types of *Lactobacillus* species associated with our indigenous fermented foods.

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Materials and Methods

Source of organisms

The *Lactobacillus* isolates were obtained from locally fermented foods. These were 'fufu' (fermented cassava), 'Kenkey' (fermented maize), 'kunu-zarki' (fermented millet), 'ogi' (fermented maize), 'iru' (fermented African locust bean), 'ugba' (fermented African oil bean) and 'wara' (fermented skimmed cow milk).

Medium used for isolation

The medium used for the isolation of the lactobacilli was de Man Rogosa Sharpe (MRS) agar (de Man *et al.*, 1960) containing per litre: beef extract, 2g; tryptone, 10g; yeast extract, 4g; glucose, 10g; $K_2HPO_4.3H2O$, 2.5g; sodium acetate, 5g; tris ammonium citrate, 2g; $MgSO_4.7H2O$, 200mg; manganase sulphate, 50mg; Tween 80, 1ml and agar 15g. The medium was prepared according to the manufacturer's direction and sterilized by autoclaving at 121°C for 15min.

Bacterial isolation and purification

The food samples were prepared in the traditional way following the methods of Odunfa

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(1985). One gram of each food item was weighed into 9 ml of sterile 0.1% peptone water to form a pulp. Serial dilutions were than made from the pulp and plated on MRS agar using spread plate method. Incubation was carried out at 37°C for 48 hrs under anaerobic condition with the use of disposable CO_2 and hydrogen generators (BBL, Cockeysville, USA). In general, colonies representing the square root of the number present were randomly selected from the plates obtained from the highest countable dilution (Harrigan and McCance, 1976). The selected colonies were isolated by streaking on MRS agar and incubated anaerobically under the same conditions as stated above. The purity of the isolated organisms was checked by streaking again on MRS agar plates, followed by microscopic examination. Subsequently they were grown on slants of MRS agar stored at 5°C prior to identification.

Characterization and identification of organisms

Pure cultures were subjected to physiological and biochemical tests (Harrigan and McCance, 1976; Sneath, 1986). Identification was based on the following examinations:(1) homolactic or heterolactic character, determined by acetic acid or ethanol production, (2) microscopic and macroscopic examination of morphology, (3) absence of catalase and oxidase, (4) absence of spore, (5) Gram stain reaction, (6) growth at 15° and 45°C, and (7) fermentation of different carbon sources. Examination of results and identification of the different strains were carried out using the Bergey's Manual of systematic Bacteriology (Sneath, 1986).

API 50-CHL test kits (Bio Merieux,France) were employed to confirm the identity of the lactobacilli (Cox and Thomsen, 1990). The test kits enables the testing of the ability of the isolates to ferment 49 carbohydrates in one test proceedure, using the computer-aided identification programme.

Determination of percentage occurrence

This was done according to the method used by Hounhouigan *et al.* (1993.)

Results and Discussion

Two hundred *Lactobacillus* isolates were obtained from seven fermented foods sampled. Out of a total 200 *Lactobacillus* isolates, 60 were obtained from 'wara' 48 from 'kenkey', 36 from 'ugba,' 30 from 'ogi', 12 from 'kunu-zarki', 9 from 'fufu' while 5 isolates were obtained from 'iru'. The isolates were found to be *L. casei, L. brevis, L. jensenii, L. plantarum, L. leichmannii, L. delbrueckii, L. cellobiosus, L. acidophilus, L. salivarius, L. fermentum and L. lactis* (Table I).

Table I: Identification of *Lactobacillus* isolates from selected fermented foods.

Fermented food	Substrate	*Lactobacillus* species identified
'Fufu'	Cassava	*Lactobacillus casei* *Lactobacillus brevis* *Lactobacillus jensenii* *Lactobacillus leichmannii* *Lactobacillus plantarum* *Lactobacillus delbrueckii*
'Ogi'	Maize	*Lactobacillus plantarum* *Lactobacillus casei* *Lactobacillus leichmannii* *Lactobacillus delbrueckii* *Lactobacillus brevis* *Lactobacillus jensenii*
'Kunu-zarki'	Millet	*Lactobacillus salivarius* *Lactobacillus casei* *Lactobacillus acidophilus* *Lactobacillus jensenii* *Lactobacillus cellobiosus* *Lactobacillus plantarum*
'Kenkey'	Maize	*Lactobacillus plantarum* *Lactobacillus fermentum* *Lactobacillus brevis* *Lactobacillus delbrueckii* *Lactobacillus acidophilus* *Lactobacillus casei* *Lactobacillus cellobiosus*
'Iru'	African locust bean	*Lactobacillus casei* *Lactobacillus plantarum*
'Ugba'	African oil bean	*Lactobacillus plantarum* *Lactobacillus casei* *Lactobacillus leichmannii* *Lactobacillus brevis;* *Lactobacillus salivarius* *Lactobacillus jensenii* *Lactobacillus acidophilus* *Lactobacillus cellobiosus*
'Wara'	Cow milk	*Lactobacillus casei* *Lactobacillus fermentum* *Lactobacillus lactis* *Lactobacillus plantarum* *Lactobacillus fermentum* *Lactobacillus jensenii* *Lactobacillus brevis*

Dominance species is *Lactobacillus plantarum.*

Among the organisms *L. plantarum* was found to be most commonly isolated species. Attempts were made to isolate and characterize the different types of *Lactobacillus* species found in assorted locally fermented foods in order (i) to have the knowledge of the different species of *Lactobacillus* involved in these fermentation processes, since the substrates used in the preparation of these foods are different and (ii) to determine which species can be improved in order to develop appropriate starter cultures for use in fermentation of local foods.

Lactobacillus plantarum, L. brevis and *L. casei* were the commonest *Lactobacillus* species isolatled. As in the earlier reports (Etchells *et al.*, 1975; Olukoya *et al*, 1993) on the occurrence of lactic bacteria spectrum, *L. plantarum* constituted the highest number of *Lactobacillus* species isolated from fermented plant mateials. The involvement of various types of *Lactobacillus* species in fermented vegatable and plant materials had earlier been reported (Cooke *et al.*, 1987; Steinkraus, 1983 and Sharpe, 1981). This lactic acid bacteria were present in fermenting foods, because of their ability to produce high levels of lactic acid as well as being able to survive under high acidic conditions.

The high percentage of *L. plantarum* recorded in the present study could be due to the fact that (1) majority of the substrates used in the preparation of the fermented foods are of plant origin. *L. plantarum* is known to be more commonly associated with the plants (Daeschel *et al.*, 1987). and (ii) *L. plantarum* requires less complex nutritional requirements compared to other *Lactobacillus* species (Hammes *et al.*, 1992).

The identification of different types of *Lactobacillus* species in the present study could be due to the fact that majority of the substrates used in the preparation of the fermented foods are of different plant origins, and each particular plant species provides a unique environment in terms of competing microorganisms, natural plant antagonists, type, availability and concentration of substrate and various physical factors. These conditions allow for the development of a characteristic epiphytic flora, from which arises a population and sequence of fermentation microorganisms when the plant material is harvested and prepared for fermentation.

The lactobacilli constitute an important group of organisms particularly in the food processing industry. The reasons for the wide spread use of *Lactobacillus* in the preparation of foods and other fermentation processes are (i) production of desired flavour or physical property such as appearance and texture in food, (ii) retardation of spoilage and reduction of contamination through the production of antimicrobial substances, (iii) enhancement of nutritional values of foods e.g. by providing vitamins, amino acids and (iv) beneficial effects on human health (Chassy, 1987; Sandine, 1987; Mckay and Baldwin, 1990).

The *lactobacillus* have been implicated in the natural fermentation of many local foods, for example they have been isolated from fermented cassava e.g. gari, lafun, fufu (Ngaba and Lee, 1979; Okafor *et al.*, 1984; Oyewole and Odunfa, 1988 & 1990), fermented maize, sorghum or millet e.g. ogi or kunu-zarki (Odunfa and Adeyele, 1985), burukutu and pito-beverages from guinea corn and cassava (Faparusi *et al.*, 1973; Ekundayo, 1969), nonofermented skimmed cow milk (Eka and Ohaba, 1977). In general, acidic cereal and cassava fermentation in Africa have *Lactobacillus* species as the predominant microorganisms. However, from the literature, it appears that up to date, there is no detailed information about the different types of *Lactobacillus* that can be found in our indigenous fermented foods. The previous studies have been geared towards gathering information on the general lactic acid bacteria, yeasts and molds. Hence the present findings present the detailed information and further expansion on the state of knowledge about the different types of *Lactobacillus* species that could be found in some of our indigenopus fermented foods.

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