

Screening of local isolates of *Lactobacillus* for characters useful in African food fermentations

Détermination de certaines activités des bactéries lactiques, importantes pour les fermentations traditionnelles

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- Abstract -

Lactobacilli are known to be involved in the production of many locally fermented foods; especially cereal and cassava based foods. It is therefore desirable to develop improved cultures for use as starter inoculum. Such starter cultures will be designed for use in small or village scale operations, as well as medium size commercial operations. The use of starter cultures would provide improved control of fermentation and lead to the production of high quality standardized products.

Lactobacilli species were isolated from 9 Nigerian fermented foods and screened for amylase production, linamarase and bacteriocin productions. Isolates of *Lactobacillus* which expressed useful characters and potentials for use as starter cultures in cassava or cereal fermentations were identified as *L. plantarum*, *L. pentosum* and *L. fermentum*.. Preliminary attempts to optimize these activities are programmed.

- Résumé -

Les *Lactobacilles* sont connus pour être impliqués dans de nombreuses fermentations alimentaires locales en particulier celles des produits à base de céréales ou de manioc. Il est donc important d'améliorer les cultures bactériennes pour qu'elles puissent être utilisées en tant qu' inoculum de départ. Ce type d'inoculum pourra être utilisé au niveau villageois ou au niveau d'unités commerciales de taille moyenne.

L'utilisation d'inoculum de départ permettra, à la fois, de mieux contrôler la fermentation et conduira à la production d' aliments standards de haute qualité.

Les *lactobacilles* ont été isolés à partir de 9 aliments fermentés Nigériens. Nous avons déterminé leurs capacités à produire de l'amylase, de la linamarase et des bactériocines. Les souches de *lactobacilles* possédant ces caractères et ayant les potentialités pour être utilisées comme inoculum de départ ont été identifiées comme appartenant aux espèces suivantes : *L. plantarum*, *L. pentosum* et *L. fermentum*. Des essais préliminaires pour optimiser ces activités sont programmés.

Introduction

Many African foods are fermented before consumption. The Micro-organisms involved in African food 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 foods. In general, acid cereal and cassava fermentation in Africa have *Lactobacillus spp.* as the predominant micro-organisms. They have been reported to be involved in the production of a wide range of products.

The indigenous fermentation operation is plagued with many problems, which include non-reproducible quality of products, lack of uniformity in taste and flavour and short shelf life. This is mainly because fermentation generally depends on chance inoculation from the environment and starter cultures are not used thus encouraging spoilage organisms, contamination and unhygienic products. In addition, cereals and cassava are generally low in nutrients. For example, cereals like corn are low in minerals. The antimetabolite phytic acid is common and condensed tannins are high in sorghum. Corn is deficient in lysine and in tryptophan (Austin, 1979). In corn meal, phytate is believed to complex with some metallic ions to form insoluble compounds. Phytate also complexes with proteins making them less soluble. The other problem with cereal-based foods in Africa is the loss of important nutrients during processing.

Cassava contains 90% fermentable carbohydrate whereas the protein content is 3.60% (Oyenuga, 1968). Since cereals and cassava play an important role in African diets, it is essential to improve their nutritive values. The inoculation of cassava or batters of cereal with one or several selected strains of bacteria would provide improved control of fermentation and lead to the production of a high quality and standardized products. The present work is aimed at improving fermented foods by screening for properties amongst useful *Lactobacillus spp.* in fermentation strains.

Materials and methods

1. Source of materials and isolation procedure

The *Lactobacillus* isolates were obtained from 9 locally fermented foods and beverages. These were *Ogi* (fermented maize or sorghum), *fufu* (fermented cassava), *Ogiri* (fermented melon seeds), *iru* (fermented African locust bean), *pito'* (fermented guinea corn and maize), *burukutu* (fermented guinea corn and maize), *nono* (fermented skimmed cow milk), *ugba* (fermented oil bean) and *kunu zarki'* (fermented millet). These foods were carbohydrate and milk based.

Samples were taken at 24h intervals under aseptic conditions or from finished products. One gram of each food item was weighed and placed into 9ml of sterile 0.1% peptone water to form a solution. Serial dilutions were then made from the solution and plated. The total viable counts were on plate count agar (PCA) (Oxoid). Yeast and mould counts were determined on Malt Extract Agar (MEA) containing 100U ml⁻¹ streptomycin. Lactic acid bacteria were isolated on de Man Rogosa Sharpe (MRS) agar incubated under anaerobic conditions (BBL Gas Pak). Plates were incubated at 30°C for 24h for the PCA and MRS media and for 4-5 days for the MEA medium.

2. Characterization and identification

Isolates were picked randomly at varying times from the PCA plates and subcultured before being subjected to physiological and biochemical tests (Harrigan and McCance, 1976; Barnett *et al.*, 1983; Sneath, 1986). Identification was based mainly on the following (1) configuration of the lactic acid produced using an enzymatic method with dehydrogenase L and D (Boehringer, Mannheim, FRG), (2) homolactic or heterolactic character; determined by acetic acid or ethanol production (3) absence of catalase (4) microscopic and macroscopic examination of morphology, mobility and spores (5) Gram stain (6) arginine deamination (7) growth at 15 and 45°C (8) fermentation of different carbob sources (AP) 50CH N°. 5030 strips, Biomerieux, Charbonnières les Bains, France). *Lactobacilli spp.* were taxonomically classified following the discriminatory schemes of Kandler and Weiss (1986) and Hammes *et al.* (1992).

2.1. Bacteriocin assay

Bacteriocin producing *Lactobacilli spp.* were identified by the formation of a clear halo on BSM in the agar drop assay as described by Tichaczek *et al.* (1992) and Olukoya *et al.* (1993). In this medium the inhibitory activity caused by organic acids and hydrogen peroxide was excluded by the low sugar content and buffering while hydrogen peroxide was removed by the inclusion of catalase. The inhibitory spectrum and sensitivity to heat and autoclaving of the bacteriocin were investigated as described by Tichaczek *et al.* (1992) and Olukoya *et al.*, (1993). Screening for general antimicrobial activity against other bacteria were done using the same procedure but on ordinary MRS agar. Most of the strains used as indicators have already been listed (Tichaczek *et al.*, 1992; Olukoya *et al.*, 1993). The other strains were from own collections.

2.2. Screening for α -amylase

This was by the addition of 1% soluble starch to MRS agar plates. After incubation at 30°C for 2-4 days, the plates were stained with iodine (0.33%

12,0.66%KI) and screened for halos. Partial characterization of the α -amylase produced was carried out as described by Amund and Ogunstina (1987).

2.3. Screening for phytase activity

This was as described by Lopez *et al.* (1983).

2.4. Screening for Fungicidal activity

Lactobacillus spp. was grown overnight on MRS agar incubated anaerobically at 37°C. This was scraped with a sterile loop into a bijou bottle containing 3ml sterile MRS broth. *Aspergillus niger* was introduced into the MRS broth and the bottle was closed and mixed thoroughly. The lid was lightly unscrewed and the bottle was incubated at 37°C. Incubated bottles were monitored daily for appearance of fungal sprouts. Aliquots were taken at intervals and cultured on SDA for fungal viability. Alternatively, the test *Lactobacillus spp.* was grown overnight in the centre of a MRS agar plate incubated anaerobically at 37°C.

This was then overlaid with soft sterile SDA (4%) seeded with *Aspergillus niger*. Plates were observed for the formation of clear halo of fungal inhibition zones around plate centres. Controls were treated in the same manner as in experiments, except that no *Lactobacillus spp.* was introduced into the medium.

Determination of the contribution of H₂O₂ and lactic acid to fungal inhibition: The test organisms were screened for catalase production. The plate assay for anti-fungal activity was done as earlier described. However, BSM (bacteriocin screening medium) containing catalase was substituted for MRS agar. In BSM the inhibitory activity caused by organic acids was excluded by the low sugar content and buffering while hydrogen peroxide was removed by catalase. (Tichaczek *et al.*, 1992).

2.5. Screening for linamarase production

The procedures described by Okafor and Ejiofor (1988) and Oyemifoe (1990) were followed for screening linamarin utilizers through hydrogen cyanide production. Because of the high cost of purified linamarin, microquantities were used in this investigation. The basal medium used was M.R.S. broth without glucose and meat extract but containing 0.05% chlorophenol red. Medium contained in a flask (250ml) and empty Eppendorf tubes were separately sterilized (121°C, 15 min.). With the aid of a micropipetter (Pipetman F - Gilson, France) with sterilized tips, 9 μ l of the basal medium was pipetted into each of the sterilized Eppendorf tubes under aseptic conditions.

Purified linamarin was purchased from Sigma Chemie GmbH, München, Germany. A stock solution of linamarin (1 µg in 10 µl) was prepared and filter-sterilized through 0.45 µm pored cellulose nitrate filter (Sartorius GmbH, Göttingen, West Germany). Linamarin solution (1 µl) was aseptically added to 9 µl basal medium. The Eppendorf tubes containing the medium were inoculated with 1 µl of the overnight culture which had earlier been washed in sterile distilled water.

Uninoculated tubes served as controls. The Eppendorf tube lids were secured with strips of filter paper (2mm width) immersed in alkaline picrate with the paper strips hanging above the inoculate media as in the modified procedure of Okafor and Ejiófor (1986) where alkaline picrate strips were placed on the inside of the top of Petri dishes containing linamarin agar. The tubes were incubated at 30°C for 24-96 hours. Change in colour of the linamarin-basal medium from red to yellow were taken as indicators of linamarin hydrolysis to produce hydrogen cyanide and acid.

Results

Of the 126 *Lactobacillus* species isolated from 9 African fermented foods, 36 (28.6%) were found to be *L. plantarum*; 25 (19.8%) *L. fermentum*; 22 (17.5%) *L. brevis*; 8 (6.3%) *L. cellobiosus*; 2 (1.6%) *L. pentosus*; 8 (6.3%) *L. acidophilus*; 2 (1.6%) *L. del. delbrueckii*; and 8 (6.3%) *Lactobacillus* species.

These isolates were screened for their (i) amylolytic activity (ii) bacteriocin production (iii) linamarase production (iv) antifungal activity (v) phytase production.

Results obtained were as follows:

- Linamarase producers 48 (38%);
- Amylase producers 4 (3.2%);
- Bacteriocin producers which showed inhibition towards one or more indicator strains were 15 (11.9%).
- Phytase producers 38 (30.2%) and
- antifungal activities 5 (3.9%).

Conclusion

Lactic acid bacteria fermentation is common in African fermented foods (Odunfa, 1985; Olukoya, 1993). *Lactobacillus* are known to possess some activities useful in local fermentation and beneficial to health. These beneficial values could be harnessed so as to improve African fermented foods (Olukoya, 1994). These

characteristics have been identified in this study and studies are presently in progress to maximize and exploit these useful characteristics. Already two fermented foods have been improved using a combination of starter cultures. These foods are Ogi (Olukoya, 1994) and Wara (Olukoya *et al.*, unpublished).

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