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MUTATION OF *LACTOBACILLUS* SP. ISOLATED FROM "KINDIRMO" A NIGERIAN LOCAL YOGHURT FOR THE IMPROVEMENT OF PROTEIN CONTENT OF *ZEA MAYS* (MAIZE) FLOUR

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ABSTRACT

Mutation has served as a good biotechnological tool for the improvement of nutrient content of fermented foods. This research undertook the mutants' selection of *Lactobacillus* spp. for protein-enrichment of the resultant fermented *Zea mays* (maize) flour. The *Lactobacillus* spp. were isolated from "Kindirmo" a local Nigerian yoghurt and identified based on morphological and physiological characteristics. The cultures obtained were subjected to ultra violet (UV) light irradiation for 20, 30, 35 seconds and the selected mutants were used for solid-state fermentation of maize flour. Finding of this study showed that protein content of wild *Lactobacillus* sp. fermented maize flour increased ($p<0.05$) from 10.5±0.5% to 11.5±0.36% after 6days of fermentation. Profile of protein content increased to highest value (11.51±0.51%) at 96h and then decreased with fermentation time. Although similar trend was observed for ash and crude fat contents however, highest peak values were observed at 48h (4.0±0.85%) and 120h (7.0±0.87.0±0.8%) of fermentation respectively. Inversely however, contents of crude fibre and carbohydrates decreased significantly ($p< 0.05$) from 2.0±0.06 to 0.65±0.1% and 70.5±1.0 to 68.0±0.6% respectively during the 6days of fermentation. Comparatively, higher ($P<0.05$) crude protein content was observed in the maize flour fermented with mutant strains of *Lactobacillus* spp. and increased ($P<0.05$) with UV irradiation exposure time. The result showed that maize flour fermented with mutant *Lactobacillus* sp.-35 had the highest protein content (10.5±0.5% to 17.50±0.5%), while *Lactobacillus* sp.-20 exhibited lowest (10.5±0.5 to 11.50±0.5%) improvement during the fermentation. The mutant strains increased protein content by 66.67%, 28.57%, and 9.5% in maize flour fermented with *Lactobacillus* sp.-35, *Lactobacillus* sp.-30 and *Lactobacillus* sp.-20 respectively. The result revealed increased protein content of the resultant fermented maize flour with mutant *Lactobacillus* sp. strain exposure duration to UV light.

Keywords: Mutants' selection, *Lactobacillus* spp, fermentation, yoghurt, maize flour

INTRODUCTION

In the past decades, agriculture has focused on meeting the food, feed and fibre needs of humans. However, challenges for the next decades, go far beyond simply addressing the nutrition of ever-growing global population in terms of sufficiency, but calls for improvement in the efficiency of agricultural products (Kishore and Shewmaker, 1999). Incidentally, biotechnology has contributed greatly as a modern tool for not only food production but also impacted positively on quality of human nutrition. This bio-engineering tool harbors broad spectrum of meritorious advances through genetic changes in protein molecules of an organism for improving quality and productivity of food. One of the most important contributions is that these minor alterations conferred amazing effects such as increased organoleptic properties and nutritional quality of food and feed several folds (Onuoha *et al*., 2017). Thus, this approach has been applied as a tool to reduce the alarming cases of widespread diseases prevailing due to malnutrition in underdeveloped and poverty-stricken world (European Food Information Council, 2013; Emily and Sherry, 2013).

One of the basic approaches to the improvement of nutritional content and safety of natural food is fermentation processes. Fermented foods represent a significant proportion and contribute to about one-third of the diet worldwide (Campbell-Platt, 1994). Of particular interest, fermentation processes have been employed to improve nutritional qualities of cereal based foods in West Africa (Cahvan *et al.*, 1988; Ogodo *et al.*, 2017). These food products are reported to possess enhanced palatability, acceptability, nutritive

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value and protein digestibility (Steinkraus, 1994; Saarela *et al.*, 2011).

Maize (*Zea mays* L.) is one of the major staple food crops in Nigeria due to its production potential and adaptability to wide range of environment. Naturally however, maize grain is deficient in lysine, tryptophan and methionine that are critical in protein synthesis, calcium absorption and biological precursor of the B-vitamin, niacin (Bantte and Prasanna, 2003; Huang *et al*., 2006). This deficiency contribute mainly to the poor nutritional profile of maize grains with only an average of 2% lysine which is less than one-half recommended for human nutrition. Since, fermentation has served as a means to improve relative protein quality and lysine availability of cereals (Hamad and Fields, 2006; Ogodo *et al.*, 2017). Several fermented maize based food products such as Doklu (indigenous to Côte d'Ivoire (Assohoun *et al*., 2013), Nsiho (white kenkey) (Anann *et al*., 2015**)**, Masa and Ogi in parts of Nigeria (Adegbehingbe, 2014a) have been traditionally produced with enhanced nutritional properties. Predominantly, *L. plantarum, L. brevis, L. casei, P. pentosaseus, P. acidilactici*, and *Lactobacillus* sp. are indigenous starter culture in the traditional fermentation of maize based product (Adegoke and Babalola, 1988; Halm *et al*., 1993; Sanni *et al.*, 1999).

Against this backdrop, strain improvement of the *Lactobacillus* spp. could serve as means to increase productivity potentials of the inherent bacteria. As a mean to achieving higher producing microbial strains, mutation techniques had tremendous appeal as the most important factor in keeping the fermentation industry. With these in mind, the researchers undertook this study to improve the protein content of maize flour through fermentation using mutant *Lactobacillus* spp. isolated from "kindirmo" a native milk product in Nigeria.

MATERIALS AND METHODS

Sample collection

Ten samples of "Kindirmo" were purchased from Fulani women vendors in Jos metropolis Nigeria. Duplicate samples of 100ml each was aseptically obtained in a sterile glass bottle and stored at 4^{o}C until analysed.

The white maize grains variety was purchased from food stuff-market in Vom-Nigeria and transported in cleaned low density polythene bags to Microbiology unit, Biotechnology Laboratory of National Veterinary Research Institute, Vom for analysis.

Isolation of Wild type Lactic Acid Bacteria

Isolation of LAB was performed by pour plate and enrichment techniques as adopted by Sudi *et al*. (2011). In this method, 10ml of Kindirmo sample was homogenized in 90ml sterile 0.1% peptone water to obtain a stock solution. The resultant mixtures was then tenfold serially diluted in sterile 0.1% peptone water and pour plated on De Mann Ragosa Sharpe (MRS oxoid) agar. The inoculated plates were incubated anaerobically at 30°C for 48h and distinct colonies formed were sub-cultured until pure isolates were obtained.

Characterization of *Lactobacillus* sp.

Identification of *Lactobacillus* spp. isolates was carried out based on growth characteristics on selective media deMan, Rogosa and Sharpe (HiMedia, India); type of colony, color, margin, elevation, opacity and presence of pigment), Gram staining, sugar fermentation test; starch, glucose, maltose, lactose, Mannitol, galactose and catalase tests as described by Koneman, (2008).

Preparation and Harvest of Inoculum

Using a sterile inoculating needle, pure *Lactobacillus* spp isolates were inoculated into 90ml of sterile tryptose soya broth and allowed to grow at 30°C until an optical density of 600 (OD_{600}) was attained corresponding to 2 X 10^8 cells/ml. The cells were then harvested by centrifugation at 4500g/10 min and washed twice with 20ml of cold sterile 0.9% NaCl solution.

Random Mutation of Isolated Strain by UV-light Irradiation

Irradiation of cultures and induction of mutations of the lactic acid bacteria was carried out following method adopted by Kutshik *et al*. (2010). Three (3) ml aliquots of the cell suspensions were aseptically transferred to sterile petri-dishes. The plates were then placed 37cm on an adjustable platform below a UV trans-illuminator lamp (at wave length 254 nm) fixed in a dark chamber for exposure periods of 20, 30 and 35 sec. Each irradiated isolate was centrifuged at 5000Xg for 15min and re-suspended in 10ml TSB and further incubated anareobically at 30°C for 18h. The cultures were then diluted serially in sterile 0.9% NaCl solution, streak

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plated on to MRS agar and incubated at 30 °C for mutants' isolation.

Solid State Fermentation of Maize flour

Maize grains were conditioned by mixing with water (500ml/kg) and then dehulled using a commercial dehuller driven engine. The dehulled grains were dry milled and sieved through a wire sieve (500 ml aperture size) to obtain maize four. The flour was then moistened with sterile distilled water (20ml/50g) aseptically and allowed to stand at room conditions for about 30min. A portion of 20g each of the moist solid was weighed into sixty (60) different conical flasks, plugged with cotton wool and autoclaved at 121°C for 15min. The sterile maize flour was labeled and aseptically inoculated with 1ml TSI broth stock culture of wild type and mutant strains of LAB. The medium was then incubated at 30°C under aerobic condition for 6 days with samples withdrawn at 2 days intervals.

Proximate analysis

Proximate parameters of moisture, ash, carbohydrates, fat and crude protein contents were determined according to AOAC standard protocols (2005).

Statistical analysis

The results obtained were expressed as means values ± standard deviation and subjected to ANOVA using SPSS version 17.0 to determine significant difference between the means at 95% level of significance.

RESULTS

Conventional taxonomy based on morphological and physiological characteristics of lactic acid bacteria are presented in Table 1. Morphological examination of the pure isolates grown on MRS agar revealed that colonies were small creamy in colour and microscopically Gram positive rods shaped. All the isolates were non-motile cells, and exhibited catalase negative activity. Carbohydrate fermentation showed that the isolates obtained produced gas from glucose, maltose, lactose, mannitol, galactose, but did not ferment starch and thus can be presumptively classified in the *Lactobacillus* genus.

The proximate composition of *Lactobacillus* spp. fermented maize flour is presented in Table 2. The results showed that protein content of maize flour increased ($P < 0.05$) from 10.5 ± 0.5% to 11.5 ± 0.36% with fermentation time. However, optimal protein content (11.51 ± 0.51%) was observed at 96h and then decreased with further extension of fermentation time. Although similar trend was observed for ash and crude fat contents however, peak values varied at 48h (4.0 ± 0.85%) and 120h (7.0 ± 0.87.0 ± 0.8%) of fermentation respectively. Inversely however, contents of crude fibre and carbohydrates decreased significantly ($P < 0.05$) from 2.0 ± 0.06 to 0.65 ± 0.1% and 70.5 ± 1.0 to 68.0±0.6% respectively during the 6 days of fermentation.

Performance of mutant *Lactobacillus* spp. on crude protein content of fermented maize flour is presented in Table 3. The profile showed increased ($P < 0.05$) protein content with fermentation time for all the strains of mutant *Lactobacillus* spp. isolated. Comparatively, higher crude protein content ($P < 0.05$) was observed in the maize flour fermented with mutant strains of *Lactobacillus* spp. and increased ($P < 0.05$) with UV irradiation exposure time. The result showed that maize flour fermented with *Lactobacillus* species-35 had the highest protein content (10.5 ± 0.5% to 17.50 ± 0.5%), least protein content (10.5 ± 0.5 to 11.50 ± 0.5%) was however, observed for *Lactobacillus* sp.-20. The protein content increased by 66.67%, 28.57%, and 9.5% for the maize flour fermented with mutant *Lactobacillus* sp.-35, *Lactobacillus* sp.-30 and *Lactobacillus* sp.-20 respectively. Comparatively, the result showed that wild *Lactobacillus* spp. fermented maize flour had its highest protein content at 4day. However, protein content of the fermented maize flour increased relatively with fermentation time and UV light exposure time of the mutants**.**

DISCUSSION

In this study, indigenous microflora of the traditional fermented dairy product "Kindirmo" suggest its richness for isolating novel lactic acid bacterial strains. Therefore, the predominance of various genera of Lactic acid bacteria in Kindirmo is in agreement with reports of co-workers (Kutshik *et al*., 2010; Sudi, 2013; Sakai *et al.,* 2014).

In Africa, lactic acid bacteria fermentation of cereals is a long established processing method used for the production of foods. Studies suggested that *Lactobacillus Lactobacillus plantarum, Pediococcus pentosaceus, L. fermentum/reuteri* and *L. brevis* dominates the maize dough fermentation and play significant role in improving nutritional value of corn flour (Nche *et al.,* 1994; Annan *et al.,* 2015).

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In this study, the wild type *Lactobacillus* spp. isolated enhanced the protein content of maize flour by 9.50%, following 6 day of fermentation. The increased protein content is consistent with the findings of Kutshik *et al.* (2010) who reported protein enrichment of potatoes flour due to *Lactobacillus* spp fermentation. Fundamentally, this trend is in conformity with the fact that protein content increases as free amino acids or nitrogen content are release when microorganisms utilize carbohydrates for energy generation during fermentation (Oyango, 2005; Frias *et al.*, 2008). Also, fermentation significantly increases small size peptides (<15 kD) essential AAs as long-chained proteins are broken down (Hirabayashi *et al.*, 1998).

The results of this study also revealed decreased crude fibre and carbohydrates contents of the maize flour with fermentation. In view of the fact that carbohydrates and crude fibre serves as carbon sources for bacterial growth, their reduction during fermentation of cereals flour is expected (Kutshik *et al.*, 2010; Onuoha *et al*., 2017). However, the lipid content of the fermented maize flour increased contrary the observation of Onuoha *et al*. (2017) where millet flour decreased with lactic acid bacteria fermentation.

This study affirmed the fact that fermentation time, impacts significantly on potentials of *Lactobacillus* spp. for protein enrichment of maize flour. Therefore, fermentation time is a very important factor for maximum protein production. In accordance to this finding, Kutshik *et al.* (2010) observed similar effect of varying fermentation periods on the efficiency of protein production of *Lactobacillus* spp. strains.

Although the wild type *Lactobacillus* sp. exhibited capacity to improve protein content in maize flour however, higher efficiency was observed in UV induced mutants. The increased potential of wild type *Lactobacillus* sp. with UV induced mutation to protein content enhancement of foods is consistent with the finding of Kutshik *et al.* (2010). In this study, mutants *Lactobacillus* sp. 35 increased protein by up to 52.4% higher than the maximum achieved for the wild type. As expected, the intensity and exposure time should reduce the survival rate of the organisms. However, high efficiency strains had been reported in the few positive mutant strains that survived mutagen lethality with increased UV exposure time (Beggs, 2002). Therefore, UV light exposure time played very critical role and increased protein content of the fermented maize flour in agreement with the finding of Geoffrey and Robert (2004). Comparatively, protein contents of the mutant *Lactobacillus* sp. strain fermented maize flour is higher than 9.0% of protein obtained for most maize cultivars (Shewry, 2007). The present study indicates that strains of *Lactobacillus* spp isolated from "Kindirmo" have great potential to improve its genome by mutagenesis for maximum improvement in protein content of maize flour.

Table 1. Morphological and Biochemical Characteristics of *Lactobacillus* spp.

Test	Characteristics
Colony size	Small
Colony shape	Circular, Irregular
Colony colour	Creamy
Cell Morphology	Rods
Gram reaction	+
Catalase activity	-
Carbohydrate fermentation	
Starch	-
Glucose	+
Maltose	+
Lactose	+
D-Manitol	+
Galactose	+

(-) negative, (+) positive

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Table 2: Proximate Content of Fermented Maize Flour with Wild Type *Lactobacillus* spp. Isolated from *Kindirmo*

Parameters (%)	Fermentation Period(days)				
	0	2	4	5	6
Crude Protein	10.5±0.5	11.5±0.35	11.51±0.51	11.5±0.29	11.5±0.36
Crude Fibre	2.0±0.06	0.8±0.08	0.7±0.05	0.7±0.72	0.65±0.1
Crude fat	5.5±1.2	6.0±0.36	6.0±0.55	7.0±0.8	7.0 ±0.8
Carbohydrates	70.5±1.0	68.5±0.6	68.5±0.5	68.5 ±0.5	68.0 ±0.6
Ash Content	1.5±0.08	4.0±0.85	3.65±0.75	3.65±0.77	3.6±0.85
Moisture Content	9.5±0.36	9.5±0.8	10.0±0.29	9.5±0.8	9.5±0.85

Values are mean± standard deviation of three (3) determinations

Table 3: Crude Proteins Content of Fermented Maize Flour with Mutants' Strains of *Lactobacillus* spp

Mutant Isolates	Crude Protein Content (%)/Fermentation time (days)				
	0	2	4	5	6
Lactobacillus spp20	10.5±0.5	10.5± 0.5	11.0±0.3	11.0±1.6	11.5±0.5
Lactobacillus spp30	10.5±0.5	10.1±0.3	9.0±3.2	12.5±0.25	13.0±0.29[a]
Lactobacillus spp35	10.5±0.5	10.5±0.35	11.5±0.35	15.5±1.25[a]	17.5±0.50[a]

Values are mean ± standard deviation of three (3) determinations and values with different superscripts within column or row varied significantly at $p < 0.05$.
Lactobacillus spp-20 isolate exposed to UV light irradiation for 20s
Lactobacillus spp-30 isolate exposed to UV light irradiation for 30s
Lactobacillus spp-35 isolate exposed to UV light irradiation for 35s

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