OPEN ACCESS Freely available online

Journal of Food: Microbiology, Safety & Hygiene

Research Article

Characterization of Probiotic Potential Lactic Acid Bacteria Found in Fermented Cereal Products from Western Kenya

Beatrice Aleyo Akweya[1*], Joseph Mwafaida Mghalu[2], Rahma Udu M. Yusuf[3], Tochi Bitange[4]

[1]Department of Pure and Applied Sciences, Technical University of Mombasa, Mombasa, Kenya; [2]Department of Biological Sciences, Pwani University, Kilifi, Kenya; [3]Department of Applied and Health Sciences, Technical University of Mombasa, Mombasa, Kenya; [4]Department of Food and Nutrition, Pwani University, Kilifi, Kenya

ABSTRACT

Cereal grains are considered as effective substrates for the production of probiotics which can be incorporated in functional foods. The natural presence of lactic acid bacteria in cereals is of great interest in producing fermented cereal products. This study aimed at biochemically characterizing probiotic potential Lactic Acid Bacteria (LAB) involved during the spontaneous fermentation of cereals (maize, sorghum and millet). The isolates were screened for growth at extreme conditions in MRS media. The temperature tested were 15°C, 37°C, 45°C and 55°C, the concentration of Sodium chloride (NaCl) tested were 2%, 4%, 7.5% and 10% (w/v), while the pH tested were 1.5, 2, 4 and 6. MAPU01, SM01, SMPU02 and SGPU02 survived a temperature of 45°C, five isolates were able to withstand salt concentration of 7.5% while only one at 10%. MAPU01 (*Lactobacillus spp*), SMPU01 (*Lactococcus spp*) and SGPU02 (*Lactococcus spp*) are possible thermophiles and can survive at low pH and moderate high salt concentration.

Keywords: Cereals; Fermentation; Lactic Acid Bacteria (LAB); Probiotic potential

INTRODUCTION

Fermented foods especially from cereals are connected to cultural diversity of Kenyans who can produce unique edible products from same raw material in different ways. Fermentation is the most outstanding technology that improves the nutritional properties of cereals [1]. Cereal products may have a range of bioactive substances with potential health benefits. Lactic Acid Bacteria (LAB) are widely used in food fermentations [2,3]. They are generally Gram positive rods or coccobacilli occurring in chain. They are non-spore former, usually non motile, nonacid fast, non-respiring, devoid of cytochrome and catalase negative. They grow well under anaerobic conditions but may grow in microaerophilic as well as aerobic conditions. They exhibit optimum growth at slightly lower acidic condition (pH 5.5-pH 6.0). They are strictly fermentative, with lactic acid as the major end product during sugar fermentation [4].

Probiotics are defined as live microorganisms in foodstuffs which, when consumed at certain levels in nutrition, stabilizes the gastrointestinal tract microflora thereby conferring health benefits on the consumer [5]. Probiotics in addition to their basic nutrients, contain biologically active components in adequate amounts, which can have a positive impact on the wellbeing of the consumer [6]. The potential health benefit depends on the characteristic profile of the probiotics. In general most probiotics are gram-positive, usually catalase-negative, rods with rounded ends, and occur in pairs, short, or long chains [7]. They are non-flagellated, non-motile and non-spore forming, and are intolerant to salt. Optimum growth temperature for most probiotics is 37°C but some strains such as *Lactobacillus casei* prefer 30°C and the optimum pH for initial growth is 6.5-7.0 [7]. The identification of specific microflora involved in indigenous cereal fermentation is needed to amplify and control positive factors and as well as to reduce negative factors such as growth and metabolism of pathogenic and toxicogenic bacteria [8].

MATERIALS AND METHODS

Sample preparation

500 g of maize, sorghum and millet grain samples was purchased from Western Kenyan main markets randomly and transported in khaki bags for subsequent preparation and analysis at the technical university of Mombasa.

50 g from mixed sample of each cereal grains was ground into flour using a mortar and pestle with proper sterilization with 85%

Correspondence to: Beatrice Aleyo Akweya, Department of Pure and Applied Sciences, Technical University of Mombasa, Mombasa, Kenya, Tel: +254720340280; E-mail: baleyo@tum.ac.ke

Received: 07-Jan-2022, Manuscript No. JFMSH-22-4931; **Editor assigned:** 10-Jan-2022, PreQC No. JFMSH-22-4931 (PQ); **Reviewed:** 24-Jan-2022, QC No. JFMSH-22-4931; **Revised:** 31-Jan-2022, Manuscript No. JFMSH-22-4931 (R); **Published:** 07-Feb-2022, DOI: 10.35248/2476-2059-22.7.171.

Citation: Akweya BA, Mghalu JM, Yusuf RUM, Bitange T (2022) Characterization of Probiotic Potential Lactic Acid Bacteria Found in Fermented Cereal Products from Western Kenya. Food Microbial Saf Hyg. 7:171.

alcohol. The pre-ferment was prepared by mixing flour with warm water in the ratio 1:2, put in a sealed bottle and allowed to ferment at room temperature for 48 hours.

Isolation

The samples were suspended appropriately and diluted in sterile saline. 10 ml of sample was homogenized with 90 ml of 0.85% (w/v) sterile sodium chloride solution to make an initial dilution (10^{-1}). Serial dilutions up to 10^{-7} were made for each sample. 1 ml sample from each of the corresponding dilutions (10^{-5} and 10^{-7}) was plated out onto [9] MRS agar by spread plate technique in duplicate. Inoculated plates were then incubated at 37°C for 48 hours under anaerobic conditions. After incubation, plates with colonies with distinct morphologies such as form, elevation, margin, surface, colour and consistency were selected randomly. The selected colonies were purified by streak plate technique. The purified strains were stored at -20°C in sterile distilled water supplemented with 10% glycerol.

Gram staining

Thin smear of bacterial cultures were made on clean slides, and then were air dried and heat fixed. Smears were covered with crystal violet for 30 seconds. Slides were then washed with distilled running water. Slides were covered with grams iodine solution for 60 seconds and then washed with 95% ethyl alcohol and then distilled water. Again the smears were covered with safranin for 30 seconds and then washed with distilled water and blot dried, air dried and were then observed under a light microscope to determine gram positives (give blue-purple color) and purified isolates. Colonies or isolates that displayed the general characteristics of Lactic Acid Bacteria (LAB) such as gram positive, were chosen from each plate for further physiological and biochemical characterization.

Catalase test

The ability to produce the enzyme catalase was checked by placing a drop of 15% (v/v) hydrogen peroxide on a clean microscopic slide. A loop full of bacteria culture was thoroughly mixed with the hydrogen peroxide on the slide. The mixture was observed for the production of gas bubbles [10].

CO_2 production from glucose

Carbon dioxide production from glucose assay was performed to determine the homo-fermentative and hetero-fermentative characteristics of the isolates. Fifty microliters of overnight cultures was transferred into 8ml of MRS broth in test tubes containing inverted durhum tubes. The tubes were then incubated anaerobically at 37°C in anaerobic jars for 48 hours. Gas accumulation in durhum tubes after incubation was taken as the evidence for CO_2 production from glucose.

Screening of potential probiotic lactic acid bacteria on various conditions

A 24 hours culture of each isolate was used as the inoculum whereby the cells were spun down, re-suspended in 0.85% normal saline and a loopful of the suspension was inoculated into each of the test tubes. The temperature tested were 15°C, 37°C, 45°C and 55°C, concentration of NaCl tested were 2%, 4%, 7.5% and 10% (w/v), while the pH tested was 1.5, 2, 4 and 6. The MRS broth was adjusted with 1M phosphoric acid and 1M NaOH to prepare the initial pH. At the end of 24 hours the colour change of each test tube was noted as a simple indication of growth or no growth.

RESULTS

The research findings indicate that potential Lactic Acid Bacteria (LAB) could be isolated from traditionally fermented cereal products. From all samples, 10 LABs were isolated 6 of which were presumptive *Lactococcus spp* and 4 *Lactobacillus spp*. Three of LAB that possessed characteristics of probiotics were isolated from maize, of which two were *Lactococcus spp* and one *Lactobacillus spp*. Millet samples had two *Lactobacillus* spp while sorghum had one (Table 1) [11] isolated three lactic acid bacteria (*Lactobacillus plantarum*, *L. fermentum* and *Lactococcus lactics*) from kunun-zaki (A Nigerian non-alcoholic cereal beverage) which they used to develop a starter culture for enhanced production of kunun-zaki. Out of the ten LAB, three isolates were found to be hetero-fermentative. Homo-fermentative LAB ferments carbohydrates with the production of only lactic acid which can lower the pH of medium close to 4.0-4.5, hetero-fermentative Lactic Acid Bacteria (LAB) on the other hand produce carbon-dioxide and other organic compounds (acetic acid, acetaldehyde, diacetyl and alcohol,) which can further lower the pH to about 3.5. These compounds impart characteristics flavour to the fermented foods [12,13].

Table 1: Cultural, morphological and biochemical characteristics of Lactic Acid Bacteria (LAB) isolated from cereals.

	Gram's reaction/Cell shape	Cultural characteristics	Catalase test	CO_2 Production from glucose	Growth at temperatures (°C)				Growth in NaCL concentration (%)				Growth at pH				Possible isolate
Organisms					15	37	45	55	2	4	7.5	10	1.5	2	4	6	
MAPU01	+Rod	PPC	-	-Homo	+	+	+	-	+	-	-	-	-	+	+	+	*Lactobacillus spp*
MAPU02	+Cocci	PPC	-	+Hetero	+	+	-	-	+	+	+	-	-	+	+	+	*Lactococcus spp*
MAPU03	+Cocci	PPC	-	-Homo	-	+	-	-	+	-	-	-	-	-	+	+	*Lactococcus spp*
SMPU01	+Cocci	PPC	-	+Hetero	+	+	+	-	+	+	+	-	+	+	+	+	*Lactococcus spp*
SMPU02	+Rod	PPC	-	-Homo	-	+	+	-	-	-	+	+	-	+	+	+	*Lactobacillus spp*
SMPU03	+Cocci	PPC	-	-Homo	+	+	-	-	+	+	-	-	-	-	-	+	*Lactococcus spp*
SMPU04	+Rod	PPC	-	-Homo	-	+	-	-	-	+	+	-	-	+	+	+	*Lactobacillus spp*
SGPU01	+Cocci	PPC	-	+Hetero	+	+	-	-	+	+	-	-	-	+	-	+	*Lactococcus spp*
SGPU02	+Cocci	PPC	-	-Homo	-	+	+	-	+	+	+	-	+	+	+	+	*Lactococcus spp*
SG03	+Rod	PPC	-	-Homo	+	+	-	-	+	+	-	-	-	-	+	+	*Lactobacillus spp*

Note: +indicate growth; -no growth; LAB isolates: -MAPU01-MAPU03 from maize, SMPU01-SMPU04 from millet, SGPU01-SGPU03 from sorghum.
Abbrevations: PPC: Pin Point Colony; Hetero-Heterofermentative; Homo-Homofermentative.

DISCUSSION

The nutritional quality and sensorial properties of cereals and their products are poor when compared to dairy products. Some of the reasons may be due to the coarse nature, lower protein content, deficiency of some essential amino acids and presence of ant nutrients such as phytic acid, tannins and polyphenols [3]. The cereal grains have a highly cross-linked water-insoluble dietary fibres which cannot be digested by the β-glucosidases and esterases produced by human microflora [14]. Lactic acid bacteria can hydrolyze complex polysaccharides in cereals to simple and biologically active compounds [15]. The proteolytic activity of probiotic culture is essential for the growth of the organisms and it is involved in the development of organoleptic properties of different fermented products [16]. They have two different metabolic pathways for hexose fermentation. In homo-fermentative pathway, lactic acid (more than 85%) is major end product whereas in hetero-fermentative pathway lactic acid, ethanol/acetone and CO_2 are the terminal products.

CONCLUSION

Traditionally fermented cereal products from Western Kenya are rich source of probiotic lactic acid bacteria. For this purpose, studies must be carried out to: Identify the bacteria molecularly, test ingredients, and screen the bacteria for possible use in developing functional cereal products and processes. This can go a long way in addressing not only malnutrition but also reaching out to lactose-intolerant and vegetarian consumers whose demand for new nourishing and palatable probiotic products is ever increasing.

REFERENCES

1. Pugalenthi M, Vadivel V. Nutritional evaluation and the effect of processing methods on antinutritional factors of sword bean *(Canavalia gladiata)*. J Food Sci Technol 2005;42(6):510-516.
2. Ng CC, Wang CY, Wang YP. Lactic acid bacterial fermentation on the production of functional antioxidant herbal *Anoectochilus formosanus Hayata*. J Biosci Bioeng. 2011;111(3):289-293.
3. Sandhu KS, Punia S, Kaur M. Fermentation of Cereals: A Tool to enhance bioactive compounds. Plant Biotechnology: Recent Advancements and Developments. 2017:157-170.
4. Kumar RS, Kanmani P, Yuvaraj N, Paari KA, Pattukumar V, Arul V, et al. Traditional Indian fermented foods: A rich source of lactic acid bacteria. Int J Food Sci and Nutr. 2013;64(4):415-428.
5. Alo MN, Uchemna LU, Elkena CN, Ukwa BN. Probiotic Potential of Nigerian fermented *Pentaclethra macrophylla* (Ugba). World J Public Health Sci. 2014;2(1):2.
6. Soccol CR, Vandernberghe LP, Spier MR, Medeiros ABP, Yamaguishi CT, Lindner JD, et al. The potential of probiotics. Food Technol Biotechnol. 2010;48(4):413-34.
7. Marazza JA, Garro MS, Savoy G. Aglycone production by *Lactobacillus rhamnosus* CRL981 during soymilk fermentation. Food Microbiol. 2009;26(3):333-339.
8. Marquart L, Cohen E. Increasing whole grain consumption. Food Technol. 2005;59(12):24-31.
9. James N, Karimi N, Thomas S. Competitiveness of kenyan and ugandan maize production: Challenges for the future. 2004.
10. Rodríguez H, Curiel JA, Landete JM. Food phenolics and lactic acid bacteria. Int J Food Microbiol. 2009;132(2-3):79-90.
11. Agarry O, Nkama I, Akoma O. Production of kunun-zaki (A Nigerian fermented cereal beverage) using starter culture. Int Res Microbiol. 2010;1(2):018-025.
12. Ajewole EA, Akoma O, Daniel AA, Nnadozie H, Njoku C. Characterization of lactic acid bacteria isolated from two fermented food products and the stomach of ruminant animal. Globl Inst Res Edu. 2014;2319-5584.
13. Steinkraus KH. Fermentations in world food processing. Compr Rev Food Sci Food Saf. 2002;1(1):23-28.
14. Dey TB, Kuhad RC. Upgrading the antioxidant potential of cereals by their fungal fermentation under solid-state cultivation conditions. Lett Appl Microbiol. 2014;59(5):493-499.
15. Duenas M, Fernández D, Hernández T. Bioactive phenolic compounds of cowpeas (Vigna sinensis L) modifications by fermentation with natural microflora and with *Lactobacillus plantarum* ATCC 14917. J Sci Food Agric. 2005;85(2):297-304.
16. Swennen K, Courtin CM, Delcour JA. Non-digestible oligosaccharides with prebiotic properties. Crit Rev Food Sci Nutr. 2006;46:459-471.