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Probiotic potential of lactic acid bacteria isolated from traditionally fermented legume products of Western Kenya

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Fermentation as a processing technique has been used for a long time by people of Western Kenya to improve the quality of raw food material. During fermentation, lactic acid bacteria (LAB) synthesize vitamins and minerals, produce biologically active peptides with enzymes such as proteinase and peptidase, and remove some ant-nutrients. The medicinal and flavor enhancing properties of fermented foods are due to the presence of probiotics. This study aims at isolating and biochemically characterizing potential probiotic lactic acid bacteria from spontaneously fermented legume-based products from two locations in Vihiga County; Emuhaya and Mbale, Kenya. The results from the findings are presumptive indicator of probiotic bacteria in fermented legumes. $RC0_{PU}2$, $SB0_{PU}3$ and $CP0_{PU}1$ isolated form Emuhaya can survive at low pH of 2 while $SB0_{PU}3$ and $CP0_{PU}1$ in addition can survive a salt concentration of 3%. $SB0_{PU}3$ and $CPO_{PU}2$ and $RC0_{PU}2$, $SB0_{PU}2$ and $CPO_{PU}3$ can grow at high temperature of 45°C. $RC0_{PU}2$, $SB0_{PU}2$, $GG0_{PU}1$, $CP0_{PU}1$ and $CPO_{PU}2$ can tolerate a salt concentration of 3%. Only two isolates $RCO_{PU}2$ and $CPO_{PU}2$ isolated from Mbale samples survived at the pH of 2. The findings from this study indicated that fermented legumes are potential sources of probiotics with unique characteristics.

Key words: Probiotic potential, legumes, lactic acid bacteria.

INTRODUCTION

Legumes are the most abundant and widely cultivated food crops in Western Kenya. They are produced in large quantities and therefore make up the staple food for Luhya people. The use of legumes as alternative sources

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of protein and other micronutrients (Margier et al., 2018; Oluwafemi et al., 2017) could be assisted in improving dietary diversity of low income rural households. The protein of legumes is rich in essential amino acid lysine but poor in sulphur containing amino acids methionine and cystine (FAO, 2016; Anon, 2017).

Fermentation is a desirable process of biological modification of primary food matrix that is brought about by microorganism and their enzymes action The utilization of fermented legume foods containing probiotics would be one avenue by which the health of the children and community at large may be improved (Huan et al., 2019). Probiotics are live microorganisms which are produced during fermentation or intentionally added to foodstuffs and when consumed at certain levels in nutrition, stabilizes the gastrointestinal tract microflora conferring health benefits on the consumer (Markowiak and Katarzyna, 2017). Probiotic organisms also contain biologically active components which can have a positive impact on the wellbeing of the consumer (Alemayehu et al., 2017; Anusha et al., 2015). Several studies have indicated that LAB plays a positive role in modulating the host immune system and displaying of antimicrobial activities against common food-borne pathogens and in preventing and treating diarrhoea (Mokoena et al., 2016). The demand for non-dairy probiotic fermented foods has increased as consumers become more health conscious and at the same time expect particular and most times exact fermented product specifications (Ranadheera et al., 2017). Most indigenous fermentation products are valued for the taste, aroma and other active components produced. The consumption of plant proteins has evolved and is driven by the influence of continued need of consumers for health foods or for partial replacement of animal proteins with plants that possess better and cheaper nutritional components (Oluwafemi et al., 2017). Based on these findings and the increasing demand for probiotic foods from plant materials, this study was carried out to isolate probiotic LAB from fermented legumes, identify the isolates and biochemically characterize them.

MATERIALS AND METHODS

The legume samples were obtained from cereal store traders from Lwanda and Mbale market in Vihiga County, Western Kenya. They were transported in kaki bags to the Food and Microbiology Laboratories, Technical university of Mombasa where the experiment was carried out.

Fermentation of legume products

The legumes (beans, soy bean, cow peas and green grams) were cleaned by winnowing to remove husks and other light foreign materials. They were then sieved to remove stones and sand. 50 g each of the legume was ground using a blender separately to obtain flour, with sterilization of the blender with 70% ethanol after every sample. The sample flour was mixed with two parts water and fermented by incubating at 30°C for 48-72 h in a sterile covered flask. 10 g sample was taken aseptically from each for lactic acid bacteria screening.

Isolation of probiotic potential lactic acid bacteria from legumes

The samples were suspended appropriately and diluted in sterile saline. From each legume fermented, 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 MRS agar plates by spread plate technique in duplicates. Inoculated plates were then incubated at 37°C for 48-72 h under anaerobic conditions.

Characteristics of the probiotic potential lactic acid bacteria isolates

MRS broth was used in these series of studies but with 0.17 g/L bromothymol blue added as pH indicator (pH 7). Universal bottle with screw caps was each filled with 20ml of the MRS broth and autoclaved. A 24 h 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 was 15, 37, 45 and 55°C, the concentration of NaCl tested was 2, 3, 6.5 and 10% (w/v), while the pH tested was 2, 3, 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 h the colour change of each test tube was noted as a simple indication of growth or no growth.

RESULTS

The results are summarized in Tables 1 and 2. This study has shown that probiotic potential lactic acid bacteria could be isolated from fermented legumes. Nine LABs were isolated from Emuhaya legume samples; 6 of these are possible *Lactococcous* species and 3 *Lactobacillus* species. The total isolates from Mbale are ten: 6 are presumptive *Lactococcous* species and 4, *Lactobacillus* species.

All of the isolates from Emuhaya and Mbale were Gram-positive and catalase-negative bacteria, generally unable to grow at 55°C and NaCl concentration of 6.5% but able to grow at pH 4.0 to 6.0. $RC0_{PU}2$, $SB0_{PU}3$ and $CP0_{PU}1$ from Emuhaya can survive at low pH of 2 while $SB0_{PU}3$ and $CP0_{PU}1$ in addition can survive at a salt concentration of 3. $SB0_{PU}3$ and $CPO_{PU}2$ (Table 1) and $RC0_{PU}2$, $SB0_{PU}2$ and $CPO_{PU}3$ (Table 2) can grow at high temperature of 45°. These lactic acid bacteria are possible thermophiles like those of yoghurt culture. $RC0_{PU}2$, $SB0_{PU}2$, $GG0_{PU}1$, $CP0_{PU}1$ and $CPO_{PU}2$ can tolerate a salt concentration of 3%. Only two isolates ($RCO_{PU}2$and $CPO_{PU}2$) from Mbale survived at the pH of 2.

DISCUSSION

There is an overwhelming increase in relation to utilization

Table 1. Phenotypic characteristics of representative strains isolated from traditionally fermented Legume from Emuhaya-Vihiga.

Organisms	Gram's reaction/cell shape	Cultural characteristics	Catalase test	Growth at temperatures (°C)				Growth in NaCl concentration (%)				Growth at pH				Possible isolate
				15	37	45	55	2	3	6.5	10	2	3	4	6	
$RC0_{PU}1$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$RC0_{PU}2$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	+	+	-	+	Lactococcus spp
$SB0_{PU}1$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$SB0_{PU}2$	+Cocci	Ppc	-	+	+	-	-	+	-	-	-	-	-	+	+	Lactococcus spp
$SB0_{PU}3$	+Cocci	Ppc	-	-	+	+	-	+	+	+	-	+	-	+	+	Lactococcus spp
$GG0_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	-	-	-	+	Lactococcus spp
$GG0_{PU}2$	+Cocci	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactococcus spp
$CP0_{PU}1$	+Cocci	Ppc	-	-	+	-	-	-	+		-	+	+	+	+	Lactococcus spp
$CPO_{PU}2$	+Rod	Ppc	-	+	+	+	-	+	-	-	-	-	-	+	+	Lactobacillus spp

+indicate growth; - no growth +; Gram positive +; Ppc: Pin Point Colony; LAB isolates: (RC0PU1- RC0PU2)-Rosecoco isolates; (SB0PU1- SB0PU3) - Soybean isolates; (GG0PU1- GG0PU2) –Green gram isolates; (CP0PU1- CPOPU2) –Cowpeas isolates.

Table 2. Phenotypic characteristics of representative strains isolated from traditionally fermented Legume from Mbale-Vihiga.

Organisms	Gram's reaction/cell shape	Cultural characteristics	Catalase test	Growth at temperatures (°C)				Growth in NaCl concentration (%)				Growth at pH				Possible isolate
			-	15	37	45	55	2	3	6.5	10	2	3	4	6	
$RCO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	-	-	+	+	Lactococcus spp
$RCO_{PU}2$	+Cocci	Ppc	-	-	+	+	-	+	-	-	-	+	+	+	+	Lactococcus spp
$RCO_{PU}3$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$SBO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactococcus spp
$SBO_{PU}2$	+Cocci	Ppc	-	-	+	+	-	+	+		-	_	+	+	+	Lactococcus spp
$GGO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	-	-	-	+	Lactococcus spp
$GGO_{PU}2$	+Rod	Ppc	-	-	+	-	-	+	-	-	-	-	-	+	+	Lactobacillus spp
$CPO_{PU}1$	+Cocci	Ppc	-	-	+	-	-	+	+	-	-	_	+	-	+	Lactococcus spp
$CPO_{PU}2$	+Rod	Ppc	-	+	+	-	-	+	-	-	-	+	-	-	+	Lactobacillus spp
$CPO_{PU}3$	+Rod	Ppc	-	-	+	+	-	+	+	-	-	-	-	+	+	Lactobacillus spp

+indicate growth; - no growth; + Gram positive; Ppc: Pin Point Colony; LAB isolates: (RCOPU1- RCOPU3)-Rosecoco isolates; (SBOPU1- SBOPU2) - Soybean isolates; (GGOPU1- GGOPU2) –Green gram isolates; (CPOPU1-CPOPU3) –Cowpeas isolates.

of commercial probiotic LAB strains isolated from indigenous and naturally fermented foods (Jawan et al., 2020). The food products are said to possess medicinal and health-promoting acid tolerant and catalase negative devoid of cytochromes (Frank, 2017). These bacteria produce lactic acid as main fermentation product of carbohydrate benefits. LABs are Gram-positive, cocci, and cocco-bacilli, fastidious, non-spore-forming rods, catabolism and other organic substances that add to the flavor, texture, and aroma that contribute to organoleptic characteristics of the products (Vishwanathan and Kadirvelu, 2016). Probiotic isolates must be tolerated and survived a stressful environment such as of low pH (acidic condition) in the stomach and high bile salt concentration in the small intestine (Prete et al., 2020). The LABs which have genes responsible for the degradation and utilization of simple sugars and complex carbohydrates are able to better survive and multiply in the gut (Conlon and Bird, 2015).

Probiotic bacteria are required to survive gastric passage, where the pH can be as low as 1.5 to 2.0 and stay alive for 4 h or more (Gupta and Sharma, 2017) before they move to the intestinal tract. The acid tolerance of bacteria is essential both for withstanding gastric stresses and also to enable the strain to survive for longer periods in high acid carrier foods without reduction in their number (Angmo et al., 2016). Most of the investigated isolates can survive low pH and high salt concentration if incubation time could have been reduced to 3 h (considering the experimental time was more, that is, about 16 h). It is therefore expected they will be good probiotics if they pass safety tests and can be recommended in the food preparations of probiotic foods. To assure viability and functionality of potential probiotic isolates, tolerance to acidic conditions is an important criterion considered. The characteristic of probiotic bacteria to exhibit resistance to acidic conditions is species and strain dependent (Papadimitriou et al., 2016). The growth of pathogenic microorganisms has been shown to be suppressed by probiotic lactic acid bacteria through the release of a variety of antimicrobial factors such as bacteriocins, hydrogen peroxide, ammonia, diacetyl and organic acids such as lactic and acetic acids. These compounds reduce the pH of the lumen, making it difficult for the growth of a variety of food-borne spoilage and pathogenic organism (Gupta and Shama, 2017). The safety analysis is an important issue during the selection and evaluation of new probiotics. Thus, characterization of the safety criteria of the probiotic strains is vital in order to avoid their side effects (Ayala et al., 2019). This study revealed in part that fermented legumes are possible sources of probiotic bacteria; however, the LABs are to be assessed for the ability to produce bacteriocin and lactic acid against a number of serious food borne and spoilage causing microorganisms. The susceptibility to selected eleven antibiotics, inability to produce gelatinase and DNase and non-hemolytic nature will reveal their safety status for further use in food and nutraceutical industry.

Conclusion

The lactic acid bacteria isolated from fermented legumes used in this study possess great potential as probiotics for human and as fermentation starter cultures. This was supported by probiotic characteristics such as survivability in acid condition and high salt concentration. The lactic acid bacteria, $RC0_{PU}2$, $SB0_{PU}2$ and $CPO_{PU}3$ that can be grown at high temperature of 45°C can be considered as thermophiles like those of yoghurt culture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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