Biotechnological characterization of lactic acid bacteria isolated from Ogi-a cereal fermented food product

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Abstract: Eleven strains of lactic acid bacteria previously isolated from the fermentation of Ogi-a Nigerian cereal fermented food product were screened for biotechnological potentials which included utilization of nondigestible α-galactosidase, bile tolerance, production of β-galactosidase, biogenic amine production, phytate utilization, production of α amylase and tannase. All eleven strains showed varying potentials. Most of the strains were observed as utilizers of nondigestible α-galactosidase. Also *Leuconostoc mesenteroides* subsp *dextranicum* was able to produce β galactosidae whereas *Leuconostoc pseudomesenteroides* and *Leuconostoc mesenteroides* were none producers. *Lactobacillus plantarum* subsp *plantarum* and *lactobacillus plantarum* had the most potential. In conclusion, all eleven strains showed varying potentials in their biotechnological activities. *Lactobacillus plantarum* subsp *plantarum* and *Lactobacillus plantarum* could be applied as starter cultures for the fermentation of indigenous foods.
[Ijeoma IO, Okerentugba PO., Oranusi NA. **Biotechnological characterization of lactic acid bacteria isolated from Ogi-a cereal fermented food product.** *N Y Sci J* 2015;8(12):21-26]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 4. doi:10.7537/marsnys081215.04.

Keywords: lactic acid bacteria (LAB), Biotechnological characterization, Ogi, Cereal, Fermented food product

1. Introduction

Lactic acid bacteria (LAB) are a group of non-pathogenic bacteria that play a vital role in our everyday life, from fermentation, preservation and production of foods to prevention of certain diseases due to their antimicrobial action (Saranya and Hemashenpagam, 2011). And mankind has exploited these bacteria for generations for the production of fermented products because of their ability to produce desirable changes in taste, flavour and texture (Derek *et al.*, 2009). However the quality and stability of these products are usually not consistent because the fermentation technology still relies on back slopping and spontaneous fermentations (Sanni, 1993; Jespersen *et al*., 1994). This hinders successful large-scale commercial processing of traditional fermented foods (Mukisa *et al.*, 2012). Application of defined starter culture is one of the approaches that could be used to standardize fermentations and ensure consistence in quality (Sanni, 1993). However the fermentation of traditional fermented foods is frequently caused by natural, wild type LAB that are a function of the environment and raw material used (Leroy and De Vuyst, 2004). These pure cultures isolated from complex ecosystems of traditional fermented foods exhibit a diversity of metabolic activities that diverge strongly from the ones of comparable strains used as industrial bulk starters (Klijn *et al.*, 1995). And in the food industry, LAB are widely applied as starter cultures (Shehata, 2012). Starter cultures for food fermentation are usually selected based on possession of useful biotechnological potentials (Dal Bello *et al*., 2007).

In this study, eleven LAB strains previously isolated from Ogi-a cereal fermented food product from Nigeria were screened for biotechnological potentials to determine their suitability as starter cultures using the following parameters utilization of nondigestible α-galactosidase, Bile tolerance, production of β-galactosidase, biogenic amine production, phytate utilization, production of α amylase and tannase.

2. Materials and Methods

2.1. Strain cultivation

The stock cultures of the 11 strains of lactic acid bacteria were stored at -80ºC in 50% glycerol (v/v). Working cultures were subculture twice in Mann Rogosa Sharpe (MRS) and M17 broth and once on MRS agar and stored at -20ºC until further use.

2.2. Screening for technological potentials of LAB strains

2.3. Bile Tolerance: Overnight strains of LAB were cultured in MRS broth enriched with 1% (v/v) of oxgall at 37ºC for 24h. The growth was monitored by spreading 0.1ml of appropriate dilutions on MRS agar (Oxoid). Control cultures contains no oxgall and cell counts were taken at 0h and was compared with cell counts after 24h. The growth of LAB was stated as CFU/ml and the percentage survival of LAB strain to bile was calculated thus initial cfu/ml at 0h minus cfu/ml 1% bile at 24h.

2.4. Utilization of Non-digestible α-galactosidase: This was determined using Yousif *et al*. (2005). Overnight cultures of strains were grown in modified MRS media which contains no glucose and meat extracts. However, it contained Sodium citrate which

replaced Diammonium hydrogen citrate. Then, the medium was supplemented with 0.004% chlorophenol red (5ml of a 0.08% ethanoic solution per litre) as pH indicator. The medium was also supplemented with α-galactosidase sugar raffinose at a concentration of $8g^{-1}$ as the sole source of carbohydrate. Cultures were incubated at 37ºC and examined for acid production over a 3 day period.

2.5. Production of β-galactosidase: β-galactosidase was tested according to the method of Ghenytanchi *et al.* (2010).

2.6. Detection of Biogenic amine production: This was carried out by the standard methods as described by Yousif *et al.* (2005).

2.7. Phytate Utilization: This was carried out using the modified method of El-Toukhy *et al.* (2013). Strains were grown on phytate screening media (PSM) and incubated at 30ºC for 2 days. Phytate utilization was measured as diameter of inhibition in millimetre.

2.8. Production of α amylase and Tannase: α-amylase and tannase production was carried out as described by Yousif *et al*. (2005). The standard methods as described by Osawa *et al*. (2000) was used to confirm the activity.

2.9. Data Analysis: Data generated was analysed using Statistical Package for Social Sciences (SPSS) 20.0 and $p \leq 0.05$ significant value was used.

3. Results

3.1. Bile Tolerance: Ability of LAB strains to survive in the GIT was determined using 1% bile salt. All LAB strains were able to grow in 1% bile salt with *Leuconostoc pseudomesenteroides* showing the highest increase in LAB counts compared to the control after 24h incubation (Table 1). No significant difference between means was observed at $p \leq 0.05$.

3.2. Utilization of Non-digestible α-Galactosidase: Screening for the utilization of α galactosidase by the LAB strains is presented in Table 2. Utilization of α galactosidase which was indicated by the zone of clearing on agar plate was observed for *Weissella cibaria*, *Lactobacillus plantarum*, *Lactococcus lactis* subsp *lactis*, *Lactobacillus fermentum* and *Lactobacillus plantarum* subsp *plantarum*. *Lactobacillus curvatus*, *Enterococcus faecalis*, *Enterococcus lactis*, *Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides* and *Leuconostoc mesenteroides* subsp *dextranicum* were observed as non-utilizers of α galactosidase.

3.3. Production of β-Galactosidase:

Screening for β-galactosidase production is presented in Table 3. *Weissella cibaria*, *Lactobacillus plantarum*, *Lactococcus lactis* subsp *lactis*, *Enterococcus faecalis*, *Enterococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus plantarum* subsp *plantarum* and *Leuconostoc mesenteroides* subsp *dextranicum* showed β-galactosidase production which was indicated by the green colonies on Xgal substrate and yellow colouration in ONPG substrate. *Lactobacillus curvatus*, *Leuconostoc pseudomesenteroides* and *Leuconostoc mesenteroides* did not produce β-galactosidase.

Table 1: Bile Tolerance of Lactic Acid Bacteria Strains

Strains	**Initial cfu/ml (10^6)**	**Control cfu/ml (10^6)**	**1% Bile cfu (10^6)**	**Survival Percentage (%)**
Weissella cibaria	2.5	2.5	2.2	26
Lactobacillus curvatus	1.7	2.2	1.6	18
Lactobacillus plantarum	1.1	0.9	1.2	15
Lactococcus lactis subsp *lactis*	1.5	1.8	1.7	69
Enterococcus faecalis	1.5	1.8	1.3	36
Enterococcus lactis	1.3	1.5	1.5	72
Leuconostoc pseudomesenteroides	1.5	2.2	6.0	30
Lactobacillus fermentum	1.9	1.7	1.5	20
Leuconostoc mesenteroides	1.3	1.7	1.6	20
Lactobacillus plantarum subsp *plantarum*	2.2	1.5	1.7	26
Leuconostoc mesenteroides subsp *dextranicum*	1.7	1.8	1.4	14
LSD	0.771	0.641	4.275	60.64
F.pr	0.001	0.001	0.001	0.485NS

Table 2: Utilization of α-galactosidase by Lactic Acid bacteria strains

Strains	Utilization of α-galactosidase (mm)
Weissella cibaria	20
Lactobacillus curvatus	0
Lactobacillus plantarum	20
Lactococcus lactis subsp *lactis*	14
Enterococcus faecalis	0
Enterococcus lactis	0
Leuconostoc pseudomesenteroides	0
Lactobacillus fermentum	16
Leuconostoc mesenteroides	0
Lactobacillus plantarum subsp *plantarum*	20
Leuconostoc mesenteroides subsp *dextranicum*	0
LSD	1.667
F.pr	0.001

Table 3: Production of β-galactosidase by Lactic Acid bacteria strains

Strains	Production of β-galactosidase
Weissella cibaria	+
Lactobacillus curvatus	-
Lactobacillus plantarum	+
Lactococcus lactis subsp *lactis*	+
Enterococcus faecalis	+
Enterococcus lactis	+
Leuconostoc pseudomesenteroides	-
Lactobacillus fermentum	
Leuconostoc mesenteroides	-
Lactobacillus plantarum subsp *plantarum*	+
Leuconostoc mesenteroides subsp *dextranicum*	+

3.4. Detection of Biogenic Amine Production: Screening for biogenic amine production using different pH adjustment is presented in Table 4. Biogenic amine production was detected for *Leuconostoc mesenteroides* subsp *dextranicum*, *Lactococcus lactis* subsp *lactis*, *Enterococcus faecalis*, *Lactobacillus fermentum* and *Lactobacillus plantarum* subsp *plantarum* at the 3 pH adjustments while *Weissella cibaria*, *Lactobacillus curvatus* and *Leuconostoc pseudomesenteroides* showed activity at pH 5.3 and 6.3. No biogenic amine production was detected *for Lactobacillus plantarum*, *Enterococcus lactis* and *Leuconostoc mesenteroides*.

Table 4: Detection of Biogenic Amine Production by Lactic Acid bacteria Strains

Strains	pH	pH	pH
Weissella cibaria	**5.3**	**5.3**	**5.3**
Lactobacillus curvatus	+	+	+
Lactobacillus plantarum	+	+	+
Lactococcus lactis subsp *lactis*	-	-	-
Enterococcus faecalis	+	+	+
Enterococcus lactis	+	+	+
Leuconostoc pseudomesenteroides	-	-	-
Lactobacillus fermentum	+	+	+
Leuconostoc mesenteroides	+	+	+
Lactobacillus plantarum subsp *plantarum*	-	-	-
Leuconostoc mesenteroides subsp *dextranicum*	+	+	+

3.5. Phytate Utilization: *Weissella cibaria*, *Lactobacillus curvatus*, *Enterococcus faecalis*, *Leuconostoc pseudomesenteroides*, *Lactobacillus fermentum* and *Leuconostoc mesenteroides* utilized phytate which was indicated by the zone of clearing on agar measured in mm. None utilization of phytate was observed for strains *Lactococcus lactis* subsp *lactis*, *Enterococcus lactis*, *Lactobacillus plantarum* subsp *plantarum* and *Leuconostoc mesenteroides* subsp *dextranicum* (Table 5).

Table 5: Phytate Utilization of Lactic Acid Bacteria Strains

Strains	**Utilization of Phytate (mm)**
Weissella cibaria	14
Lactobacillus curvatus	30
Lactobacillus plantarum	0
Lactococcus lactis subsp *lactis*	0
Enterococcus faecalis	13
Enterococcus lactis	0
Leuconostoc pseudomesenteroides	20
Lactobacillus fermentum	12
Leuconostoc mesenteroides	16
Lactobacillus plantarum subsp *plantarum*	0
Leuconostoc mesenteroides subsp *dextranicum*	0
LSD	5.380
F.pr	0.001

Table 6: α-Amalyse Production by Lactic Acid Bacteria Strain

Strains	**α-Amalyse Production (mm)**
Weissella cibaria	0
Lactobacillus curvatus	8
Lactobacillus plantarum	6
Lactococcus lactis subsp *lactis*	9
Enterococcus faecalis	0
Enterococcus lactis	0
Leuconostoc pseudomesenteroides	0
Lactobacillus fermentum	6
Leuconostoc mesenteroides	0
Lactobacillus plantarum subsp *plantarum*	14
Leuconostoc mesenteroides subsp *dextranicum*	0
LSD	2.591
F.pr	0.001

3.6. Production of α Amylase and Tannase: *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactococcus lactis* subsp *lactis*, *Lactobacillus fermentum* and *Lactobacillus plantarum* subsp *plantarum* showed a positive result for α amylase production. No activity was observed *Weissella cibaria*, *Enterococcus faecalis*, *Enterococcus lactis*, *Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides* and *Leuconostoc mesenteroides* subsp *dextranicum* (Table 6).

All LAB strains showed activity for tannase production except *Lactobacillus curvatus*, *Lactococcus lactis* subsp *lactis* and *Lactobacillus plantarum* subsp *plantarum* (Table 7).

Table 7: Tannase Production by Lactic Acid Bacteria Strains

Strains	Tannase Production (mm)
Weissella cibaria	13
Lactobacillus curvatus	0
Lactobacillus plantarum	11
Lactococcus lactis subsp *lactis*	0
Enterococcus faecalis	10
Enterococcus lactis	19
Leuconostoc pseudomesenteroides	13
Lactobacillus fermentum	11
Leuconostoc mesenteroides	9
Lactobacillus plantarum subsp *plantarum*	0
Leuconostoc mesenteroides subsp *dextranicum*	10
LSD	2.466
F.pr	0.001

4. Discussion

The eleven LAB strains were assessed for biotechnological potentials using various parameters for possible selection as starters and biopreservatives. *Leuconostoc pseudomesenteroides* which had the highest counts compared to the control showed possibility of surviving in the gastrointestinal tract.

Stachyose and raffinose are resistant to cooking and other processing techniques due to possession of α-D- galactosidase bond (Yousif *et al*., 2005). This bond can be removed by bacteria associated with food fermentation having oligosaccharides (Yousif *et al*., 2005) and in this study *W. cibaria*, *Lb. plantarum*, *Lc lactis* subsp *lactis*, *Lb fermentum* and *Lb plantarum* subsp *plantarum* showed such potentials.

Alleviation of lactose intolerance by probiotic strain producing β-galactosidase have been reported (Ljungh and Wadstrom, 2005). Most of the strains produced green colonies on X-gal substrate which was confirmed by the yellow production in ONPG substrate. In this study *Lb curvatus* did not produce β galactosidase which contradicted reports by Gheytanchi *et al*., (2010) who in a study observed production of β galactosidase by all *Lactobacillus* species isolated. Biogenic amine occurrence in many foods as a result of microbial activities and raw material containing proteins have been reported (Yousif *et al*., 2005) Most of the strains identified in this study decarboxylated the amino acids (lysine, tyrosine, histidine and ornithine) used. *Lb curvatus* tested positive for biogenic amine production which agreed with studies by Mohammed and Mayo, (2006). Occurrence of biogenic amine positive had previously been reported by Omafuvbe and Enyioha, (2011) and it agreed partially with results of this present study.

Most strains were positive for phytate and α amylase production. Previously Yousif *et al*., (2005) reported absence of α amylase producers for Hussuwa a fermented sorghum product while Omafuvbe and Enyioha, (2011) reported the occurrence of a few strain producing amylase enzyme from African fermented maize and cassava products. Also strains *Lb curvatus*, *Lc lactis* subsp *lactis* and *Lb plantarum* subsp *plantarum* were negative for tannase production. Studies by Osawa *et al*., (2000) reported *Lb plantarum*, *Lb paraplantarum* and *Lb pentosus* positive for tannase activity while the rest of the 14 *Lactobacillus* sp were negative for tannase. Also this trait have been reported to be of ecological advantage for *Lactobacillus* sp as they are often associated with fermentation of plant materials (Osawa *et al*., 2000). In conclusion, all eleven strains showed varying potentials in their biotechnological activities. *Lactobacillus plantarum* subsp *plantarum* and *Lactobacillus plantarum* could be applied as starter cultures for the fermentation of indigenous foods.

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12/8/2015