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α-galactosidase and β-glucosidase Enzyme Activity of Lactic Strains Isolated from Traditional Fermented Foods of West Garo Hills, Meghalaya, India

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A B S T R A C T

In the study, α-galactosidase released by Lactic acid bacteria catalyse hydrolysis of galactose containing oligosaccharide. Similarly, β-glucosidase of lactic acid bacteria are making a significant contribution to the dietary and sensory attributes of fermented foods as by deglucosylation, they can release flavour compounds from glucosylated precursors and could increases the bioavailability of health-promoting secondary metabolites. Therefore, the aim of this study was to select strains of lactic acid bacteria isolated from ethnic fermented foods of West Garo Hills of Meghalaya state. Out of forty nine fermented food-derived isolates, *Lactobacillus rhamnosus* (K4) was selected as the maximum producer of α-galactosidase and *Lactobacillus helveticus* (K14) produced maximum β-glucosidase. Hence, these strains can be used as producer of both, α-galactosidase and β-glucosidase enzymes during fermentation of complex sugars.

Introduction

Lactic acid bacteria (LAB) of the taxonomic order Lactobacillus (phylum Firmicutes) play an indispensable role in most societies and have been exploited throughout recorded history (Makarova *et al*., 2006). They are an integral part of traditional food processing and preservation technologies, including the fermentation of dairy products, vegetables and meat. The evolution of LAB is tightly connected to the consumption of fermented foods and many LAB species were 'domesticated' and passed down the generations in the course of emerging food processing technologies.

Lactobacillus sp. possesses glycohydrolases like the enzyme α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) hydrolyses terminal, non-reducing α-D-galactose residual in the a-galactosides, as

well as galactose oligosaccharides such as melibiose, raffinose and stachyose (Dey and Pridham, 1972). α- Galactosidase is not synthesized by humans, and thus the presence of these oligosaccharides could hinder digestion and cause flatulence, since they are utilized by the gas-generating intestinal microorganisms. α-Galactosidase can be used to clear these oligosaccharides and upgrade the nutrition of legume food (Thananunkul *et al*., 1976). Enzyme treatment with microbial a-galactosidase would be promising for the elimination of these oligosaccharides (Thananunkul *et al*., 1976). *Lactobacillus* species have been found in large numbers as part of the intestinal flora of humans and other animals, where they are thought to increase resistance to common intestinal disorders, especially those with a microbial pathogenesis, for example, gastroenteritis (Casas and Dobrogosz, 2000). They can achieve this by fortifying the normal micro-flora either through their fermentation products or by the production of glycosidases, which degrade carbohydrates, thereby supplying energy for the growth of other bacteria (Sandine *et al*., 1979).

By definition, *β*-D-glucosidases (EC 3.2.1.21) remove glucopyranosyl residues from the non-reducing end of *β*-D-glucosides by catalysing hydrolysis of the glycosidicbond. *β*-D-Glucosidases occur abundantly in all domains of life and exert diverse biological functions (Cairns and Esen, 2010). LAB involved in plant food fermentations have been investigated extensively with regard to the release of plant metabolites through *β*-glucosidase activities. Oleuropein is a phenolic glucoside responsible for the bitterness of unprocessed olives, and partial degradation of oleuropein is required before table olives can be consumed. The most important species for the fermentation of olives is *Lactobacillus plantarum*. Most of these isolates were able to hydrolyse oleuropein through *β*-glucosidase activity (Ghabbour *et al*., 2011). Soybeans contain high concentrations of their b-glucosides genistin and daidzin. These were reported to be hydrolysed by b-glucosidase activities of LAB during soy milk fermentations (Chin *et al*., 2006). *β*-Glucosidase is considered to be the key enzyme for the bioconversion of isoflavone forms in fermented soy products. It has superior activity for hydrolyzing acetylglycoside and malonylglycoside isoflavones (Park *et al*., 2006). If *β*-glucosidase can effectively convert acetylglycoside and malonylglycoside to their aglycones, it can lead to an enrichment of isoflavone aglycones in soy foods such as soy-based functional fermented food products. Soy isoflavones have been implicated in health benefits, including the potential to reduce the risk of age-related and hormone-related disorders such as cancer, menopausal symptoms, cardiovascular disease and osteoporosis.(Cheng *et al*., 2013; Okabe *et al*., 2011; Zhang *et al*., 2014; Martins *et al*., 2013; Bedani *et al*., 2014)

Our present investigation deals with the analysis of α-galactosidase and β-glucosidase enzyme activities of the Lactic acid bacterial cultures obtained from the traditional fermented foods of the West Garo Hills region of Meghalaya with an approach to justify their probiotic potentiality for designing a functional fermented food further.

Materials and Methods

Sampling and Isolation

Various samples of fermented foods were obtained from different regions of West Garo Hills in Meghalaya state and 1 ml of a mix of the fermented food samples and poured into 50 ml sterile MRS broth (HiMedia, India) and incubated at 37˚C for (2-5) days (Abbas and Mahasneh, 2014). The enriched fermented

food samples were serially diluted in sterile normal saline. Aliquots of 100 µl from each dilution were then plated on MRS media supplemented with 0.01% bromocresol purple as a pH indicator. Plates were incubated at 37˚C for 24 h. Presumptive *Lactobacillus* colonies with yellow halos were randomly picked up from the MRS plates and were further subcultured onto fresh plates of the same medium to ensure purity.

Determination of α-galactosidase activity of lactic cultures

Enzyme extracts from the lactic cultures were assayed for α-galactosidase activity according to the method of Hati *et al*., (2012).The pure lactic cultures were inoculated in 100ml of sterilized MRS medium and incubated at 37^0C for 24 h in an incubator. Then the cultures are centrifuged at 3100-3200rpm for 12 min. Supernatant was discarded and 20ml of 50mM sodium citrate buffer (pH-5.5) was added to the cell pellet for washing and mixed through vortexing. Then again centrifuge was done at 3100-3200 rpm for 15 min. The washing step was repeated twice. After that, the cell pellets were suspended in 20ml of 50mM sodium citrate buffer (pH 5.5) and mixed by vortexing. Sonication of the cells upto 15min for 3times at 2^0C under optimized conditions at 20 KHz frequency, pulses: 1 minute after 5 minutes intervals and Amplitude: 60% was carried out. Then, the sonicated mixtures were further centrifuged at 11000rpm for 30 min at 4^0C. 500µl supernatant from sonicated mixtures and 750 µl of p-nitrophenyl-α-D-galactoside (substrate) was mixed homogenously and incubated at 37^0C for 1 h. After that, 500 µl of 0.2M sodium carbonate was added to the mixture to stop the reaction and yellow colour was developed gradually during the addition. Immediately, the absorbance at 410 nm was measured through double beam UV-VIS spectrophotometer (Systronics, India).

Soybean sample

Soybeans were obtained from a local market in Anand, Gujarat (India).

Soymilk preparation

To prepare soymilk, 100 g of soybeans were soaked for 14-16 h in 1 L of distilled water at room temperature (28^0C) in a 2 L beaker as stated by Hati *et al*., (2014). The soak water was drained from the soybeans, which were then blanched at 98^0C in boiling distilled water for 15-20 min. The beans were hand washed thoroughly to remove their testa. The soybeans and 500 mL of boiled distilled water were placed in a blender (Philips, India) and blended for 10-12 min. The boiled water inactivated lipoxygenase enzyme during blending. The resulting slurry was filtered through two layers of muslin cloth. Approximately 500 mL of soymilk was obtained per 100 g of soybeans in 500 mL of water.

Development of Inoculum

The pure MRS grown lactic cultures obtained from fermented foods were transferred into sterile rehydrated skim milk and glycerol placing 1 mL aliquots in cryovials and storing at -20 ^{o}C. After two successive transfers of the test organisms in MRS broth (HiMedia, India) and incubation at 37^0C for 24 h, each activated culture was inoculated into MRS broth and incubated at 37^0C for 16 h. Theses working cultures were then transferred into soymilk medium to check their activity in this medium. *Lactobacillus* cultures were selected because of their potential use for making traditional indigenous fermented food product to improve health by providing various health benefits (e.g. antimicrobial, antihypertensive, antioxidative, antidiarrheal, etc.) (Hati *et al*., 2014).

Determination of β-glucosidase activity of lactic cultures

The β-glucosidase activity of lactic cultures were determined by estimating the rate of hydrolysis of p-nitrophenol β-D-glucopyranoside (pNPG) (HiMedia, India) as stated by Scalabrini *et al*., (1998) and Otieno *et al*., (2006) with some modifications. Lactic isolates were adapted by two successive transfers in soy milk (Nelson *et al*., 1976) by incubating at 37^0C for 24 h. Subsequently, they were added (1% v/v) in 10ml of soy milk and incubated at 37^0C for 12 h. For enzymatic activity, 5mM pNPG (substrate) was prepared in 100mM sodium phosphate buffer with pH 7.0. Subsequently, 500 µl of this substrate was added to 5ml of each aliquot sample and incubated at 37^0C for 30 min. Thereafter, 250 µl of cold 0.2M sodium carbonate was added to stop the reaction. The mixture thus obtained was centrifuged at 15000 rpm for 30 minutes using a centrifuge (Hermle Z 216 MK, Germany) and filtered through a syringe filters (0.45µ; Milllipore, USA). The amount of p-nitrophenol released was the indicator of β-glucosidase activity which was determined by using a spectrophotometer (Systronics UV Vis double beam Spectrophotometer, India) at 410nm. The enzyme assay is based on the principle that when β-glucosidase enzyme acts on the substrate pNPG, it releases p-nitrophenol in the medium changing its colour to yellow. One unit of the enzyme activity is defined as the quantity of β-glucosidase action that released one nanomole of p-nitrophenol from pNPG per milliliter per min at 37^0C under the assay conditions (Hati *et al*., 2014).

Biochemical characterization

The isolates were evaluated for carbohydrate fermentation properties, fermentation efficacy of sugars, and enzyme activities were analyzed using API 50 CH kits according to the manufacturer's instructions. Results were scored after incubating at 37^oC for 24 and 48 h. These results were put on the apiweb™ identification software with database (V5.1) which used the phenotypic data to predict genus and species of the isolates. Interpretations of the fermentations profiles were facilitated by comparing all results obtained for the tested isolates with information from the computer aided database, apiweb™ (https://apiweb.biomerieux.com).

Results and Discussion

A total of forty nine fermented food-derived strains were evaluated for the α-galactosidase and β-glucosidase activity at an absorbance of 410nm.

Biochemical characterization

On the basis of both the above enzymatic activities, eight best isolates were selected for their biochemical characterisation. Biochemical tests of all the isolates were carried out by API CH 50 Microbial Identification kit (*bioMerieux, India).*

α-galactosidase activity of the lactic isolates

The lactic acid bacterial isolates that possessed enzymatic activity showed a yellow colour during the reaction due to the release of p-nitrophenol from p-nitrophenyl-α-D-galactoside (substrate).The highest enzyme activity was exhibited by *Lactobacillus rhamnosus* (K4) as 0.407 enzyme units followed by *Lactobacillus fermentum* (K3) with 0.399 enzyme units and then *Lactobacillus fermentum* **(**K16) with 0.357 enzyme units. Hence in majority, these specific three isolates were found to be predominant over the rest (Figure 1). Hati *et al*., (2012) previously reported that α-galactosidase activity depends on the strain

and amount of oligosaccharides present in growth medium. *L. rhamnosus* C6 strain was selected for the development of soy dahi (curd) as it produced maximum amount of α-galactosidase enzyme which in concordance to our study relating to *L. rhamnosus* K4 strain being adequate producer of α-galactosidase.

As reported by Milica *et al*., (2016) in terms of α - galactosidase activity, enzyme extracted from *L. reuteri* was the most active one (1.27 IU/mL), while notably less active α-galactosidases were extracted from *L. acidophilus* (0.255 IU/ml) and *S. thermophilus* (0.181 IU/ml).

Fig.1 Alpha galactosidase activity of forty nine lactic isolates from the fermented foods of West Garo Hills, Meghalaya

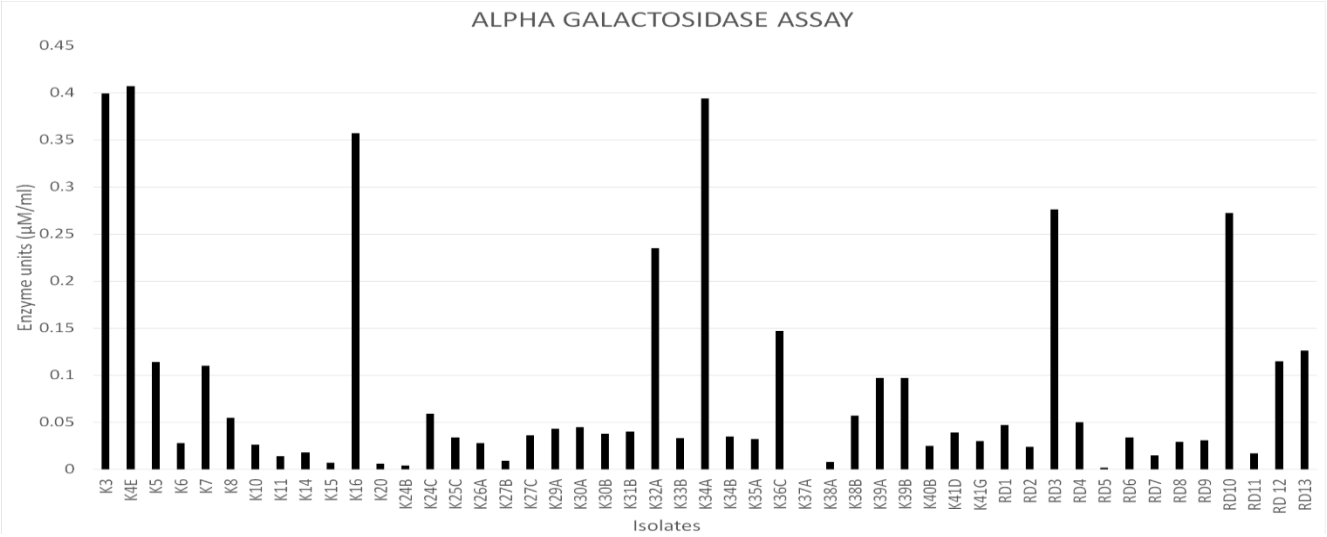

Fig.2 Beta glucosidase activity of forty nine lactic isolates from the fermented foods of West Garo Hills, Meghalaya

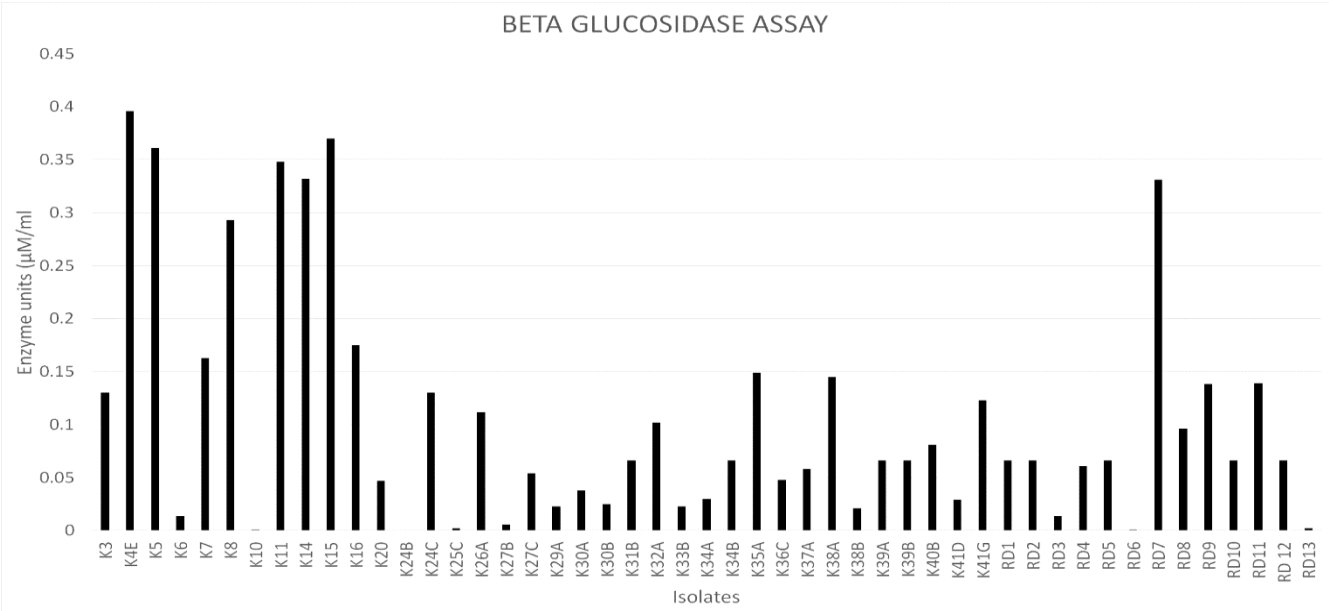

Table.1 Biochemical tests of LAB isolates through API 50CH kit

	API 50 CH	K3A	K4E	K5	RD7	K7	K14	K16	K32A
0	Control	-	-	-	-	-	-	-	-
1	Glycerol	-	-	-	-	-	-	-	-
2	Erythritol	-	-	-	-	-	-	-	-
3	D-Arabinose	-	-	-	-	-	-	-	-
4	L-Arabinose	-	-	-	+	+	-	+	+
5	D-Ribose	+	+	+	+	+	-	+	+
6	D-Xylose	-	-	-	-	-	-	-	+
7	L-Xylose	-	-	-	-	-	-	-	-
8	D-Adonitol	-	-	-	-	-	-	-	-
9	Methylβ-D-Xylopyranoside	-	-	-	+	-	-	-	-
10	D-Galactose	+	+	+	+	+	-	+	-
11	D-Glucose	+	+	+	+	+	+	+	-
12	D-Fructose	+	+	+	+	+	-	+	+
13	D-Mannose	+	+	+	-	+	-	+	-
14	L-Sorbose	-	+	-	-	+	-	-	-
15	L-Rhomnose	-	-	-	-	+	-	-	-
16	Dulcitol	-	+	-	-	-	-	-	-
17	Inositol	-	-	-	+	-	-	-	-
18	D-Mannitol	-	+	-	-	+	-	+	-
19	D-Sorbitol	-	+	-	+	+	-	-	-
20	Methyl α-D-Mannopyranoside	-	-	-	-	+	-	-	-
21	Methyl α-D-Glucopyranoside	-	-	-	+	-	-	-	-
22	N-Acetyl Glucosamine	-	+	-	+	+	+	+	-
23	Amygdalin	-	-	-	+	+	-	-	-
24	Arbutin	-	+	-	+	+	-	+	-
25	Esculin Ferric citrate	+	+	+	+	+	-	+	+
26	Salicin	-	+	-	+	+	-	+	-
27	D-Celiobiose	-	+	-	+	+	-	+	-
28	D-Maltose	+	+	+	+	+	-	+	-
29	D-Lactose (bovine origin)	+	+	+	+	+	+	+	-
30	D-Melibiose	+	+	+	+	+	-	+	-
31	D-Saccharose (Sucrose)	+	+	+	+	+	-	+	-
32	D-Trehalose	+	+	+	-	+	-	+	+
33	Inulin	-	-	-	+	-	-	-	-
34	D-Melezitose	-	+	-	-	+	-	-	-
35	D-reffinose	+	+	+	-	+	+	+	-
36	Amidon(starch)	-	-	-	-	-	-	-	-
37	Glycogen	-	-	-	-	-	-	-	-
38	Xylitol	-	-	-	+	-	-	-	-
39	Gentiobiose	-	+	-	+	+	-	+	-
40	D-Turanose	-	-	-	-	+	-	-	-

41	D-Lyxose	-	-	-	-	-	-	-	-
42	D-Tagatose	-	+	-	-	+	-	+	-
43	D-Fucose	-	-	-	-	-	-	-	-
44	L-Fucose	-	-	-	+	-	-	-	-
45	D-Arbitol	-	-	-	-	-	-	-	-
46	L-Arbitol	-	+	-	+	-	-	-	-
47	Potassium Gluconate	+	+	+	-	+	-	+	-
48	Potassium2-KetoGluconate	-	-	-	-	-	-	-	-
49	Potassium 5-keto Gluconate	-	-	-		-	-	-	-
50	Catalase Test	-	-	-	-	-	-	-	-
(+) = positive; (-)= negative									

β-glucosidase activity of lactic isolates

The rate of hydrolysis of the substrate, p-nitrophenol β-D-glucopyranoside (pNPG) by the lactic isolates determined the β-glucosidase activity of them (Figure 2). The highest enzyme activity was exhibited by *Lactobacillus rhamnosus* (K4) as 0.396 enzyme units followed by *Lactobacillus fermentum* (K5) with 0.361 enzyme units and *Lactobacillus helveticus* (K14) with 0.332 enzyme units. Hence, these specific three isolates proved to be the maximum producer of β-glucosidase enzyme as compared to the rest of the other isolates.

Previously, Hati *et al*., (2014) reported *β*-Glucosidase activity of six *Lactobacillus* cultures that exhibited different levels of *β*-Glucosidase activity during their growth under optimal conditions and *Lactobacillus rhamnosus* C6 showed the highest activity (1.66 U mL−1) among the others. Matsuda *et al*., (1994) reported *L. rhamnosus* can be used to increase isoflavone concentrations in fermented soy foods as it possesses *β*-Glucosidase activity. Our study is in agreement with Jose *et al*., (2009) who also reported that *L. rhamnosus* strains are generally active, showing higher *β*-Glucosidase specific activity compared with that of other *Lactobacillus* strains.

In conclusion, in this study, it was clearly shown that some strains of lactic acid bacteria obtained from the traditional fermented foods of West Garo Hills are simultaneous producers of both, α-galactosidase and β-glucosidase but among them *Lactobacillus rhamnosus* (K4) was the most potent producer of α-galactosidase and β-glucosidase followed by *Lactobacillus fermentum* (K3) and *Lactobacillus helveticus*(K14). These cultures could be used for the production of fermented soy based foods.

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