SCREENING AND CHARACTERIZATION OF α-GALACTOSIDASE PRODUCING BACTERIAL STRAINS ISOLATED FROM INDIAN HOT SPRING

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***Abstract*:** α-Galactosidase hydrolyze 1,6-linked-α-galactose residues from raffinose, stachyose, and melibiose. Bacteria show great potential for production of α-galactosidases due to elevated expression levels, extracellular secretion and scope for improvement of yield by optimization of culture conditions. Thermostable α-galactosidase has been obtained from extremophilic bacteria can withstand the high-processing temperatures employed in industries. Biochemical characterization of the enzyme is important for their successful utilization in numerous biotechnological applications**.** In the present study, Manikaran hot springs (Parvati valley, Kullu district, Himachal Pradesh, India) were selected to screen bacterial strains producing α-galactosidase followed by its characterization. Culture dependent approach was used to screen 39 α-galactosidase positive isolates using X-gal plate assay. Morphological, microscopic and biochemical characterization was performed on positive isolates.

Keywords: α-Galactosidase, hot springs, thermophiles, bacterial diversity

1. Introduction

α-D-Galactosidases (α-D-galactosidegalactohydrolase, E.C.3.2.1.22) are commercially significant exo-glycosidases, which hydrolyze the terminal α-galactosyl units from glycoproteins and glycolipids. The enzyme acts on the non-reducing ends of galactose, oligosaccharides, galactomannans and galactolipids. This enzyme also possess feature of catalyzing transgalactosylation reactions employing number of natural and synthetic substrates where galactose moiety is being transferred to an acceptor molecule instead of water (Goulas *et al*., 2009). On the basis of the amino acid sequence, α-galactosidase has been classified into six unique glycoside hydrolases (GH) families namely GH4, GH27, GH36, GH57, GH97 and GH110. It cleaves the α-1,3 and α-1,6 glycosidic linkages by following inversion and/or retention mechanism. The enzyme from GH4, GH27, GH36, GH57, GH97 follows the retention mechanism and GH110 follows inversion mechanism (Katrolia *et al*., 2014).

The enzyme have been isolated and characterized from microbial, plant and animal sources. Bacterial sources include *Bacillus stearothermophilus, Bacteroides ovatus, Bifidobacterium breve, Streptococcus pneumoniae, Thermotoga maritima, Thermus thermophilus etc.* Fungal sources include *Aspergillus niger, Candida albicans, Penicillium chrysogenum etc.* Plant sources comprise of *Ceretonia siliqua, Cicer arietinum, Coffea arabica, Nicotiana tabacum, Vicia faba* whereas humans, pigs, rats, rabbit *etc.* are major animal sources. Both solid state and submerged fermentation techniques can be employed for large scale production of the enzyme. α-Galactosidase is an exceptionally important industrial enzyme used in food/feed, guar gum and biomass processing. In food processing industry, the enzyme is employed in removal of raffinose family oligosaccharides (RFOs) as they are not hydrolyzed by the humans and monogastric animals due to lack of pancreatic α-galactosidase in their intestine. It is used in removal of antinutritional factors from animal feed thus improving its nutritional value. Functional food ingredients galactooligosaccharides (GOS) are produced by α-galactosidase through transgalactosylation. These α-GOS improved host's overall health by encouraging the growth of probiotic bacteria, undergoing fermentation in the colon of the host and preventing growth of potential enteric pathogens (Torres *et al*., 2010).

The enzyme has been studied extensively for its role in Fabry disease and its treatment. Enzyme replacement therapy (ERT) involves the administration of recombinant α-galactosidase available in commercial formulations (Alegra *et al*., 2012). Several innovative studies holding a promising future are being conducted on the enzyme's ability for conversion of blood group "universal donor" and prevention of xenograft rejection (Choi *et al*., 2012; Goldstein *et al*., 1982).

Novel microflora is inhabiting in extreme environments like hot water springs, acting as a source of thermozymes. Around 400 hot springs are located in India (Zimik *et al*., 2017). Parvati Valley region in Himachal Pradesh contains six hot springs within an area of 40 km including Manikaran geothermal springs having temperatures upto 95ºC. These sites were chosen for this study to characterize α-galactosidase producing bacterial strains.

2. Materials and methods

All chemicals/reagents used were of analytical grade including X-gal (5-Bromo-4-chloro-3-indolyl α-D-galactopyranoside: 20 mg/ml in DMSO), Nutrient agar, Raffinose. Crystal violet (primary stain), Gram's iodine (mordant), ethanol (decolourizer), Safranin (counter stain), Nigrosin (10% w/v), Formalin (0.5% v/v), Malachite green, Safranin, Hydrogen peroxide (10% v/v), Tetramethyl-p-phenylenediamine dihydrochloride (TMPD) (1% v/v), Tryptophan broth (1%), Kovac's Reagent (0.5% v/v), MR-VP broth, Methyl red indicator (0.4% w/v), Barritt's reagent, Carbohydrate utilization kit [HiCarbo^{TM} kit KB009, Sodium phosphate buffer (0.1 M; pH 7.0), p-Nitrophenyl-α-galactopyranoside (pNPG: 5 mM), Na_2CO_3 (0.25 mM), Raffinose, Nutrient broth, Phosphate buffer, Distilled water.

2.1 Sample Collection and Screening of α-galactosidase producing bacteria.

Sampling was done from five sites of Manikaran hot springs, Himachal Pradesh, India). Water samples (A, B, C) and soil samples (SA, SB) were collected in sterile containers.. Temperature and pH were recorded for wthe sites. Nutrient agar medium plates supplemented with 1% raffinose were prepared and X-gal was spread on it. Samples (soil and water) were serially diluted (upto10^{-6}) or water samples were directly spread plated followed by overnight incubation at 50ºC.

2.2 Culture characterization and microscopic observations

Fresh cultures were grown on Nutrient agar and raffinose plates. Colonies were observed for color, shape, size and margins under light microscope. Gram staining was performed using overnight grown bacterial cultures. A thin smear was formed by putting a drop of culture on slide followed by air drying and heat fixation. Smear was flooded for 1 min with crystal violet followed by distilled water washing. Further, for one minute, Gram's iodine was added on smear and then washed off with distilled water. Ethanol (95% v/v) was used to decolorize primary stain followed by washing with distilled water. Safranin was then flooded and kept for 45 sec which was then washed off, slides were air dried and observed under microscope. To observe the morphology and shape of bacterial sample, negative staining was performed. On one end of a slide, a drop of nigrosin stain was placed. A loop of overnight grown bacterial culture was mixed with stain. The mixture was spread from one end to another end of slide to form smear. Slide was air dried and observed under microscope. To visualize bacterial endospores, smear was prepared and heat fixed. Blotting paper was placed over it, saturated with malachite green and was heated for 5 min. It was then cooled, counterstained with safranin (2 min), washed with distilled water and observed under microscope.

2.3 Biochemical characterization

Isolate were grown on Nutrient agar slants and flooded with 1 ml H_2O_2 to observe catalase activity. While, oxidase test was performed via transfer of bacterial colony to filter paper saturated with freshly prepared 1% aqueous (TMPD). Indole production medium (1% tryptone broth) was inoculated and incubated at 37°C for 24 h. Test was performed by adding Kovac's reagent to the broth. Two hundred microlitres of culture grown in MR-VP broth was tested by adding Barritt's reagents (A & B). To one part of the culture grown in medium, one-drop methyl red solution was added.

2.4 Carbohydrate utilization test

Isolates grown in nutrient broth were transferred (50 µl) to the wells containing carbohydrate substrates. They were incubated overnight at 37°C and colour change in each well was observed and compared to the standard of the kit.

2.5 Estimation of Enzyme

To determine α-Galactosidase activity, the isolates were grown in liquid nutrient medium containing 1% raffinose at 50°C for 24 hours at 150 rpm. The samples was taken and centrifuged at 10,000 rpm for 10 minutes. For extracellular enzyme, supernatant was used as source of enzyme for estimation of enzyme activity (U/ml). The enzyme activity was measured using pNPG as substrate.

One hundred microlitres of appropriately diluted enzyme, 800 µl of phosphate buffer (pH 7.0) and 100 µl of pNPG was added. The reaction mixture was incubated at 60°C for 30 minutes and Na_2CO_3 (1 ml) was added to stop the reaction. The liberated p-Nitrophenol was estimated spectrophotometrically at 405 nm. One unit of enzyme was defined as amount of enzyme that liberates 1 µM of product per minute under given assay conditions. (Courtois and Petek, 1966).

3. Results and Discussions

Development of blue colour in α-galactosidase positive colonies due to hydrolysis of X-gal was observed in X-gal plates **(Fig. 1).** Thirty nine positive bacterial isolates (pure) were obtained after streaking and were preserved as glycerol stocks for future use. The isolates from the 5 sites were obtained **(Table 1).** Similar screening techniques were used for isolation of α-galactosidase producing *Geobacillus* sp. (Obeidat *et al*., 2012), *Geobacillus yumthangensis* AYN sp. (Najar *et al*., 2018), *Meiothermus taiwanensis* (Chen *et al*., 2002) from hot springs of Jordan, Yumthang (Sikkim, India) and Taipei (Taiwan) respectively. In another study, genome mining was done to detect α-galactosidase producing ability of *Anoxybacillus gonensis* $G2^T$, isolated from Gonen hot springs of Turkey (Lim *et al*., 2015).

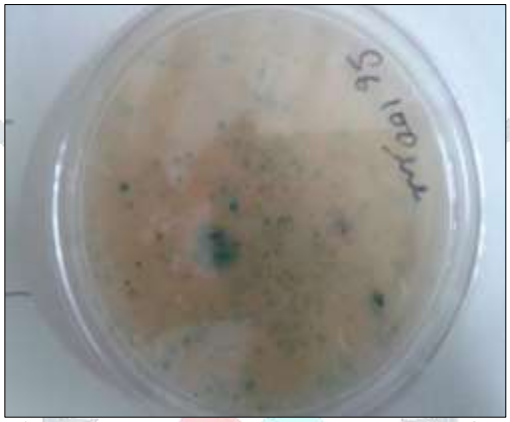

Fig. 1: Screening of α-galactosidase producing bacteria on X-gal.

Table 1: α-Galactosidase positive isolates from water and soil samples.

Samples	Sample codes	Total isolates	Isolate codes
Water samples	A	07	SB1, SB2, SB3, SB4, SB5, SB6, SB7
	B	04	SB8, SB9, SB10, SB11
	C	06	SB12, SB13, SB14, SB15, SB16, SB17
Soil samples	SA	10	SB18, SB19, SB20, SB21, SB22, SB23, SB24, SB25, SB26, SB27
	SB	12	SB28, SB29, SB30, SB31, SB32, SB33, SB34, SB35, SB36, SB37, SB38, B39

Colony morphological features like colour, shape and size were observed. They displayed some variations in colour like off white, cream, pale yellow, yellow and orange. Shape of colonies varied as circular, irregular and rhizoid. Different elevation patterns were observed like raised, flat, convex and umbonate. Colony margins were observed for positive isolates showing different margins such as entire, curled, lobate and undulate **(Table 2).** Rod shape was depicted by most of the bacterial isolates (27) and some (12) were cocci in shape. Majority of the isolates were Gram-positive (SB1-13, SB15-17, SB19, SB21-28, SB30-32, SB34, SB35, SB38 and SB39). Only a few were found to be Gram-negative (SB14, SB18, SB20, SB29, SB33, SB36 and SB37). Twenty one endospore forming isolates were seen in SB1-3, SB6, SB7, SB11-13, SB16, SB21-SB25, SB27, SB28, SB30, SB31, SB34, SB35 and SB39 **(Table 3A and 3B).** Similar observations were reported in *Bacillus megaterium* VHM1 forming round, elevated colonies and were microscopically identified as Gram-positive spore forming rods (Patil *et al*., 2010). *Geobacillus* sp. from Jordan hot springs was Gram-positive rod shaped bacteria forming light yellow, circular to rhizoid colonies (Obediet *et al*., 2012).

Table 2: Colony morphology of thirty nine α-galactosidase positive bacterial isolates.

Isolate/s	Colour	Form	Elevation	Margin
SB1	Cream	Circular	Raised	Entire
SB2	Off white	Circular	Raised	Entire
SB3	Cream	Irregular	Raised	Undulate
SB4	Cream	Circular	Convex	Entire
SB5	Pale yellow	Irregular	Raised	Lobate
SB6	Off white	Circular	Flat	Entire
SB7	Off white	Circular	Raised	Lobate
SB8	Cream	Irregular	Raised	Entire
SB9	Cream	Irregular	Raised	Lobate
SB10	Cream	Circular	Convex	Entire
SB11	Pale yellow	Irregular	Raised	Undulate
SB12	Orange	Circular	Umbonate	Curled
SB13	Cream	Irregular	Flat	Entire
SB14	Cream	Irregular	Flat	Undulate
SB15	Cream	Circular	Convex	Entire
SB16	Off white	Circular	Convex	Entire
SB17	Off white	Rhizoid	Flat	Undulate
SB18	Off white	Circular	Raised	Entire
SB19	Off white	Circular	Raised	Lobate
SB20	Off white	Irregular	Raised	Lobate
SB21	Cream	Irregular	Raised	Entire
SB22	Yellow	Irregular	Flat	Entire
SB23	Cream	Irregular	Flat	Curled
SB24	Cream	Circular	Raised	Entire
SB25	Cream	Circular	Raised	Entire
SB26	Cream	Irregular	Flat	Entire
SB27	Off white	Circular	Raised	Entire
SB28	Pale yellow	Irregular	Umbonate	Lobate
SB29	Cream	Circular	Raised	Entire
SB30	Cream	Circular	Raised	Entire
SB31	Cream	Irregular	Flat	Curled
SB32	Off white	Rhizoid	Flat	Curled
SB33	Off white	Irregular	Flat	Lobate
SB34	Cream	Rhizoid	Raised	Lobate
SB35	Cream	Irregular	Raised	Entire
SB36	Pale yellow	Irregular	Raised	Entire
SB37	Pale yellow	Circular	Convex	Entire
SB38	Pale yellow	Irregular	Raised	Lobate
SB39	Cream	Circular	Flat	Entire

Table 3A: Microscopic/biochemical characterization of different isolates (SB1-20).

Characteristics	Isolates																			
	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	SB12	SB13	SB14	SB15	SB16	SB17	SB18	SB19	SB20
Gram Staining	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-
Endospore Staining	+	+	+	-	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-	-
Indole test	+	+	+	+	+	+	-	-	-	-	+	+	-	+	+	+	+	-	+	+
Methyl red	-	+	+	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	-	+
VP test	-	-	-	-	-	+	+	-	+	-	+	+	-	-	+	+	-	+	+	+
Citrate utilization	+	+	+	-	-	-	-	+	+	+	-	-	+	-	+	+	+	+	+	-
Catalase test	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Oxidase test	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-

Table 3B: Microscopic/biochemical characterization of different bacterial isolates (SB21-39).

Characteristics	Isolates																		
	SB21	SB22	SB23	SB24	SB25	SB26	SB27	SB28	SB29	SB30	SB31	SB32	SB33	SB34	SB35	SB36	SB37	SB38	SB39
Gram Staining	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+
Endospore staining	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	-	-	-	+
Indole test	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+
Methyl red	-	+	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+
VP test	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	+	+	-	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+
Catalase test	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase test	+	-	+	+	+	-	+	+	+	+	-	+	+	+	-	+	-	+	+

α-Galactosidase producing bacterial isolates were characterized biochemically by performing various tests. Release of oxygen bubbles indicated the production of catalase. Turning of colony into purple or deep- bluish in colour (5-10 sec) indicated positive oxidase isolates. Pink to red color ring was observed at the top of the medium in case of positive result on addition of Kovac's reagent whereas no color change indicated negative indole test. Appearance of a cherry red colour indicated positive reaction for VP test while, a magenta red colour indicated positive for methyl red test and yellow colour is noted as negative result. Isolates showing positive results included 26 for indole, 36 for catalase, 30 for oxidase, 27 for methyl red and 17 for Voges Proskauer test. Bacterial isolates were tested on thirty five sugars and their carbohydrate utilization profile is shown in **Table 4A and 4B.** Isolates utilized variable carbohydrates including complex ones like raffinose, melibiose *etc*. Eight isolates from Jordan hot springs belonging to *Geobacillus* were analyzed biochemically (Obediet *et al*., 2012). α-Galactosidase positive *Meiothermus taiwanensis* showed utilization of melibiose, galactose, xylose *etc*. in carbohydrate profiling (Chen *et al*., 2002).

Table 4A: Carbohydrate utilization profile of bacterial isolates (SB1-20).

Carbohydrate	Isolates																			
	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	SB12	SB13	SB14	SB15	SB16	SB17	SB18	SB19	SB20
Lactose	+	-	+	-	+	+	-	+	+	+	+	+	+	-	-	-	+	-	-	-
Xylose	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	-	+
Maltose	+	+	+	-	+	-	-	+	+	-	-	+	-	+	-	+	+	+	-	+
Fructose	+	-	-	+	+	-	+	+	+	+	+	+	-	-	-	+	-	+	+	+
Dextrose	+	-	-	-	+	-	-	+	-	+	-	-	+	-	-	+	+	-	+	-
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
Trehalose	-	+	-	-	+	-	-	+	+	+	-	-	-	+	-	+	+	+	-	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+
Sucrose	+	+	-	+	+	-	+	+	+	-	+	+	+	-	-	-	-	+	-	+
L-Arabinose	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	+	+	+
Mannose	+	-	-	+	-	-	-	+	-	+	-	-	+	+	-	+	-	-	+	-
Inulin	-	-	-	+	-	+	+	-	-	+	-	+	-	-	-	-	-	-	+	-
Sodium Gluconate	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	+	-	+
Glycerol	+	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	+	-	+
Salicin	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-
Dulcitol	+	+	-	-	-	-	-	+	-	-	+	+	+	+	+	+	-	+	+	+
Inositol	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	+	-
Sorbitol	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	+

Carbohydrate	Isolates																			
	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	SB12	SB13	SB14	SB15	SB16	SB17	SB18	SB19	SB20
Mannitol	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-
Adonitol	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	+	-	-	+	-
α-methyl-D glucoside	+	-	+	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-
Ribose	-	+	-	-	+	+	+	-	-	+	+	-	+	-	+	+	-	+	+	+
Rhamnose	-	-	+	-	-	-	-	+	-	+	-	-	-	+	+	-	+	-	-	+
Cellobiose	-	-	-	-	+	+	-	-	-	+	+	-	+	-	-	+	+	+	+	-
Melezitose	-	-	+	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+
α -methyl-D-mannoside	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-
Xylitol	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	+	+	-	-	-
Esculin Hydrolysis	-	+	+	-	+	-	-	-	+	-	-	-	-	+	+	-	-	+	+	+
D-Arabinose	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-
Citrate Utilization	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	+	-	-
Malonate Utilization	-	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-	-	-	+	+
Sorbose	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-

Table 4B: Carbohydrate utilization profile of bacterial isolates (SB21-39).

Carbohydrate	Isolates																		
	SB21	SB22	SB23	SB24	SB25	SB26	SB27	SB28	SB29	SB30	SB31	SB32	SB33	SB 34	SB 35	SB 36	SB37	SB38	SB 39
Lactose	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+	-	-	+	+
Xylose	-	+	-	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
Maltose	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+
Fructose	+	+	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+
Dextrose	-	+	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Trehalose	-	+	+	+	-	+	-	+	+	-	-	-	+	-	-	-	-	+	+
Melibiose	+	+	+	+	+	+	+	-/+	+	+	+	+	+	+	+	-	+	-	-
Sucrose	-	+	-	+	+	-	-	+	+	-	+	+	-	-	-	-	+	+	+
L-Arabinose	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+
Mannose	+	+	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	-	-
Inulin	-	-	-	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+
Sodium Gluconate	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-
Glycerol	+	-	-	-	+	+	-	-	-	+	-	+	+	+	-	-	-	+	-
Salicin	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+
Dulcitol	+	+	-	-	-	-	+	+	-	-	-	+	-	+	+	+	-	-	-
Inositol	+	-	+	-	-	-	+	-	+	-	-	+	+	-	-	-	-	+	+
Sorbitol	-	+	-	+	-	-	-	-	+	+	+	-	-	+	-	+	+	+	+
Mannitol	+	+	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	+	-
Adonitol	-	-	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	+	+
α -methyl-D glucoside	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-
Ribose	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+
Rhamnose	+	-	-	+	-	+	+	-	-	+	+	+	-	+	-	-	-	+	-
Cellobiose	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+
Melezitose	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-
α -methyl-D-Mannoside	-	+	-	+	+	-	-	-	-	+	+	-	-	-	+	-	+	+	+
Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-
Esculin Hydrolysis	+	-	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	+	+
D-Arabinose	-	-	-	+	+	+	-	-	+	+	+	-	-	+	+	-	-	-	-
Citrate Utilization	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-
Malonate Utilization	-	-	+	+	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-
Sorbose	-	+	+	-	-	+	+	-	-	+	+	+	-	+	-	-	-	+	+

Thirty nine isolates positive for α-galactosidase were grown individually for 48 h at 40°C. Extracellular enzyme activity was measured as per standard protocol. Different isolates produced variable quantities of enzyme as shown in **Table 5.**

Table 5: Production of extracellular α-galactosidase by thirty nine bacterial Isolates and their selection.

Isolate/s	Enzyme activity (U/ml)	Isolate/s	Enzyme activity (U/ml)	Isolate/s	Enzyme activity (U/ml)
SB1	146.12	**SB14**	**326.09**	**SB27**	**355.12**
SB2	153.23	**SB15**	057.06	**SB28**	145.65
SB3	098.26	**SB16**	026.32	**SB29**	198.16
SB4	129.58	**SB17**	179.15	**SB30**	**315.78**
SB5	**384.13**	**SB18**	**277.86**	**SB31**	107.21
SB6	145.98	**SB19**	168.25	**SB32**	132.12
SB7	**385.34**	**SB20**	141.78	**SB33**	196.24
SB8	187.98	**SB21**	054.29	**SB34**	125.06
SB9	168.23	**SB22**	**299.89**	**SB35**	198.26
SB10	053.65	**SB23**	089.20	**SB36**	187.24
SB11	**383.76**	**SB24**	154.26	**SB37**	164.21
SB12	178.96	**SB25**	156.12	**SB38**	029.23
SB13	184.25	**SB26**	191.52	**SB39**	179.12

***Red marked eight bacterial isolates were selected based on their activities**

Each data point represents mean of three independent experiments/ assays.

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