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Biotransformation of banana pseudostem extract into a functional juice containing value added biomolecules of potential health benefits

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Banana is one of the most widely grown fruit crops, with the second largest produced fruit in the world. India is the largest producer of banana in the world. Banana cultivation generates huge residual biomass, which is generally wasted after harvesting of fruit. The present study represents a novel method for biotransformation of banana pseudostem extract into a functional juice, containing high value nondigestible oligosaccharides, and rare monosaccharide of nearly zero caloric value-D-allulose. The bioprocess involves employment of membrane separation techniques, and the biocatalysts executing glucosyltransferase and D-fructose epimerization activities. The bioprocessed banana pseudostem juice was estimated to contain prebiotic glucooligosaccharides (~5 g L^{-1}) and D-allulose (~7 g L^{-1}). Thus, the study represents a simple and innovative bioprocess for transformation of banana pseudostem extract into a functional juice possessing high value biomolecules that exert multifarious health benefits.

Keywords: D-Allulose, Biomass, Glucooligosaccharides, Prebiotic.

India is the largest producer of banana in the world. In India, banana is cultivated in about 846 thousand ha area producing about 29 million tons of banana fruits annually (National Horticulture Board, 2015-16). Banana plantation requires a large land area, and after the harvesting of banana fruit, banana tree plants are generally left as such in the field as a waste. Banana cultivation generates a considerable amount of agricultural wastes, equalling about 88% (w/w) in the form of leaf and trunk or pseudostem after fruit harvesting[1]. In India, approximately 51 million tons of banana pseudostem is wasted annually[2,3]. The banana plant left-over is source of fungal contamination, and if incinerated in open field, it creates air pollution related issues[4].

Banana pseudostem is comprised of concentric layers of leaf sheaths, and this biomass is reported to be rich in nutrient such as minerals, sugars, resistant starch, dietary fibres, and antioxidant compounds[5,6]. Thus, wastage of this residual biomass is loss of the nutrient value therein. Efforts have been made to develop approaches for transformation of banana plant biomass into value-added biomolecules such as cellulosic fibres as raw materials for paper[7], organic fertilizer[8], bioethanol[9], and lactic acid[10], etc. Banana pseudo-stem extract was demonstrated to inhibit the growth of food borne pathogens[11]. The antimicrobial compounds present in pseudo-stem extract such as tannic acid can significantly reduce the chances of food spoilage[11]. The inner core of banana pseudostem is also used as vegetables and pickles by local population in India. During the process of bread making, use of about 10% of banana pseudo-stem flour in wheat flour gives a property of higher moisture, ash, crude fibre, soluble, insoluble and total dietary fibre contents, higher level of phenolics, and antioxidant properties; but lower protein, fat and carbohydrate contents as compared to the conventional wheat flour bread[12]. A nutritious preparation was made using banana pseudo-stem extract and jaggery, as a low-cost beverage[13].

Traditionally, all parts of banana plant are known to have medicinal applications[14]. Banana pseudostem has been reported as a potential source of polyphenols or antioxidants, such as gentisic acid, (+)-catechin, protocatechuic acid, caffeic acid, ferulic acid, and cinnamic acid[15]. Banana pseudo-stem extract has been found very effective in exerting antidiabetic effects[16-18]. Banana pseudostem consumption ameliorates the diabetes and advanced glycation end-products (AGE) related complications, such as hyperglycemia,

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polyuria, polyphagia, polydipsia, urine sugar, renal failure and AGE accumulation[19]. Traditionally, people used to drink banana pseudo-stem juice to prevent urinary disorders and stone formation in gall bladder[20]. CSIR-Central Food Technological Research Institute, Mysore, India[21] has overcome the problem of browning in banana pseudostem juice, and developed a process for making a beverage preparation by blending it with other fruit juices or their concentrates.

As banana pseudostem biomass is a low-lignin-content lignocellulosic biomass, it has been suggested to be a useful resource for methane production[22]. The banana tree biomass (pseudostem and leaves) was utilized as suitable substrate for lignolytic and cellulolytic enzyme production by fungi under solid substrate fermentation[23]. The potential of banana pseudo-stem juice has also been identified as a natural coagulant for pre-treatment of waste water[24]. The use of banana pseudostem extract in the bark extract of *Acacia pennata* results in better dyeing of wool, as compared to *Acacia pennata* alone[25]. Banana pseudostem extract is also used as liquid fertilizer and plant nutrient spray[26]. ICAR-Central Institute for Research on Cotton Technology[27], has established a process to use banana pseudostem extract as mordant for dyeing cotton fabrics with natural dyes. Further, banana tree biomass has been attempted to be transformed into animal feed[28-30].

The objective of this study was to develop a bioprocess for transformation of banana pseudostem extract into a functional juice containing prebiotic glucooligosaccharides and nearly calorie free rare monosaccharide, D-allulose.

Materials and Methods

Preparation of feedstock

Banana pseudostem was harvested from Grande Naine variety of banana (*Musa acuminata*) plants cultivated in NABI-CIAB campus, Sector 81 (Knowledge City), Mohali, Punjab, India. The banana pseudo-stem was washed with water, and then cut into 40 cm long vertical pieces using chopper. The juice was extracted using mechanical juice extractor (table top sugarcane crusher) at ambient temperature. The insoluble particles were removed by centrifugation for 10 min at 6000 rpm. The supernatant was subjected to membrane filtration with 50 kDa molecular weight cut-off (Cleansep Systems, India). The filtrate was further concentrated as retentate using 50 Da membrane. The filtered/concentrated banana pseudostem juice were sterilized by heating to 90°C for 10 min in a water bath, and used as feedstock.

The concentration of sugars (sucrose, fructose, and glucose) in the banana pseudostem juices were determined using HPLC (Agilent Model-1260) under the following chromatographic conditions: column Agilent Hi-plex Ca (7.7×300) mm, 8 µm; sample injection volume 20 µL; mobile phase deionized water (100%), flow 0.6 mL min^{-1}; column temperature: 85°C; refractive index detector (RID) at 50°C.

Enzyme production

The bacterial strain *Leuconostoc mesenteroides* MTCC 10508 was cultivated in a sterile medium (pH 6.5), comprised of peptone 10 g L^{-1}, yeast extract 5 g L^{-1}, sucrose 20 g L^{-1}, sodium acetate 5 g L^{-1}, tween-80 0.1 %, K_2HPO_4 2 g L^{-1}, $MgSO_4.7H_2O$ 0.2 g L^{-1}, $MnSO_4.4H_2O$ 0.01 g L^{-1}, NaCl 0.01 g L^{-1}, and $CaCl_2.2H_2O$ 0.02 g L^{-1} at 24°C with 120 rpm for 24 h. About 2% of this culture was used as primary inoculums into a sterile medium of the aforementioned composition, and incubated at 24 °C with 120 rpm for 24 h to achieve extracellular enzyme production, as described previously[31,32,33]. Bacterial cells were harvested by centrifugation at 10,000 rpm for 20 min, and discarded after autoclaving. The cell-free supernatant was mixed with 40 % (v/v) polyethylene glycol (PEG-400) and incubated at 4°C for 12 h. Then, dextransucrase was precipitated by centrifugation at 10,000 rpm for 20 min. at 4°C, and the pellet was dissolved in ice cooled 20 mM sodium acetate buffer (pH 5.2). The protein was dialyzed overnight against 20 mM sodium acetate buffer (pH 5.2) by using a membrane (Sigma Aldrich, USA) with 14 kDa cut-off. The enzyme activity of dextransucrase was determined as described previously[33].

Heterologous expression of the engineered enzyme, Smt3-D-psicose 3-epimerase, was done in *Escherichia coli*. The recombinant *E. coli* cells were cultivated in Luria-Bertani (LB) medium at 37°C and 220 rpm, under antibiotic (Kanamycin) selection, and protein expression was induced by addition of 0.1 mM Isopropyl-β-D-thiogalactopyranoside (IPTG) in the culture, after attaining ~0.6 OD (at 600 nm). The induced culture was incubated at 16°C for 24 h with a shaking at 150 rpm. The cells were harvested and lysed by using sonication on ice for 2 min. The purification of Smt 3-D-psicose 3-epimerase was done by using nickel-nitrilotriacetic acid (Ni-NTA) agarose affinity chromatography matrix (Qiagen),

followed by Q-Sepharose ion exchange column, as described previously[34].

Treatment of banana pseudo-stem juice with dextransucrase enzyme

The filtered or concentrated banana pseudo-stem juice were treated with 50 mg L^{-1} dextransucrase in the presence of maltose (4 g L^{-1}) as acceptor molecule. The reaction samples were incubated at 30°C for about 3 h. The qualitative production of glucooligosaccharides was examined by Thin Layer Chromatography (TLC), determining the degree of polymerization (DP). The reaction samples (0.2 µL) were spotted onto TLC aluminum plates pre-coated with silica and fluorescent indicator F254 (Merck Millipore, Germany). The spots were dried using hairdryer and the plates were run in a solvent system consists of ethyl acetate: acetic acid: 1-butanol: H_2O (4: 3: 2: 2). After air-drying, a solution of 0.5% (w/v) 1-naphthyl ethylenediamine dihydrochloride in methanol and 5% sulphuric acid was sprayed over the plate. The plate was then heated at 120°C in oven for about 10 min. The relative estimation of oligosaccharide yield in treated banana pseudo-stem juice samples was done as described previously[33]. HPLC (Agilent Model-1260) was run under the following chromatographic conditions: column Zorbax NH2 (4.6 ×250 mm), 5 µm; sample injection volume 5 µL; mobile phase acetonitrile : water (60:40), flow 1.4 mL min^{-1}, column temperature 25°C, and refractive index detector (RID) 45°C.

Immobilization of dextransucrase enzyme

The purified dextransucrase (0.05 mg of protein mL^{-1}) was mixed with 2 % (w/v) sodium alginate and 2 % (w/v) pectin in 20 mM sodium acetate buffer (pH 5.2), and the solution was dropped into 0.2 M $CaCl_2$ solution. The beads so formed were kept for 12 h in 0.2 M calcium chloride solution at 4°C in order to harden the beads. The beads with immobilized dextransucrase enzyme were washed with 20 mM sodium acetate buffer pH 5.2 and kept in buffer for further uses. For oligosaccharide synthesis, the immobilized enzyme (50 mg L^{-1}) was added in the sample (e.g. banana pseudo-stem juice), along with maltose (4 g L^{-1}), and incubated at 30°C and 120 rpm for 3 h. The alginate beads with immobilized enzyme were recovered by simple filtration. The post-reaction samples were analyzed by TLC and HPLC, as explained above, for oligosaccharide synthesis.

Microbial bioprocessing of banana pseudo-stem juice

The banana stem juice (pH adjusted at 6.0) was inoculated with *L. mesenteroides* cells (2% of primary inoculum) and incubated at 30°C with 120 rpm for 12 h. After this, the cells were harvested by centrifugation at 10,000 rpm for 20 min, and discarded after autoclaving. The supernatant was analyzed by TLC and HPLC, as explained above, for qualitative and quantitative analyses of oligosaccharides.

Treatment of banana pseudo-stem juice with Smt3-d-psicose 3-epimerase

The dextransucrase treated banana pseudo-stem juice samples (pH maintained at 7) were contacted with Smt3-D-psicose 3-epimerase enzyme. The reaction sample was incubated at 50°C for 1 h, followed by denaturation of the enzyme as explained previously[34]. The magnetic nanoparticle immobilized Smt3-D-psicose 3-epimerase enzyme[35] was also employed in the study for complete recovery of enzyme from the reaction sample. The qualitative and quantitative analyses of D-allulose was done by using HPLC (Agilent Model-1260) under the following chromatographic conditions: column Agilent Hi-plex Ca (7.7×300) mm, 8 µm; sample injection volume 20 µL; mobile phase deionized water (100%), flow 0.6 mL min^{-1}; column temperature: 85°C; refractive index detector (RID) at 50°C.

Digestibility analysis of oligosaccharides

The banana stem juice derived oligosaccharides was subjected to digestion by simulated gastric juice and pancreatic α-amylase. The simulated gastric juice was prepared in HCl buffer of pH 1, 2, and 3, containing 0.3% (w/v) pepsin from porcine gastric mucosa (Sigma Aldrich, USA), NaCl 8 g L^{-1}, KCl 0.2 g L^{-1}, Na2HPO4.2H2O 8.25 g L^{-1}, NaH2-PO4 14.35 g L^{-1}, CaCl2.2H2O 0.1 g L^{-1}, and MgCl2.6H2O 0.18 g L^{-1}. The modified banana pseudostem juice containing prebiotic oligosaccharide and simulated gastric juice was mixed in a ratio of 1:1 and incubated at 37°C for 3 to 24 h. The modified banana pseudostem juice was treated with α-amylase (2 unit mL^{-1}) from porcine pancreas (Sigma Aldrich, USA), dissolved in 20 mM sodium phosphate buffer (pH 7.0) and 6.7 mM sodium chloride, and incubated at 37°C for 3 to 24 h. The effects of gastric juice and α-amylase on the integrity of oligosaccharides were analyzed by spotting the samples onto TLC plate as explained above.

Effect of banana pseudo-stem juice derived oligosaccharides on growth of probiotics

The yeast strain of *Saccharomyces cerevisiae* MTCC No. 36 was cultured in a sterile medium (pH 6.2), composed of yeast extract 3 g L^{-1}, malt extract 3 g L^{-1}, glucose 10 g L^{-1} and peptone 5 g L^{-1} at 25°C and 120 rpm for 48 h. This culture was used as primary inoculums (1%) in dextransucrase treated (modified) banana pseudostem juice, followed by incubation at 25°C and 120 rpm for 48 to induce utilization of free monosaccharide sugars (glucose and fructose) present in the juice. The cells were harvested by centrifugation, and the juice was freeze-dried. The powder so obtained was used as banana pseudostem derived oligosaccharides in the study.

The probiotic bacteria e.g. *Lactobacillus fermentum* MTCC No. 903 and *Lactobacillus acidophilus* MTCC No. 10307 were cultured in a sterile medium (pH 6.5) comprised of peptone 10 g L^{-1}, yeast extract 5 g L^{-1}, glucose 20 g L^{-1}, sodium acetate 5 g L^{-1}, tween-80 0.1 %, K_2HPO_4 2 g L^{-1}, $MgSO_4.7H_2O$ 0.2 g L^{-1}, $MnSO_4.4H_2O$ 0.01 g L^{-1}, NaCl 0.01 g L^{-1}, and $CaCl_2.2H_2O$ 0.02 g L^{-1} at 37°C and 120 rpm for 12 h. About 1 mL of overnight grown probiotic cultures were harvested by centrifugation at 3200 g for 15 min at 4°C. The cell pellets were washed twice with phosphate buffered saline (PBS, pH 7.4) and re-suspended in 1 mL PBS, and then inoculated into banana pseudo-stem juice derived oligosaccharides solution (1% w/v). The glucose solution (1% w/v) and untreated banana pseudo-stem juice were taken as control samples. The growth of probiotics was monitored by measuring the absorbance (at 600 nm) of the culture broth at different time intervals.

Results

Clarification of banana pseudostem juice

Mechanical extraction of banana pseudostem yielded about 0.15 L juice per kg of fresh biomass. The banana pseudostem extract was clarified by employing centrifugation followed by membrane filtration (50 kDa). The filtrate was estimated to contain about 27.03 g L^{-1} sugars (sucrose 11.25 g L^{-1}, glucose 6.04 g L^{-1} and fructose 9.73 g L^{-1}). The clarified banana pseudostem juice, concentrated as retentate from 50 Da membrane, exhibited 50.14 g L^{-1} sugars, comprised of 20.45 g L^{-1} sucrose, 11.37 g L^{-1} glucose, and 18.31 g L^{-1} fructose (Fig. S1. On web only). Sucrose concentration in the filtered banana pseudostem juice was maintained upto 20 g L^{-1} by addition of table sugar. Both the filtered and concentrated banana pseudostem juice were used as feedstock.

Enzymatic bioprocessing of banana pseudo-stem juice

The extracellularly produced dextransucrase was fractionated from the culture medium of *L. mesenteroides* MTCC 10508 by PEG purification method. The dextransucrase enzyme from *L. mesenteroides* MTCC 10508 has been shown to prime oligosaccharide synthesis most efficiently in the presence of maltose or isomaltose as acceptor molecules[33]. In our previous study, the ratio of 5:1 (sucrose:maltose) was found appropriate for oligosaccharides synthesis[33]. Enzyme dosage optimization was done, and dextransucrase concentration of 50 mg L^{-1} yielded the maximum oligosaccharides in presence of 20 g L^{-1} sucrose and 4 g L^{-1} maltose in the reaction (Table S1. On web only). The banana pseudostem juice, containing 20 g L^{-1} sucrose and 4 g L^{-1} maltose, was treated with dextransucrase enzyme (50 mg L^{-1}) at 30°C for about 3 h to induce the catalysis of oligosaccharide biosynthesis. TLC and HPLC analysis revealed bioconversion of about 95% sucrose, yielding about 5 g L^{-1} oligosaccharides (DP3-DP6) in the juice (Fig. 1; Table 1).

Immobilization of dextransucrase enzyme and oligosaccharide production

To achieve easy recovery of enzyme, avoiding the contamination of banana pseudo-stem juice, dextransucrase was immobilized by entrapment method in calcium alginate-pectin beads (Fig. 2A). Examining enzyme activity in the filtrate, obtained after harvesting the beads, tested enzyme leaching. Almost nil activity was obtained in the filtrate, indicating immobilization of the entire soluble enzyme to the alginate-pectin matrix. The immobilized dextransucrase enzyme (50 mg L^{-1}) was

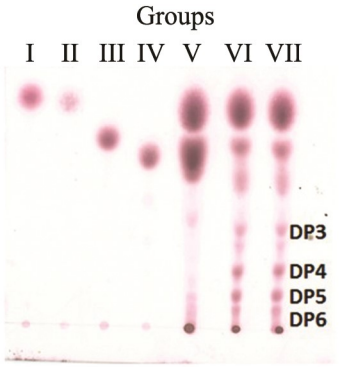

Fig. 1 — Thin layer chromatogram of oligosaccharides in banana pseudo-stem juice after enzymatic bioprocessing using free dextransucrase enzyme. [I: Fructose, II: Glucose, III: Sucrose, IV: Maltose, V: BS juice + Maltose + IDsr, VI: BS juice + Maltose + Dsr, VII: BS juice + Maltose + Dsr, BS: Banana pseudostem, Dsr: dextransucrase, IDsr: heat inactivated dextransucrase, DP: degree of polymerization]

tested for oligosaccharide synthesis in table sugar solution (20 g L^{-1}) in 20 mM sodium acetate buffer (pH 5.2) in the presence of maltose (4 g L^{-1}). The catalytic action of entrapped enzyme resulted in biosynthesis of about 5 g L^{-1} oligosaccharides in the range of DP3-DP6 in 3 h (Fig. 2B). The reusability of the beads with immobilized enzyme was examined by reusing them for ten duty cycles. No appreciable loss was observed in the oligosaccharide yield, and thus, in the oligosaccharide biosynthetic efficiency of the entrapped dextransucrase enzyme.

Banana pseudostem juice was treated with the immobilized dextransucrase enzyme (50 mg L^{-1}) in presence of maltose (4 g L^{-1}), and biosynthesis of about 5 g L^{-1} oligosaccharides (DP3-DP6) was achieved in 3 h (Fig. 3). The immobilized enzyme was recovered from the juice sample by simple filtration.

Microbial bioprocessing of banana pseudo-stem juice using *L. mesenteroides*

An alternative approach of microbial bioprocessing was attempted for *in situ* production of prebiotic oligosaccharides in banana pseudo-stem juice. The banana pseudostem juice (pH adjusted at 6.0) was inoculated with 2% of the primary culture of *L. mesenteroides* MTCC 10508 cells, which equals to about ~12×10^8 cells of the primary inoculum, and incubated at 30°C with 120 rpm for 12 h. Any other nutrient was not supplemented in banana pseudostem juice, which suggests that banana pseudostem juice can be a replacer of synthetic medium for cultivation of *L. mesenteroides*. The extracellularly produced dextransucrase enzyme catalyzed acceptor primed oligosaccharide biosynthesis in presence of maltose added in banana pseudo-stem juice during cultivation of *L. mesenteroides* for 12 h. The qualitative and quantitative estimations were done by using TLC and HPLC, which revealed production of ~5 g L^{-1} oligosaccharides in the banana pseudo-stem juice with about 90% sucrose conversion in 12 h (Fig. 4; Table 1).

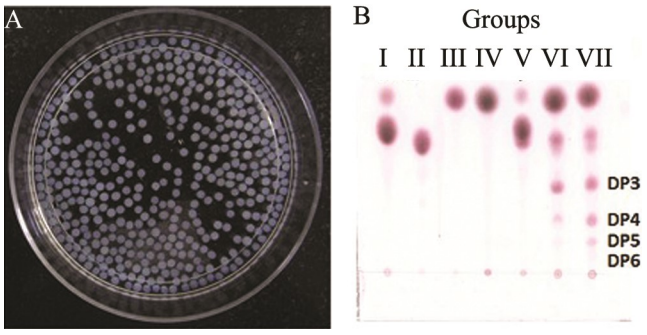

Fig. 2 — (A) Alginate-pectin beads with immobilized dextransucrase enzyme, and (B) Thin layer chromatogram of oligosaccharides obtained after enzymatic bioprocessing of table sugar solution using immobilized dextransucrase. [I: Table sugar, II: Maltose, III: Glucose, IV: Fructose, V: Table sugar + Maltose (Control), VI: Table sugar + Maltose + Immobilized Dsr (1 h), VII: Table sugar+ Maltose+ Immobilized Dsr (3 h), Dsr: dextransucrase, DP: degree of polymerization].

Digestibility of oligosaccharides in modified banana pseudo-stem juice by simulated gastric juice and pancreatic α-amylase

In vitro digestibility test was performed to assess the resistance of oligosaccharides in banana pseudostem juice against the hydrolytic actions of simulated

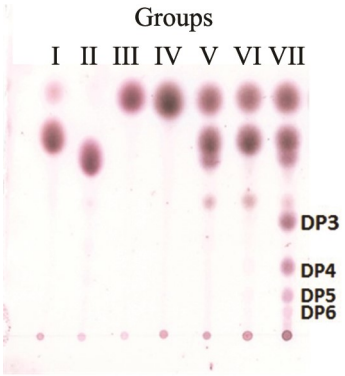

Fig. 3 — Thin layer chromatogram of oligosaccharides in banana stem juice after enzymatic bioprocessing using immobilized Dextransucrase enzyme. [I: Table sugar, II: Maltose, III: Glucose, IV: Fructose, V: BS juice + Maltose (Control), VI: BS juice + Maltose+ Immobilized Dsr (1 h), VII: BS juice + Maltose + Immobilized Dsr (3 h), BS: Banana pseudostem, Dsr: dextransucrase, DP: degree of polymerization].

Table 1 — Oligosaccharides yield in banana pseudo-stem juice after enzymatic bioprocessing using free dextransucrase enzyme and *L. mesenteroides*

Reaction	Time of Reaction (h)	Total sugar [a] (g L^{-1})	Sucrose (g L^{-1})	Sucrose conversion (%)[c]	Oligosaccharides yield (g L^{-1})
Dextransucrase enzyme					
Before	0	54.15±0.004	21.19±0.018	0	0
After	3	49.53±4.06	0.56±0.79	96.38±5.10	5.02±1.23
L. mesenteroides					
Before	0	80.56±5.15	27.80±3.02	0	0
After	12	50.45±4.03	2.60±0.37	90.64±1.36	5.40±0.20

[a]Total sugar = sucrose + glucose + fructose + maltose; [b]Maltose is added in the reaction at a final concentration of 4 g L^{-1}; [c]Sucrose conversion (%) = (sucrose before reaction –sucrose after reaction)/sucrose before reaction * 100

gastric juice of low pH (pH 3 to 1). After several hours of treatment with simulated gastric juice of low pH, the oligosaccharides were found more or less intact in TLC chromatogram analysis (Fig. 5). HPLC analysis estimated about 10% hydrolysis of banana pseudostem oligosaccharides after treatment with simulated human gastric juice for 12 h.

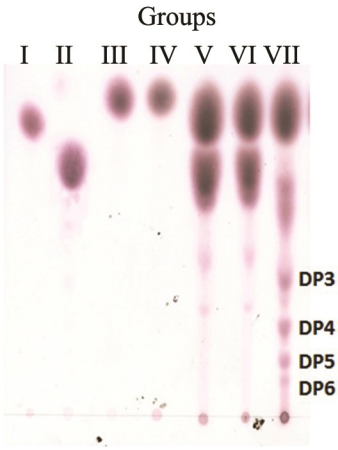

Fig. 4 — Thin layer chromatogram of oligosaccharides in banana pseudostem juice after microbial bioprocessing using *L. mesenteroides*. [I:Sucrose, II:Maltose, III:Glucose, IV:Fructose, V:BS juice + Maltose (Control), VI:BS juice + Maltose + *L. mesenteroides* (0 h), VII:BS juice + Maltose + *L. mesenteroides* (12 h), BS:Banana pseudostem, and DP:degree of polymerization]

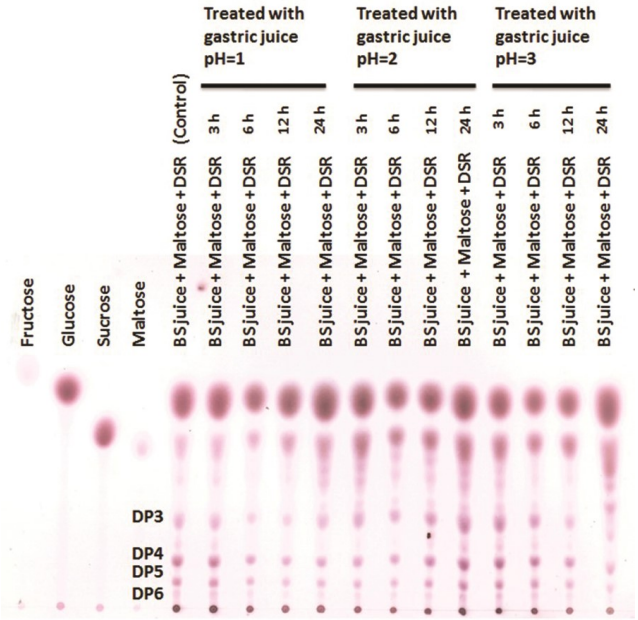

Fig. 5 — Thin layer chromatogram of banana pseudo-stem juice containing oligosaccharides after treatment with simulated human gastric juice for different time intervals. [BS: Banana pseudo-stem, DSR: dextransucrase, DP: degree of polymerization]

The modified banana stem juice containing oligosaccharides was treated with α-amylase from porcine pancreas for a longer period of time. The spots of oligosaccharides (DP3-DP6) in the TLC chromatogram of 24 h of treated banana pseudostem juice suggested resistance of the oligosaccharides against α-amylase (Fig. 6). The HPLC analysis revealed about 12% hydrolysis in oligosaccharides after 24 h treatment. Thus, the results suggested fairly good resistance of oligosaccharides in banana pseudostem juice against hydrolysis by gastrointestinal enzymes.

Growth stimulation of probiotics by banana pseudostem derived oligosaccharides

The oligosaccharides derived from the bioprocessing of banana pseudo-stem juice were tested for its effect on growth of probiotic bacterial strains, *L. acidophilus* and *L. fermentum*. The growth of probiotics was remarkable with due course of time intervals in presence of banana pseudo-stem derived oligosaccharides, as compared to glucose and untreated banana pseudostem juice taken as control (Fig. 7).

Production of D-allulose in the dextransucrase treated banana pseudostem juice

Our group developed a chimeric Smt3-D-psicose 3-epimerase enzyme with improved operational stability and kinetic properties, and its immobilization on magnetic nanoparticles[34,35]. The dextransucrase treated banana pseudostem juice samples (pH maintained at 7) were contacted with free or immobilized

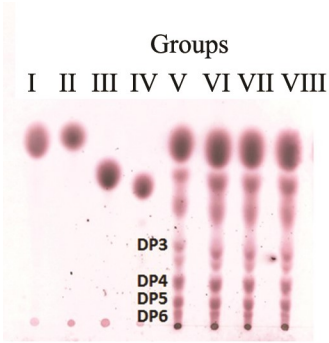

Fig. 6 — Thin layer chromatogram of banana pseudo-stem juice containing oligosaccharides after treatment with pancreatic α-amylase for different time intervals. [I: Fructose, II: Glucose, III: Sucrose, IV: Maltose, V: BS juice + Maltose + Dsr (Control), VI: pseudo-stem juice containing oligosaccharides + α-amylase (3 h), VII: pseudo-stem juice containing oligosaccharides + α-amylase (6 h), VIII: pseudo-stem juice containing oligosaccharides + α-amylase (12 h), BS: Banana pseudo-stem, Dsr: dextransucrase, DP: degree of polymerization]

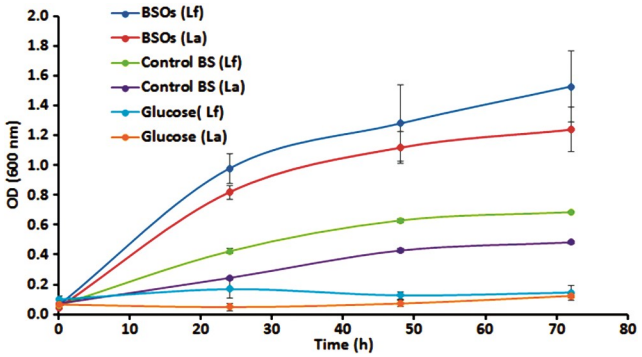

Fig. 7 — Growth profile of *Lactobacillus fermentum* and *Lactobacillus acidophilus* in banana stem juice derived oligosaccharides, glucose and control (not treated with dextransucrase) banana pseudo-stem juice. [BSOs (Lf): growth of *L. fermentum* in banana stem juice derived oligosaccharides; BSOs (La): growth of *L. acidophilus* in banana stem juice derived oligosaccharides; Control BS (Lf): growth of *L. fermentum* in control banana stem juice; Control BS (La): growth of *L. acidophilus* in control banana stem juice; Glucose (Lf): growth of *L. fermentum* in glucose; Glucose (La): growth of *L. acidophilus* in glucose]

Smt3-D-psicose 3-epimerase enzyme. The reaction sample was incubated at 50°C for 1 h, followed by denaturation of free enzyme or recovery of immobilized enzyme under magnetic field. The quantitative analysis of monosaccharides by using HPLC revealed D-fructose to D-allulose conversion yield of 25-30%, which amounts to production of about 7 g L^{-1} D-allulose in the treated banana pseudo-stem juice samples.

Discussion

In developing countries such as India, biomass is used as a significant source of energy and other materials of traditional uses. The excessive agricultural biomass is being promoted to be used as feedstock for development of products with value addition. Novel bioprocessing approaches for biotransformation of biomass into biovalue can promote new and growing industries in rural areas, and thus, would be helpful in rural development, greenhouse gas reduction, and sustainability transition. The aim of this study was to establish a bioprocess for transformation of banana pseudostem extract into a value added product possessing prebiotic and functional biomolecules.

Banana fruit is one the most extensively consumed fruits worldwide. However, banana cultivation yields vast quantities of bio-mass residues, which is discarded as wastes in the fields or nearby rivers or lakes or roads or at fruit packaging sites etc., causing environmental problems in the lack of suitable technology for its alternative economic use. Traditional folklore medicinal aspects, useful in the treatment of various diseases including diabetes, intestinal and urinary disorders etc., and several additional health benefits of banana pseudostem are known[16,18]. The nutraceutical properties in banana pseudostem juice make it a potential feedstock for healthful beverages. The present study represents a bioprocess for nutritional enhancement of clarified banana pseudo-stem juice with non-digestible glucooligosaccharides and nearly calorie-free sugar, D-allulose.

The acceptor primed glucosyltransferase reaction of Dextransucrase transfers D-glucosyl moiety of sucrose into α-(1-6) linked oligosaccharides in presence of acceptor such as maltose. The oligosaccharides are non-digestible and promotes growth and activity of health beneficial microbiota i.e. probiotics in human intestine. Thus, dextransucrase is a potential biocatalytic tool for biosynthesis of the functional oligosaccharides i.e. prebiotics by utilizing the simple sugars, sucrose and maltose, in the feedstock[33]. However, it is desirable to recover the enzyme from the sample for its reuse, making the catalytic reaction economically useful, and to avoid contamination of the enzyme in the sample especially in case of dietary supplements. Calcium alginate has been explored as one of the most reliable carriers for glucansucrase immobilization or microencapsulation, in terms of immobilization yield, recovery, and operational stability[36,37]. In the present study, dextransucrase enzyme entrapped in calcium alginate-pectin beads was shown to be useful for multiple duty-cycle use in the treatment of banana biomass. The considerable functional stability of the biocatalyst could be attributed to the intermolecular cross linking established by interactions between carboxylate groups of pectin, the natural anionic polysaccharide, and the positive amino acid residues of dextransucrase[38,39].

In view of the traditional uses of *L. mesenteroides* in dairy industry and the poor pathogenic incidences in human beings, it is considered to be reasonably safe microorganism[40]. The successful cultivation of *L. mesenteroides* cells in banana pseudo-stem juice indicates its utilization as a biocatalyst for microbial bioprocessing of banana plant biomass. Moreover, banana pseudostem juice can be used as an alternative carbon source for cultivating *L. mesenteroides* and dextransucrase enzyme production for food and health applications.

The dextransucrase based biocatalytic reaction transforms almost all the sucrose into prebiotic oligosaccharides in banana pseudo-stem juice. As the oligosaccharides in modified banana pseudo-stem juice were resistant to both acidic and neutral pH, the transformed bioproduct could be a potential functional ingredient for supplementation in foods and dairy products of acidic to neutral pH. Furthermore, the considerable resistance against gastric and pancreatic enzymes indicates persistence of oligosaccharides in the intestine in more or less intact form. The non-digestible glucooligosaccharides are selective substrates of beneficial microbes, and therefore, helpful in improving the intestinal microbiota as prebiotics[41]. The acceptor primed glucose-transfer reaction in banana pseudo-stem juice utilizes sucrose as substrate, and releases free fructose in equimolar concentration. The D-fructose was targeted for catalytic conversion into D-allulose, employing the recently developed fructose epimerizing biocatalyst, Smt3-d-psicose 3-epimerase[34], immobilized onto magnetic nanoparticles[35]. D-allulose is a C-3 epimer of D-fructose. It is a rare sugar with almost zero caloric value but having 70% of the relative sweetness of sucrose[42]. The demand of rare sugar, D-allulose, is rapidly advancing in food and medical industries due to multifarious positive impact on health such as anti-hyperglycemic, anti-dyslipidemic, anti-diabetic, anti-obesity, antioxidant, and neuroprotective effects[42-44]. Further, it has been shown to enhance stability, texture and antioxidant property of the food products[45]. Thus, the major proportion of calorie sugars in the modified banana pseudo-stem juice was transformed into nutritionally beneficial molecules- prebiotic glucooligosaccharides and D-allulose. However, the downstream processing for separation of these high-value bioproducts is usually not very economical, resulting higher cost to consumers. Herein, a bioprocess has been developed for transformation of the underutilized banana biomass extract into a low calorie functional juice of prebiotic nature. As employment of immobilized biocatalysts avoids the contamination of the proteins used for biotransformation, the modified banana pseudostem juice containing *in vitro* synthesized prebiotic glucooligosaccharides and functional sugar, D-allulose, can be used as feedstock for functional beverages or as food ingredient in other products.

Conclusion

The study reported a method for biotransformation of banana pseudostem extract into a healthy juice with functional properties by employing immobilized biocatalyst with dextransucrase activity. The bioprocessing of juice resulted in production of oligosaccharides with considerable resistance to enzymatic hydrolysis and capability of stimulating growth of probiotics, indicating its nondigestible nature and prebiotic potential. The free D-fructose generated as byproduct in the acceptor primed oligosaccharide synthesis was subjected to epimerization reaction to produce nearly calorie-free functional rare sugar, D-allulose, by employing an engineered catalyst with D-psicose 3-epimerase activity. Moreover, banana stem juice was demonstrated as replacer of synthetic culture medium for cultivation of *L. mesenteroides*, dextransucrase enzyme production, and microbial bioprocessing. To the best of our knowledge, this is the first report on bioprocessing of underutilized banana biomass (banana pseudo-stem extract) as feedstock for the preparation of functional beverages and ingredients augmented by prebiotic glucooligosaccharides and rare sugar, D-allulose.

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Reference

1 Elanthikkal S, Gopalakrishnapanicker U, Varghese S & Guthrie JT, Cellulose microfibres produced from banana plant wastes: Isolation and Characterization. *Carbohydr Polym*, 80 (2010) 852.

2 Chauhan S & Sharma AK, Enzyme treatment in improving the quality of pseudo-stem fiber of banana plant to use this bioresource for making handmade paper. *Int J Fibre Text Res*, 4 (2014) 57.

3 Preethi P & Balakrishna Murthy G, Physical and chemical properties of banana fibre extracted from commercial banana cultivars grown in Tamilnadu state. *Agrotechnol*s, 11 (2013) 008.

4 Gumisiriza R, Hawumba JF, Okure M & Hensel O, Biomass waste-to-energy valorisation technologies: a review case for banana processing in Uganda. *Biotechnol Biofuels*, (2017) 10:11 doi: 10.1186/s 13068-016-0689-5.

5 Abdul Aziz NA, Ho LH, Azahari B, Bhat R, Cheng LH & Mohamad Ibrahim MN, Chemical and functional properties of the native banana (*Musa acuminata* x balbisiana colla cv. awak) pseudo-stem and pseudo-stem tender core flours. *Food Chem*, 128 (2011) 748.

6 Ho LH, Noor Aziah AA & Bhat R, Mineral composition and pasting properties of banana pseudo-stem flour from *Musa*

acuminata x *balbisiana* cv. Awak grown locally in Perak, Malaysia. *Int Food Res J*, 19) (2012) 1479.

7 Li W, Zhang Y, Li J, Zhou Y, Li R & Zhou W, Characterization of cellulose from banana pseudo-stem by heterogeneous liquefaction. *Carbohydr Polym*, 132 (2015) 513.

8 de Carvalho CS, de Aguiar LV, Sales-Campos C, de Almeida Minhoni MT & de Andrade MC, Applicability of the use of waste from different banana cultivars for the cultivation of the oyster mushroom. *Braz J Microbiol*, 43 (2012) 819.

9 Ingale S, Joshi SJ & Gupte A, Production of bioethanol using agricultural waste: banana pseudo-stem. *Braz J Microbiol*, 45 (2014) 885.

10 Idrees M, Adnan A, Malik F& Qureshi FA, Enzymatic saccharification and lactic acid production from banana pseudo-stem through optimized pretreatment at lowest catalyst concentration. *Excli J*, 12 (2013) 269.

11 Ray PR & Ghatak PK, Phytochemical characterization and antimicrobial property of banana (*Musa paridasiaca*) pseudo-stem juice. *Int J Agric Sc & Vet Med*, 1 (2013) 87.

12 Ho LH, Abdul Aziz NA & Azahari B, Physico-chemical characteristics and sensory evaluation of wheat bread partially substituted with banana (*Musa acuminata* x *balbisiana* cv. Awak) pseudo-stem flour. *Food Chem*, 139 (2013) 532.

13 Kumar SK & Reddy KD, Utilization of plantain stem pseudo-stem of banana tree *(Musa cavendish)*. *J Agro Natl Res Mgmt*, 2 (2015) 102.

14 Sampath Kumar KP, Bhowmik D, Duraivel S & Umadevi M, Traditional and medicinal uses of banana. *J Pharmacogn Phytochem*, 1 (2012) 53

15 Saravanan K & Aradhya SM, Polyphenols of pseudostem of different banana cultivars and their antioxidant activities. *J Agric Food Chem.,* 59 (2011) 3613.

16 Bhaskar JJ, Kumar AP & Salimath PV, Effect of banana (*Musa* sp. var. elakki bale) flower and pseudo-stem on antioxidant and lysosomal enzyme activities in streptozotocin-induced diabetic rats. *J Pharm res*, 4 (2011) 1087.

17 Jamuna JB & Nandini CD, Feeding of banana flower and pseudo-stem to diabetic rats results in modulation of renal gluts, TGFβ, PKC and extracellular matrix components. *Nutr Metab Cardiovasc*, 24 (2014) 623

18 Ramu R, Shirahatti PS, Zameer F & Prasad NM, Investigation of antihyperglycaemic activity of banana (*Musa sp.* var. *nanjangud rasa bale*) pseudo-stem in normal and diabetic rats. *J Sci Food Agric*, 95 (2015) 165.

19 Bhaskar JJ, Shobha MS, Sambaiah K & Salimath PV, Beneficial effects of banana (*Musa* sp. var. elakki bale) flower and pseudo-stem on hyperglycemia and advanced glycation end-products (ages) in streptozotocin-induced diabetic rats. *J Physiol Biochem*, 67 (2011) 415.

20 Pillai RG, The core of the pseudo-stem of *Musa* in the treatment of urinary stones. *Ancient Sci Life*, 1 (1995) 2.

21 CSIR-Central Food Technological Research Institute. http://www.cftri.com/ttbdtechs/bananastemjuice.pdf.

22 Li C, Liu G, Nges IA, Deng L, Nistor M & Liu J, Fresh banana pseudo-stems as a tropical lignocellulosic feedstock for methane production. *Energy Sustain Soc*, 6 (2016) 27.

23 Reddy GV, Babu PR, Komaraiah P, Roy KRRM & Kothari IL, Utilization of banana waste for the production of lignolytic andcellulolytic enzymes by solid substrate fermentation using two pleurotus species (*P. ostreatus* and *P. sajor-caju*). *Process Biochem*, 38 (2003) 1457.

24 Alwi H, Idris J, Musa M & Hamid KHK, A preliminary study of banana stem juice as a plant-based coagulant for treatment of spent coolant wastewater. *J Chem*, http://dx.doi.org/10.1155 (2013) 165057.

25 Shukla SR, Shinde SC, Banye AS & Patil SM, Dyeing of wool with *Acacia pennata. Indian J Fibre Text*, 29 (2004) 350.

26 Mohiuddin AKM, Saha MK, Hossian MS & *Ferdoushi A*, Usefulness of banana (*Musa paradisiaca*) wastes in manufacturing of bio-products. *The Agriculturists,* 12 (2014) 148.

27 Central Institute for Research on Cotton Technology. http://circot.res.in/hindi/images/downloads/annual_reports/annual_report_2012_13.pdf.

28 Ghosh M, Mukherjee R & Nandi B, Production of extracellular enzymes by two pleurotus species using banana pseudo-stem biomass. *Eng Life Sci*, 18 (1985) 243.

29 Marie-Magdeleine C, Liméa L, Etienne T, Lallo CH, Archimède H & Alexandre G, The effects of replacing dichantium hay with banana (*Musa paradisiaca*) leaves and pseudo-stem on carcass traits of ovin martinik sheep. *Trop Anim Health Prod*, 41(7) (2009) 1531.

30 Duguma B & Janssens GP, Assessment of feed resources, feeding practices and coping strategies to feed scarcity by smallholder urban dairy producers in jimma town, Ethiopia. *Springerplus*, 5 (2016) 717.

31 Purama RK & Goyal A, Identification, effective purification and functional characterization of dextransucrase from Leuconostoc mesenteroides NRRL B-640. *Bioresour Technol*, 99 (2008) 3635.

32 Purama RK & Goyal A, Purified Dextransucrase from Leuconostoc mesenteroides NRRL B640 Exists as Single Homogeneous Protein: Analysis by Nondenaturing Native PAGE. *Internet J Microbiol*., 6 (2008) 1.

33 Sharma M, Patel SN, Lata K, Singh U, Krishania M, Sangwan RS & Singh SP, A novel approach of integrated bioprocessing of cane molasses for production of prebiotic and functional bioproducts. *Bioresour Technol*, 219 (2016) 311.

34 Patel SN, Sharma M, Lata K, Singh U, Kumar V, Sangwan RS & Singh SP, Improved operational stability of D-psicose 3-epimerase by a novel protein engineering strategy and D-psicose production from fruit and vegetable residues. *Bioresour Technol*, 216 (2016) 121.

35 Singh SP, Sangwan RS, Patel SN & Singhal N, A process for magnetic particle immobilization of Smt3-d-psicose 3-epimerase enzyme and post-reaction recovery and recycled use of the immobilized enzyme for production of D-psicose from biomass or bioresource or agro-industrial products or residues, and uses of the same. (2016) Patent Application No. 201611044752.

36 Tanriseven A & Dogan S, Production of isomalto-oligosaccharides using dextransucrase immobilized in alginate fibres. *Process Biochem*, 37 (2002) 1111.

37 Kothari D, Baruah R & Goyal A, Immobilization of glucansucrase for the production of gluco-oligosaccharides from *leuconostoc mesenteroides*. *Biotechnol Lett*, 34 (2012) 2101.

38 Gomez L & Villalonga R, Functional stabilization of invertase by covalent modification with pectin. *Biotechnol Lett*, 22 (2000) 1191.

39 Satar R, Matto M, & Husain Q, Studies on calcium alginate-pectin gel entrapped concanavalin a-bitter gourd (*Momordica charantia*) peroxidase complex. *J Sci Ind Res*, 67 (2008) 609.
40 Ogier JC, Casalta E, Farrokh C & Saïhi A, Safety assessment of dairy microorganisms: the *Leuconostoc* genus. *Int J Food Microbiol*, 126 (2008) 286.
41 Gibson GR & Roberfroid MB, Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr*, 25 (1995) 1401.
42 Chung MY, Oh DK & Lee KW, Hypoglycemic health benefits of d-psicose. *J Agric Food Chem*, 60 (2012) 863.
43 Hossain A, Yamaguchi F, Matsuo T, Tsukamoto I, Toyoda Y, Ogawa M, Nagata Y & Tokuda M, Rare sugar d-allulose: potential role and therapeutic monitoring in maintaining obesity and type 2 diabetes mellitus. *Pharmacol Ther*, 155 (2015) 49.
44 Nagata Y, Kanasaki A, Tamaru S & Tanaka K, D-psicose, an epimer of D-fructose, favorably alters lipid metabolism in Sprague-Dawley rats. *J Agric Food Chem*, 63 (2015) 3168.
45 Zeng Y, Zhang H, Guan YP, Zhang LL & Sun XX, Comparative study on the effects of D-psicose and D-fructose in the Maillard reaction with b-lactoglobulin. *Food Sci Biotechnol*, 22 (2013) 341.