Microbial Synthesis Of Novel Prebiotic Oligosaccharides Production By Pichia Cecembensis SI-15 From Anacardium Occidentale

S.Priyatharsini, C. Uma and P.Sivagurunathan*

Abstract: Cashew pseudo fruits (Anacardium occidentale L.) are a wealthy supply of antioxidants, ascorbic acid, organic acids, carbohydrates and minerals. These fruits contain many healthful properties and have long been used in ancient drugs for the treatment of many diseases. Pichia cecembensis yeast strain was extracted from the raw cashew fruit crush. The cashew fruit crush might be a wise offer of reducing sugar and it's going to be used owing to the substrate for the assembly of novel oligosaccharide. The food-grade of sucrose, yeast extract, and K2HPO4 was supplemental to the juice to push microbial growth. This study aimed to measure the assembly of prebiotic oligosaccharides from cashew potable. The isolation of oligosaccharides was characterized by NMR and FT-IR analysis.

Keywords: Oligosaccharides, Cashew apple juice, FT-IR, NMR, and yeast strain

1. INTRODUCTION

The cashew may be tropical and sub-tropical tree happiness to the family Anacardiaceae, the Anacardium Linn, and therefore, the species nut tree Linn var. nanum. The cashew apple is that the peduncle of the cashew fruit that's created in minerals, reducing sugars (fructose and glucose), vitamins, and a number of amino acids. The cashew grows even on poor soils with low rain and it's cultivated in cardinal countries round the world. Brazil, India, Vietnam, and the Federal Republic of Nigeria are the foremost producers. Though cashew fruits are consumed as juice, ice-cream, and different foodstuffs, and cashew cultivation in Brazil is an associate activity that aims within the main to supply cashew batty. The nuts represent solely 100 percent of the overall fruit weight, and huge amounts of cashew juices area unit left within the field when the removal of the nut. Cashew fruits are made in laevulose, glucose, and minerals. These sugars will act as acceptors to supply oligosaccharides exploitation glycosyltransferases enzymes [6]. The diversity of commercial products derived from cashew apples generates an oversized quantity of waste that may trigger serious environmental issues. Throughout juice and pulp production, concerning four-hundredth of a load of most fruits like mango, acerola, edible fruit, and cashew apple end in agro-industrial residues. Such residues, consisting of peel and seeds, typically concentrate relevant quantities of bioactive phytochemicals, which regularly contain inhibitor capability that's over that found within the fruit pulp [1].

- *S.Priyatharsini. Department of Microbiology, Faculty of Science, Annamalai University, Chidambaram, Tamil Nadu tharshufamilys@gmail.com*
- *C.Uma, Department of Microbiology, Faculty of Science, Annamalai University, Chidambaram, Tamil nadu umasaravanan1@gmail.com*
- *Corresponding Author :P.Sivagurunathan, Department of Microbiology, Faculty of Science, Annamalai University, Chidambaram, Tamil Nadu. Sivaguru1981@yahoo.com*

When prebiotic oligosaccharides are value-added as new purposeful ingredients to food, they probably improve the standard of foods together with milk drinks and performance symbiotically. the subsequent criteria are necessary for a food component to be classified as a prebiotic: • It mustn't undergo chemical reaction and may not be absorbed within the upper epithelial duct tract; • Should be a selective substrate for a restricted range of probably helpful microorganism within the colon, that stimulates its growth and develop metabolic activities; • Should be ready to promote a healthy viscus collection and, consequently, induce effects within the lumen that profit the host [21]. In the presence of saccharose, the introduction of alternative carbohydrates (acceptors) shifts the accelerator pathway from the assembly of oligosaccharides [5, 15, 16, 18, 20]. It can be a prebiotic indigestible food element that may affect the host's ability to stimulate body expansion and / or the limited variety of microorganisms in the colon.[9]. Prebiotic carbohydrates act by increasing the expansion activity of bifidobacteria in human intestines, with the advantage of being by selection used by bifidobacteria and, not by infective microorganisms like enteric and Escherichia. Bifidobacteria are thought of as helpful microorganisms that are ready to maintain human health and stop a rise in infective microorganisms. Oligosaccharides are very well recognized as 'functional food ingredients' because of their positive effects on human health. This analysis work focuses on the employment of cashew juice as a substrate for the production of prebiotic oligosaccharide by the exploitation of native microbes.

2. MATERIALS AND METHODS

2.1. Sample collection

Cashew apples were collected from completely different places in Cuddalore District (Tamil Nadu, India). The cashew apple samples were transported to the laboratory where the fruits were separated from their round the bend. The apples were washed totally with clean water. Then the apples were cut and ground employing a Mixer Grinder. The juice obtained by pressing the mash was filtered

through a 0.5 mm mesh sieve. it had been then holding on and frozen at–80°C for analyses. Cashew potable contains a high level of tannic acid that was removed with the use of processed albuminoid.

2.2. Isolation and cultivation of oligosaccharide-producing being strain

To isolate a being strain that produces novel oligosaccharides, samples from raw juices like yellow and red varieties were collected. The cashew fruit crush was serial diluted and placed on an agar plate. Morphologically distinguishable colonies were transferred on the contemporary culture medium and polite at 37C for twenty-four hours. a complete of seventy-three strains were differentiated individually and hold on as frozen stock cultures at -20C within the nutrient broth medium. All microorganism strains were cultured polite within the nutrient broth medium for 2–3 days at37C.

2.3. Oligosaccharides production

A strain of SI-15 (Pichia cecembensis) was activated in an optimized synthetic medium composed of sucrose, 50 g/L (food grade); yeast extract, 20 g/L (Himedia); MgSO4.7H2O, 0.20 g/L; MnSO4.2H2O, 0.01 g/L; FeSO4.7H2O, 0.01 g/L; CaCl2.2H2O, 0.02 g/L; NaCl, 0.01 g/L; and K2HPO4 (anhydrous), 20 g/L [17, 16] in an orbital shaker set at 30C and 150 rpm for 12 h. The initial pH scale of the substance was adjusted to 6.5 with the addition of phosphoric acid. The 10% of this cell culture was used as inoculums to the cashew potable fermentation medium. The cashew fruit crush fermentation medium in substitution to the artificial medium was prepared to dilute the processed fruit crush to achieve fifty g/L of total reducing sugar content and adding food-grade sucrose to reach fifty g/L, yeast extract, 4 g/L, ammonium sulfate 4g/L and K2HPO4 20 g/L. Sucrose was added to induce the assembly of oligosaccharides. The initial pH was adjusted to 6.5 and also the matter was sterilized at 121 ^{o}C for fifteen min. The fermentation used as a control was carried out as a similar artificial medium used to activate the strain. Fermentations were administrated in 250 milliliter Erlenmeyer's flasks containing 100 ml of the culture medium placed in an orbital shaker at 30C and150 rpm for twenty-four h. After harvest the cell culture by action at 7000rpm for 10min, the collected supernatant was accustomed to confirm the catalyst activity. The crude catalyst within the soured cashew fruit juice was applied and used to synthesize the oligosaccharides. They determined the quantified initial rate of the release of reducing the sugar by the victimization of the DNS technique [14]. The crude soured broth contains1ml was created up to 3ml with water. After adding 2ml of the DNS chemical agent, it was heated at 80C for fifteen min during a boiling water tub. When cooling, the reducing sugars were liberated and it absolutely was measured spectrophotometrically at 580 nm and expressed as an aldohexose equivalent. aldohexose was taken as a control.

2.4. Detection of oligosaccharides

The oligosaccharides created from the fermentation of CAJH were detected and characterized concerning the degree of chemical change by thin-layer natural process on silicon oxide plates of 250µm thickness. Samples of ten µL were absorbed onto the plates on a line regarding 1.5 cm on top of the lower plate edge. When drying with a hairdryer, the plate was irrigated for 2 ascents in an exceedingly solvent mixture composed of n-butanol:acetone: H2O (50:40:10 v/v). To render sugars visible, plates were sprayed to saturation with a solute containing 0.3 g/100 metric capacity unit of N* (1-naphthyl) ethylamine dihydrochloride throughout a solvent system composed of methanol: sulfuric acid (95:5 v./v)[4].

2.5.Internal transcribed spacer (ITS) amplification, sequencing, and treeing program

2.5.1. Preparation of template DNA:

It is important to use a pure culture of yeast for identification. The culture was suspended into a 0.5 milliliter of sterile saline unbroken in a 1.5-milliliter centrifuge tube. The contents were centrifuged at 10 thousand revolutions per minute for 10 min. once removal of the supernatant, the pellet was suspended in 0.5 milliliters of InstaGene Matrix (Bio-Rad, USA) incubated 56 ^{o} C for thirty min then heated for ten min. when heating, the supernatant is employed for PCR. For taking off the PCR, Add one µl of model polymer in twenty µl of PCR reaction answer. The primers as ITS1 and ITS4 were used for fungal or yeast and then performed 35 amplification cycles at 94 ^{o} C for 30 sec, 55 ^{o} C for 30 sec, and 72 ^{o} C for 40 sec. DNA fragments amplified about 400 bp in the case of yeast or fungal sample. Include a positive control (fungal DNA) and negative control (no DNA template) was also included in the PCR. Non-PCR primers and dNTPs from PCR products are extracted using the PCR purifier (Millipore).

2.5.2. Sequence

The sublimate PCR product of about 400 bp was sequenced with the misuse of ITS1F and ITS4 universal ITS primers. Sequestration was performed using Sequential Slider Rotation Serial Assay Kit (Applied Biosystems, USA). In addition, the serial product applied Biosystems were installed on the 3730XL DNA Automated System (Apply Biosystems, USA). The culture sequence obtained were subjected to BLAST analysis, the phylogenetically similar kind strains sequence and alternative phylogenetic related sequence were designated from the GenBank and they were subjected to several sequence alignment so align sequences were cut to similar length in nucleotides and were subjected to phylogenetic tree (neighbor-joining) construction using MEGA 6.

2.6. Nucleotide sequence accession number

The nucleotide sequence data have been deposited in GenBank under accession number MG460473.

2.7. Determination and characterization of oligosaccharides

2.7.1. Nuclear resonance spectroscopy (NMR) analysis

Nuclear resonance spectroscopy(NMR)complete assignments of the glycosidic linkage signals were carried

out using 1H and 13C NMR (coupled and decoupled) by programs in a Bruker Avance III HD four hundred MHz spectrometers with a 5mm Inverse probe. The refined samples were dissolved in the DMSO solvent [20].

2.7.2. Fourier transforms – infrared spectroscopy analysis (FT-IR)

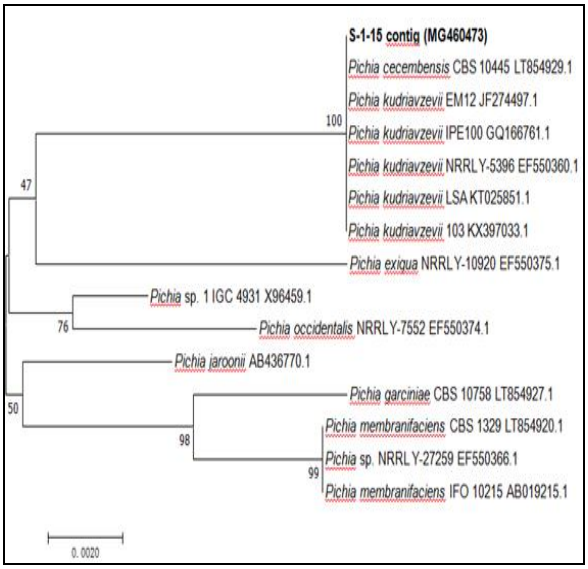
The IR spectra of TLC purified compound of powder extracts were recorded in an FT-IR spectrometer (Thermo Nicolet, AVATAR, 330 FT-IR system, Madison, wi 53711-4495) within the 4000-400 cm-1spectral region using potassium bromide (KBr) solid cells. The targeted samples of crude extract were refined with potassium bromide salt (Sigma –Aldrich) to remove scattering effects from giant crystals. This fine mixture was then ironed in a very press to make a clear pellet through that the beam of the spectrometer is passed. The spectra were recorded for the various pellets and analyzed using the quality methodology represented by the previous authors [7, 11, 12].

3. RESULT AND DISCUSSION

The present study was investigated a unique yeast strain from cashew fruit juice that produces a new oligosaccharide exploitation saccharose, the only supply of carbon. Out of seventy-three microorganism strains isolated from cashew fruit juice, six strains (SII-6, SI-8, SII-14, SI-15, SII-16, and SI-15) were elect within the 1st screening exploitation TLC analysis, result of the created a singular spot expecting a replacement carbohydrate throughout three days incubation among the cashew apple juice fermentation medium. Therefore, they were monitored for the assembly of a singular carbohydrate. Once each strain was severally incubated with the medium containing a combination of sucrose, the culture supernatants were detected and developed on a TLC plate. Supported the microscopic identification of the strain SI-15 was discovered to be an oval-shaped. ITS issue sequence of strain SI-15 exhibited a high degree of similarity with sequences of the genus Pichia. Multiple sequence alignment of ITS factor sequences discovered a close process association between strain SI-15 and Pichia cecembensis (>99% similarity). (Fig.1).

Figure –20

The phylogram showing the position of Pichia cecembensis (SI-15) with other Pichia species based on ITS region-based characterization partial gene sequence and phylogenetic tree analysis.



Raw nucleotide sequences of Pichia cecembensis (SI-15)strain

AGCATTATACGGTGAAACTGCGAATGGCTCATTAAATC
AGTTATCGTTTATTTGATAGTTCCGTTCTACATGGATAA
CCGTGGAAAATCTAGAGCTAATACATGCGTAAAGCCCC
GACTTCGGGAGGGGTGTATTTATTAGATAAAAAATCAA
T
GCCCTCGGGCCTTTTGATGATTCATAATAACTTTTCGAA
GCTCATGGCCTTGCGCC
GGAGCTGGTTCATTCAAATTTCTGCCCTATCAACTTTC
GATGGTAGGATAGAGGCCTACCATGGTTTTCACGGGT
AACGGGGAATAAGGGTTCGATTCCGGAGAGGGAGCCT
GAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCG
CGCAAATTACCCAATCCTGACACAGGGAGGTAGTGAC
AATATATAACGATACAGGGCCTTTGGTCTTGTAATTGG
AATGAGTACAATGTAAATACCTTAACGAGGAACAATTG
GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCA
GCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAG
CTCGTAGTTGAACTTTGGGCCTGGGCGGACGGTCTAC
CTATGGTAAGCACTGTTGCGGCCGGGTCTTTCCTTCTG
GCTAGCCCTCGGGCGAACCAGGACGATTACTTTGAGG
AAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATATA
TTAGCATGGAATAATAGAATAGGACGCATGGTTCTATTT
TGTTGGTTTCTAGGACCATCGTAATGATTAATAGGGAC
GGTCGGGGGCATCAGTATTCAGTCGTCAGAGGTGAAA
TTCTTGGATTGACTGAAGACTAACTACTGCGAAAGCAT
TTGCCAAGGACGTTTTCATTAATCAAGAACGAAAGTTA
GGGGATCGAAGATGATCAGATACCGTCGTAGTCTTAAC
CATAAACTATGCCGACTAGGGATCGGGTGGTGCTACTT
TGCCCACTCGGCACCTTACGAGAAATCAAAGTTTTTGG
GTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAA
AGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCT
GCGGCTTAATTTGACTCAACACGGGGAAACTCACCAG
GTCCAGACGTAATAAGGATTGACAAGTTAGAGACTTCT
CTTGATCTTACGGGTGGTGGTGCATGGCCGTTTTTAGT
CCTTGGAGTGATTTGTCTGCTTAATTGCGATAACGGAC
GAGACCTTAACCTGCTAAATAGGGCTGCGAGCATCTG
CTCGGGTGCTCTTCTTAGAGGGACTATGGGTATCAAAC
CCATGGAAGTTTGAGGCAACAACAGGTCTGTGATGCC
CTTAGACGTTCTGGGCCGCACGCGCGCTACACTGACG
GAGCCAGCAAGTCCAACCTTGGTCGAGAGGCCCGGGT
AATCTCGTGAAACTCCGTCGTGCTGGGGATAGAGCATT
GTAATTTTTGCTCTTCAACGAGGAATTCCTAGTAAGCG

CAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTT
GTACACACCGCCCGTCGCTACTACCGATTGAATGGCTT
AGTGAGGCTTCAAGATTGGCGCCGCGGGAGGGGCAA
CTTTCCCATGGGGCCGAGAATC

The bands in the region 3371cm-1 indicated O-H stretching vibration and 2937cm-1 indicated C-H aliphatic stretching vibration as a peak. The band 2117cm-1-1923cm-1 indicated the C-C stretching vibration. The absorption peak at 1651 cm-1 indicated the C-O found in the glycosidic linkage. The absorption 1433cm-1 indicated C=H bonds glucopyranose units, and 1049 cm-1 indicated C-C bond within the glucose linkage from the monosaccharide units. This data showed that figure-1.In the FT-IR spectrum, fractions absorption band OH bending modes and sugar OH stretching vibrations together in the region from3386 to 3495cm-1are resulted from hydroxyl that closely related to polysaccharides. Common to all spectra were vibration due to the carbohydrate backbone[19]. Bonds between 1416 and 1433cm-1 are assigned to C=O with the glycosidic linkage from the glycosyl units. Bonds between 1200 and 1100cm-1 are allotted to C–C and C–O modes likewise as a shoulder attributable to the C–O–C of the glycosidic linkage (1135 cm-1)whilst the foremost absorption is attributable to C–O feeble coupled to gamma C–O–H[13]. The relatively strong absorption peak is about 1646cm-1, indicating the IR absorption properties of polysaccharides. [3]. The FT-IR spectra of the substance indicate the presence of carboxyl groups, which may possibly perform binding sites for voltage cations [2]. The anomeric resonances for the protons unit of measurement sometimes found at intervals the chemical shift vary of 4.3-5.1 ppm, with the a-anomeric protons between four.8-5.1 ppm. The anomeric resonances for the carbons unit of measurement found at intervals the chemical shift vary of 98-107 ppm, the ring protons unit of measurement sometimes found at intervals the 3.3-4.5ppm region. The methyl group teams from 6-deoxy sugars and from O- and N-acetyl teams area unit found within the vary of 2.0 ppm. The ring carbons and element connected can seem at around 56.5 ppm. The carbon signals from the methyl group teams area unit found in 19.01ppm, This characteristic 1 H and 13 C chemical shifts are shown in (Fig 3,4). These signals confirm the presence of sugar with organic compound rings of anomeric protons. The range from 0.5ppm to 1.5ppm represents methyl group configuration in the carbohydrates. The signal at 1.0–1.25ppm arouses from methyl proton of the 6-deoxy sugars [10].

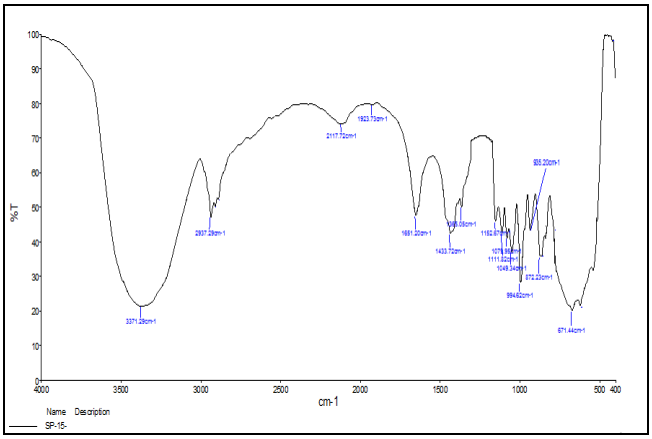


Figure2: *FT-IR spectrum analysis of oligosaccharide fraction produced by Pichia cecembensis.*

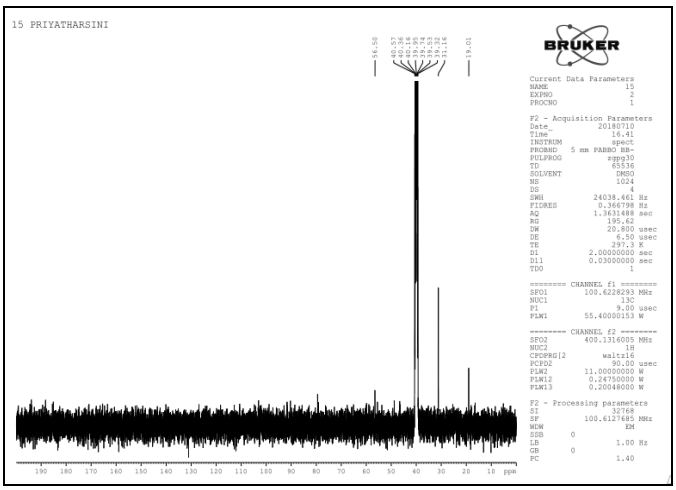


Figure 3:*1H Nuclear magnetic resonance spectroscopy of the oligosaccharide produced by Pichia cecembensis SI-15.*

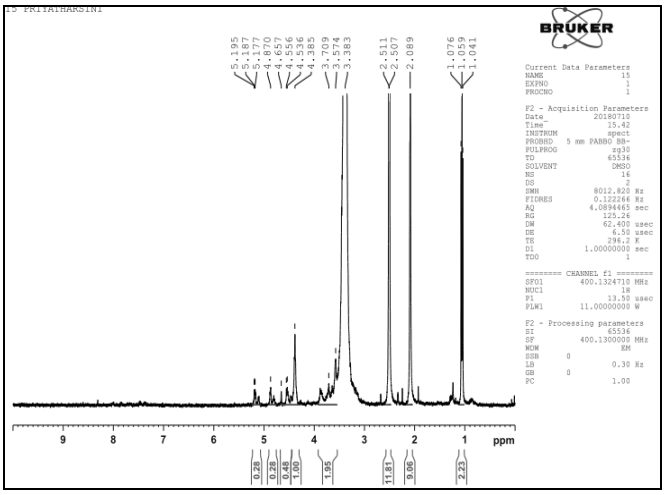


Figure 4:*13C Nuclear magnetic resonance spectroscopy spectrum of the oligosaccharide produced by Pichia cecembensis SI-15.*

4. CONCLUSION

Cashew fruits were made in sugars and minerals that act as acceptors to supply oligosaccharides. The rise in consumption of cashew nuts ends up in an increase in wastage of cashew apple fruit which may be employed in a product utilized in as discussed above. This analysis concludes that there are factors for prebiotic oligosaccharides in cashew fruit crush. This study forms a platform for several researchers on productive usage of biowaste

ACKNOWLEDGMENT

This work was supported by the Rajiv Gandhi National Fellowship, UGC, New Delhi.India

REFERENCES

[1] Ajila, C. M., Bhat, S. G., &Prasada Rao, V. J. S. (2007). Valuable parts of raw and ripe peels from 2 Indian Mango varieties.Food Chemistry, 102(4), 1006-1011.

[2] Bramhachari, P.V., Kishor, P.B.K., Devi, R.R., Kumar, R., Rao, B.R., & Dubey, S.K.(2007). Isolation and characterization of mucous exopolysaccharide(EPS) produced by Vibriiofurnissii strain VB0S3. J. Microbiol. Biotechnol. 17: 44–51.

[3] Bremer, P.J., & Geesey, G.G. (1991). Associate analysis of biofilms development utilizing non-destructive attenuated total coefficient Fourier remodels infrared spectrometry.

[4] Biofouling. 3: 89–100. Cote, G., & Leathers, T.D. (2005). A method for measuring and classifying Leuconostoc spp. glucanases per strain-dependent acceptor product patterns.J. Ind. Microbiol. Biotechnol. 32: 53–60.

[5] Heincke, K., Demuth, B., Jordening, H.J., & Buchholz, K. (1999). Kinetics of the dextransucrase acceptor with maltose-experimental results and modeling. Enzyme Microb. Technol. 24: 523–534.

[6] Honorato, T.L., & Rodrigues, S. (2010). Dextransucrase stability in cashew juices. Food Bioprocess. Technol. 3: 105–110.

[7] Jenny, K., Othmar, K.,&Fiechter, A. (1988). Biosurfactants from Bacillus licheniformis: structural analysis and characterization. Appl. Microbiol. Biotechnol. 36: 5–13.

[8] Kohmoto, T., Fukui, F., Takaku, H., Machida, Y., Arai, M.,& Mitsuoka, T. (1988). Effect of isomalto-oligosaccharides on human fecal flora. Bifidobact Microflora. 7: 61–69.

[9] Kolida, S., Thuhy, K., & Gibson, G.R. (2002). Prebiotic of inulin and oligofructose. Brit. J. Nutrit. 87: S193–S197.

[10] Kumar, C.G., Joo, H.S., Choi, J.W., Koo, Y.M., &Changa, C.S. (2004). Purification and characterization of extracellular polysaccharides from haloalkalophilicBacillus sp.I-450. Enzyme and microbial Technol. 34: 673–681.

[11] Lang, S.,& Wagner, F. (1987). Structures and Properties of biosurfactant. Biosurf. Biotechnol. pp.21–47. Lin,

[12] S.C., Minton, M.A., Sharma, M.M.,& Georgiou, G. (1994). Structural and immunologic characterization of a biosurfactant made by Bacilli licheniformis JF-2. Appl. Environ. Microbiol. 60: 31–38.

[13] Malfait, T., Van Dael H., Van Cauwelaert F., (1987). Raman spectrum analysis of the metallic element salt of kappa-carrageenan and related compounds in resolution. Carbohydr. Res. 163: 9-14.

[14] Miller, R. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar.Anal.Chem. 31,426–428.

[15] Periera, A.M., Costa, F.A.A., & Rodrigues, M.I. (1998). Invitro synthesis of oligosaccharides by the receptor reaction of dextransucrase from Leuconostocmesenteroides. Biotechnol. Lett. 20: 397–401.

[16] Rabelo, M.C., Honorato, T.L., Gonçalves, L.R.B., Pinto, G.A.S., & Rodrigues, S. (2006).Enzymatic synthesis of prebiotic oligosaccharides.Appl. Biotechnol. Biochem. 133 (2006): 31–40.

[17] Minton, M.A., Sharma, M.M.,& Georgiou, G. (1994). Structural and medical specialty characterization of a biosurfactant created by eubacterium licheniformis JF-2. Applied Environmental Microbiology. 60: 31–38.

[18] Malfait, T., Van Dael H., Van Cauwelaert F., (1987). Raman chemical analysis of the atomic number 11 salt of kappa-carrageenan and related compounds in answer. Carbohydr. Res. 163: 9-14.

[19] Schrader, B. (1995). IR spectroscopy: Methods and applications.

[20] Wiley-VCH, Weinheim. Seymour, F.R., Knapp, R.D., Bishop, S.H., (1976). Determination of the structure of dextran by 13C nuclear resonance spectroscopic analysis. Carbohydr Res., 51: 179-194.

[21] Tsuchiya, H.M., Koepsell, H.J., Corman, J., Bryant, G., Bogard, M.O., Feger, V.H., Jackson, R.W. (1952). The impact of sure cultural factors on the production of dextransucrase by Leuconostocmesenteroides. J. Bacteriol. 64: 521–527.

[22] Vergera, C.M.A.C., Honorato, T.L., Maia, G.A., & Rodrigues, S. (2010). Prebiotic effect of cashew apple (Anacardium Occidentale L.) juice. LWT - Food Sci. Technol. 43: 141–145.v