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INVESTIGATION OF THE FUNCTIONAL GROUPS, MOLECULAR WEIGHT AND TOPOGRAPHY OF *PEDIOCOCCUS PENTOSACEUS (*PH3)POLYSACCHARIDE

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ABSTRACT

Polysaccharide dextran produced in the sucrose media by Pediococcus pentosaceus(PH3) was extracted and purified through ethanol precipitation. An investigation of its functional groups, molecular weight and topography was done by the application of spectroscopic analysis FT-IR, HPLC and SEM. FT-IR analysis results showed that there were similarities between in - house dextran and standard dextran. Further more in comparison to the standard dextran (Mw-670,000 Da), the molecular weight of the polysaccharide produced by the organism when sucrose was used as a substrate, was greater than the standard (Mw>670,000Da). From the surface morphology studied, using various magnifications of SEM, its porous structure revealed high water holding capacity and dextran to be a potent candidate for industrial and pharmaceutical applications.

Key Words: Dextran, *Pediococcus pentosaceus*, functional groups, FT-IR, HPLC, Scanning electron microscope

INTRODUCTION

Polysaccharides are long carbohydrate molecules of monosaccharide units joined together by glycosidic bonds. Recently, lactic acid bacteria (LAB) have received attention for their exopolysaccharides (EPSs) producing ability, indicating that a broad range of EPSs from LAB

with variable functionality can be applicable for a wide range of industries (Cerescenzi 1995). Dextran, xanthan, gellan, pullulan, yeast glucan and bacterial alginate are the examples of industrially important microbial exopolysaccharides (Cerning and Marshall 1999, Shamala and Prasad 1995). Dextran $(C_6H_{10}O_5)n$ belongs to the group of homopolysaccharides consisting of glucose monomers linked mainly (95%) by α-1,6 glycosidic bonds together with a few α-1,2 and α -1,3 branched glycosidic linkages depending on the specificity of the particular dextran producing enzyme, namely dextransucrase (Robyt and Wathes 1978). Dextran has various industrial applications in food, pharmaceutical and chemical industries as emulsifier, carrier, stabilizer, etc. Dextran is a generic name for several α-glucans produced by LAB that belong to the *Leuconostoc, Lactobacillus, Streptococcus, Pediococcus* or *Weissella* genera (Smitinont *et al.* 1999, Naessens *et al*. 2005; Bounaix *et al*. 2009). When the organism is grown on sucrose rich media, the production of an enzyme, dextransucrase (EC 2.4.1.5), is induced. Dextransucrase produces dextran using two pathways, first, hydrolyzing the sucrose and binding the glycosyl moiety, thereafter, building up the dextran chain by an insertion mechanism (Tecante *et al* 1986):

$$C_{12}H_{12}O_{11} \longrightarrow (C_6H_{10}O5)\ n + n\ C_6H_{12}O_6$$

Structural and functional attributes of dextran are dependent on the dextransucrase produced by the strain (Leathers 2003). Dextran hydrogels have biomedical applications in contact lens, cell encapsulation for drug delivery, tissue engineering and scaffold, burn dressing and spinal cord regeneration (Hoffman 2002, Van Tomme & Hehnink 2007). Dextrans serve as one of the most promising macromolecular carrier candidates for a wide variety of therapeutic agents due to their excellent physico-chemical properties and physiological acceptance.

In drug delivery, polysaccharides have been used to generate controlled release,function as matrices, coatings, films, hydrogels, microspheres, and nanoparticles. (Krogar *et al* 2002, Palviainen *et al* 2001, Wierik *et al* 1997, Bjork and Edman 1990, Hamdy *et al* 2001, Liu *et al* 2008, Felt *et al* 1998, Chen and Jo 1995). From a pharmaceutical standpoint, polysaccharides possess many favorable characteristics such as cost effectiveness, availability, low toxicity, biocompatibility, and biodegradability. Moreover, polysaccharides have a large number of reactive groups, a wide range of molecular weights, and varying chemical composition, which contribute to their diverse structural properties. Due to the presence of various derivable groups

on molecular chains (e.g. hydroxyl), polysaccharides can be easily modified chemically, resulting in many kinds of polysaccharide derivatives. Various chemical and physical modifications such as cross-linking, grafting, esterification, oxidation, and combination with a variety of other molecules can be performed to produce semi-synthetic and composite materials with extended functionality. Another advantage of polysaccharides is bioadhesion, especially for mucosal surfaces, which has been used for targeting specific organs or cells and prolonging drug residence time (Mizrahy and Peer 2011). All of these qualities have led to the growing use of polysaccharides like dextran, starch and xanthan in drug delivery systems.

Dextrans differ in the type of glycosidic linkage, degree and types of branching, length of chain, molecular mass and their confirmation (Majumdar *et al* 2009a). The physical properties such as surface morphology, structure are crucial in determining the application potency of dextrans.

Considering their immense industrial and pharmaceutical application, the production, purification, structural and functional study of dextran is of great interest.

In this regard the physiochemical attributes has been referred. The molecular mass, functional groups and structure were analyzed by HPLC, FT-IR and SEM.

MATERIALS AND METHOD

*Pediococcus pentosaceus (*PH3*)* isolated from brined cucumbers was used for the studies on its dextran. Following confirmation of organism, its enzyme production and optimization, the isolate was used as a source for the production of polysaccharide dextran (Addala Lakshmi Bhavani and Sundar, 2014).

This dextransucrase producing isolate was used for dextran synthesis with sucrose as the inducer.

Stock Culture Maintenance

Pediococcus pentosaceus (PH3) isolate was maintained in test tubes containing MRS medium (Peptone 1 g**,** Beef extract 1 g**,**Yeast extract 0.5g, Glucose 2.0 g, Tween 80 0.1 ml, Na_2HPO_4 0.2 g, Sodium acetate 0.5 g, Triammonium citrate 0.5 g, $MgSO_4$ $7H_2O$ 0.02g, $MnSO_4$ $4H_2O$ 0.02 g, Agar 2.0 gms, Distilled water 100ml, pH 6.2 – 6.6), at 15^oC, and was constantly sub-cultured at regular intervals into fresh medium.

Production and Extraction of Dextran

Modified media of Tsuchiya *et al* 1952 was used for production of dextran from the isolate *Pediococcus pentosaceu (PH3).* For fermentation process the organism was grown at 25^0C, in a medium(g/100ml) containing Sucrose , 2.0; yeast extract ,0.5; K2HPO4, 2.0; MgSO4(7H2O) , 0.02; MnSO4(4H2O) 0.001; FeSO4(7H2O) , 0.001; CaCl2 ,0.001;NaCl , 0.001.The pH of the medium was adjusted to 6.9 before sterilization at 121^oC for 15 minutes The isolate was grown at 25^oC under shaking condition at 180 rpm for 18 Hrs. After incubation the fermentation media was centrifuged at 12,000 rpm for 10 min at 4°C. The pellet obtained was discarded and to the supernatant, twice the volume of absolute ethanol was added and incubated overnight at 4°C. Centrifugation at 12,000 rpm for 10 minutes at 4°C was performed to pellet out the slimy Dextran. The pellet was washed thrice with distilled water by repeated centrifugation at 12,000 rpm for 10 minutes at 4°C. The samples were kept in hot air oven at 80°C for 2 h to attain proper drying of the samples. Finally, dried sample were weighed and the total yield obtained (modified Qader *et al*, 2001).

Spectroscopic analysis

Fourier transforms infrared spectroscopy (FT-IR)

The FT-IR spectrum of the produced sample was obtained at a resolution of 4 cm^{-1}. The sample was incorporated into KBr (spectroscopic grade) and pressed into a 2 mm pellet.IR spectra (Marzieh Moosavi-Nasab *et al* 2010) were recorded in the transmittance mode from 4000 to 400 cm^{-1}

Determination of Molecular weight

Dextran standard and Dextran sample were dissolved in distilled water at a concentration of 2.0 mg/mL and analyzed on an WATERS 1525 Binary HPLC pump system equipped with a Empower software and a UV detector (WATERS -Dual Absorbance- range 190nm-700nm), Gel column (4.6 x 250mM Length, particle size 5micrometer) and the sample injection valve with size of loop of 20ul. The column and detector compartment were maintained at 30°C and 35°C, respectively. Distilled water was used as mobile phase at a flow rate of 1.0 mL/min and injection volume was 20 µL. Eluent used was acetonitrile and water (90:10) at a flow rate of 0.5mL/Min with a pressure maintained at 500psi. The molecular weight of sample Dextran was determined by comparing with the standard.

SEM Analysis

A little amount of dried sample of dextran was applied to the SEM stub by means of an adhesive tape and coated with 10nm Au in a sputter coater. The surface of the sample was visualized in SEM (JEOL, Model JSM 6390, Japan), operated at different accelerated voltages 10.0, 20.0 kV at various magnifications.

RESULTS AND DISCUSSION

The dextransucrase producing isolate of *Pediococcus pentosaceus* from brined cucumber was used for dextran synthesis. For which the isolate was grown in the modified enzyme production media. Cucumbers are laden with numerous and variable microflora, but a small number of LAB that are responsible for fermentation (Etchells *et al* 1973).Pediococci are Gram-positive LAB commonly found on fermenting plant material and are commercially used as starters in meat, vegetable and silage fermentation (Fleming and McFeeters 1981; Smith and Palumbo 1983) .

The commonly known dextransucrase is also known as Glucansucrase that is responsible for dextran (glucan) synthesis from sucrose and it catalyzes the transfer of glucosyl residue from sucrose to the growing glucan polymer, liberating fructose as a byproduct (Sidebotham, 1974).The free glucosyl moieties are polymerized to form the homopolysachharide dextran ,which has numerous industrial and medical applications (Neubauer *et al* 2003).

Dextran production

After 18 hours of incubation, in fermentation broth, the colour and viscosity of the media changed. The colour of the media changed from pale yellow to dark yellow. The watery media became more viscous. As the incubation time, prolonged after 24 hours the viscosity of the medium decreased.

Precipitation of dextran

After ethanol precipitation, dextran was obtained in a gelatinous form, which was slightly whitish yellow coloured, rather sticky and had high elasticity; the gelatinous dextran was obtained as a pale yellow gummy mass. Its weight was 0.10g/ ml.

The purified dried dextran sample was used for the investigation of its functional groups, molecular weight and surface morphology.

FT - IR

This technique was used to investigate the nature of functional groups of Dextran produced in terms of monomeric units and their linkage. In the present study the spectral data obtained (Fig.1) for dextran produced by *Pediococcus pentasaceus* exhibited major characteristic peaks which are destined for α (1 -6) linkage in dextran (Purama *et al*, 2009; Seymour *et al* 1980 ; Shingel 2002; Wang *et al* 2007). The spectrum of dextran was studied in the region 400$cm^{-1}$and 4000cm^{-1} .The band in the region of 33940.7 cm^{-1} is due to the hydroxyl stretching vibrations of polysaccharides. The band in the region of 2932 cm^{-1} is due to C-H stretching vibration and the band region of 1599.64 cm-1 is due to the carboxyl group.

It was evidenced that the bands around 3400cm^{-1}, 2939cm^{-1}, 990-1200cm^{-1} are common to all polysaccharides representing O-H stretching: C-H stretches of the $–CH_2$ groups and saccharides respectively (Freitas *et al* 2009). The bands in the regions of 3400cm^{-1} represent the hydroxyl stretching vibration of the polysaccharide (Liu *et al* 2008).

The main characteristic bands found in the spectra of Dextran at 1157$cm^{-1}$and 1041cm^{-1} are due to the valent vibrations of C-O and C-C bonds and deformation vibrations of -CCH and –HCO bonds. The band at 1157 cm-1 is assigned to valent vibrations of C-O-C bond and glycosidic bridges.

It was suggested that the peak at 1103cm^{-1} is due to the vibration of the C-O bond at the C-4position of D- glucose (Shingel 2002). It was also observed that absorption peaks at 916.19 cm-1 and 862.18 cm^{-1} are characteristics of (1-3)-α-D glucan. FTIR spectra analysis of dextran contains both α-(1-6) and α–(1-3) linkages.It was reported that peaks at 928.90, 846.34 and 820.86 cm-1 region are specifically distinctive for α – (1-3) glycosidic bond (Seymour *et al* 1980, Wang *et al* 2007)

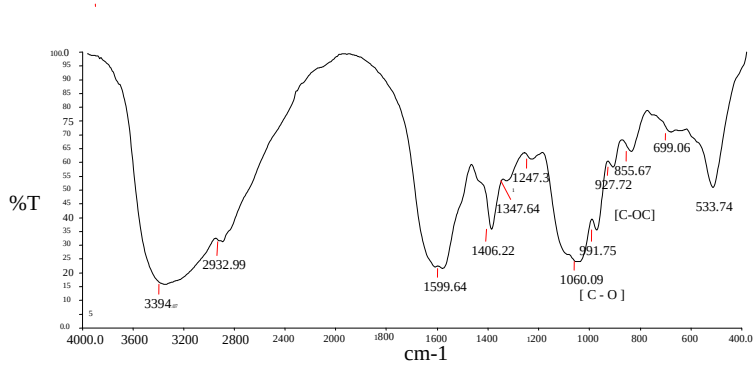


Figure 1 - FTIR spectrum of in house Dextran

Molecular weight of Dextran

The results suggest (Table 1, Fig.2 and 3) that dextran produced by *Pediococcus pentosaceus* has higher molecular weight (MW > 670,000 Da) based on the molecular mass of standard dextran (670,000 Da). The presence of higher concentration of sucrose in the culture media had a significant effect on molecular weight of the in-house produced dextran.
The average molecular weight of the dextran produced by *L. mesenteroides* was determined by gel permeation chromatography on LKB gel filtration system using blue dextran 2000 (average Mw 200 KD) as a standard in a study. The results suggested that dextran produced from sucrose had higher molecular weight (MW > 2000 KDa) based on the molecular mass distribution of blue dextran (approximately 2000 KDa) (Moosavi – Nasab 2009)

A simple method had been developed for determination of molecular weight distribution and average molecular weight by a HPLC system in a study. A common C18 column and a mobile phase of high water content (90%) were used in this system, and the oligosaccharide sample was derivatized with ABEE (4-aminobenzoic acid ethyl ester). In this system, a larger solute elutes faster than a smaller solute. The merit of this method is the fact that standards are not required since the signal intensity of each ABEE-oligosaccharide is generally proportional to its molar concentration regardless of its molecular weight. The applicability of this method has been examined for a synthetic sample, a dextran sample and beer samples (Yoon Suk Baik and Won Jo Cheong, 2007)

Table No.1

S.No	Name	Retention Time	Injection Volume	RT	Area	Height	Molecular weight
1.	Std. Dextran	25.00 Min	20.00µl	5.847	1401425	98194	670,000Da
2.	Sample Dextran	25.00Min	20.00µl	5.951	2015255	275795	≥ 670,000Da

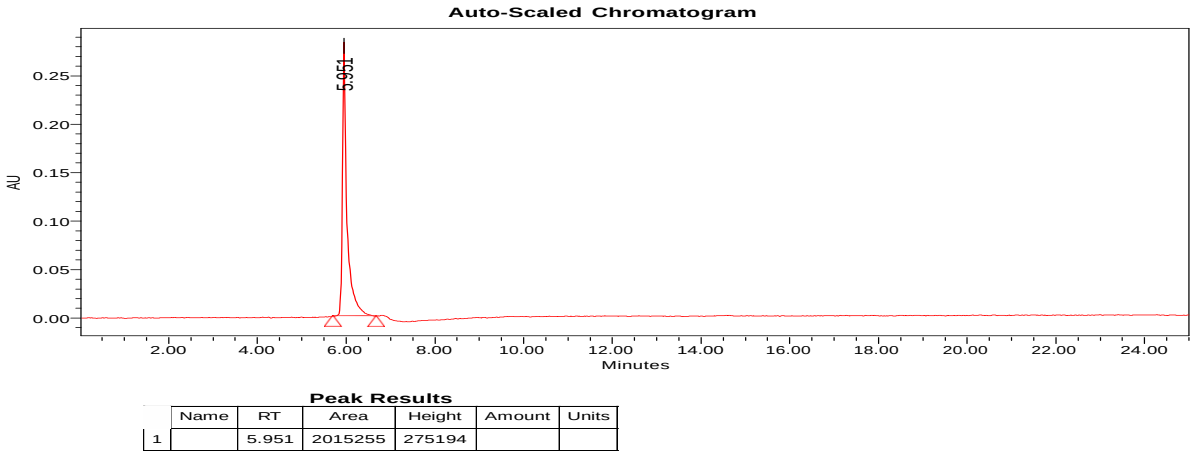
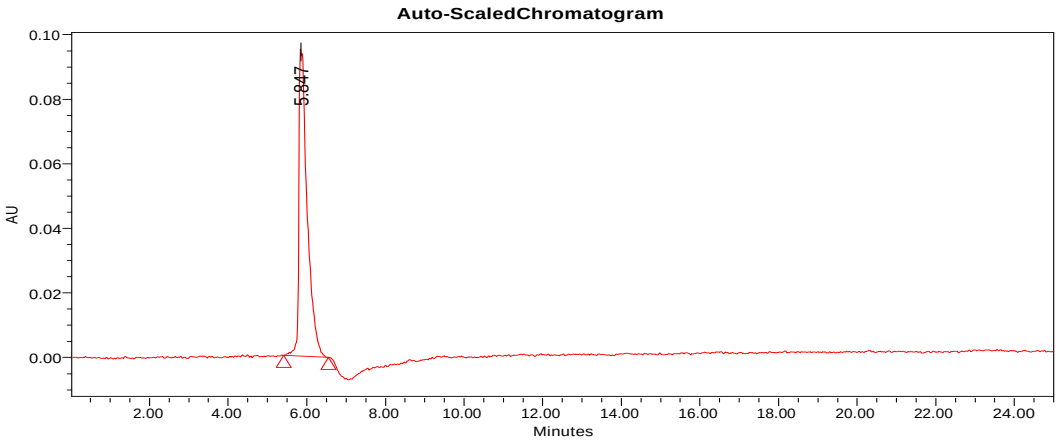


Fig. 2 The chromatogram of in house dextran



PeakResults

	Name	RT	Area	Height	Amount	Units
1		5.847	1401425	98194		

Fig. 3 The chromatogram of standard dextran

Scanning Electron Microscopy

The surface homogeneity and morphology of dextran had been investigated using SEM. The surface morphology of dextran of the natural isolate *Pediococcus pentosaceus* was revealed by the results of the scanning electron micrograph at various magnifications. Numerous pores were observed in the reticular surface of dextran (Fig.4).At different resolutions a highly compact mesh was observed with small pore size distribution predicting that the dextran can seize substantial amount of water molecules in order to facilitate an even texture when solubilized in water based solution. The water soluble dextran which gives a smooth creamy texture is most likely utilized in food industry as texturizer, thickener, gelling, stabilizing and emulsifying agents (Khan, Park and Kwon, 2007).

The porous structure promises high water holding capacity and consequent potential to be used in industry. From the porous dextrans, hydrogels can be created by either physical or chemical crosslinking taking advantage of the abundant hydroxyl groups present on the (1-6) linked D-Glucose residues (Levesque *et al* 2005). Hydrogels being composed of hydrophilic polymeric network can absorb considerable amount of the water and exhibit compatibility with proteins and living tissues (Hoffman 2002). Hydrogels designed for tissue engineering scaffolds should also contain pores large enough to all migration, penetration and proliferation of living cells into the

wound bed. The key factor controlling the pore size volume, fraction and the inter connections are the composition of the network polymer chain and cross link density (Hoffman, 2002).

Structural and morphological analysis of dextran produced by *Leuconostoc mesenteroides* BA08 in a whey- supplemented media using SEM showed granular and porous or weblike structure (Vaibhaok Lule *et al* 2016). Similarly scanning electro-micrographs of glucan produced by *Leconostoc dextranicum* NRRL B-1146 showed small porous or weblike structure that facilitates water holding capacity, thus can be used as a texturizing agent in food industry (Majumdar, Goyal 2009).

It was recently reported that the dextran from *Pediococcus pentosaceus* holds potential usage as gelling agent in food industry and as drug delivery carriers (Patel *et al* 2010)

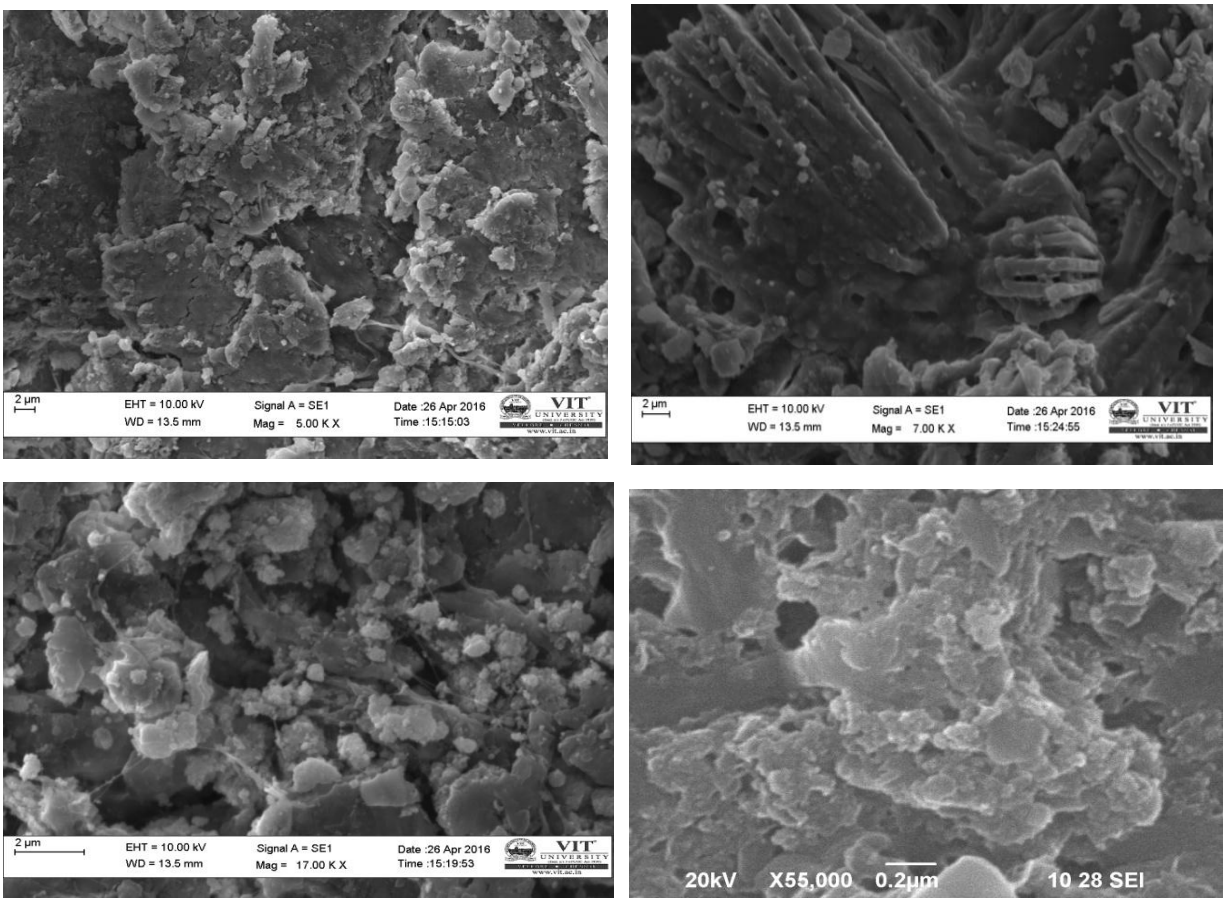


Fig.4 SEM images of dextran at various magnifications.

CONCLUSION

The polysaccharide produced by *Pediococcus pentosaceus* was dextran and its structural properties were analysed. The FT-IR analysis confirmed that the polysaccharide is linear dextran with α (1 -6) linkage and α – (1 -3) branching. The molecular mass determination supports the confirmation of the presence and production of polysaccharide. The surface morphology of dry powder form shows that its fibrous structure helps dextran to be used as a support and carrier material for drugs.

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