ISSN 1330-9862
(FTB-2746)

original scientific paper

Structural Characterization of Insoluble Dextran Produced by *Leuconostoc mesenteroides* NRRL B-1149 in the Presence of Maltose

*Rishikesh Shukla[1], Shraddha Shukla[1], Veselin Bivolarski[2], Ilia Iliev[2], Iskra Ivanova[3] and Arun Goyal[1]**

[1]Department of Biotechnology, Indian Institute of Technology Guwahati, 781 039 Guwahati, Assam, India

[2]Department of Biochemistry and Microbiology, Plovdiv University, 24 Tzar Assen Str, BG-4000 Plovdiv, Bulgaria

[3]Department of General and Applied Microbiology, Biological Faculty, Sofia University, 8 Dragan Tzankov Str, BG-1164 Sofia, Bulgaria

Received: September 13, 2010
Accepted: January 28, 2011

Summary

Leuconostoc mesenteroides NRRL B-1149 is known to produce dextran with 52 % α-(1→6) and 40 % α-(1→3) linkages. Low solubility of dextran in water is associated with the presence of high percentage of α-(1→3) linkages. *Leuconostoc mesenteroides* NRRL B-1149 produces two types of enzymes, dextransucrase and fructansucrase, which are active with sucrose and raffinose, respectively, as confirmed by the activity staining. The insoluble dextran was synthesized using partially purified dextransucrase in the presence of maltose. A water-soluble dextran was also produced by dextransucrase from *Leuconostoc mesenteroides* NRRL B-1149. The produced insoluble dextran was purified by alcohol precipitation, and then structurally characterized using FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopic techniques. From the spectral analysis, it was confirmed that the insoluble dextran produced by *Leuconostoc mesenteroides* NRRL B-1149 contained dextran with α-(1→6) linkages and α-(1→3) branched linkages. The surface morphology of dried and powdered dextran studied using scanning electron microscopy revealed its fibrous structure.

Key words: dextransucrase, *Leuconostoc mesenteroides* NRRL B-1149, dextran, insoluble dextran, FTIR, NMR, SEM

Introduction

Dextransucrase is a glucosyltransferase (EC 2.4.1.5) which catalyses the synthesis of polysaccharide called dextran from sucrose (*1*) according to the following equation:

$$n(\text{sucrose}) \rightarrow \text{dextran} + n(\text{fructose}) \qquad /1/$$

Dextrans feature substantial number of consecutive α-(1→6) linkages in their main chain, usually comprising more than 50 % of the total linkages. These α-D-dextrans also possess side chains with α-(1→4), α-(1→3), or α-(1→2) branched linkages. A survey was done of 96 strains of dextran-producing bacteria to classify dextrans by their structure and properties and identify a suitable strain for pharmaceutical industry (*2*). Different types of dextrans of various size and structure were synthesized depending on the dextransucrase produced by the strain (*3–5*). The nature and the frequency of the other linkages present are highly dependent on the glucosyltransferase-producing strain (*2*). In this way, the most widely stud-

*Corresponding author; Phone: ++91 361 258 2208; Fax: ++91 361 258 2249; E-mail: arungoyl@iitg.ernet.in

ied polymer, dextran from *Leuconostoc mesenteroides* NRRL B-512F, contains about 95 % of α-(1→6) linkages and only 5 % of α-(1→3) branched linkages (*2*), while dextran from *L. mesenteroides* NRRL B-1299 is composed of 65 % α--(l→6) linkages, 30 % α-(1→2) linkages and 5 % α-(1→3) branched linkages (*6,7*). *Leuconostoc mesenteroides* NRRL B-1149 is known to produce branched dextran with 52 % α-(1→6) and 40 % α-(1→3) linkages (*2*).

Polysaccharides exist widely in all living organisms and play extensive biological roles (*8*). As compared to other polysaccharides, the (1→3)-α-D-glucan isolated from the cell wall of fungi, especially the (1→3)-β-D-glucan, has not been investigated much (*9,10*). The β-D-glucan had received considerable attention due to its immunomodulatory properties, such as adjuvant, antimicrobial, antitumour, and radiation-protective activities. The structure and solution properties of a few (1→3)-α-D-glucans from different sources have been studied by some groups, for example, a few short side chains of (1→6)-α- or (1→4)-α-linked D-glucose residues attached to the O6 of the (1→3)-α-D-glucan backbone are produced by *Streptococcus mutans* (*11*) and *Streptococcus salivarius* (*12*); (1→3)--α-D-glucan with a few (1→6) branched linkages by *Lentinus edodes* (*13*); linear (1→3)-α-D-glucan by *Poria cocos* mycelia (*14*). Some bioactive and medicinal properties of (1→3)-α-D-glucan derivatives have also been reported. The antitumour activity of sulphated linear (1→3)-α-D-glucan from the fruiting body of *Agrocybe cylindracea* (DC. ex Fr.) Maire (Bolbitiaceae) was obtained, and its antitumour activity against Ehrlich ascites carcinoma was investigated (*15*). In addition, a carboxy-methylated derivative of (1→3)-α-D-glucan with the activity against sarcoma 180 was synthesized by substitution with chloroacetic acid (*16*). The relationship between the nature of functionalized groups and physicochemical properties of the chemically modified derivatives of linear (1→3)-α-D-glucan, obtained from the spores of *Ganoderma lucidum*, and their immunomodulating activity were reported (*8*). In the present study, a water-insoluble dextran produced by dextransucrase of *Leuconostoc mesenteroides* NRRL B-1149 has been purified by alcohol precipitation. The structure of the dextran was analyzed by Fourier transform infrared (FTIR), nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectroscopic and scanning electron microscopy (SEM) techniques.

Materials and Methods

Microorganism

Leuconostoc mesenteroides NRRL B-1149 was procured from Agriculture Research Service (ARS Culture Collection), USDA, Peoria, USA.

Identification of fructansucrase and dextransucrase by activity staining

The cell-free supernatant (CFS) of *Leuconostoc mesenteroides* NRRL B-1149 was analyzed for fructansucrase activity by running 7 % acrylamide gels using denaturing (SDS) discontinuous method. Electrophoresis was performed on a mini gel unit (Bio-Rad, Hercules, CA, USA) using 1.5-mm thick gels, following the method of Laemmli (*17*). After the run, the gel was cut in three parts. One part contained the molecular mass markers and bovine serum albumin (BSA) and was stained with Coomassie Brilliant Blue R250. The other two parts containing the lanes with cell-free supernatant were washed three times with a solution of 20 mM sodium acetate buffer (pH=5.4) containing 0.1 % Triton X-100 and 0.005 % calcium chloride for 20 min each to remove SDS. Then, one part of the gel was incubated with 5 % raffinose solution in 20 mM sodium acetate buffer (pH=5.4), and the other part of the gel was incubated with 10 % sucrose solution in 20 mM sodium acetate buffer (pH=5.4) for 10–12 h (*18,19*). After the incubation, the gels were washed twice with 75 % ethanol for 20 min and incubated in a solution with 0.7 % periodic acid in 5 % acetic acid for 20 min at room temperature. The gels were then washed three times with 0.2 % sodium bisulphate in 5 % acetic acid solution and finally stained with Schiff's reagent (0.5 % by mass per volume basic fuchsin, 1 % sodium bisulphite and 0.1 M HCl) until the discrete magenta bands appeared within the gels to confirm the presence of both forms of enzymes, fructansucrase and dextransucrase.

Production and purification of insoluble dextran

For the production of insoluble dextran, 10 % sucrose and 5 % maltose were added to 30 mL of 20 mM sodium acetate buffer (pH=5.4) containing 0.1 % sodium azide (*18*) using 600 μL of purified dextransucrase (0.2 mg/mL, 13 U/mg) (*20*). The reaction mixture was then incubated for 24 h at 28 °C and 180 rpm. After incubation, the mixture was put in a boiling water bath for 10 min and then centrifuged at 10 000 rpm for 10 min. The pellet containing insoluble dextran was washed three times, precipitated with 65 % ethanol as final concentration and then lyophilized.

FTIR and NMR spectroscopic analyses of insoluble dextran

The FTIR spectrum using spectrometer (Spectrum One FTIR spectrometer, PerkinElmer Instruments, San Jose, CA, USA) was recorded for purified dextran and insoluble dextran in a KBr pellet. Nuclear magnetic resonance (NMR) was performed in a Varian AS400 spectrometer (Agilent Technologies, Palo Alto, CA, USA). The insoluble dextran was vacuum dried and then exchanged with deuterium by successive lyophilization steps in D_2O (99.6 % atom ^{2}H, Sigma-Aldrich, St. Louis, MO, USA) and 15 mg were dissolved in 0.4 mL of D_2O for ^{1}H NMR and 30 mg were dissolved in 0.4 mL of D_2O for ^{13}C NMR. Tetramethyl silane (TMS) was used as an internal reference.

Scanning electron microscopy of insoluble dextran

A sample of the dried dextran was attached to the SEM stub with a double-sided tape. The sample was coated with ~10 nm Au in a sputter coater (SC 7640, Leo Electron Microscopy Ltd, Cambridge, UK). The surface of the sample was viewed in scanning electron microscope (Leo 1430VP, Leo Electron Microscopy Ltd) operated at 10.0 kV.

Results and Discussion

Confirmation of enzymes by activity staining

The activity staining of the gel showed positive results with both raffinose and sucrose. Two magenta colour bands appeared on the gel incubated with 5 % raffinose, showing the existence of two molecular forms of fructansucrase (Fig. 1, lane 4). Three magenta colour

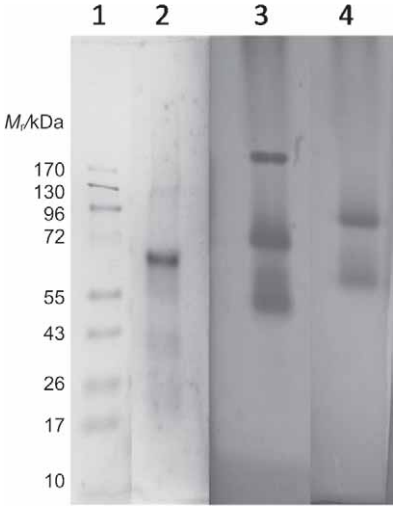

Fig. 1. Activity staining for enzymes from *L. mesenteroides* NRRL B-1149. Lane 1: molecular mass marker, lane 2: BSA, lane 3: activity staining using CFS with 10 % sucrose, lane 4: activity staining using CFS with 5 % raffinose

bands appeared on the gel incubated with 10 % sucrose due to the formation of dextran on the gels (Fig. 1, lane 3), which confirmed three active molecular forms of dextransucrase. These results showed that *Leuconostoc mesenteroides* NRRL B-1149 produced both fructansucrase and dextransucrase, although the former at a lower level.

Production and purification of dextran

Insoluble dextran was synthesized by extracellular dextransucrase using sucrose as the glucosyl donor along with maltose as an acceptor molecule. The insoluble dextran was purified by alcohol precipitation. The ethanol-precipitated insoluble dextran was lyophilized and further structurally characterized using FTIR and NMR spectroscopic techniques.

FTIR analysis

This technique was used to investigate the functional groups of commercial dextrans and pullulans and their nature in terms of monomeric units and their linkages (*21*). Some glucans with anticancer properties were also characterized using FTIR spectral data (*22,23*). The FTIR spectrum of insoluble dextran is shown in Fig. 2. The band in the region of 3434 cm^{-1} is due to the hydroxyl stretching vibration of the polysaccharide (*23*). The band in the region of 2928 cm^{-1} is due to C–H stretching vibration and the band in the region of 1641 cm^{-1} is due to carboxyl group (*22,23*). The main characteristic bands found in the spectra of dextran at 1146 and 1021 cm^{-1} are due to valent vibrations of C-O and C-C bonds and deformation vibrations of the CCH and HCO bonds.

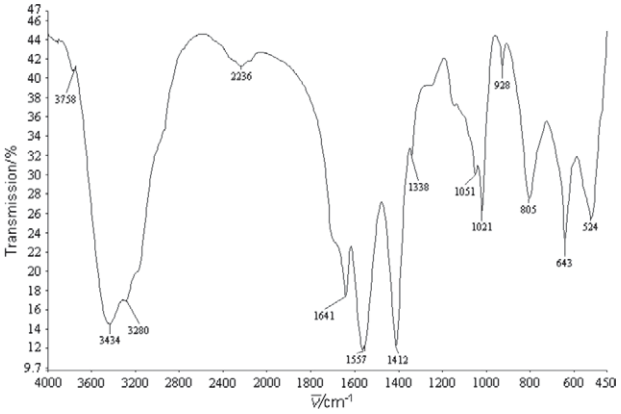

Fig. 2. FTIR spectrum of insoluble dextran from *Leuconostoc mesenteroides* NRRL B-1149

The band at 1146 cm^{-1} is assigned to valent vibrations of C–O–C bond and glycosidic bridge. The presence of a peak at 1021 cm^{-1} is due to the great chain flexibility present in dextran around the glycosidic bonds, as shown earlier (*21*). It was also observed that the absorption peaks at 928.90, 846.34 and 820.86 cm^{-1} were characteristic of (1→3)-α-D-glucan (*24,25*). FTIR spectral analysis of dextran from *L. mesenteroides* NRRL B-1149 showed that it contains both α-(1→6) and α-(1→3) linkages. This was further confirmed by ^{1}H NMR and ^{13}C NMR analyses.

^{1}H NMR analysis of insoluble dextran

The anomeric proton resonances for the 400 MHz ^{1}H NMR spectrum of dextran are shown in Fig. 3. *L. mesenteroides* NRRL B-1149 was shown to produce dextran with linear linkages having α-(1→3) branch points. It has been reported that various dextrans have ^{1}H NMR spectral resonances (C-2, C-3, C-4, C-5 and C-6) in the 3–4 ppm region and the hemiacetal C-1 resonance in 4–6 ppm region (*26*). The ^{1}H NMR spectral region for anomeric carbon of dextran from *L. mesenteroides* NRRL B-1355 contained a resonance at 4.95 ppm and the branched

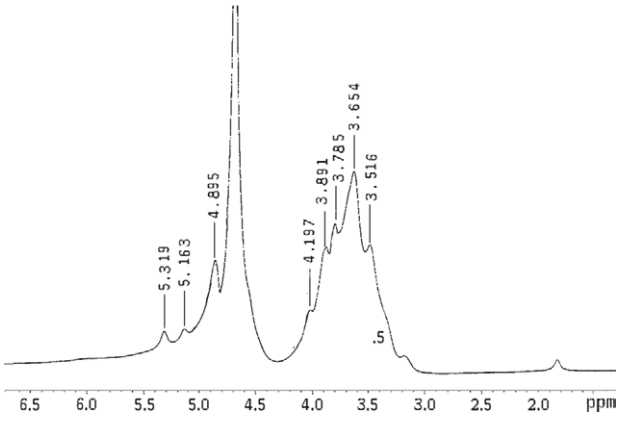

Fig. 3. ^{1}H NMR (400 MHz, D_2O) spectrum of insoluble dextran from *L. mesenteroides* NRRL B-1149

linkages contained the resonance peak at 5.3 ppm (*27*). In this study, the ^{1}H NMR spectra contained a resonance peak in the region of 4.895 ppm and branching at 5.319 and 5.163 ppm (Fig. 3). Seymour *et al.* (*28*) observed the distribution of ^{1}H NMR spectral resonances between 3 and 6 ppm for different dextrans. The resonance at 4.89 ppm is due to the H-1 of α-(1→6) glucosyl residues of the main chain (*28*). Table 1 shows the assignments for different resonances of ^{1}H and ^{13}C NMR.

Table 1. ^{1}H and ^{13}C NMR chemical shifts of dextran from *L. mesenteroides* NRRL B-1149

Atoms	1	2	3	4	5	6
^{1}H	4.89	3.65	3.78	3.51	3.89	4.19
^{13}C	97.89	71.65	73.55	69.80	70.39	65.71

^{13}C NMR analysis of dextran

For the carbohydrate compounds, it has been previously demonstrated that C-2, C-3, C-4 and C-5 chemical shifts are normally found in 70–75 ppm region and the C-1 carbon, which is an anomeric carbon, shows a downfield chemical shift of about 90 ppm, while C-6 displays an upfield chemical shift (60 ppm) (*29*). The insoluble dextran from *L. mesenteroides* NRRL B-1149 showed six prominent ^{13}C NMR resonances at 100 MHz: 97.946, 71.581, 73.572, 69.652, 70.460 and 65.754 ppm (Fig. 4), which are the characteristics of linear dextran (*27,30*). Apart from these six peaks, which correlate with six signals of linear dextran, the spectra of insoluble dextran also contain minor peaks indicative of branching. According to Seymour *et al.* (*31*), the resonances of C-2, C-3, C-4 and C-5 are displaced downfield into the 75–85 ppm region, which is the resonance region known for branched linkages. There was a weak signal at 100.946 ppm, which was downfield of peak at 98.24 (97.89), meaning that the branching occurred only through 3,6-di-*O*-substituted residues (*32*). The peak that occurred at 80.969 ppm in continuation with six peaks of linear dextran indicated the presence of α-(1→3) linkage (*33*).

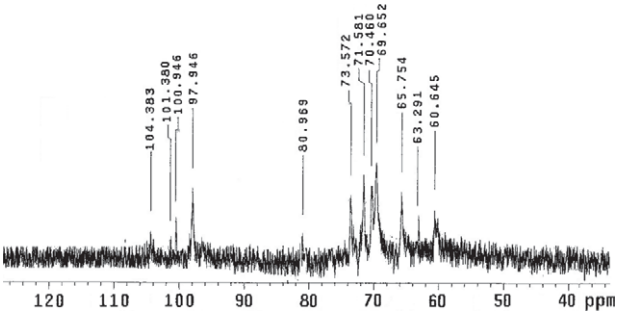

Fig. 4. ^{13}C NMR (100 MHz, D_2O) spectrum of insoluble dextran from *L. mesenteroides* NRRL B-1149

It was also observed that the β-anomeric carbon atoms have signals found downfield from 102 ppm (*28*) (104.383 in present case). The peak at 104.383 showed that the insoluble dextran from this strain may contain β-anomeric carbon which was formed due to the presence of fructansucrase. The resonance peaks obtained with the insoluble dextran from *L. mesenteroides* NRRL B-1149 were compared to dextrans from other strains using the data reported earlier (Table 2; *31*). The letters A–F in Table 2 refer to six major resonance peaks of the linear dextrans from the strains *L. mesenteroides* NRRL B-640, *L. mesenteroides* NRRL B-1399, *L. mesenteroides* NRRL B-1355 and *Streptobacterium dextranicum* B-1254 (*31*). It has been shown earlier that all dextrans contain six major resonance peaks for linear linkages and additional peaks for branching (*27,28,31,34*). The anomeric carbon and C-6 were involved in the chain that elongates α-(1→6) linkages and assigned to peaks A and F, respectively, while peaks B, C, D and E represent C-3, C-2, C-5 and C-4, respectively, which were not involved in the branching (*35*). The resonance spectrum obtained from the insoluble dextran from *L. mesenteroides* NRRL B-1149 was comparable to the three strains (Table 2), and the results show that the insoluble dextran may also contain α-(1→2) and α-(1→4) linkages along with α-(1→6) linked linear chain and α-(1→3) branching.

SEM analysis of insoluble dextran

The surface morphology of dried and powdered insoluble dextran formed by the enzyme was analyzed by scanning electron microscopy, as shown in Fig. 5. The SEM analysis revealed a porous structure. The insoluble dextran in the present study has less pores and hence low water holding capacity as compared to the other soluble dextrans, which may be because of more branching.

Table 2. ^{13}C NMR spectral data comparison of insoluble dextran from *L. mesenteroides* NRRL B-1149 with other strains

	α(1→6) linear dextran B-640	α(1→6) and α(1→2) linked D-glucan from B-1399*	α(1→6) and α(1→3) linked D-glucan from B-1355*	α(1→6) and α(1→4) linked D-glucan from B-1254*	Insoluble dextran from *L. mesenteroides* B1149 (this work)
A (C-1)	98.67	98.71, 97.22, 96.37	100.55, 100.29, 99.02	101.03, 100.76, 99.38, 98.70	101.38, 100.94, 97.94
B (C-3)	74.36	74.40	74.36	74.33	73.57
C (C-2)	72.37	72.40	72.62	72.42	71.58
D (C-5)	71.14	71.18	71.16	71.26	70.46
E (C-4)	70.52	70.54	70.62	70.44	69.65
F (C-6)	66.56	66.59, 61.38	66.13	66.55, 61.55	65.75, 60.64

A-F refer to six major resonance peaks of the linear dextran
*taken from Seymour *et al.* (*31*)

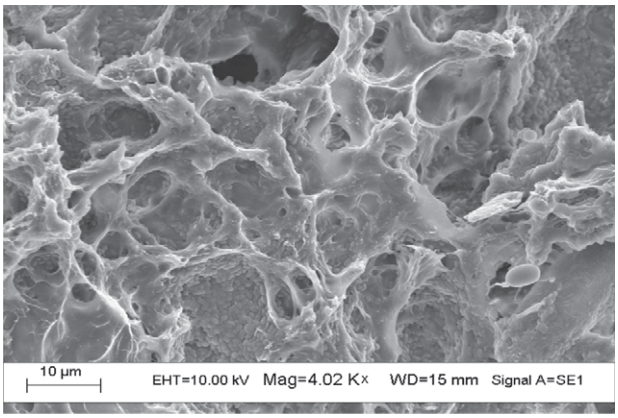

Fig. 5. SEM image of insoluble dextran from *L. mesenteroides* NRRL B-1149

Being insoluble, the dextran from *L. mesenteroides* NRRL B-1149 can be used as support material for enzymes and drugs.

Conclusions

The activity staining of enzymes produced by *Leuconostoc mesenteroides* NRRL B-1149 confirmed the presence of both dextransucrase and fructansucrase, which form dextran with the α-anomeric carbons and fructan with the β-anomeric carbons, respectively. The insoluble dextran produced by *L. mesenteroides* NRRL B-1149 was purified and its structural properties were analyzed. The FTIR, ^{1}H and ^{13}C NMR spectral analyses confirmed that the polysaccharide produced from *L. mesenteroides* NRRL B-1149 contains both linear dextran with α-(1→6) linkages and with α-(1→3) branching. However, the comparison of ^{13}C NMR results of other strains also showed the presence of α-(1→2) and α-(1→4) linkages. The presence of β-anomeric carbon was also observed in the results of ^{13}C NMR. The branched dextrans with α-(1→3) linkage have shown various applications such as adjuvant, antimicrobial, antitumour and radiation-protective activities. The surface morphology of dry powdered form of insoluble dextran showed that it has fibrous structure and can be used as support material for enzymes and drugs.

Acknowledgements

The authors would like to thank the Council of Scientific and Industrial Research (CSIR), India, and Department of Science and Technology (DST) of the Ministry of Science and Technology, New Delhi, India (for Indo--Bulgarian project) and Ministry of Education and Science, Bulgaria, for project grants.

References

1. E.J. Hehre, Polysaccharide synthesis from disaccharides, *Methods Enzymol. 1* (1955) 178–192.
2. A. Jeanes, W.C. Haynes, C.A. Wilham, J.C. Rankin, E.H. Melvin, M.J. Austin *et al.*, Characterization and classification of dextrans from ninety-six strains of bacteria, *J. Am. Chem. Soc. 76* (1954) 5041–5052.
3. T.D. Leathers: Dextran. In: *Biopolymers*, *Vol. 5*: *Polysaccharides I: Polysaccharides from Prokaryotes*, E.J. Vandamme, S. DeBaets, A. Steinbüchel (Eds.), Wiley-VCH, Weinheim, Germany (2002) pp. 229–321.
4. J.F. Robyt, Mechanism in the glucansucrase synthesis of polysaccharides and oligosaccharides from sucrose, *Adv. Carbohydr. Chem. Biochem. 51* (1995) 133–168.
5. F.R. Seymour, R.D. Knapp, Structural analysis of dextrans, from strains of *Leuconostoc* and related genera that contain 3-*O*-α-D-glucosylated-α-D-glucopyranosyl residues at the branch points, or in consecutive, linear position, *Carbohydr. Res. 81* (1980) 105–129.
6. M. Kobayashi, K. Shishido, T. Kikuchi, K. Matsuda, Fractionation of the *Leuconostoc mesenteroides* NRRL B-1299 dextran and preliminary characterization of the fractions, *Agr. Biol. Chem. 37* (1973) 357–365.
7. F.R. Seymour, M.E. Slodki, R.D. Plattner, A. Jeanes, Six unusual dextrans: Methylation structural analysis by combined g.l.c.-m.s. of per-*O*-acetyl-aldononitriles, *Carbohydr. Res. 53* (1977) 153–166.
8. S. Pérez, M. Kouwijzer, K. Mazeau, S.B. Engelsen, Modeling polysaccharides: Present status and challenges, *J. Mol. Graphics, 14* (1996) 307–321.
9. X. Bao, C. Liu, J. Fang, X. Li, Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst, *Carbohydr. Res. 332* (2001) 67–74.
10. T.R. Størseth, K. Hansen, J. Skjermo, J. Krane, Characterization of a β-D-(1→3)-glucan from the marine diatom *Chaetoceros mülleri* by high-resolution magic-angle spinning NMR spectroscopy on whole algal cells, *Carbohydr. Res. 339* (2004) 421–424.
11. S. Ebisu, A. Misaki, K. Kato, S. Kotaini, The structure of water-insoluble glucans of cariogenic *Streptococcus mutans*, formed in the absence and presence of dextranase, *Carbohydr. Res. 38* (1974) 374–381.
12. Y. Tsumuraya, A. Misaki, Structure of the water-insoluble α-D-glucan of *Streptococcus salivarius* HHT, *Carbohydr. Res. 74* (1979) 217–225.
13. P. Zhang, P.C.K. Cheung, Evaluation of sulfated *Lentinus edodes* α-(1→3)-D-glucan as a potential antitumor agent, *Biosci. Biotechnol. Biochem. 66* (2002) 1052–1056.
14. Y. Jin, L. Zhang, Y. Tao, C. Zeng, Y. Chen, P.C.K. Cheung, Solution properties of a water-insoluble (1→3)-α-D-glucan isolated from *Poriacocos* mycelia, *Carbohydr. Polym. 57* (2004) 205–209.
15. L. Zhang, M. Zhang, Q. Zhou, J. Chen, F. Zeng, Solution properties of antitumor sulfated derivative of α-(1→3)-D--glucan from *Ganoderma lucidum*, *Biosci. Biotechnol. Biochem. 64* (2000) 2172–2178.
16. T. Kiho, I. Yoshida, K. Nagai, S. Ukai, C. Hara, (1→3)-α-D--glucan from an alkaline extract of *Agrocybe cylindracea*, and antitumor activity of its *O*-(carboxymethyl)ated derivatives, *Carbohydr. Res. 189* (1989) 273–279.
17. U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature, 227* (1970) 680–685.
18. T. Vasileva, A. Kirilov, V. Bivolarski, M.S Bounaix, V. Gabriel, H. Robert *et al.*, Characterization of glycansucrase activities from *Leuconostoc mesenteroides* Lm17 and Ure 13 strains, *Biotechnol. Biotechnol. Eq. 23* (2009) 698–701.
19. A.W. Miller, A.W. Robyt, Detection of dextransucrase and levansucrase on polyacrylamide gels by the periodic acid--Schiff stain: Staining artifacts and their prevention, *Anal. Biochem. 156* (1986) 357–363.
20. R. Shukla, I. Iliev, A. Goyal, Purification and characterization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-1149, *Biotechnol. Biotechnol. Eq. 24* (2010) 576–580.

21. K.I. Shingel, Determination of structural peculiarities of dextran, pullulan and γ-irradiated pullulan by Fourier-transform IR spectroscopy, *Carbohydr. Res. 337* (2002) 1445–1451.

22. W. Cao, X.Q. Li, L. Liu, T.H. Yang, C. Li, H.T. Fan, Structure of anti-tumor polysaccharide from *Angelica sinensis* (Oliv.) Diels, *Carbohydr. Polym. 66* (2006) 149–159.

23. C. Liu, Q. Lin, Y. Gao, L. Ye, Y. Xing, T. Xi, Characterization and antitumor activity of a polysaccharide from *Strongylocentrotus nudus* eggs, *Carbohydr. Polym. 67* (2007) 313–318.

24. F.R. Seymour, R.L. Julian, A. Jeanes, B.L. Lamberts, Structural analysis of insoluble D-glucans by Fourier transform infrared difference-spectrometry: Correlation between structures of dextrans from strains of *Leuconostoc mesenteroides* and of D-glucans from strains of *Streptococcus mutans*, *Carbohydr. Res. 86* (1980) 227–246.

25. P. Zhang, L. Zhang, S. Cheng, Chemical structure and molecular weights of α-(1→3)-D-glucan from *Lentinus edodes*, *Biosci. Biotechnol. Biochem. 63* (1999) 1197–1202.

26. R.L. Sidebotham, Dextrans, *Adv. Carbohydr. Chem. Biochem. 30* (1974) 371–444.

27. F.R. Seymour, R.D. Knapp, E.C.M. Chen, A. Jeanes, S.H. Bishop, Structural analysis of dextrans containing 2-*O*-α-D--glucosylated α-D-glucopyranosyl residues at the branch points, by use of ^{13}C-nuclear magnetic resonance spectroscopy and gas–liquid chromatography–mass spectroscopy, *Carbohydr. Res. 71* (1979) 231–250.

28. F.R. Seymour, R.D. Knapp, S.H. Bishop, A. Jeanes, High temperature enhancement of ^{13}C-NMR chemical-shifts of unusual dextrans, and correlation with methylation structural analysis, *Carbohydr. Res. 68* (1979) 123–140.

29. P.A.J. Gorin, Carbon-13 nuclear magnetic resonance spectroscopy of polysaccharides, *Adv. Carbohydr. Chem. Biochem. 38* (1981) 13–104.

30. S. Uzochukwu, E. Balogh, R.T. Loefler, P.O. Ngoddy, Structural analysis by ^{13}C-nuclear magnetic resonance spectroscopy of glucan extracted from natural palm wine, *Food Chem. 76* (2002) 287–291.

31. F.R. Seymour, R.D. Knapp, S.H. Bishop, Determination of the structure of dextran by ^{13}C-nuclear magnetic resonance spectroscopy, *Carbohydr. Res. 51* (1976) 179–194.

32. F.R. Seymour, R.D. Knapp, E.C.M. Chen, S.H. Bishop, A. Jeanes, Structural analysis of *Leuconostoc* dextrans containing 3-*O*-α-D-glucosylated α-D-glucosyl residues in both linear--chain and branch-point positions, or only in branch-point positions, by methylation and by ^{13}C-N.M.R. spectroscopy, *Carbohydr. Res. 74* (1979) 41–62.

33. F.R. Seymour, R.D. Knapp, E.C.M. Chen, A. Jeanes, S.H. Bishop, Structural analysis of dextrans containing 4-*O*-α-D--glucosylated α-D-glucopyranosyl residues at the branch points, by use of ^{13}C-nuclear magnetic resonance spectroscopy and gas liquid chromatography–mass spectrometry, *Carbohydr. Res. 75* (1979) 275–294.

34. F.R. Seymour, R.D. Knapp, A. Jeanes, Structural analysis of levans by use of ^{13}C nuclear magnetic resonance spectroscopy, *Carbohydr. Res. 72* (1979) 222–228.

35. P. Colson, H.J. Jennings, I.C.P. Smith, Composition, sequence and conformation of polymers and oligomers of glucose as revealed by carbon-13 nuclear magnetic resonance, *J. Am. Chem. Soc. 96* (1974) 8081–8087.