Water Kefir grain as a source of potent dextran producing lactic acid bacteria

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Abstract

Water kefir is a beverage fermented by a microbial consortium captured in kefir grains. The kefir grains matrix is composed of polysaccharide, primarily dextran, which is produced by members of the microbial consortium. In this study, we have isolated lactic acid bacteria (LAB) from non-commercial water kefir grains (from Belgrade, Serbia) and screened for dextran production. Among twelve LAB isolates three produced slime colonies on modified MRS (mMRS) agar containing sucrose instead of glucose and were presumed to produce dextran. Three LAB were identified, based on morphological, physiological and biochemical characteristics and 16S rRNA sequencing, as *Leuconostoc mesenteroides* (strains T1 and T3) and *Lactobacillus hilgardii* (strain T5). The isolated strains were able to synthesize a substantial amount of dextran in mMRS broth containing 5% sucrose. Maximal yields (11.56, 18.00 and 18.46 g/l) were obtained after 16, 20 and 32 h for T1, T3 and T5, respectively. Optimal temperature for dextran production was 23°C for two *Leuconostoc mesenteroides* strains and 30 °C for *Lactobacillus hilgardii* strain. The produced dextrans were identified based on paper chromatography, while the main structure characteristics of purified dextran were observed by FT-IR spectroscopy. Our study shows that water kefir grains are a natural source of potent dextran producing LAB.

Keywords: dextran, water kefir grains, *Lc. mesenteroides*, *Lb. hilgardii*.

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Water kefir is a beverage produced by fermentation of sucrose solution supplemented with lemon slice and dry fruits, best figs [1]. Beverage is slightly carbonated, with refreshing and moderately sour taste. The fermentation is performed by a consortium of symbiotic microorganisms embedded in kefir grains. The microbiota of kefir grains varies depending on its origin and culture media used for fermentation [2]. The composition of the kefir grains' microbiota has been excessively studied [3–7]. It includes yeasts, acetic acid bacteria and lactic acid bacteria (LAB), with the last being the most abundant [6]. LAB of the kefir grains belong to the following genera: *Lactobacillus*, *Lactococcus* and *Leuconostoc* [3,4,8].

The main component of water kefir grain matrix is dextran [9]. This exopolysaccharide (EPS) is synthesized by indigenous LAB. Different authors reported different bacterial species as the predominant EPS producers in water kefir grains and these include *Lactobacillus casei* (two subspecies) [4], *Leuconostoc mesenteroides*, *Lactobacillus nagelii* and *Lactobacillus hordei* [6], as well as *Lactobacillus hilgardii* [3,8].

Dextran is a high-molecular-mass homopolysaccharide made of glucose. The glucose molecules in the main chain of dextran are linked by alpha 1-6 glycosidic bonds. Branching can occur at positions 2, 3 or 4 [10]. The proportion of branching and the chain length affects the rheological properties of EPS [11].

Due to its relative stability and good solubility, dextran is widely used in different branches of industries, such as medical, pharmaceutical, food, textile and chemical industries [12–16]. Insoluble dextran could be applied as matrix for immobilization of biomolecules [17].

Because dextran is an important polysaccharide that can be applied in various branches of industries, the aim of this study was to isolate LAB which produces dextran from water kefir grains. The present paper describes characteristics of three LAB isolates from water kefir grains that are able to produce dextran in large quantities. We describe conditions for the production of dextran by *Leuconostoc mesenteroides* originating from this source for the first time. Purified dextran samples were preliminary characterized by paper chromatography and FTIR spectroscopy.

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EXPERIMENTS

Isolation and screening for dextran producing LAB

Non-commercial water kefir grains (from a household in Belgrade, Serbia) were used for isolation of LAB. The grains (1 g) were homogenized in 9mL saline and used as inoculum for two different media: MRS and M17 agar. After two days of incubation at 30 °C, five to ten colonies from each agar plate were inoculated in MRS broth. They were re-streaked and purified strains were routinely propagated in MRS broth at 30 °C in micro-aerofilic condition for further characterization.

The first screening for dextran production was performed on modified MRS (mMRS) agar having 5% sucrose instead of glucose. Selected cultures were streaked on surface of agar plates. Agar media were incubated in micro-aerofilic conditions at 30 °C for 48 h. The slimy colonies were identified and propagated in mMRS broth. The amount of produced dextran was estimated colorimetrically by phenol–sulfuric acid method using glucose as a standard [18]. The absorbance was measured at 490 nm in spectrophotometer (Ultrospec 3300 *pro*Amersham Bioscience).

Identification of dextran-producing isolates

The three selected strains (T1, T3 and T5) were identified by phenotypic and molecular methods. The first tentative identification as LAB was performed according to morphological characteristics (colony morphology, Gram staining and cells shape) and physiological characteristics (catalase test, growth at different temperatures, growth in media with different concentration of NaCl). Further, isolates were examined for fermentation of different carbohydrate using the API 50 CHL system (BioMérieux, France).

The species level identification of selected LAB dextran-producing strains was confirmed by sequencing of the 16S rRNA encoding gene, using the following procedure: the total DNA extraction from pure cultures was done by phenol-chloroform method [19]. The 16S rRNA gene was amplified using the total DNA as template for PCR with primers UNI16SF (5'-GAG AGT TTG ATC CTG GC-3) and UNI16SR (5'-AGG AGG TGA TCC AGC CG-3'). PCR mixture contained 2.5 µL 10xPCR buffer (0.5 M KCl, 0.1 M Tris-HCl, pH 8.8 at 25°C and 0.8% Nonidet P40), 1.5 µL $MgCl_2$ (25 mM), 18.25 µLultrapure double distilled H_2O, 0.25 µLTaq polymerase (Fermentas, Lithuania) and 1 µL genomic DNA of the isolate. The reactions were carried out in GeneAmp 2700 PCR Cycler (Applied Biosystems, Foster City, CA, USA) programmed as follows: the initial denaturation of DNA for 5 min at 96 °C, 30 cycles of 30 s at 96 °C, 30 s at 55 °C, and 30 s at 72 °C; and the extension of incomplete products for 5 min at 72 °C. Resulting PCR amplicons were sequenced at Macrogen in Amsterdam, the Netherlands. The BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST) was used to determine the most related sequence in the NCBI nucleotide sequence database.

Production of dextran

Effect of temperature and fermentation time on dextran yield

To evaluate the influence of temperature and incubation time on the growth and dextran production, the three high yield dextran-producing strains (T1, T3 and T5) were grown in 100 mL of mMRS broth containing 50 g/L of sucrose in 300 mL Erlenmeyer flasks at 30 °C for 24 h. This overnight culture was used as inoculum in further experiments.

To study the influence of various temperatures on dextran production the strains were incubated at 23, 30 and 37 °C. Incubation lasted 16h for *Lc. mesentroides* T1, 20 h for *Lc. mesentroides* T3 and 32h for *Lb. hilgardii* T5.

To study the effect of time on dextran production, the culture media were incubated for different time intervals ranging from 0 to 48h. Fermentation was carried out at 23 °C for *Lc. mesenteroides* strains T1 and T3 and 30 °C for *Lb. hilgardii* T5. Dextran concentration, cell biomass and final pH of fermented culture broth were determined.

Isolation and purification of dextran

Samples of the fermented media for dextran quantification were treated in boiling water for 15 min to inactivate bacteria. Bacterial cells were removed by centrifugation at 7000 rpm for 15 min at 4 °C. Dextran was precipitated from the supernatant by addition of double volume ice cold 96% ethanol and kept overnight at –20 °C. The samples were centrifuged at 13500 rpm for 15 min at 4 °C. Dextran pellets were separated from the supernatant and dissolved in hot distilled water. The precipitation procedure was repeated twice. The final precipitate was dissolved in hot distilled water. Dextran samples were additionally purified by dialysis through 10 kDa membrane against distilled water at 4 °C for 48 h with 3–4 changes per day. The purified dextrans were then recovered by spray-drying (BÜCHI Mini Spray Dryer B-290, inlet temperature 140 °C, outlet temperature 80 °C) and stored at room temperature.

Characterization of dextran

Study of monomer composition

Thin layer chromatography (TLC) was performed to determine monomer composition of isolated dextrans. TLC was carried out using paper chromatography of the hydrolyzed polymers according to method described by

Bailer and Oxford [20]. Glucose, fructose and galactose were used as standards.

Study of Fourier-transformed infrared spectroscopy

The major structural groups of the purified dextrans were detected by Fourier-transformed infrared (FTIR) spectroscopy using KBr method.

FT-IR spectroscopy (Bomem MB100, Canada) was performed by mixing the sample, dextran powder (0.6 mg) with 200 mg of KBr. The mixture was tableted under a pressure of 350 MPa, and dried at 110 °C for 48 h before analysis. The analysis was performed at room temperature (27 °C) in the range of wavelengths of 400–4000 cm^{-1}, with a resolution of 4 cm^{-1}.

RESULTS

Identification of dextran-producing isolates

In the present study, 12 lactic acid bacteria were isolated from water kefir grains and were denoted as T1 to T12. Isolated bacteria were able to grow on MRS and M17 plates at 30 °C. All tested strains were catalase-negative and Gram-positive with various shapes ranging from long rods to coccoid rods (Table 1). Three isolates formed mucous colonies on mMRS agar (Fig. 1). They were selected for further studying and dextran production quantification and characterization.

Testing the growth at different temperatures showed that optimal temperature for all three strains was 30 °C. They all could grow at 37 °C but none could grow at 45 °C. The isolate T3 could grow at 15 °C, while T5 showed weak growth at this temperature. The isolate T3 was tolerant to NaCl at all tested concentrations (4, 6.5 and 9.6%), while T1 and T5 grew at 4 and 6.5% NaCl and did not grow in the presence of 9.6% NaCl. All three strains fermented glucose with gas production (Table 1).

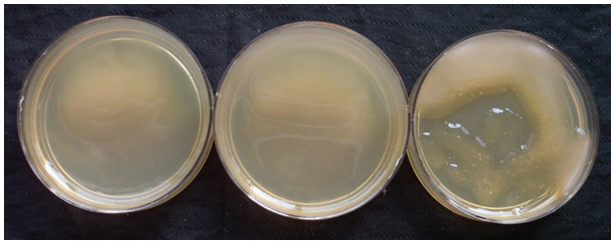

Figure 1. Picture of isolates T1 (left), T3 (middle) and T5 (right) growing on the surface of the mMRS agar with 5% sucrose plates.

API test was used for determination of fermentation pattern of isolates (Table 2).

According to physiological characteristics the isolates T1 and T3 belong to the genus *Leuconostoc*. The sugar fermentation patterns differed for several carbohydrates including arbutin, fructose, galactose, lactose, melibiose, salicin, trechalose, methyl-α-D-glucopyranoside, *N*-acetylglucosamine, gentibiose, inuline and turanose (Table 2). Based on the fermentation profile of the isolate T5 (Table 2), this isolate was preliminarily identified as *Lactobacillus hilgardii* according to Bergey's Manual of Systematic Bacteriology [21]. All three strains could ferment sucrose which is the main carbohydrate available during water kefir preparation.

Based on the 16S rRNA gene encoding sequence of the isolates T1 and T3 were identified as *Leuconostoc mesenteroides* (the isolates shared >99% sequence similarity with *Lc. mesenteroides* strain NCFB 529, acc NR_040817), while the isolate T5 was identified as *Lactobacillus hilgardii* (with 99% 16S rRNA encoding sequence similarity to strain NBRC 15886, acc NR_113817.1). Based on the 16S rRNA gene sequences the isolates and their close relatives, a phylogenetic tree was constructed by the use of ARB software [22] (Fig. 2).

Table 1. Morphological and physiological characteristics of the bacterial isolates; +: positive reaction, –: negative reaction and ±: weakly positive reaction

Characteristic		Isolate		
		T1	T3	T5
Gram reaction		+	+	+
Cell form		Elongated cocci	Elongated cocci	Long rods
Cell size, μm		0.76-0.92	0.86-1.00	15.56-18.30
Catalase		–	–	–
Growth at different temperatures, °C	15	–	+	±
	30	+	+	+
	37	+	+	+
	45	–	–	-
Growth in the present of different concentrations of NaCl, %	4	±	+	±
	6.5	±	±	±
	9.6	–	±	-
Glucose fermentation/gas		+/+	+/+	+/+

Table 2. Carbohydrates fermentation profiles of dextran-producing LAB strains after 48 h of incubation at 30 °C; +: positive reaction, –: negative reaction and ±: weakly positive reaction

Carbohydrate	Bacteria			Carbohydrate	Bacteria		
	Lc. mesenteroides		*Lb. hilgardii*		*Lc. mesenteroides*		*Lb. hilgardii*
	T1	T3	T5		T1	T3	T5
Control	–	–	–	Esculin	+	+	+
Glycerol	–	–	–	Salicin	+	–	–
Erythritol	–	–	–	Cellobiose	+	+	–
D-Arabinose	–	–	–	Maltose	+	+	+
L-Arabinose	–	–	+	Lactose	+	–	–
Ribose	–	w	+	Melibiose	+	–	–
D-Xylose	–	+	+	Sucrose	+	+	+
L-Xylose	–	–	–	Trehalose	–	+	–
Adonitol	–	–	–	Inulin	–	–	–
methyl-β-D-xylopyranoside	+	–	–	Melezitose	–	–	–
Galactose	+	–	–	Raffinose	–	–	–
Glucose	+	+	+	Amidon	w	–	–
Fructose	+	+	+	Glycogen	–	–	–
Mannose	–	+	–	Xylitol	–	–	–
Sorbose	–	–	–	β-Gentibiose	–	w	–
Rhamnose	–	–	–	Turanose	–	+	–
Dulcitol	–	–	–	Lyxose	–	–	–
Inositol	–	–	–	Tagatose	–	–	–
Mannitol	–	w	–	D-Fucose	–	–	–
Sorbitol	–	–	–	L-Fucose	–	–	–
methyl-α-D-mannopyranoside	–	–	–	D-Arabitol	–	–	–
methyl-α-D-glucopyranoside	–	+	–	L-Arabitol	–	–	–
N-Acetylglucosamine	–	+	–	Gluconate	–	w	+
Amygdalin	+	–	–	2-Ketogluconate	–	–	–
Arbutin	–	–	–	5-Ketogluconate	–	–	–

Dextran production

The temperature of incubation significantly influenced the amount of produced dextran during growth of all three isolates (Fig. 3). The isolates were grown on three temperatures of 23, 30 and 37 °C. For *Lb. hilgardii* T5 strain, the amount of synthesized dextran varied between the tested temperatures and was the highest at 30 °C, while both *Lc. mesenteroides* strains showed maximal dextran production at the lowest tested temperature. The maximum dextran amount for *Lc. mesenteroides* T1 and T3 strains was 11.56 and 18.00 g/l, respectively. There was a strong decrease in dextran yield at higher temperatures. T5 strain showed maximal dextran production of 18.46 g/L at the optimal growth temperature (30 °C).

In this study, dextran production by *Lc. mesenteroides* T1 and T3 strains and *Lb. hilgardii* T5 strain was monitored during 48 h in mMRS broth. The T1 and T3 strains were incubated at 23 °C and T5 strain was incubated at 30 °C.The production of dextran increased with time during the exponential growth phase and in the stationary started to decrease (Fig. 4). Dextran yield reached maximum after 16h and 20h of incubation for *Lc. mesenteroides* T1 and T3 strains, respectively. Prolonged time of fermentation had positive effect on dextran yield only for isolate T5 which could be explained by slower growth rate of this bacterium. The maximum dextran production for the isolate T5 was observed after 32 h of fermentation, which was the beginning of stationary phase of growth of this bacterium. The pH of the fermented broth decreased from the initial pH of 7.2 to 4.3 during fermentation (Fig. 4), due to the metabolic activity of isolates, primarily lactic acid production. Common for all three strains was that when the pH value dropped to 4.3, the degradation of dextran started, and the yield was lower.

Characterization of dextran

TLC was performed using paper chromatography with glucose, fructose and galactose as standards. Results show that glucose is monomer unit of poly-

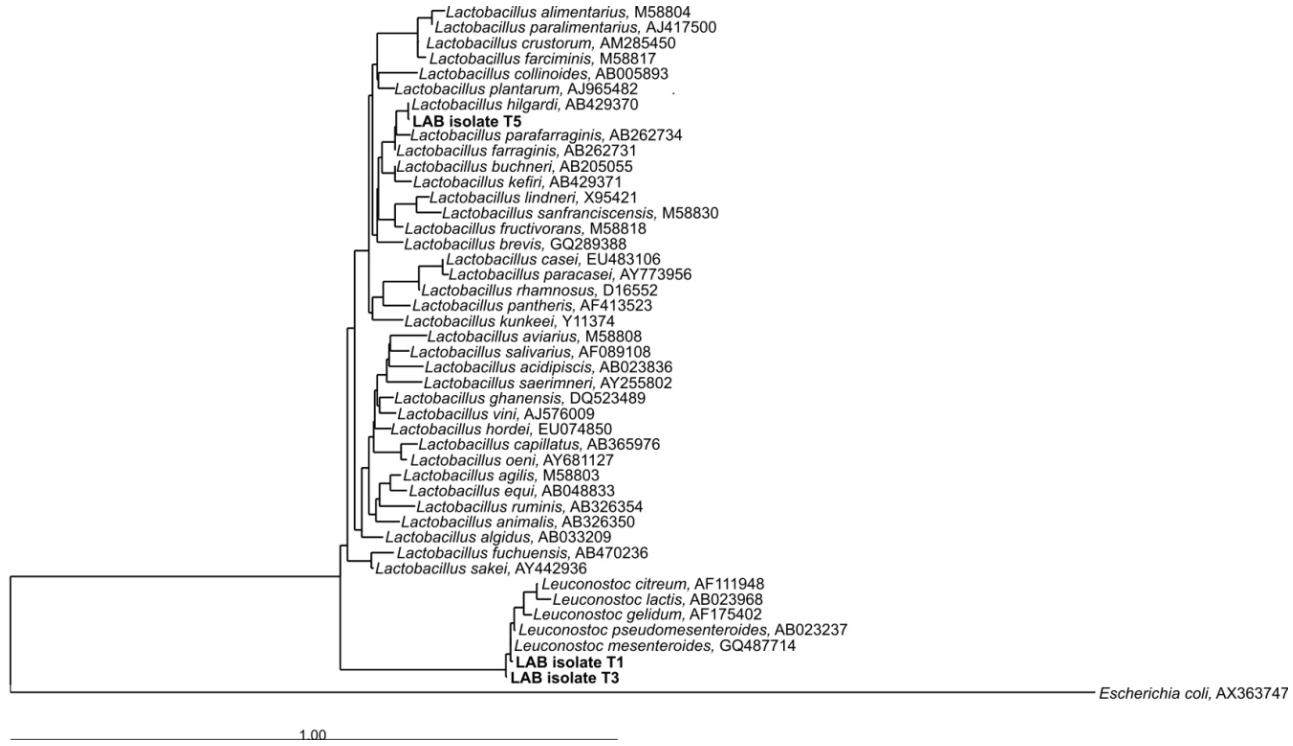

Figure 2. The phylogenetic tree of the LAB strains (T1, T3 and T5) isolated from water kefir grain and other members of Lactobacillus and Leuconostoc genera, constructed using the 16S rRNA gene sequences. The tree was constructed using the neighbor joining algorithm as implemented in ARB software. Escherichia coli is the root of the tree.

saccharides produced by all three studied isolates (T1, T3 and T5).

FTIR spectroscopy analysis confirmed polysaccharide nature of the extracellular material synthesized by the tested isolates (Fig. 5).

All samples analyzed on FT-IR had similar appearance in the area of 2000–4000 cm^{-1}. Intense and broad band in 3200–3500 cm^{-1} originated from the O–H stretching vibrations due to the influence of hydrogen bonds which is characteristic for polysaccharides molecules [23]. Peak around 2927 cm^{-1} is characteristic for the C-H stretching vibration.

There are slight differences in these spectra in the range of 400–2000 cm^{-1}. Broad signal from C–O–C and

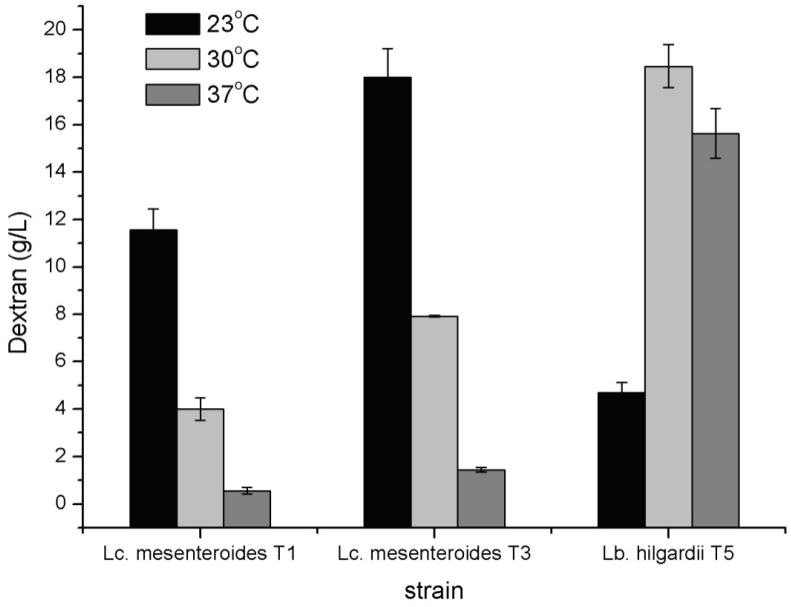

Figure 3. Effect of temperature on dextran production. The bars show the mean value of three replicates, while the vertical lines show the standard deviation of the measurements.

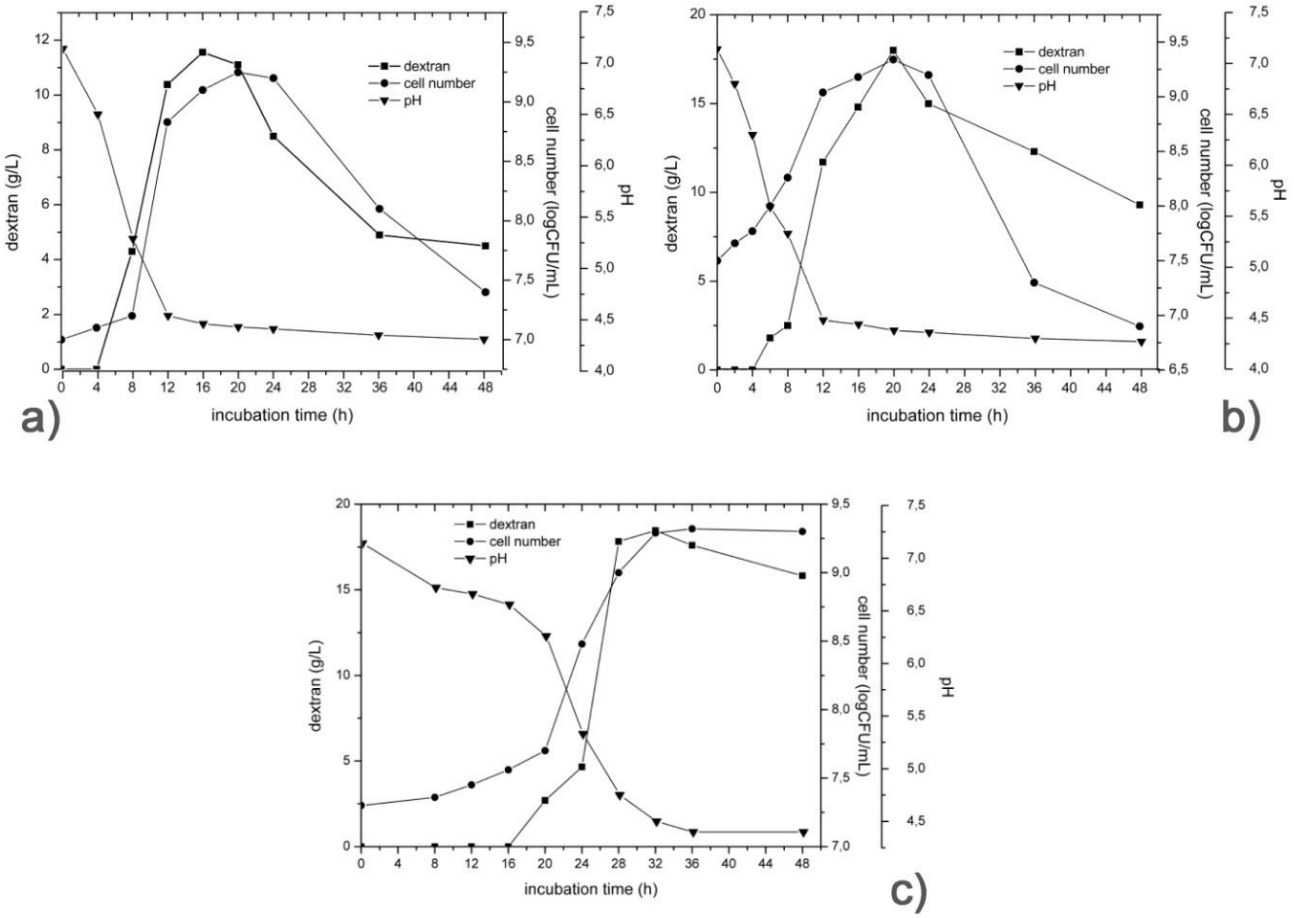

Figure 4. Effect of incubation time on dextran yield, final pH and cell growth for isolates T1 (a), T3 (b) and T5 (c) grown in mMRS broth with 5% sucrose at 23 °C (Lc. mesenteroides T1 and T3 strains) and 30°C (Lb. hilgardii T5 strain).

C–O at 1000–1200 cm^{-1} indicated the presence of the characteristic bonds of carbohydrates [24]. In this range the most intense peak for all samples are in the 1016–1020 cm^{-1} and 1030–1040 cm^{-1} which confirmed that the tested compounds are polysaccharides [25].

A characteristic absorption at 825–850 cm^{-1} indicates α-configuration of monomeric units. The absence of the signal at 900 cm^{-1} indicates the absence of β-configuration [26]. Based on the observed spectra, it was concluded that EPSs produced by all three analyzed isolates were of the same nature: homo-polysaccharides of glucose in α-configuration, and the EPS was identified as dextran.

DISCUSSION

The present study describes three new LAB strains isolated from water kefir grains that are able to produce dextran. Dextran production is a prevalent feature among LAB [27]. Dextran has numerous applications in food as well as in non-food industries [12–16], although a greater commercial use is limited by relatively small yields [28]. Most of LAB produce homopolysaccharides in quantities lower than 1g/L when growing under conditions that are not optimized for EPS production [29].

We have selected 3 among 12 LAB strains isolated from water kefir grain based on the slimy colony formation as potential dextran producers .The preliminary screening for dextran production showed that these three strains were potent dextran producers with yields ranging from 11.56 to 18.46 g/L. Dextran production yields obtained with our isolates is similar or exceeding the maximal yields of other reported dextran-producing bacteria [30–33]. In the study of Van der Meulen and colleagues, the total of 174 strains were isolated from cereal and dairy products, of which only 9 produced glucans with yields in range 0.8–17.2 g/L [30]. Majumder and coworkers reported dextran yield of 12 g/L produced by *Lc. mesenteroides* NRRL B-640 under optimized conditions [32]. This yield is similar with our strain *Lc. mesenteroides*T1, while strain *Lc. mesenteroides*T3 produced 33% more dextran from the same amount of sucrose in the medium. The strain *Lc. mesenteroides* T3 converted 36% of sucrose into dextran, which is higher than that reported by Sarwat and colleagues [31] in the study of *Lc. mesenteroides* CMG713 (32.5). Qader and coworkers optimized conditions for

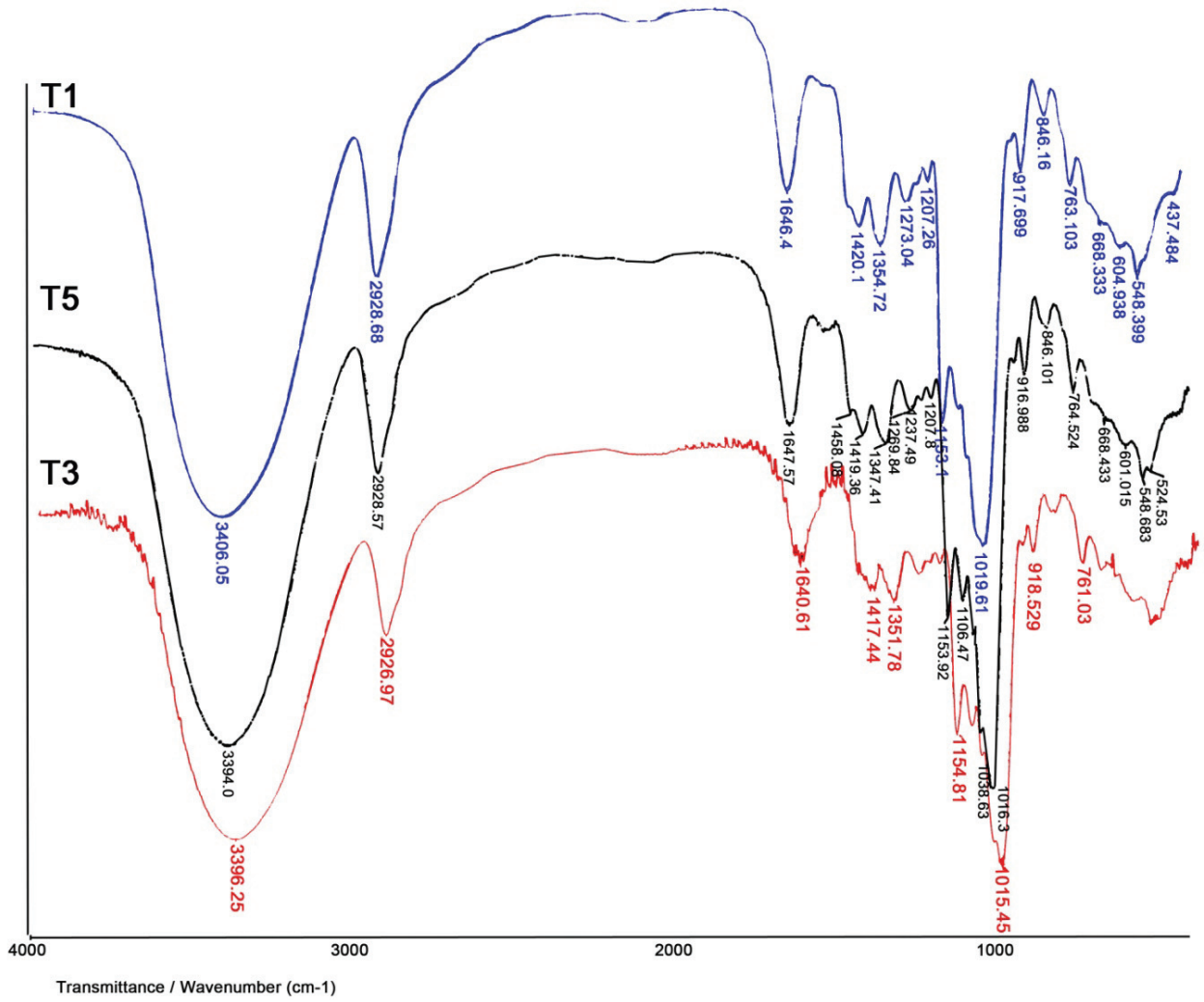

Figure 5. FT-IR spectra of the exopolysaccharides produced by the isolates Lc. mesenteroides T1, Lc. mesenteroides T3 and Lb. hilgardii T5.

dextran production by *Lc. mesenteroides* PCSIR-4 and achieved 47.8% conversion [34].

We determined the optimal temperature and incubation time for dextran production for the three analyzed strains. The optimal dextran production for *Lb. hilgardii* T5 was achieved at 30 °C, which is in agreement with previously published data [8]. *Lb. hilgardii* is responsible for dextran production and grain formation [8]. Glucose molecules in this dextran are linked by alpha 1-3 and alpha 1-6 glycosidic bonds in the main chain with additional alpha 1-3 bonds in the side chain. The presence of alpha 1-3glycosidic bonds in dextrans is known to render them insoluble in water [35].

The optimal temperature for dextran production by *Leuconostoc* strains is strain-dependent and varies from 23–30 °C [31,36,37]. Both *Leuconostoc mesenteroides* strains presented in this paper produced the highest amount of dextran at 23 °C.

Since conditions for dextran production are specific for each strain, they could be further optimized. Several factors including sucrose concentration, nitrogen source, mineral salts, initial pH medium and aeration can influence dextran production [32,33,38,39]. Further optimization of conditions that favor dextran production for our strains has a potential to achieve yields higher than ever reported in the literature, given that under tested conditions, the dextran yields were at level of exceeding those reported by others. Also, the product itself should be more detailed characterized. First, molecular mass should be determined, as well as factors affecting production of dextran desired mass, which is important for its application.

CONCLUSION

Our results show that water kefir grains are rich natural source of potent dextran-producing LAB. Our attempt to isolate dextran producing LAB from a non-commercial water kefir grains generated three strains, which yields of dextran production are exceeding those

reported in the literature, because of which these strains can be considered as candidates for commercial exploitation.

Acknowledgements

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REFERENCES

[1] J. Reiß, Metabolic activity of Tibi grains. Zeitschrift für Lebensmittel Untersuchung und Forschung **191** (1990) 462–465.

[2] H.H. Hsieh, S.Y. Wang, T.L. Chen, Y.L. Huang, M.J. Chen, Effects of cow's and goat's milk as fermentation media on the microbial ecology of sugary kefir grains, Int. J. Food Microbiol. **157** (2012) 73–81.

[3] M. Pidoux, The microbial flora of sugary kefir grain (the gingerbeer plant): biosynthesis of the grain from *Lactobacillus hilgardii* producing a polysaccharide gel, Mircen J. **5** (1989) 223–238.

[4] A. Galli, E. Fiori, L. Franzetti, M.A. Pagani, G. Ottogalli, Composizione microbiologica e chimica deigranuli di Kefir "di frutta", Ann. Microbiol. Enzimol. **45** (1995) 85––95

[5] L. Franzetti, A.Galli, M.A. Pagani, I. De Noni, Microbiological and chemical investigations on "sugar kefir" drink, Ann. Microbiol. Enzimol. **48** (1998) 67–80.

[6] A. Gulitz, J. Stadie, M. Wenning, M.A. Ehrmann, R.F. Vogel, The microbial diversity of water kefir, Int. J. Food Microbiol. **151** (2011) 284–288.

[7] M.G. da C.P. Miguel, P.G. Cardoso, K.T.Magalhães, R.F. Schwan, Profile of microbial communities present in tibico (sugary kefir) grains from different Brazilian States, World J. Microb. Biot. **27** (2011) 1875–1884.

[8] F.W. Waldherr, V.M. Doll, D. Meißner, R.F. Vogel, Identification and characterization of a glucan-producing enzyme from *Lactobacillus hilgardii* TMW 1.828 involved in granule formation of water kefir, Food Microbiol. **27** (2010) 672–678.

[9] M.Horisberger, Structure of the dextran of the tibi grain, Carbohyd. Res. **10** (1969) 379–385.

[10] J.F. Robyt, Mechanisms in the glucansucrase synthesis of polysaccharides and oligosaccharides from sucrose, Adv. Carbohydr. Chem. Biochem. **51** (1995) 133–168.

[11] S.J.F. Vincent, E.J. Faber, J.R. Neeser., F. Stingele, J.P. Kamerling, Structure and properties of the expolysaccharide produced by *Streptococcus macedonicus* Sc 136, Glycobiology **11** (2001) 131–139.

[12] P.T. Murphy, R.L. Whistler, in: R.L. Whistler and J.N. BeMiller (Eds.), Industrial Gums. Polysaccharides and Their Derivatives, 2nd ed, Academic Press, New York, 1973, pp. 513–542.

[13] M. Auerbach, D. Witt, W. Toler,M. Fierstein, R.G. Lerner, H. Ballard, Clinical use of the total dose intravenous infusion of iron dextran, J. Lab. Clin. Med. **111** (1988) 566–570.

[14] M. Naessens, A. Cerdobbel, W. Soetaert, E. Vandamme, Leuconostoc dextransucrase and dextran: production, properties and applications, J. Chem. Technol. Biotechnol. **80** (2005) 845–860.

[15] G. Lacaze, M. Wick, S. Cappelle, Emerging fermentation technologies: Development of novel sourdoughs, Food Microbiol. **24** (2007) 155–160.

[16] R. Sen, Biotechnology in petroleum recovery: The microbial EOR, Prog. Energ. Combust. **34** (2008) 714–724.

[17] R. Shukla, S. Shukla, V. Bivolarski, I. Iliev, I. Ivanova, A. Goyal, Structural Characterization of Insoluble Dextran Produced by *Leuconostoc mesenteroides* NRRL B-1149 in the Presence of Maltose, Food Technol. Biotechnol. **49** (2011) 291–296.

[18] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. **28** (1956) 350–356.

[19] J. Marmur, A procedure for the isolation of deoxyribonucleic acid from microorganisms, J. Mol. Biol. **3** (1961) 208–218.

[20] R.W. Bailer, A.E. Oxford, The Nature of the Capsular Polysaccharides of the Dextran-Producing Organisms *Leuconostoc mesenteroides*, *L. dextranicum* and *Streptococcus bovis*, J. Gen. Microbiol. **20** (1959) 258–266.

[21] P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.H. Schleifer, W.B. Whitman,Bergey's manual of systematic bacteriology, vol 3. Springer, Dordrecht, Heidelberg, London, New York, 2009.

[22] W. Ludwig, O. Strunk, R. Westram, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Förster, Brettske, S. Gerber, A.W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. König, T. Liss, R.Lüßmann, M. May, B. Nonhoff, B. Reichell, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, K.H. Schleifer, ARB: a software environment for sequence data, Nucleic Acids Res. **32** (2004) 1363–1371.

[23] K.J. Howe, K.P. Ishida, M.M. Clark, Use of ATR/FT-IR spectrometry to study fouling of microfiltration membranes by natural waters, Desalination **147** (2002) 251––255.

[24] P.J. Bremer, G.G. Geesey, An evaluation of biofilm development utilizing non-destructive attenuated total reflectance Fourier transform infrared spectroscopy, Biofouling **3** (1991) 89–100.

[25] S. Nataraj, R.Schomacker, M.Kraume, M.I. Mishra, A. Drews, Analyses of polysaccharide fouling mechanisms during crossflow membrane filtration, J. Membrane Sci. **308** (2008) 152–161.

[26] R.G. Zhbankov, V.M. Andrianov, M.K. Marchewka, Fourier transform IRand Raman spectroscopy and structure of carbohydrates, J. Mol. Struct. **436–437**(1997) 637–654.

[27] P. Ruas-Madiedo, C.G. de los Reyes-Gavilán, Methods for the Screening, Isolation, and Characterization of Exopolysaccharides Produced by Lactic Acid Bacteria, J. Dairy Sci. **88** (2005) 843–856.

[28] L. De Vuyst, B. Degeest, Expolysaccharides from lactic acid bacteria: technological bottlenecks and practical solutions, Macromol. Symp. **140** (1999) 31–41.

[29] M.L. Werning, S. Notararigo, M. Nácher, P. Fernández de Palencia, R.Aznar,P.López, Biosynthesis, Purification and Biotechnological Use of Exopolysaccharides Produced by Lactic Acid Bacteria. Food Additive, Prof. Yehia El-Samragy (Ed.), (2012) ISBN: 978-953-51-0067-6, InTech, (available from: http://www.intechopen.com//books/food-additive/biosynthesis-purification-and-iotechnological-use-ofexopolysaccharides-produced-by-lactic-acid-bac).

[30] R. Van der Meulen, S.Grosu-Tudor, F. Mozzi, F.Vaningelgem, M. Zamfir, G.Font de Valdez, L.De Vuyst, Screening of lactic acid bacteria isolates from dairy and cereal products for exopolysaccharide production and genes involved, Int. J. Food Microbiol. **118** (2007) 250–258.

[31] F. Sarwat, S.A.U. Qader, A. Aman, N. Ahmed, Production & Characterization of a Unique Dextran from an Indigenous *Leuconostoc mesenteroides* CMG713, Int. J. Biol. Sci. **4** (2008) 379–386.

[32] A. Majumder, S.Bhandari, R.K. Purama, S. Patel, A. Goyal, Enhanced production of a novel dextran from *Leuconostoc mesenteroides* NRRL B-640 by Response Surface Methodology, Ann. Microbiol. **59**(2) (2009) 309––315.

[33] A. Majumder, A. Singh, A. Goyal, Application of response surface methodology for glucan production from *Leuconostoc dextranicum* and its structural characterization, Carbohyd. Polym. **75** (2009) 150–156.

[34] S.A. Qader, L. Iqbal, A. Aman, E. Shireen, A. Azhar, Production of dextran by newly isolated strains of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9, Turk. J. Biochem. **31** (2006) 21–26.

[35] M. Pidoux, G. A. De Ruiter, B. E. Brooker, I. J. Colquhoun, V. J. Morris, Microscopic and chemical studies of a gelling polysaccharide from *Lactobacillus hilgardii*, Carbohyd. Polym. **13** (1990) 351–362.

[36] E.J. Hehre, J.Y. Sugg, Serologically reactive polysaccharides producedthrough the action of bacterial enzymes: Dextran of *Leuconostoc mesenteroides* from sucrose, J. Exp. Med. **75** (1942) 339–353.

[37] A. Aman, N.N. Siddiqui, S.A.U. Qader, Characterization and potential applications of high molecular weight dextran produced by *Leuconostoc mesenteroides* AA1, Carbohyd. Polym. **87** (2012) 910–915.

[38] S.D. Sawale, S.S. Lele, Statistical Optimization of Media for Dextran Production by*Leuconostoc* sp., Isolated from Fermented Idli Batter, Food Sci. Biotechnol. **19** (2010) 471–478.

[39] P. Kanmani, R. Satishkumar, N.Yuvaraj, K.A. Paari, V. Pattukumar, V. Arul, Production and purification of a novel exopolysaccharide from lactic acid bacterium *Streptococcus phocae* PI80 and its functional characteristics activity in vitro, Bioresource Technol. **102** (2011) 4827–4833.

IZVOD

ZRNO VODENOG KEFIRA KAO IZVOR BAKTERIJA MLEČNO KISELINSKOG VRENJA POTENTNIH PRODUCENATA DEKSTRANA

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Vodeni kefir je napitak koji nastaje fermentacionom aktivnošću bakterija mlečno-kiselinskog vrenja, kvasaca i sirćetnih bakterija. Ova kompleksna smeša mikroorganizama smeštena je unutar polisaharidnog matriksa – kefirnog zrna. Glavnu šećernu komponentu matriksa čini dekstran koji sintetišu bakterije mlečno-kiselinskog vrenja. Dekstran je homo polisaharid velike molekulske mase čija je monomerna jedinica glukoza. Zahvaljujući dobroj rastvorljivosti i stabilnosti, dekstran ima široku primenu u različitim granama industrije, kao što su medicina, farmacija, prehrambena, tekstilna i hemijska industrija. Nerastvorni dekstran može da posluži kao nosač za imobilizaciju biomolekula. Imajući u vidu veliku primenjivost dekstrana, cilj ovog rada je bio izolovati bakterije mlečno-kiselinskog vrenja iz zrna vodenog kefira koje produkuju ovaj egzopolisaharid sa visokim prinosima. U radu je prikazana izolacija i karakterizacija tri selektovana soja i optimizacija uslova za produkciju dekstrana. Na osnovu morfoloških, fizioloških i biohemijskih osobina i 16S rRNK sekvenciranja utvrđeno je da su izolati T1 i T3 sojevi vrste *Leuconostoc mesenteroides*, dok je izolat T5 identifikovan kao *Lactobacillus hilgardii*. Za produkciju dekstrana korišćena je modifikovana MRS podloga sa 5% saharoze, kao jedinim izvorom ugljenika. Maksimalni prinosi dekstrana (11,56, 18,00 i 18,46 g/l) su dobijeni nakon 16, 20 i 32 h fermentacije, za T1, T3 i T5, redom. Optimalne temperature za produkciju dekstrana su 23 °C za dva *Leuconostoc mesenteroides* soja, a za *Lactobacillus hilgardii* 30 °C. Uzorci sintetisanih polisaharida su identifikovani kao dekstrani na osnovu papirne hromatografije, dok su glavne strukturne karakteristike prečišćenih dekstrana utvrđene na osnovu FTIR spektroskopije.

Ključne reči: Dekstran • Zrno vodenog kefira • *Lc. mesenteroides* • *Lb. hilgardii*