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The Ability of a Novel Strain *Scheffersomyces* (Syn. *Candida*) *shehatae* Isolated from Rotten Wood to Produce Arabitol

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Abstract

Arabitol is a polyalcohol which has about 70% of the sweetness of sucrose and an energy density of 0.2 kcal/g. Similarly to xylitol, it can be used in the food and pharmaceutical industries as a natural sweetener, a texturing agent, a dental caries reducer, and a humectant. Biotechnological production of arabitol from sugars represents an interesting alternative to chemical production. The yeast *Scheffersomyces shehatae* strain 20BM-3 isolated from rotten wood was screened for its ability to produce arabitol from L-arabinose, glucose, and xylose. This isolate, cultured at 28°C and 150 rpm, secreted 4.03 ± 0.00 to 7.97 ± 0.67 g/l of arabitol from 17–30 g/l of L-arabinose assimilated from a medium containing 20–80 g/l of this pentose with yields of 0.24 ± 0.00 to 0.36 ± 0.02 g/g. An optimization study demonstrated that pH 4.0, 32°C, and a shaking frequency of 150 rpm were the optimum conditions for arabitol production by the investigated strain. Under these conditions, strain 20BM-3 produced 6.2 ± 0.17 g/l of arabitol from 17.5 g/l of arabinose after 4 days with a yield of 0.35 ± 0.01 g/g. This strain also produced arabitol from glucose, giving much lower yields, but did not produce it from xylose. The new strain can be successfully used for arabitol production from abundantly available sugars found in plant biomass.

Key words: *Scheffersomyces shehatae*, arabitol production, biotechnological process optimization, biotransformation, yeast identification

Introduction

The pentitol L-arabitol, similarly to its enantiomer xylitol, has been included in the list of the top twelve biomass-derivable chemicals designated for further biotechnological research (Erickson *et al*., 2012). Just like xylitol, arabitol can be used as a natural sweetener that offers a number of health benefits, including its ability to reduce dental caries and prevent the formation of adipose tissue and accumulation of fat in the digestive tract (Mingguo *et al*., 2011). Because arabitol is a low-calorie product (only 0.2 kcal/g) with low glycemic and low-insulinemic indices and anticariogenic and prebiotic effects (Koganti *et al*., 2011), it is an excellent sugar substitute for diabetic patients. This polyol can also be used as a texturing agent, a humectant, a softener, and a colour stabilizer in the production of foods and pharmaceuticals (Kumdam *et al*., 2013). Biotechnological production of arabitol from monosaccharides, such as L-arabinose and glucose, or from waste substrates (*e.g.* glycerol) by yeasts such as *Candida* spp., *Pichia* spp., *Debaryomyces* spp., *Wickerhamomyces* spp., and *Saccharomycopsis* spp. represents an efficient and cost-effective alternative to chemical production (Saha and Bothast, 1996; Koganti *et al*., 2011).

Among the yeasts screened for their ability to produce arabitol from L-arabinose, some of the most frequently reported are those from the genus *Candida* (McMillan and Boynton, 1994; Saha and Bothast, 1996; Kordowska-Wiater *et al*., 2008). McMillan and Boynton (1994), for example, showed that L-arabinose was metabolized to arabitol, among others, by the yeasts *Candida shehatae* and *Candida tropicalis*, with the latter being the best producer of arabitol, giving a yield of 1.02 g/g during cultivation in a medium containing yeast nitrogen base and arabinose. In a screening study of 49 L-arabinose-utilizing yeast strains, Saha and Bothast (1996) demonstrated that *Candida entomaea* NRRL Y-7785 was a superior secretor of L-arabitol (a yield of about 0.7 g/g). Kordowska-Wiater *et al*.

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(2008) reported that *Candida parapsilosis* DSM 70125 and *C. shehatae* ATCC 22984 produced arabitol at yields of 0.28–0.78 g/g and 0.25–0.5 g/g, respectively, depending on the shaking speed (100–200 rpm), in a medium containing 20 g/l of L-arabinose and yeast- and malt extracts. Watanabe *et al*. (2010) selected from over 1600 yeast strains, a single strain, closely related to *Candida subhashii*, designated NY7122, which was able to produce L-arabitol and ethanol from L-arabinose as the sole carbon source (Watanabe *et al*., 2010).

C. shehatae (syn. *Scheffersomyces shehatae*) is mainly known as a yeast able to assimilate the pentose D-xylose and metabolise it to ethanol or xylitol, especially in processes that use hemicellulosic hydrolysates (Girio *et al*., 1989; Jeffries and Kurtzman, 1994; Tanimura *et al*., 2012; Antunes *et al*., 2014). Kurtzman and Suzuki (2010) have proposed the new genus *Scheffersomyces* to accommodate some *Pichia* and *Candida* species (among others *C. shehatae*) connected with woody habitats on the basis of phylogenetic relationships among these species. *S. shehatae* is an environmental species isolated from wood and soil. Apart from D-xylose, it also ferments glucose, galactose and trehalose (Kurtzman *et al*., 2011). Its fermentative effectiveness depends on oxygen availability, with optimum levels of ethanol and xylitol production being achieved under oxygen-limited and anaerobic conditions. The ability of *S. shehatae* to produce arabitol from pentoses and hexoses has been much less investigated.

In the present study, eleven yeast strains isolated from rotten wood were screened for their ability to assimilate L-arabinose. A single strain which was found to be able to produce arabitol from L-arabinose and glucose was subjected to molecular identification and morphological and biochemical characterization, and tested for arabitol production efficiency.

Experimental

Material and Methods

Isolation, preliminary identification and maintenance of the yeast isolates. Yeast strains were isolated from samples of rotten wood (as a source of pentose sugars) collected from Polish forests. Five grams of wood sample was shaken with 45 ml of physiological saline in Erlenmeyer flasks on a reciprocal shaker for 10 min. The suspensions were diluted decimally and plated onto Petri dishes containing YGC agar (yeast extract 5 g/l, glucose 20 g/l, chloramphenicol 0.1 g/l, agar 15 g/l) (BTL, Łódź, Poland). The Petri dishes were incubated at 28°C for 5 days, and the single colonies of yeasts obtained were transferred separately to new Petri dishes with YGC agar and incubated. The purification procedure was repeated twice. The microorganisms forming colonies were identified microscopically, then inoculated onto YPG agar slants (yeast extract 10 g/l, peptone 20 g/l, glucose 20 g/l, agar 20 g/l) (BTL, Łódź, Poland) and, after incubation at 28°C for 3 days, they were maintained at 4°C. They were deposited in the Culture Collection of the Department of Biotechnology, Human Nutrition and Science of Food Commodities of the University of Life Sciences in Lublin, Poland. *C. shehatae* ATCC 22984 was used as a reference strain.

Screening of arabinose-assimilating yeasts. Yeast isolates were screened using a modified method by Subtil and Boles (2011). Petri dishes with selective YNB-A agar (yeast nitrogen base 6.7 g/l, L-arabinose 20 g/l, agar 20 g/l) were inoculated with ten microlitres of three decimal dilutions of all the yeasts studied. The Petri dishes were incubated at 28°C for 5 days. Strains which showed growth on YNB-A were selected for the second stage of the screening procedure.

Optimization experiment of arabitol production from arabinose. The inoculation medium was composed of L-arabinose 20 g/l, yeast extract 3 g/l, malt extract 3 g/l, $(NH_4)_2SO_4$ 5 g/l, and KH_2PO_4 3 g/l, pH 5.5. The cultivation medium was composed of L-arabinose 20 g/l, 50.0 g/l, or 80.0 g/l, yeast extract 3 g/l, malt extract 3 g/l, $(NH_4)_2SO_4$ 5 g/l, and KH_2PO_4 3 g/l. The pH was adjusted to 5.5. In the study of the effect of pH on arabitol production, the pH of the medium was adjusted to 3.5, 4.0, 4.5, 5.0, or 6.0.

For each strain, a loopful of cells from a slant was transferred into a tube with the inoculation medium and incubated at 28°C for 24 h. Then, the cultivation medium was inoculated with 2% (v/v) of the inoculum and incubated in a rotary shaker (Infors HT Minitron, Infors AG, Switzerland) at 150 rpm and 28°C for 4 or 5 days. A study of the effect of rotational speed on arabitol production was conducted at shaker speeds of 100 and 200 rpm. The impact of temperature was investigated by incubating yeast cells at 24 and 32°C. On the basis of the results obtained from the optimization experiments, a verification experiment was carried out in inoculation and cultivation media containing 20 g/l of arabinose under the following conditions: pH 4.0, 32°C, and 150 rpm. Incubation was continued for four days The samples were collected to measure pH, biomass concentration, L-arabinose utilization, and production of L-arabitol, and ethanol (where applicable) every 24 h. The biotransformation experiment was performed in triplicate.

Cultivation experiment of arabitol production from glucose or xylose. The inoculation medium was composed of D-glucose 50 g/l, yeast extract 3 g/l, malt extract 3 g/l, $(NH_4)_2SO_4$ 2 g/l, and KH_2PO_4 3 g/l. The cultivation medium was composed of glucose 50 g/l or 100 g/l, yeast extract 3 g/l, malt extract 3 g/l, $(NH_4)_2SO_4$

2 g/l, and KH_2PO_4 3 g/l. The medium for D-xylose metabolism was composed of D-xylose 20 g/l, yeast extract 3 g/l, malt extract 3 g/l, $(NH_4)_2SO_4$ 5 g/l, and KH_2PO_4 3 g/l. The pH of all the media was adjusted to 5.5. The inoculum and productive cultures were prepared as above. The cells were cultivated for 5 days at 28°C and 150 rpm. The cultures were analysed for pH, biomass concentration, D-glucose or D-xylose utilization, and production of arabitol and other products (glycerol, ethanol, xylitol, and ribitol) every 24 h. The cultivation experiment was performed in triplicate.

Morphological characterization. For macroscopic morphology observations, the yeast strains were grown on YPG agar (BTL, Łódź, Poland) at 28°C for 2–4 days. Intravital microscopy imaging was performed at a magnification of 1000 × with a Delta Optical Evolution 300 optical microscope (Delta Optical, Poland) equipped with an HDCE-50B camera using ScopeImage Dynamic Pro (Delta Optical, Poland) software. Then, the colonies and the cells were characterized according to Yeasts of the World software (ETI-Biodiversity Center, Amsterdam, Netherlands) and Kurtzman *et al*. (2011).

Biochemical characterization and identification of the yeast strains by the Biolog System™. The Biolog System™ (Biolog YT MicroPlate™; Biolog Inc. Hayward, CA, USA) is designed for classification of yeasts on the basis of their carbon source utilisation profiles (assimilation of 59 substrates and oxidation of 35 substrates). The system used in this study was equipped with a multichannel pipette, a computer-linked absorbance and turbidity growth reader, and Biolog Microlog System 3 (5.2) software for data management. Before use, the selected yeast strain was cultured on Biolog Universal Yeast Agar (BUY™) and incubated at 25°C for two days. Next, cells were removed from agar surface with a sterile swab and suspended in sterile water at a specified density (47% T). One hundred microliters of the cell suspension was inoculated into each of the 96 wells of the Biolog YT MicroPlate, which was then incubated in the dark at 27°C for 72 h. The data were recorded with a plate reader at 590 nm every 24 h up to 72 h. For identification, the MicroPlate was read in the MicroStation™ reader and compared to the YT database. The percent of total carbon source utilisation response following oxidation (metabolism) tests (%) was calculated using optical density (OD 590 nm) data for each well, corrected by subtracting the values for the blank well (inoculated, but without a carbon source). The carbon substrates were divided into the following groups: amino acids, carbohydrates, carboxylic acids, polymers, polyalcohols, and miscellaneous. For each series, the corrected absorbance values of a particular group of substrates were summed up and expressed as percent of the average absorbance value of this substrate group for selected yeast strain.

For assimilation tests, turbidity was measured in wells containing carbon substrates assembled into the following groups: carbohydrates, carboxylic acids, polymers, polyalcohols, glycosides, miscellaneous, and others. The rate of assimilation of each substrate was measured by optical density (OD 590 nm), and positive, negative and partial responses were recorded as +, −, and +/− respectively.

Genetic identification. DNA was extracted from yeast cells (20–30 mg) which had been transferred to 2-ml Eppendorf tubes and disintegrated by grinding in liquid nitrogen to a fine powder. DNA was obtained using a Plant and Fungi DNA Purification Kit (EURx, Poland), according to the manufacturer's instructions. After spectrophotometric analysis of DNA purity and concentration (NanoDrop, ThermoScientific, USA), DNA samples were stored at −20°C. ITS1 (5-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers were used for the identification of the yeasts according to White *et al*. (1990). PCR reactions were run on a SensoQuest Labcycler (SensoQuest GmbH, Germany) in a 25 µl volume using 2 × PCR Master Mix (Thermo Scientific Fermentas, Lithuania) with 20 pmol of each primer and 20 ng of DNA. Thermal cycling conditions were as follows: an initial step at 95°C for 3 min followed by 40 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and finally 72°C for 8 min. Then, the amplification product obtained after electrophoresis in a 1.5% (wt/v) agarose gel with ethidium bromide in 1 × TBE was visualised using the GelDoc 2000 gel documentation system (Bio-Rad, USA), and the size of the PCR product was determined using the GeneRuler 100 bp plus DNA Ladder (Thermo Scientific Fermentas, Lithuania).

The purified PCR product obtained using ITS1 and ITS4 primers was directly sequenced at Genomed, Poland, by the Big dye Terminator method using an ABI 3730xl Genetic Analyzer. The DNA sequence obtained was compared with the sequences found in the NCBI GenBank database and then submitted to NCBI GenBank under accession number KP783503.1.

Analysis of sugars and polyols. Samples of cultures were centrifuged at 9 000 × g for 15 min, and the supernatants were used for HPLC analysis. In each sample, after deproteinization by acetonitrile, the concentrations of L-arabinose, glucose, xylose, arabitol, glycerol, ribitol, and xylitol were determined using a chromatograph from Gilson Inc. (Middleton, WI, USA) equipped with a Bio-Rad Aminex Carbohydrate HPX 87H column (Bio-Rad Laboratories Inc., Hercules, CA, USA), and a refractive index detector (Knauer GmbH, Berlin, Germany). 0.05 M sulphuric acid was used as the mobile phase at a flow rate of 0.5 ml/min, and the temperature of separation was 42°C. Chromax 2007 software version 1.0a (Pol-lab, Poland) was used for the integration and

analysis of chromatograms. Qualitative and quantitative analysis of the examined substrates and products was performed by comparison of peaks' areas and retention times with those obtained for pure chemicals.

Arabitol (glycerol, ribitol) yield was calculated as grams of product per grams of sugar consumed. Productivity was calculated as grams of product per grams of sugar consumed per hour.

Ethanol analysis. The deproteinised supernatants were analysed using a gas chromatograph/mass spectrometer (GC/MS) (Model GC2010, Shimadzu, Japan) coupled with an MS-EI apparatus (model QP 2010Plus, Shimadzu, Japan) and an auto injector (model AOC-20i, Shimadzu, Japan). The gas chromatograph was equipped with a 25 m × 0.32 mm (0.3 μm film thickness) CP-WAX 57 CB (Agilent J&W) column operated in the splitless mode with the valve closed for 0.3 min. The carrier gas was helium at a flow rate of 1.8 ml/min. The injector and detector temperature was 200°C. The column oven temperature was set at 50°C for 6 min, then increased at a rate of 3°C/min to 120°C and held for 3 min, and finally raised at a ramp rate of 15°C/min to 190°C, and held for 2 min. Data were acquired in the SCAN-mode (20–400 m/z). GC peaks were identified by comparing the MS fragmentation pattern and the relative retention time with those of the reference compounds. Quantitative determination of a constituent was performed on the basis of the calibration curve of the dose-peak area of a corresponding pure compound. Ethanol yield and productivity were calculated in the same way as arabitol yield and productivity.

Biomass and pH measurement. The biomass was determined by measuring the optical density (OD) at 600 nm using a BioRad Smart Spec Plus spectrophotometer (BioRad, USA). Then, the relationship between OD and dry cell weight was calculated on the basis of a previously prepared calibration curve. The biomass yield was calculated as grams of dry cell weight per grams of sugar consumed. pH of the cultures was monitored every day using an electronic pH-meter (Hanna Instruments, Poland).

Statistical analysis. Data on arabitol production by the investigated yeasts were expressed as mean ± standard deviation. Differences among mean arabitol concentrations, yields, and productivities were tested for statistical significance at $p < 0.05$ using the analysis of variance and Fisher's test for univariate groups (STATISTICA 8.0, StatSoft Inc., Tulsa, USA).

Results and Discussion

Identification and characterization of yeast strains. During the isolation procedure, eleven yeast strains was obtained. It was shown that only four isolates were able to assimilate L-arabinose on YNB-A agar, and only one strain, designated 20BM-3, produced arabitol from arabinose in liquid medium at a yield above 0.2 g/g. This strain was characterized morphologically and biochemically. After three days of incubation on YPG agar, strain 20BM-3 formed 3 mm-diameter colonies which were white-cream, convex, smooth, glistening and butyrous with a smooth edge (Fig. 1). The cells of this strain were round and oval (2–4 × 3.5–6 μm) with multilateral budding. They occurred singly or in pairs and started to form a pseudomycelium (Fig. 2).

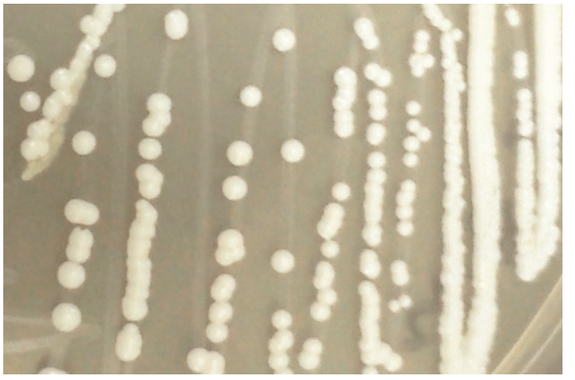

Fig. 1. Colonies of *S. shehatae* strain 20BM-3 on YPG agar.

In the genetic identification procedure, the region containing the 3' end of 18S rDNA, ITS1, 5.8S rDNA, ITS2 and the 5' end of 26S rDNA was amplified by PCR using ITS1 and ITS4 primers. On the basis of the DNA

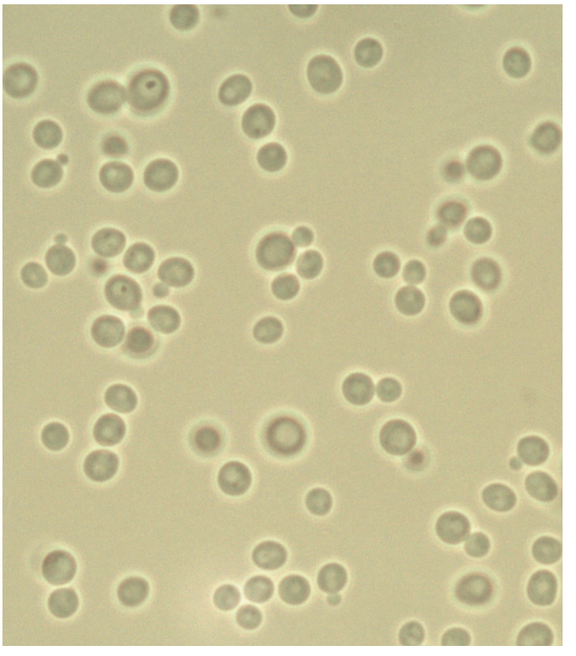

Fig. 2. Morphology of cells of the yeast *S. shehatae* strain 20BM-3, 1000×.

Table I
Ability of *S. shehatae* strain 20BM-3 to assimilate different substrates acc. the Biolog system after 48 h.

Group of substrates	Carbon substrate	Result	Group of substrates	Carbon substrate	Result
Carbohydrates	D-Cellobiose	(+)	Miscellaneous	Succinic Acid Methyl Ester + D-Xylose	(+/–)
	Gentiobiose	(+/–)		N-Acetyl-L-Glutamic Acid + D-Xylose	(+/–)
	Maltose	(+)		Quinic Acid + D-Xylose	(+/–)
	Maltotriose	(+)		D-Glucuronic Acid + D-Xylose	(–)
	D-Melezitose	(+/–)		Dextrin + D-Xylose	(+/–)
	D-Melibiose	(+/–)		α-D-Lactose + D-Xylose	(+/–)
	Palatinose	(+)		D-Melibiose + D-Xylose	(–)
	D-Raffinose	(+/–)		D-Galactose + D-Xylose	(+/–)
	Stachyose	(+/–)		m-Inositol + D-Xylose	(+/–)
	Sucrose	(+/–)		1,2 Propanediol + D-Xylose	(–)
	D-Trehalose	(+)		Acetoin + D-Xylose	(–)
	Turanose	(+/–)	Others	Succinic Acid Mono-Methyl Ester	(–)
	α-D-Glucose	(+)		N-Acetyl-D-Glucosamine	(+/–)
	D-Galactose	(+)		D-Glucosamine	(–)
	D-Psicose	(+/–)		Tween 80	(+)
	L-Rhamnose	(+/–)	Polyalcohols	Maltitol	(+/–)
	L-Sorbose	(+/–)		D-Mannitol	(+/–)
	L-Arabinose	(–)		D-Sorbitol	(+/–)
	D-Arabinose	(–)		Adonitol	(+/–)
	D-Ribose	(–)		D-Arabitol	(+/–)
	D-Xylose	(+/–)		Xylitol	(+/–)
Carboxylic acids	Fumaric Acid	(+/–)		i-Erythritol	(+/–)
	L-Malic Acid	(+/–)		Glycerol	(+/–)
	Bromo-Succinic Acid	(–)	Poly-mers	Dextrin	(+/–)
	L-Glutamic Acid	(+/–)		Inulin	(+)
	Amino-Butyric Acid	(+/–)	Glycosides	α-Methyl-D-Glucoside	(+/–)
	α-Keto-Glutaric Acid	(+/–)		β-D-Methyl-Glucoside	(+/–)
	2 Keto-D-Gluconic Acid	(+)		Amygdalin	(–)
	D-Gluconic Acid	(+/–)		Arbutin	(–)
				Salicin	(+/–)

sequence of the amplified product (656 basepairs), which was compared to DNA sequences in NCBI GenBank, the isolate 20BM-3 was identified as *Scheffersomyces shehatae* (syn. *C. shehatae*).

Identification performed by the Biolog system on the basis of the growth profile called the metabolic fingerprint confirmed that isolate 20BM-3 belonged to the genus *Candida*. The Biolog system was additionally used as a rapid and convenient tool for detailed biochemical characterization of the investigated yeast. Table I summarizes the results of the carbon source assimilation tests for the isolated strain, and Fig. 3 shows the results of the oxidation tests. Some scientists (Praphailong *et al*., 1997; Foshino *et al*., 2004; Wang *et al*., 2008) have used the Biolog system for the identification of marine yeasts and yeast strains in food and beverages. It should be noted that there are several differences between the metabolic profile of *S. shehatae* obtained using the Biolog system and the biochemical characteristics of this yeast according to Kurtzman *et al*. (2011) and Yeasts of the World software. These differences, regarding the assimilation of pentoses, especially L-arabinose and D-xylose, are probably due to insufficient aeration of the Biolog plates. As the screening experiment demonstrated, the strain reported here was able to assimilate L-arabinose and D-xylose, but it needed oxygen and growth factors supplied in the cultivation medium. The key role of aeration in arabinose assimilation has been highlighted by Fonseca *et al*. (2007). On the other hand, there are some reports about strains of *S. shehatae* not able to assimilate arabinose (Antunes *et al*., 2014).

Arabitol production from L-arabinose. The screening procedure using the medium containing 20 g/l of L-arabinose suggested that the isolate identified as

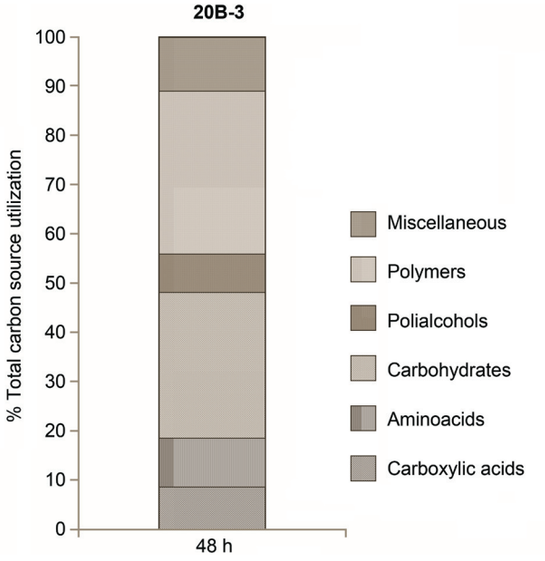

Fig. 3. Ability of *S. shehatae* strain 20BM-3 to oxidize different substrates after 48 h according to the Biolog system.

S. shehatae strain 20BM-3 was a promising arabitol producer and so, in further stages of the study, this strain was cultivated under different conditions using the "one variable at a time" optimization method. In this set of experiments, media containing different initial concentrations of L-arabinose as the carbon source (20.0, 50.0, and 80.0 g/l) and different initial pH values (3.5–6.0) were used. The yeast was incubated at different temperatures (24–32°C) and different rotational speeds (100–200 rpm), which had been selected on the basis of previous literature reports by different scientists (Kordowska-Wiater, 2015). The higher concentrations of the sugar (50.0 and 80.0 g/l) were chosen to check the ability of the yeast to produce arabitol in media of higher osmolarity. The results of the experiments are presented in Table II. The novel strain assimilated about 20.0 g/l of arabinose when cultured for 4 and 5 days, respectively, in media containing 20 and 50 g/l of the pentose, and was able to use about 30 g/l of this sugar in the medium containing 80 g/l of arabinose over 5 days.

Table II
Parameters of L-arabitol production by the novel *S. shehatae* strain 20BM-3 depending on biotransformation conditions.

Conditions (variables)	Production parameters							
	Cult. time (h)	Residual arabinose (g/l)*	Maximum $C_{A\text{-}ol}$ (g/l)*	$Y_{A\text{-}ol}$ (g/g)*	$p_{A\text{-}ol}$ (g/g×h)*	C_B (g/l)*	Y_B (g/g)*	Final pH
Initial C_A (g/l)[1]								
20	96	3.05 ± 0.41	4.03 ± 0.00^{b}	0.24 ± 0.03^{a}	0.002 ± 0.00^{a}	1.94 ± 0.01	0.11 ± 0.00	3.9
50	120	32.40 ± 0.06	6.42 ± 0.28^{a}	0.36 ± 0.02^{b}	0.003 ± 0.00^{a}	2.04 ± 0.04	0.12 ± 0.00	4.85
80	120	49.90 ± 0.07	7.97 ± 0.67^{a}	0.26 ± 0.02^{a}	0.002 ± 0.00^{a}	1.76 ± 0.02	0.06 ± 0.00	4.50
Initial pH[2]								
3.5	96	5.42 ± 0.46	4.82 ± 0.51^{bc}	0.33 ± 0.05^{ac}	0.003 ± 0.00^{b}	2.31 ± 0.06	0.16 ± 0.04	3.1
4.0	96	4.04 ± 0.21	5.88 ± 0.09^{de}	0.37 ± 0.00^{a}	0.004 ± 0.00^{a}	2.81 ± 0.09	0.17 ± 0.06	3.44
4.5	96	4.18 ± 0.58	5.28 ± 0.33^{cd}	0.33 ± 0.03^{ac}	0.003 ± 0.00^{b}	2.47 ± 0.14	0.16 ± 0.09	4.46
5.0	96	4.33 ± 0.63	4.35 ± 0.38^{ab}	0.28 ± 0.01^{bc}	0.003 ± 0.00^{b}	2.42 ± 0.23	0.15 ± 0.00	5.13
5.5	96	3.83 ± 0.06	4.04 ± 0.06^{a}	0.25 ± 0.00^{b}	0.003 ± 0.00^{b}	2.86 ± 0.31	0.18 ± 0.02	4.23
6.0	96	5.83 ± 0.23	3.13 ± 0.42^{f}	0.22 ± 0.03^{b}	0.002 ± 0.00^{b}	2.42 ± 0.14	0.17 ± 0.01	4.95
Shaker rot. speed (rpm)[3]								
100	96	12.94 ± 0.79	2.32 ± 0.06^{a}	0.33 ± 0.03^{a}	0.003 ± 0.00^{a}	1.49 ± 0.04	0.21 ± 0.00	5.8
200	96	6.98 ± 0.30	2.22 ± 0.26^{a}	0.17 ± 0.02^{b}	0.002 ± 0.00^{a}	1.66 ± 0.01	0.13 ± 0.00	5.5
Temp. (°C)[4]								
24	96	13.05 ± 0.55	1.72 ± 0.07^{b}	0.25 ± 0.01^{a}	0.003 ± 0.00^{a}	1.59 ± 0.02	0.23 ± 0.03	5.85
32	96	4.93 ± 0.86	4.65 ± 0.47^{a}	0.31 ± 0.01^{b}	0.003 ± 0.00^{a}	2.27 ± 0.06	0.15 ± 0.00	4.8
Verific. experim.^	96	2.48 ± 0.04	6.2 ± 0.17^{e}	0.35 ± 0.01^{a}	0.004 ± 0.00^{a}	2.59 ± 0.13	0.15 ± 0.01	3.6

* mean values ± standard deviations;
$C_{A\text{-}ol}$ – arabitol concentration; $Y_{A\text{-}ol}$ – arabitol yield; $p_{A\text{-}ol}$ – arabitol productivity;
C_A – arabinose concentration; C_B – biomass concentration; Y_B – biomass yield;
[1] stable conditions: pH 5.5; 28°C; 150 rpm;
[2] stable conditions: 20 g/l arabinose; 28°C; 150 rpm;
[3] stable conditions: 20 g/l arabinose; pH 5.5; 28°C;
[4] stable conditions: 20 g/l arabinose; pH 5.5; 150 rpm;
^ stable conditions: 20 g/l arabinose; pH 4.0; 32°C 150 rpm;
Values with the same superscript letters within a column and one variable are not significantly different ($p < 0.05$).

Production of arabitol was quite effective, with yields of 0.24–0.36 g/g. It is worth noting that biomass production was similar in all cultures within the whole incubation period. The pH of all cultures decreased to 3.9–4.85 during the incubation time. A study of the effect of the initial pH of the medium on arabitol secretion by the novel strain demonstrated that maximum product concentrations and yields were obtained at pH in the range of 3.5–4.5 (Table II), with an optimum observed at pH 4.0. This shows that the yeast preferred lower values of this variable for effective production of arabitol, and, accordingly, acidified the medium during the experiments. By contrast, the effect of pH on biomass production was rather negligible, with the maximum biomass concentration, obtained at pH 5.5, exceeding only slightly the concentration obtained at pH 4.0. The effects of rotational speed and temperature of cultivation on arabinose consumption and arabitol and biomass production by *S. shehatae* strain 20BM-3 were much more salient. A high concentration and a high yield of the polyol were obtained during incubation of the yeast at 32°C and 150 rpm, whereas rotational speeds of 100 and 200 rpm were unfavourable for this yeast (Table II). It is known that rotational speed strongly affects the availability of oxygen to the cells during arabinose catabolism. The initial metabolic pathway for L-arabinose degradation involves redox transformations: L-arabinose is reduced by an unspecific NADPH-linked aldose reductase to arabitol, which is then converted to L-xylulose by NAD-linked dehydrogenase before entry to the pentose phosphate pathway. Under low oxygen conditions, arabitol accumulates because of poor regeneration of NAD necessary for the conversion by arabitol dehydrogenase, and the further stages of the catabolic pathway are stopped or slowed down. Conversely, high oxygenation favours biomass production because the metabolic pathways are more energy-efficient (Fonseca *et al.*, 2007). The analysis of all the combinations of culture conditions for the novel strain *S. shehatae* 20BM-3 suggested that the optimal conditions for this strain were as follows: concentration of arabinose in the medium 20 g/l (because higher concentrations of arabinose were not assimilated completely), pH 4.0, incubation temperature 32°C, and rotational speed 150 rpm. Worth noting is the fact that these conditions were not only conducive to arabitol production, but also promoted yeast growth. For comparison, *C. shehatae* ATCC 22984 cultivated under the same conditions was, generally, a less effective arabitol producer, especially during incubation in the medium with 80 g/l of L-arabinose and at lower pH values of the media (3.5–4.5). Table III compares the effectiveness of the novel strain to that of the reference strain cultivated under the same conditions and over the same incubation time. In another study on *C. shehatae* ATCC 22984, Kordowska-Wiater *et al.* (2008) obtained similar arabitol yields from 20 g/l L-arabinose at 28°C and 100 rpm (0.3–0.35 g/g) or 200 rpm (0.2–0.25 g/g); lower yields were obtained at 32°C or 24°C and 150 rpm, and better results were observed at 28°C and 150 rpm (0.5 g/g), which may be explained by the different volumes of the cultures. Experimental verification of the results of the optimization study confirmed that pH 4.0, 32°C and 150 rpm exerted a positive effect on arabitol production by the new strain *S. shehatae* 20BM-3, which secreted 6.2 ± 0.17 g/l of the product after 4 days with a yield of 0.35 ± 0.01 g/g and a productivity of $0.004\ g/g \times h$. Under these conditions, the yeast assimilated arabinose slightly faster than at 28°C, producing similarly low amounts of biomass (Table II). Taking into account the results obtained at 28°C and 32°C, it may be concluded that both temperatures are acceptable and which of them is used for industrial-scale production of arabitol will ultimately depend on economic considerations.

Arabitol production from xylose and glucose. The ability of the novel strain to produce arabitol from glucose and xylose was investigated in shaken cultures. The yeast assimilated both sugars. D-xylose was transformed to xylitol (0.2 g/l after 2 days) and ethanol (0.45 g/l after 3 days); arabitol was not detected. Bideaux *et al.* (2016), who constructed a metabolic network for

Table III
The comparison of selected parameters of arabitol production by strain 20BM-3 with parameters obtained for reference strain *C. shehatae* ATCC 22894 expressed as % of growth (+) or decrease (–) of parameter value.

	% of growth of production parameters		
	Maximum $C_{A\text{-}ol}$*	$Y_{A\text{-}ol}$*	$p_{A\text{-}ol}$*
Initial C_A (g/l)[1]			
20	9.21	10.23	0
50	42	28.57	50
80	4428	1300	1333
Initial pH[2]			
3.5	53.5	65	59.01
4.0	47.74	37	36.37
4.5	13.55	6.45	7.63
5.0	–21.76	–20	–25
5.5	6.88	1.21	13.33
6.0	–5.15	29.41	28.72
Shaker rotation speed (rpm)[3]			
100	–3.29	–5.71	–6.40
200	149.28	–10.52	–8.40
Temp. (°C)[4]			
24	–9.37	13.63	11.36
32	–12.89	6.89	6.82

* results calculated on the basis of mean values; other designations as in Table II.

Table IV
Parameters of L-arabitol, glycerol, ethanol and ribitol production from glucose by the novel *S. shehatae* strain 20BM-3.

Initial C_G (g/l)[1]	Production parameters				
	Cult. time (h)	Residual glucose (g/l)*	Maximum $C_{A\text{-}ol}$ (g/l)*	$Y_{A\text{-}ol}$ (g/g)*	$p_{A\text{-}ol}$ (g/g × h)*
50	48	1.81 ± 0.01	0.77 ± 0.41	0.016 ± 0.00	0.0003 ± 0.00
100	120	41.28 ± 0.36	4.00 ± 0.36	0.07 ± 0.00	0.0006 ± 0.00
			Maximum $C_{G\text{-}ol}$ (g/l)*	$Y_{G\text{-}ol}$ (g/g)*	$p_{G\text{-}ol}$ (g/g × h)*
50	24	10.43 ± 0.99	1.21 ± 0.35	0.03 ± 0.05	0.001 ± 0.00
100	96	50.24 ± 5.76	3.09 ± 0.33	0.06 ± 0.04	0.0006 ± 0.00
			Maximum $C_{E\text{-}ol}$ (g/l)*	$Y_{E\text{-}ol}$ (g/g)*	$p_{E\text{-}ol}$ (g/g × h)*
50	72	1.81 ± 0.05	0.78 ± 0.03	0.016 ± 0.00	0.0002 ± 0.00
100	120	41.28 ± 0.36	1.15 ± 0.07	0.02 ± 0.01	0.0002 ± 0.00
			Maximum $C_{R\text{-}ol}$ (g/l)*	$Y_{R\text{-}ol}$ (g/g)*	$p_{R\text{-}ol}$ (g/g × h)*
50	72	1.81 ± 0.05	0.12 ± 0.05	0.0025 ± 0.0	0.00003 ± 0.00
100	96	50.24 ± 5.76	2.44 ± 0.12	0.05 ± 0.01	0.0005 ± 0.00

* mean values ± standard deviations;
C_G – glucose concentration; $C_{A\text{-}ol}$ – arabitol concentration; $Y_{A\text{-}ol}$ – arabitol yield; p, $_{A\text{-}ol}$ – arabitol productivity;
$C_{G\text{-}ol}$ – glycerol concentration; $Y_{G\text{-}ol}$ – glycerol yield; $p_{A\text{-}ol}$ – glycerol productivity; $C_{E\text{-}ol}$ – ethanol concentration;
$Y_{E\text{-}ol}$ – ethanol yield; $p_{E\text{-}ol}$ – ethanol productivity; $C_{R\text{-}ol}$ – ribitol concentration; $Y_{R\text{-}ol}$ – ribitol yield; $p_{R\text{-}ol}$ – ribitol productivity;
[1] stable conditions: pH 5.5; 28°C; 150 rpm.

xylose conversion in *C. shehatae* ATCC 22984 on the basis of metabolic fluxes, also found that arabitol was not secreted during xylose catabolism. In our experiment, we obtained a biomass concentration of 2.22 g/l, which was similar to that obtained in the yeast culture grown on arabinose. In this present study, *S. shehatae* 20BM-3 assimilated glucose at concentrations of 50 or 100 g/l, secreting arabitol into the broth, at concentrations of 0.77 ± 0.41 and 4.00 ± 0.36 g/l after 2 and 5 days, respectively. A second product was glycerol at concentrations of 1.21 ± 0.35 and 3.09 ± 0.33 g/l, respectively. This yeast strain also produced small quantities of ethanol and ribitol. The parameters of arabitol, glycerol, ribitol, and ethanol production from glucose are presented in Table IV. Biomass production was quite low, reaching, after 5 days, 3.2 and 1.83 g/l from 50 or 100 g/l of glucose, respectively, which confirms that strain 20BM-3 preferred lower sugar concentrations. To compare, *C. shehatae* ATCC 22984 produced 0.8 and about 3 g/l of arabitol from glucose within the same time and under identical conditions. The pathway of glucose catabolism for conversion of glucose to arabitol leads *via* the glucose phosphorylation stage and conversion to D-ribulose-5-phosphate (or D-xylulose-5-phosphate) followed by dephosphorylation and reduction of D-ribulose (or D-xylulose) to arabitol, which may either enter the pentose phosphate pathway or be secreted by the cells (Kordowska-Wiater, 2015). It is clear that the yields and productivities obtained by strain 20BM-3 are rather low, but there exist methods of improving the strain's production of arabitol from glucose, which is an abundant and cheap source of carbon. For example, Fromanger *et al.* (2010), who investigated *C. shehatae* ATCC 22984 in fed-batch cultures in media containing xylose or glucose in aerobic conditions, observed that this yeast strain produced large quantities of biomass and CO_2 and lower quantities of ethanol, glycerol, xylitol, and ribitol, but not arabitol, from xylose, whereas glucose was metabolized mainly to biomass and CO_2 with small amounts of glycerol, arabitol, ethanol, and ribitol, but not xylitol, which was similar to our findings. In oxygen-limited conditions, they detected the same products, but at different concentrations, with ethanol being the main metabolite of both xylose and glucose. The ability of *C. shehatae* FPL-Y-049 to produce traces of arabitol from glucose during ethanol production from wood hydrolyzate has been reported by Sreenath and Jeffries (2000).

Conclusion

The novel yeast strain isolated from rotten wood, designated 20BM-3, was found to be able to produce arabitol efficiently from arabinose and much less efficiently from glucose. This strain was genetically identified as *S. shehatae* and was characterized morphologically and biochemically using the Biolog system. It secreted arabitol in batch cultures at average concentrations of 4.03–7.97 g/l, depending on the initial concentration of arabinose in the medium, under moderate aeration conditions at 28°C and an initial pH 5.5, with average yields in the range of 0.24–0.36 g/g. It was observed that the yeast used about 20–30 g/l of L-arabinose in the medium. The initial pH of the medium (in the range of 3.5–6.0) was found to have an impact

on arabitol production, with pH 4.0 being the preferred value. The analysis of all the combinations of culture conditions for the novel strain *S. shehatae* 20BM-3 demonstrated that optimal growth was obtained at arabinose concentration in the medium 20 g/l, initial pH 4.0, incubation temperature 32°C, and rotational speed 150 rpm. Further experiments showed that this novel strain was also able to produce arabitol from glucose, but only at low yields. The results obtained in this work encourage continuation of screening studies in search of novel yeast strains possessing the ability to produce arabitol from different sugars *e.g.* those abundant in plant biomass. They also point to the importance of optimization studies and genetic modification studies in looking for new ways of obtaining this polyol.

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