ZBORNÍK PRÍSPEVKOV
3. KONFERENCIE
CENTRA EXCELENTNOSTI

Aplikácia OMICS nástrojov v štúdiu vzniku chorôb a ich prevencie

Chemický ústav SAV, v. v. i., Dúbravská cesta 9, Bratislava

30. november 2022

Recenzent

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Editor

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ISBN 978 – 80 – 971665 – 4 - 0

Biotransformation of maltose to maltobionic acid by *Bacillus* sp. PDD-3b-6, bacterium isolated from cloud water

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Introduction

The capacity of microorganisms to biotransform low value and waste compounds to high added value products is a topic with wide industrial and environmental interests. Aldobionic acids (ABA), lactobionic (LBA), maltobionic (MBA) and cellobionic (CBA), are attractive due to their properties and numerous applications in different branches of industry, health sector or cosmetology, and their market is growing. ABA are prepared from corresponding disaccharides (lactose, maltose and cellobiose, respectively) by oxidation of the aldehyde group of the reducing end glucose to a carboxyl. Oxidation can be achieved by many ways; electro-chemically, chemically, through heterogeneous catalysis, enzymatically or by biocatalytic oxidation. Industrially used oxidation processes are tackling many problems such regeneration of costly metal catalysts (Pd, Au) or enzymes, impurities present in the form of residual solvents or metals, and random oxidation processes leading to a mixture of oxidized products (Vedovato et al. 2020). Separation techniques, inevitable to obtain pure products or a well-defined mixture, increase products costs. Selective biocatalytic oxidation by microorganisms can lead straightforward to a desired product. Bioproduction of lactobionic acid (LBA) is the most studied due to a cheap starting substrate for its production, which influence its emerging applications.

Both MBA (α-D-glucopyranosyl-(1→4)-D-gluconic acid) and CBA (β-D-glucopyranosyl-(1→4)-D-gluconic acid) have similar physico-chemical properties as LBA. Due to its humectant properties and dermato-protective effect, MBA has important applications in dermatology and cosmetology, in the medical field due to its strong radical scavenging property it is used as a preservative for organs intended for transplantation, and recent studies revealed other health benefits (Tanabe et al. 2020; Suehiro et al. 2020, 2022). Demand for MBA on the market is growing due to its numerous applications in textile, oil and refining industry, for household cleaning products, in food industries for food preservation as well as in building industry. Depending on the application, different grades of MBA purity (cosmetic or industrial) are required. Cheaper MBA production solutions by bio-fermentation of low-cost substrates are also investigated, from high maltose corn syrup (Oh et al. 2020a, 2022a) or waste cooked rice (Oh et al. 2022b).

Some bacterial strains, most frequently affiliated with *Pseudomonas*, can directly oxidize lactose and maltose to LBA and MBA, respectively, and then use these sugars as carbon sources (Kluyver et al. 1951). Such reutilization of LBA and MBA as a source of carbon was not observed in *B. cepacia* indicating distinct functioning (Murakami et al. 2002).

Bacillus sp. PDD-3b-6, a bacterial strain isolated from cloud water phase of tropospheric clouds (Genbank accession number DQ512741) has numerous metabolic properties of interest regarding sugar catabolism, contaminants such as herbicides (Durand et al., 2006), or volatile

compounds present in the atmosphere (Husarova et al. 2011). Special properties of *Bacillus* sp. PDD-3b-6 were discovered during a screening of its capacity to metabolize carbohydrates on sucrose or high concentration of glucose were already described in Matulova et al. 2011 and Matulova et al. 2014.

Study of degradation of different sugars in pure water by *Bacillus* sp. PDD-3b-6 sugars resulted in a revelation of its capacity to biotransform some of these sugars to ABA. Here we describe MBA production by this bacterium in pure water on different sugar substrates, which were the only carbon source.

Key words: aldobionic acids, maltobionic acid, *Bacillus* sp. PDD-3b-6, sugar metabolism, NMR

Material and methods

Already described in Matulova et al. 2011 and Matulova et al. 2014.

Results and Discussion

The ability of Bacillus sp. PDD-3b-6 to degrade a variety of saccharides was studied by in situ ^{1}H NMR spectroscopy (Matulova et al. 2014). Diverse saccharides were examined, including pentoses and hexoses, oligo- and polysaccharides as well as acyclic alditols. According degradation rates the tested substrates could be divided into 3 groups: i) substrates completely degraded within 24 – 48 hours of incubation; ii) slowly degraded substrates: their concentration slowly decreased, and only traces of substrates were present at the end of the incubation (96 - 128h); iii) substrates not degraded.

In some cases, the presence of new metabolites with a disaccharide structure was observed. In sample taken after 4h incubation on maltose signals of new metabolite appeared at δ_{H1} 5.167 ppm. They became the only ones in the sample taken after 39h, used for detailed NMR analysis. It was identified as MBA (Table 1). MBA was further used by bacterium when all maltose was consumed.

Table 1 NMR data of MBA and CBA in D_2O at 25ºC.

		Position H/C	**1**	**2**	**3**	**4**	**5**	**6**	**6'**
Glcα (1→4)	MBA	δ_H	5.167	3.586	3.768	3.46	3.93	3.85	3.80
		$^3J_{HH}$ (Hz)	4.1	10.3	9.9	9.9	2.2	4.8	12.0
		δ_C	101.36	72.68	73.90	70.32	73.42	61.34	
GlcA		δ_H		4.137	4.156	3.92	4.013	3.83	3.697
		$^3J_{HH}$ (Hz)		2.6	6.2	3.4	3.8	7.7	11.9
		δ_C	179.35	73.60	73.35	83.30	73.37	63.1	
Glcβ (1→4)	CBA	δ_H	4.62	3.34	3.50	3.40	3.45	3.90	3.72
		δ_C	105.68	76.10	78.23	72.16	76.10	63.27	
GlcA		δ_H	-	4.15	4.08	4.00	3.98	3.85	3.75
		δ_C	180.96	75.07	74.22	84.48	74.47	64.47	

COSY and HSQC spectra of the sample containing only MBA (on maltose at 39h) showed characteristic spectral patterns of cross peak signals due to MBA (Fig. 1, spectra C and F, respectively). The same spectral patterns were revealed in COSY and HSQC of samples on sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) and turanose (α-D-glucopyranosyl-(1→3)-α-D-fructopyranose) giving an evidence about MBA formation also on these substrates. Screening of COSY and HSQC spectra of all samples, taken at

different time intervals and for different sugars, was performed. Fig. 1 shows also as an example COSY (A, B, C) and HSQC (D, E, F) spectra of incubation media with fructose and glucose (Glc 39h; B, E). In these samples MBA was the only/nearly only metabolite, showing characteristic spectral patterns of MBA.

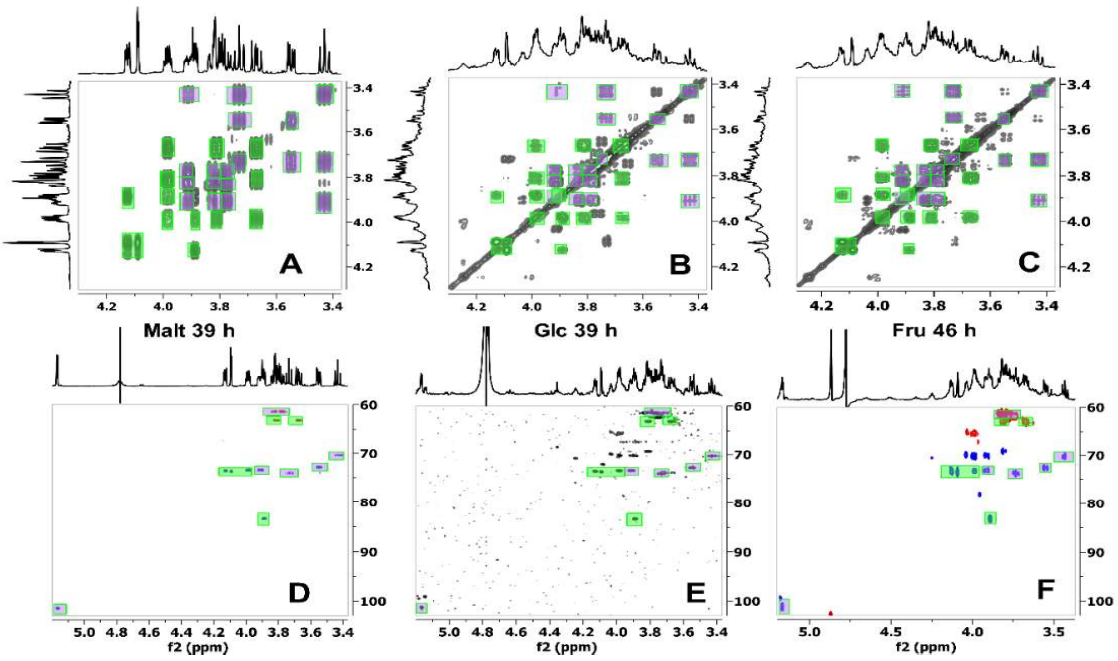

Fig. 1 Characteristic MBA spectral pattern in COSY (A, B, C) and HSQC (D, E, F) spectra: incubation media of *Bacillus* sp. PDD-3b-6 with: fructose after 46 h of incubation (Fru, A and D, respectively); glucose after 39 h (Glc, B and D, respectively); maltose after 39 h (Malt, C and F, respectively). Colours: Green - acyclic part of the molecule (aglycon), violet – cyclic part of the molecule (at the not reducing end).

Six other strains of *Bacillus* spp. (*B. amyloliquefaciens* CIP 103256T*, *B. cereus* ATCC 14579*, *B. licheniformis* ATCC 21733, *B. megaterium* DSM32*, *B. sphaericus* ATCC 10208*, *B. subtilis* CIP 52.65*) and *Pseudomonas fluorescens* CIP 69.13 have been tested under identical incubation conditions for their capacity to produce MBA from maltose. MBA was detected only in the incubation medium of *Bacillus* sp. PDD-3b-6 and *P. fluorescens* CIP 69.13.

Fig. 2 shows structures of carbohydrate substrates (glucose, fructose, maltose, sucrose, trehalose, and turanose) on which *Bacillus* sp. PDD-3b-6 has the capacity to produce MBA. These sugars were directly transformed into MBA without any intermediate compound. On maltose, trehalose, sucrose and turanose the non-reducing glucose unit has an α-configuration. On cellobiose, with β-configuration of the non-reducing glucose unit, bacterium produced CBA as the only metabolite present in the medium. Furthermore, biotransformation of lactose led only to a mixture of

metabolites including LBA. These observations indicate that during syntheses of these ABA no cleavage of the inter-glycosidic linkage takes place.

The main difference between disaccharide structures in Fig. 2 resides in the reducing end sugar: glucose in maltose and trehalose; fructose in sucrose and turanose. This indicates that different enzymes should be involved into their transformation into a gluconic acid to form MBA. However, the situation is different in trehalose as in its molecule two α-glucose units are C1-C1′ inter-glycosidically linked. Here, anomeric centres are blocked for an oxidation.

Further studies are necessary to explain the mechanism of MBA production or type of enzymes involved in MBA production by *Bacillus* sp. PDD-3b-6. Nevertheless, the sugar metabolism study of *Bacillus* sp. PDD-3b-6 led to surprising information: the capacity of this bacterium to produce MBA on different sugars and CBA on cellobiose in pure water. Obtained results are of great biotechnological interest because of a simple process of MBA isolation and purification.

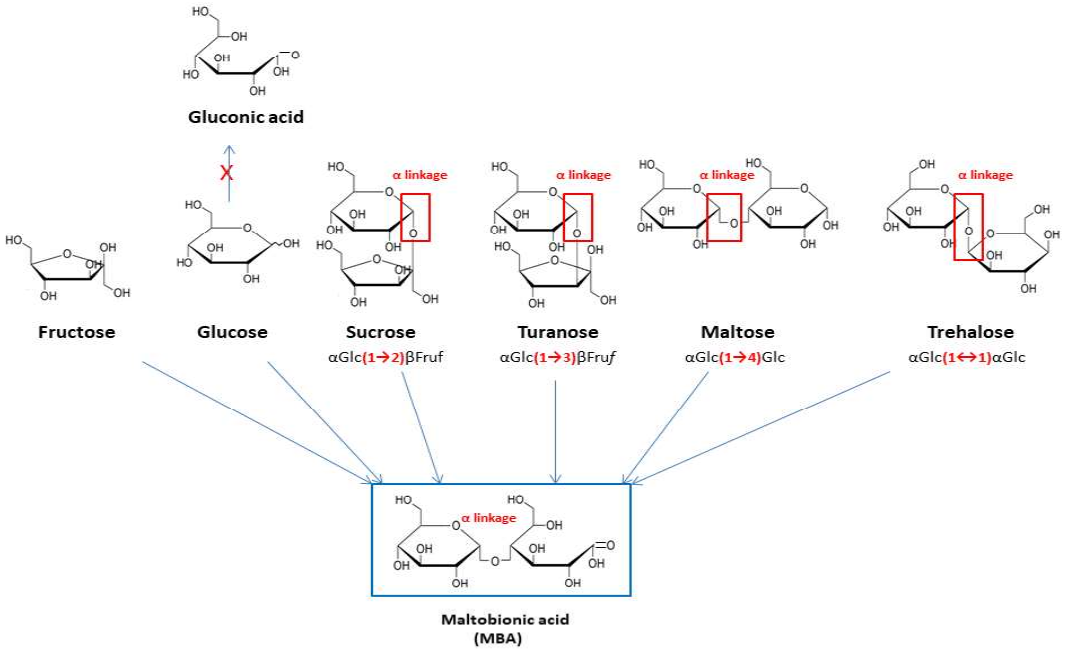

Fig. 2. Sugars directly biotransformed to MBA by *Bacillus* sp. PDD-3b-6 in pure water.

Acknowledgements

This publication was created with the support of the Operational Program Integrated Infrastructure for the project: Study of structural changes of complex glycoconjugates in the process of inherited metabolic and civilization diseases, ITMS: 313021Y920, and project: Integration of multiomics study results and biotechnological production of biologically important substances, ITMS 313011T560, co-financed by the European Regional Development Fund.

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