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Cellobionic and Lactobionic acids produced by *Bacillus* sp. PDD-3b-6, bacterium isolated from cloud water

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Introduction

Aldobionic acids (ABA), lactobionic (LBA), maltobionic (MBA) and cellobionic (CBA) are attractive in different branches of industry due to their interesting properties for numerous applications. Due to their similar structures they have similar physico-chemical properties.

LBA (β-D-galactopyranosyl-(1→4)-D-gluconic acid) takes an important place in food, medicine, cosmetics and chemical industries due to its metal chelating, moisturizing effect, biocompatibility, biodegradability, antioxidant, antimicrobial, and many other properties (Gutiérrez et al. 2012; Alonso et al. 2013). LBA is produced by many bacteria, but the screening for new LBA producing bacterial strains continues (Murakami et al. 2002; Lee et al. 2022, Han et al. 2022). For biotechnological largescale application of LBA production high yields are desirable and thus optimal incubation conditions are searched including genetical engineering methodologies (Alonso et al. 2013, 2017; Sarenkova et al. 2018; Oh et al. 2020a, 2020b, 2022a, 2022b). Some research group deal with optimisation of CBA bioproduction from cheap cellulosic biomass conversion to cellobiose and its subsequent biotransformation to cellobionate (Zhou M et al., 2022; Industrial Crops and Products 2022, 188:115650.). 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).

Keywords: aldobionic acids, *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

Bacillus sp. PDD-3b-6, a bacterial strain was isolated from cloud water phase of tropospheric clouds (Genbank accession number DQ512741). It has numerous metabolic properties of interest regarding sugar catabolism, contaminants (Durand et al., 2006), or volatile compounds present in the atmosphere (Husarova et al. 2011). Particular were those observed on sucrose or high concentration of glucose in pure water (Matulova et al. 2011 and Matulova et al. 2014). Study of sugar metabolism revealed further surprising information. The ^{1}H, COSY and HSQC NMR spectral patterns enabled identification of MBA production in pure water on low concentration of glucose, fructose, maltose, sucrose, turanose and trehalose without any intermediate compound. However, when all substrate was exhausted MBA was further used by bacterium as a source of energy.

The rate of cellobiose biotransformation by *Bacillus* sp. PDD-3b-6 was similar as that of trehalose and maltose and it resulted in the CBA production. CBA was the only metabolite present in its incubation media after 46h (cellobiose was totally consumed by bacterium), and its signal

intensities in NMR spectra (Table 1) gradually decreased. They disappeared after 55 h. Lactose degradation was much slower than maltose and cellobiose. Signals of LBA (Murakami et al. 2002) appeared early (after 4 h), its amount increased slowly and reached a maximum after 22 h of incubation, then it remained constant and gradually disappeared after 55 h. However, LBA never remained the only metabolite in the medium. At the end of the incubation (96 h) only 35% of LB was degraded. These observations indicate that during syntheses of these ABA no cleavage of the inter-glycosidic linkage takes place.

Table 1 NMR data of CBA in D_2O at 25°C, TSP-d_4, 500MHz.

		Position H/C	**1**	**2**	**3**	**4**	**5**	**6**	**6'**
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	

The main structural difference between disaccharide transformed to MBA 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. Free gluconic acid was not identified in the incubation media. Not reducing end sugar in both, lactose and cellobiose, were in β configuration and thus biotransformation led to LBA and CBA respectively.

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 and CBA isolation and purification. Rich enzymatic system of this bacterium is promising for a development of new strategies in biosynthesis of ABA.

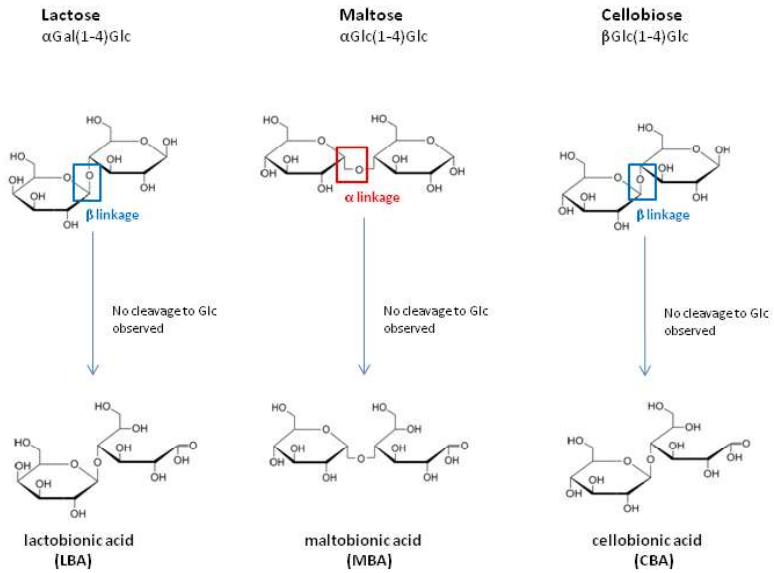

Fig. 1 Disaccharides directly biotransformed to ABA by *Bacillus* sp. PDD-3b-6 in pure water.

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