Beta-Galactosidase Activity in Psychrotrophic Microorganisms and their Potential Use in Food Industry

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Abstract

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Twenty-one psychrotrophic resp. psychrophilic bacterial strains were screened for presence of β-galactosidase activity which showed 8 of them. β-Galactosidase activity of these strains was determined for 2 substrates – synthetic substrate (ONPG) and lactose – and also temperature profile of this enzyme was measured. β-Galactosidase from *Arthrobacter sp.* C2-2 not only proved the typical properties of cold-active enzyme, but it also preferred lactose as a substrate. Therefore, it was chosen for further isolation and purification and was found that it contains two β-galactosidase isoenzymes. One of them had strong preference for lactose and was able to catalyse transglycosylation reactions at low temperature. It has, thus, potential use in food technology.

Keywords: psychrotrophic microorganism; cold-active enzyme; β-galactosidase; lactose hydrolysis; transglycosylation

Psychrophilic and psychrotrophic microorganisms are important in global ecology as a large proportion of our planet is cold (below 5°C). These microorganisms have potential uses in low-temperature biotechnological processes (OKUYAMA *et al*. 1998), but on the other hand they are responsible for the spoilage of chilled food.

In order to retain rates of metabolic processes at low temperatures, psychrophiles must contain enzymes that have a high specific activity in the cold. This is generally reflected in a relatively low apparent optimum temperature for activity compared with the corresponding enzymes from mesophiles/thermophiles. These enzymes are also generally thermolabile. Due to their high specific activity and their rapid inactivation at higher temperatures, along with their producers they offer a great potential as biocatalysts in biotechnology and in food processing.

Glycosidases are enzymes that are able to hydrolyse glycosidic bonds in oligo- or polysaccharides and heteroglycosides. They are widely distributed in all organisms but their physiological function is not always fully recognised. In some cases they are able to catalyse the opposite direction of hydrolysis, i.e. (trans)glycosylation.

One of the glycosidases, β-galactosidase, is the enzyme widely used especially in dairy technologies. This enzyme provides two benefits that make its use attractive for dairy industry: preparation of lactose-free milk and biosynthesis of galactooligosaccharides that are interesting from the technological as well as health point of view.

Low activity of β-galactosidase causes digestive insufficiency, called lactose intolerance in grave cases (PAIGE & DAVIS 1985). In the Czech Republic the lactose intolerance is not as common as in Far East or Africa, but decreased activity of β-galactosidase is rather frequent especially with seniors. Lactose-free milk is manufactured for those patients (SUAREZ *et al*. 1995).

There are also technological reasons for partial removal of lactose from milk: higher solubility, suppression of lac-

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tose crystallisation in sweet condensed milk and ice creams, increase of sweetness, decrease in the hydroscopicity of dried dairy products (MAHONEY 1985) etc. Commercially available β-galactosidases used in dairy technologies have a temperature optimum at 37°C (Lactozym – Novo Nordisk, Maxilact – Gist Brocades). It is not very convenient because dairy processes often go at this temperature. Under these conditions the enzyme activity decreases 5–10 times; to achieve the desirable activity, the enzyme concentration should be increased and time of hydrolysis should be prolonged.

β-galactosidase belongs to the glycosidases that show both hydrolysation and transglycosylation activity, i.e. they are able to synthesise oligosaccharides during hydrolysis of natural substrates. It was found that two models – transglycosylation and reverse hydrolysis (RASTALL & BUCKE 1992) – could be used for oligosaccharide synthesis. Oligosaccharides synthesised by enzymes are mainly used as food additives with activity to improve physicochemical characteristics (CRITTENDEN & PLAYNE 1996). Oligosaccharides are frequently added to beverages, infant milk powders, yogurts and other dairy products, chewing gums etc.

Oligosaccharides are water soluble and mildly sweet in comparison with the commonly used mono- and disaccharides. Their relatively low sweetness is useful in food production if enhancement of other food flavours is desirable.

In food industry, oligosaccharides are of great interest due to their possible health benefits for consumers. These oligosaccharides are not utilised by the mouth microflora (*Streptococcus mutants*) and, therefore, limit the formation of caries. Many of them are not digested in the small intestine and this makes them suitable for use as a low-calorie sweetener and for consumption by individuals with diabetes.

In recent years, the ability of many oligosaccharides to promote the proliferation of bifidobacteria in the colon has been recognised. These oligosaccharides are selectively fermented by bifidobacteria, thus the growth of undesirable bacteria is suppressed (MODLER 1994). Currently, the oligosaccharides are added as pure compounds to dairy products.

In food industry the most frequently used oligosaccharides are produced by enzymes. An estimated amount of oligosaccharides manufactured in 1995 was about 85 000 t (CRITTENDEN & PLAYNE 1996). A relatively high increase is to be expected. Major companies dealing with oligosaccharide production are in Japan.

In our department, glycosidases e.g. α-glucosidases have been studied for a long time. The methods and experience obtained before are now used for the studies of glycosidases promising for the food industry. Our goal is to find microbial producers of cold-active β-galactosidase, especially among the psychrophilic and psychrotrophic Antartica isolates and lactic acid bacteria.

MATERIAL AND METHODS

Bacterial Strains and Culture Conditions

The strains came from a collection of Imperial College University of London and from the Czech Collection of Microorganisms in Brno. All strains were cultivated in suitable medium (BHI, minimal medium containing lactose, MRS medium and medium for *Enterococcus*). The incubation was carried out at 15°C on a platform shaker at 150 RPM. X-press was used for disintegration.

β-Galactosidase Screening

The strains were grown on suitable plates (solid medium) containing 0.01% X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and 0.1 mM IPTG (isopropyl-thio-β-D-galactopyranoside) as an inducer. Plates were incubated at 15°C for approximately 3 days. Colonies producing β-galactosidase were blue.

Enzyme Assay

β-Galactosidase hydrolytic activity was determined by measuring the release of *o*-nitrophenol from ONPG (*o*-nitrophenyl-β-D-galactopyranoside) at 420 nm. Enzyme reactions were performed by diluting the enzyme in phosphate buffer at 30°C and starting the reaction by the addition of ONPG at a final concentration of 1.5% (w/v) in DMF (dimethylformamide). After 20 min the reaction was stopped by the addition of 10% (w/w) Na_2CO_3.

Activity with lactose as a substrate was determined in phosphate buffer at 30°C by the addition of lactose up to 40 mM. After 30 min the reaction was stopped by heating the sample to 95°C. Concentration of glucose was determined using a glucoseoxidase-peroxidase assay.

Gel Electrophoresis

Protein samples were analysed on 6.8% polyacrylamide gels by the method of Laemmli. Staining for β-galactosidase activity was done by incubation of nondenaturing gels in an assay buffer containing X-gal instead of ONPG to detect *in situ* activity.

Transglycosylation Reactions

Experiments on the transglycosylation activity of β-galactosidase were carried out under the following conditions: 0.1M phosphate buffer pH 7.5, 15°C. The enzyme (0.2 U in reaction mixture) was incubated with lactose (15% [w/w] in reaction mixture). Samples (30 ml) were taken at various times from the reaction mixture and 30 µl of internal standard was added. The mixture was heated for 3 min at 100°C to denature the enzyme. After filtration, 20 µl aliquots were analysed by HPLC.

High Performance Liquid Chromatography (HPLC)

Oligosaccharide synthesis was monitored by HPLC (Waters, USA). All reaction products were identified and quantified on Supelcogel Ca column by elution with deionised water at a flow rate 0.5 ml/min at 80°C. Elution was monitored with a differential refractometer.

RESULTS

Psychrophilic and psychrotolerant strains from the Czech Collection of Microorganisms (in Brno) able to metabolise lactose and grow at the temperature below 30°C, and strains from the Imperial College of London Collection originating from Antarctica (mainly the genus *Arthrobacter*) were tested for β-galactosidase activity at a low temperature, for hydrolytic activity toward lactose and for ability to catalyse transglycosylation reactions. Tested strains were cultivated in solid medium containing X-gal and IPTG at 15°C. Their ability to grow at the above-mentioned temperature and to hydrolyse X-gal (chromogenic substrate) present in the medium was studied (Table 1).

Table I. Ability of different microbial strains to grow at 15°C and to hydrolyse synthetic substrate X-gal

Strain	Grow at 15°C	X-gal
Leuconostoc mesenteriodes subsp. *dextranicum*	+	+
Leuconostoc mesenteriodes subsp. *mesenteroides*	+	+
Lactobacillus plantarum	+	+
Lactobacillus paracasei subsp. *paracasei* 1753	+	+
Lactobacillus paracasei subsp. *paracasei* 1752	+	–
Lactobacillus casei	+	–
Lactococcus lactis	+	–
Lactobacillus rhamnosus	+	–
Enterococcus malodoratus	+	–
Enterococcus faecium	+	–
CH07	+	+
C1-2a	+	+
C2-2	+	+
CO85	+	+
GY 24	-	–
GY 26	+	–
Arthrobacter globiformis ncimb 8907	+	–
GY 15	+	–
GY 21	–	–
BOS 76	+/–	–
GY23	+	–

Two of 21 tested strains were not able to grow at 15°C, another one was growing only very slowly. In the given conditions 13 of these strains were not able to hydrolyse X-gal. Therefore, they were excluded from subsequent studies. For 8 strains that were able to grow and hydrolyse X-gal in the given conditions, temperature optimum and the relation between β-galactosidase activity at low temperature and the activity at temperature optimum (in %) was measured (Table 2). The strains *Leuconostoc mesenteroides* subsp. *mesenteroides*, CH07, C2-2 and C1-2a proved to show the highest ratio of β-galactosidase activity at low temperature. According to another parameter, high specific activity towards lactose, only strain C2-2 was selected for future studies.

Native PAGE (polyacrylamide electrophoresis) of enzyme extracts from C2-2 cultures grown either in BHI liquid medium or in minimal medium containing 3% of lactose proved that two isoenzymes were responsible for β-galactosidase activity of the strains (C2-2-1 and C2-2-2). Formation of C2-2-1 isoenzyme was induced by lactose (Fig. 1).

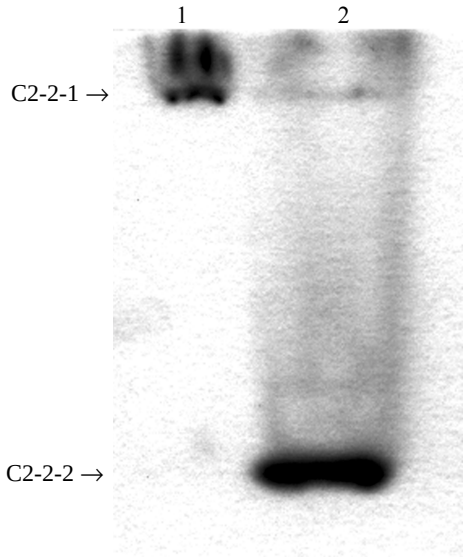

Fig. 1. Native polyacrylamide electrophoresis of raw cellular extracts from the strain *Arthrobacter* sp. C2-2 grown either in minimal medium with 3% of lactose (1) or in BHI medium (complete medium). Protein with β-galactosidase activity was detected using X-gal

Temperature profiles and specific activities towards ONPG and lactose for both isoenzymes were compared and it was found that the isoenzyme induced by lactose (C2-2-1) had a lower temperature optimum (25°C) and a higher proportion of β-galactosidase activity at 10°C (42 %) than the other one (C2-2-2) (Fig. 2). It also showed higher specific activity for lactose.

The ability of isoenzyme C2-2-1 to catalyse the transglycosylation reaction was also tested. However, the ex-

Table 2. Temperature optima and proportion of β-galactosidase activity at 10°C in comparison with the activity at optimum temperature for selected strains

	Temperature optimum (°C)	% activity at 10°C	Ratio $a_{spec.}$ ONPG:lactose
CH07	35	45	5:1
C1-2a	35	32	24:1
C2-2	35	28	3:1
CO85	45	14	1:nd
Leuconostoc mesenteriodes subsp. *dextranicum*	50	11	1:nd
Leuconostoc mesenteriodes subsp. *mesenteroides*	30	46	1:nd
Lactobacillus plantarum	55	11	1:nd
Lactobacillus paracasei subsp. *paracasei* 1753	60	10	1:nd

Strains were cultivated on BHI medium and disintegrated (nd – not determined)

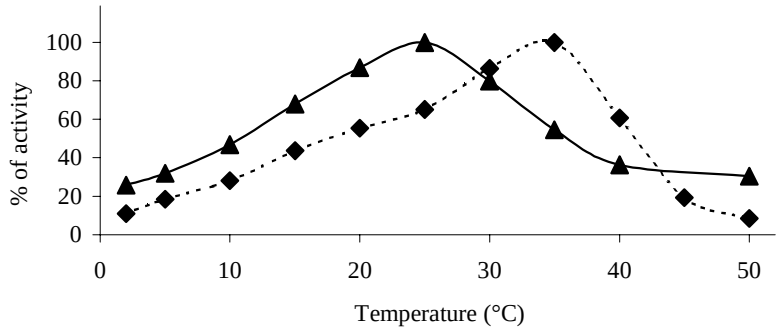

Fig. 2. Temperature profiles of β-galactosidase of both isoenzymes of strain C2-2 in raw extracts

periment was carried out only with desalted crude enzyme preparation and with lactose as an acceptor of freed galactose. This preparation was able to transform more than 15% of present lactose to higher galactooligosaccharides (tetra- and trisaccharides) at 15°C and with original lactose concentration 15% in 7.5 hours.

CONCLUSION

More than 20 bacterial strains were screened for the presence of cold-active β-galactosidase. Out of them the strain *Arthrobacter* sp. C2-2 was chosen for further isolation and purification. It contains two β-galactosidase isoenzymes (C2-2-1 and C2-2-2), one of them (C2-2-1) is able to catalyse transglycosylation reactions at a low temperature.

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Souhrn

KARASOVÁ P., SPIWOK V., MALÁ Š., KRÁLOVÁ B., RUSSELL N.J. (2002): **Aktivita β-galactosidasy v psychrotrofních mikroorganismech a její možné použití v potravinářském průmyslu**. Czech J. Food Sci., **20**: 43–47.

Z 21 testovaných psychrotrofních, resp. psychrofilních mikrobiálních kmenů osm prokazovalo β-galaktosidasovou aktivitu. Aktivita β-galaktosidasy z těchto kmenů byla kvantifikována, a to vzhledem k syntetickému substrátu (ONPG) a k laktose, a byl proměřen její teplotní profil. β-galaktosidasy ze čtyř kmenů prokazovaly typické vlastnosti chladově aktivních enzymů. Podle poměru aktivity k ONPG a k laktose byl k dalšímu studiu vybrán kmen *Arthrobacter* sp. C2-2. U tohoto kmene byly nalezeny dva isoenzymy s β-galaktosidasovou aktivitou, které vedle hydrolytické aktivity mají i aktivitu transglykosylační. Jeden z těchto isoenzymů má převažující aktivitu vůči laktose a je tedy potenciálně využitelný v potravinářském průmyslu.

Klíčová slova: psychrotrofní mikroorganismy; chladově aktivní enzymy; β-galactosidasa; hydrolýza laktosy; transglykosylace

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