研究成果報告書

事業名（補助金名）　：　基盤的研究開発育成事業（若手研究補助金）
研究開発テーマ名　：　食品産業に応用可能な新規低温性乳糖分解酵素の探索と開発
研 究 代 表 者 名　：　中川　智行【東京農業大学生物産業学部食品科学科／講師】

目　的

ほとんどの成人日本人は乳糖を処理する能力が低い「乳糖不耐性」であるが、乳中の乳糖を事前に分解することで乳糖不耐症でも牛乳を安心して飲むことができる。また、チーズ加工中に生産される食品廃棄物の一つである乳清はそれに含まれる乳糖を新たな発酵原料として利用可能であり、乳清を産業廃棄物から再資源化できるものと考えている。

一方、牛乳、乳製品および乳清は常温で非常に劣化しやすいため、その加工は低温で迅速に行うことが好ましい。つまり、上記の乳加工において低温条件下で機能する乳糖分解酵素は大きなメリットを持ち、これら酵素が北海道の乳産業に大きな利益を与えるものと考えている。

そこで、本研究では、牛乳、乳製品および乳清中の乳糖を低温下で充分に分解可能な新規低温性乳糖分解酵素の開発を目指した。

結果・考察

① F2 株由来低温性乳糖分解酵素の精製とその諸性質の検討

A. psychrolactophilus F2 株の菌体内画分から低温性乳糖分解酵素を Table 1 の手順で SDS-PAGE にて単一バンドを示すまで精製した（Fig. 1）。

得られた精製酵素は 130kDa のサブユニットからなる 4 量体酵素であった。本酵素は 10ºC に最大活性が認められ、かつ 0ºC においてもその 80%以上の活性を有することから、低温性酵素であるものと判断した（Fig. 2A）。また、本酵素の ONPG およびラクトースに対する親和性（K_m）は 10℃において、それぞれ 2.8mM および 50mM であった。一方、本酵素は pH7.0-10.0 の広い範囲で安定であるものの、45ºC-10 分、50ºC においては 5 分以内の処理で完全に失活した（Fig. 2B）。食品への酵素製剤を添加する際、酵素反応後、速やかに添加酵素を失活させる必要があり、F2 株由来低温性乳糖分解酵素は低温殺菌条件でも十分失活させることが可能である点においても食品産業への応用が期待できる。

Table 1. Summary of purification of the β-galactosidase from *A. psychrolactophilus* strain F2.

	Total protein (mg)	Total activity (Units)	Specific activity (U/mg)	Purification (Fold)
Crude extract	1470	75.3	0.0511	1
$(NH_4)_2SO_4$ (40%)	695	297	0.427	8.37
DEAE-Cellulose DE52	30.7	63.1	2.05	40.2
Butyl TOYOPEARL 650S	0.474	10.2	21.6	423
HW-75F	0.131	4.37	33.3	652

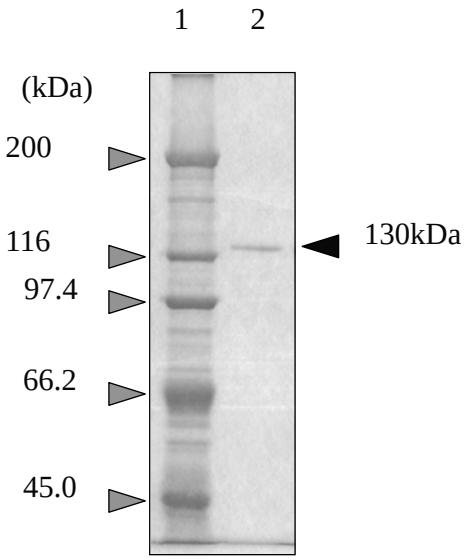

Fig. 1. SDS-PAGE analysis of the purified β-galactosidase from *A. psychrolactophilus* strain F2. Lanes: 1, molecular standard high-range markers (Bio-Rad Laboratories, Hercules, CA); 2, purified β-galactosidase.

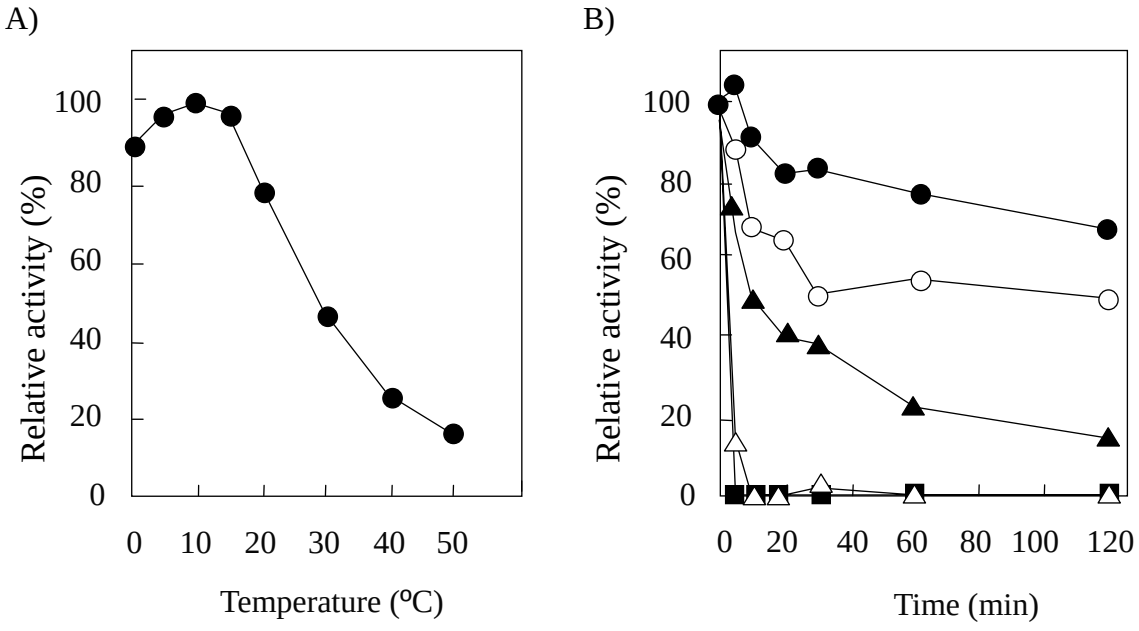

Fig. 2. Effects of temperature on the activity (A) and stability (B) of the purified β-galactosidase. In the stability test,

the enzyme was incubated under the indicated conditions and remaining activity was assayed under the optimum conditions for β-galactosidase. Symbols for the stability test (B): ●, 30ºC; ○, 35ºC; △, 40ºC; ▲, 45ºC; and ■, 50ºC.

② F2 株由来低温性乳糖分解酵素の一次構造の解析

A. psychrolactophilus F2 株由来低温性乳糖分解酵素の精製タンパク質の N 末端アミノ酸配列の情報をもとに、本酵素をコードする遺伝子 *bglA* をカセット PCR 法およびインバース PCR 法を用いて F2 株のゲノム DNA より獲得した。

BglA は 3,084bp からなる ORF を持ち、1,028 アミノ酸残基をコードしていた（Fig. 3）。*BglA* は、(1) F2 株由来乳糖分解酵素の N 末端アミノ酸配列を有すること、(2) 推定分子量が精製酵素と一致すること（111 kDa）などから、F2 株の乳糖分解酵素をコードする遺伝子であることが示唆された。*BglA* の遺伝子産物である BglAp は *Arthrobacter* strain B7 株 isozayme 15 と 77.5%、*Arthrobacter* sp. C2-2 株由来酵素と 79%、*Arthrobacter* SB 株由来酵素とは 62.5%の高い相同性を示し、アミノ酸配列の前半に Glycosyl Hydrolase Family 2（GH2）に関連したモチーフが、後半には GH42 に関連したモチーフが保持されており、GH2 と GH42 のキメラ構造をとっていると考えられた。

また、ゲノムサザン解析により、*bglA* は F2 株における唯一の乳糖分解酵素遺伝子であることが明らかになり、本株がアイソザイムを持たないという結果と一致した。

以上のように *A. psychrolactophilus* F2 株由来低温性乳糖分解酵素をコードする遺伝子を明らかに出来たことから、本酵素の一次構造が明らかになったことに加え、今後、*A. psychrolactophilus* F2 株由来低温性乳糖分解酵素の大量発現、さらには部位特異的変異等の手法を用いて、より有効でかつ高活性を示す組み換え酵素の開発へとつながるものと考えている。

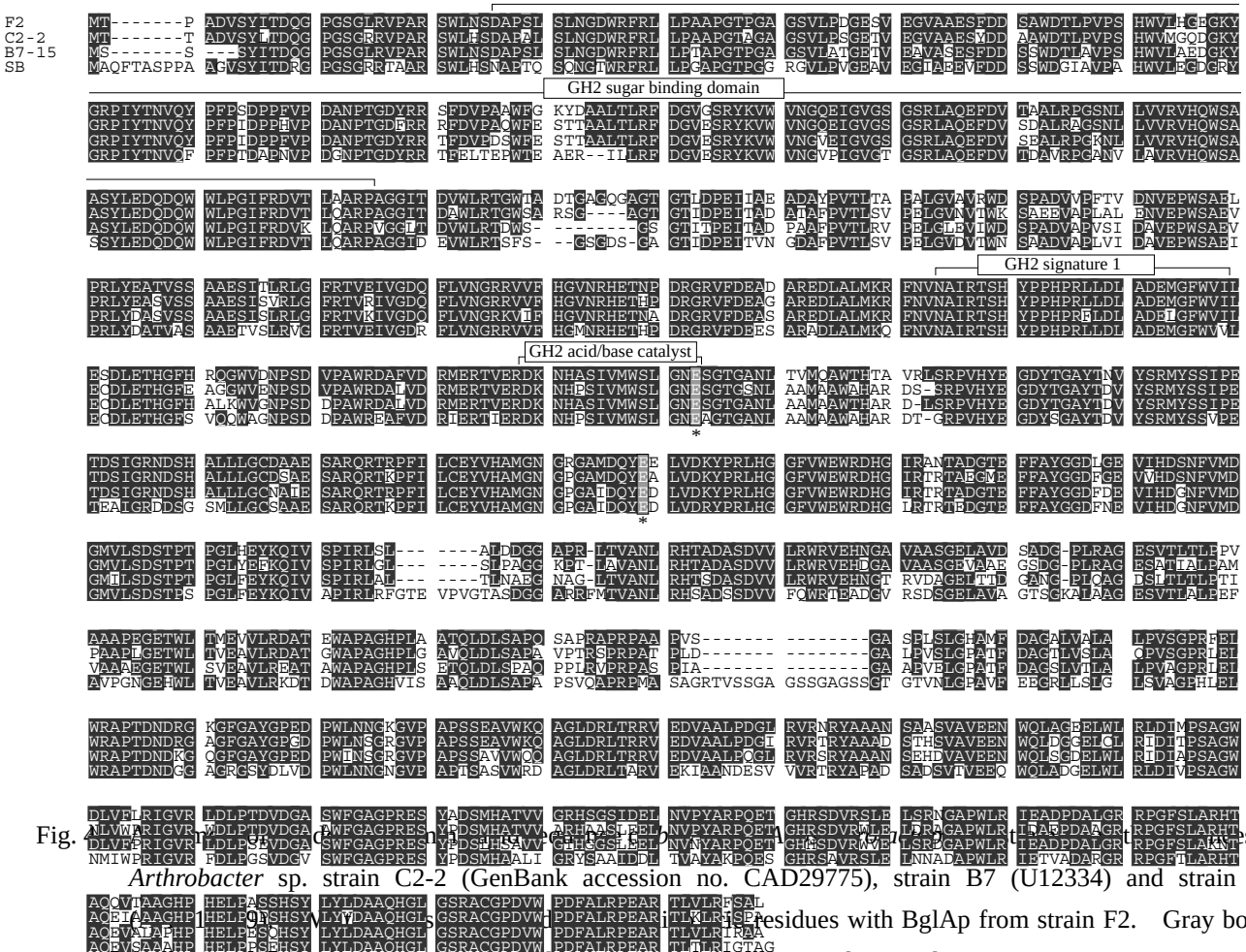

Fig. 4 Alignment of amino acid sequences of *Arthrobacter* sp. strain C2-2 (GenBank accession no. CAD29775), strain B7 (U12334) and strain SB residues with BglAp from strain F2. Gray boxes with asterisk indicate glutamic acid residues involved in catalysis in the *E. coli* LacZ enzyme.

③ F2 株由来低温性乳糖分解酵素の生乳中の乳糖分解能

A. psychrolactophilus F2 株由来乳糖分解酵素の応用を目指して、部分精製酵素を用いて生乳中のラクトース分解を試みた。生乳 1ml に対して 1Unit の酵素を添加し、10ºC にて酵素処理を実施した結果、乳中のラクトースを 24 時間で 20%まで低下させることができた（Fig. 5）。この結果は、本酵素が食品加工へ適用できる可能性を有することを示している。

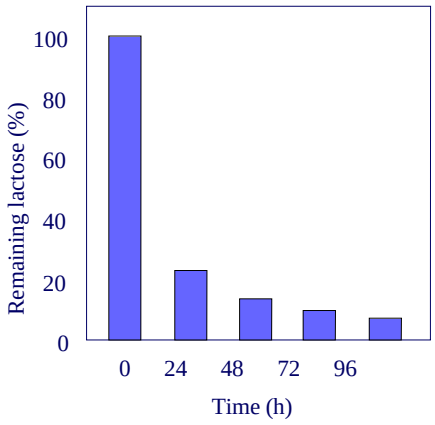

Fig. 4. Hydrolysis of lactose in raw milk after 24h at 5ºC.

今後の展望

本研究にて、牛乳、乳製品および乳清中の乳糖を低温下（10ºC）で充分に分解可能な新規低温性乳糖分解酵素を*A. psychrolactophilus* F2株の菌体内画分に見出し、その酵素化学的諸性質およびその一次構造を明らかにした。本酵素は低温で充分な活性を示すのみならず、中温域での失活が可能であり、さらには1Unitの本酵素にて生乳1ml中のラクトースを10ºC-24時間で20%まで低下させる能力を有していた。これらの性質からF2株由来低温性乳糖分解酵素は将来的に食品産業に応用できる可能性を秘めているものと考えている。

最後になりましたが、私共の研究に対しましてご理解とご援助を頂きましたノーステック財団に心より御礼申し上げます。

また、本研究成果の一部は学術雑誌「*Appl. Microbiol. Biotechnol* 72:720-725」に掲載された。

Appl Microbiol Biotechnol (2006) 72: 720–725
DOI 10.1007/s00253-006-0339-0

Tomoyuki Nakagawa · Yuji Fujimoto · Ryoko Ikehata · Tatsuro Miyaji · Noboru Tomizuka

Purification and molecular characterization of cold-active β-galactosidase from *Arthrobacter psychrolactophilus* strain F2

Received: 5 December 2005 / Revised: 12 January 2006 / Accepted: 13 January 2006 / Published online: 11 April 2006

Abstract In this study, we purified and molecularly characterized a cold-active β-galactosidase from *Arthrobacter psychrolactophilus* strain F2. The purified β-galactosidase from strain F2 exhibited high activity at 0°C, and its optimum temperature and pH were 10°C and 8.0, respectively. It was possible to inactivate the β-galactosidase rapidly at 45°C in 5 min. The enzyme was able to hydrolyze lactose as a substrate, as well as *o*-nitrophenyl-β-D-galactopyranoside (ONPG), the K_m values with ONPG and lactose being calculated to be 2.8 mM and 50 mM, respectively, at 10°C. Moreover, the *bglA* gene encoding the β-galactosidase of strain F2 was cloned and analyzed. The *bglA* gene consists of a 3,084-bp open reading frame corresponding to a protein of 1,028 amino acid residues. BglAp, the gene product derived from *bglA*, had several conserved regions for glycosyl hydrolase family 2, e.g., the glycosyl hydrolase 2 (GH2) sugar binding domain, GH2 acid-base catalyst, GH2 triosephosphate isomerase barrel domain, GH2 signature 1, and several other GH2 conserved regions. From these facts, we conclude that the β-galactosidase from *A. psychrolactophilus* strain F2, which is a new member of glycosyl hydrolase family 2, is a cold-active enzyme that is extremely heat labile and could have advantageous applications in the food industry.

Introduction

In low temperature environments, many microorganisms adapt to the cold conditions and can grow. Among cold-adapted microorganisms, a psychrophile is a microorganism exhibiting optimal growth at low temperature (Morita 1975). Therefore, it is possible that psychrophiles produce cold-adapted enzymes, which exhibit high catalytic activities at low temperature, to adapt to cold habitats, and thus, psychrophiles have attracted attention as sources of enzymes with potential for low-temperature catalysis (Hoyoux et al. 2004). Indeed, a variety of cold-active enzymes have been found in psychrophiles (Feller et al. 1996; Gerday et al. 2000; Marshall 1997), and our group has reported a variety of psychrophiles and their cold-active enzymes (Nakagawa et al. 2002, 2003, 2004b, 2005a,b, 2006).

Cold-active enzymes could have important applications in food processing, biomass conversion, molecular biology, environmental biosensors, bioremediation, cleaning of contact lenses, and several other processes. In particular, they are attractive for the food industry, e.g., for the processing of fruit juices and milk, because (1) there is an increasing industrial trend to treat food stuffs under mild conditions to avoid spoilage and changes in taste and nutritional value at ambient temperature, and (2) cold-active enzymes can be inactivated at moderate temperatures without heating treatment (Margesin and Schinner 1994; Russell 1998). Among cold-active enzymes, β-galactosidase (EC 3.2.1.23), which hydrolyzes lactose to glucose and galactose, is one of the important food–industrial enzymes. It can be used to degrade lactose for several purposes: e.g., (1) removal of lactose from refrigerated milk for people who are lactose intolerant, (2) conversion of lactose to glucose and galactose, which are more fermentable sugars than lactose, in whey, and (3) removal of lactose from pollutants of the dairy industry (Triveni 1975).

In a previous study, we isolated several psychrophilic microorganisms that produce cold-active β-galactosidase, from soil from Hokkaido, Japan, for application to the food industry (Nakagawa et al. 2003, 2006). Among the strains isolated, strain F2, which was identified as *Arthrobacter psychrolactophilus*, was able to hydrolyze lactose at below 5°C, and there was only one type of cold-active β-galactosidase in intracellular fractions of strain F2, although

T. Nakagawa (✉) · Y. Fujimoto · R. Ikehata · T. Miyaji · N. Tomizuka
Department of Food Science and Technology,
Faculty of Bioindustry, Tokyo University of Agriculture,
196 Yasaka, Abashiri,
Hokkaido 099-2493, Japan
e-mail: t-nakaga@bioindustry.nodai.ac.jp
Tel.: +81-152-483845
Fax: +81-152-483845

some other strains have at least two β-galactosidase isozymes (Nakagawa et al. 2003). Moreover, a cell-free extract of the F2 strain exhibited an optimum temperature of 10°C and showed high specific activity of β-galactosidase at 0°C, although cold-active β-galactosidases previously reported exhibited optimum temperatures of around 20 to 35°C and practically low activities at 0°C (Cieslinski et al. 2005; Coker et al. 2003; Fernandes et al. 2002; Hoyoux et al. 2001; Karasova-Lipovova et al. 2003; Loveland et al. 1994). Therefore, it seems that strain F2 has a novel type of cold-active β-galactosidase, and that the β-galactosidase from strain F2 can be applied to the food industry.

The present study was conducted for enzymatic and molecular characterizations of the cold-active β-galactosidase from *A. psychrolactophilus* strain F2. Therefore, we attempted purification of the cold-active β-galactosidase from an intracellular fraction of strain F2 and cloning of the *bglA* gene encoding the enzyme from genomic DNA of strain F2.

Materials and methods

Bacterial strain and cultivation conditions

Arthrobacter psychrolactophilus strain F2 was cultivated in a mineral synthetic medium (Nakagawa et al. 2002; Sakai et al. 1998) containing 1% (w/v) lactose as the sole carbon source. The initial pH of the medium was adjusted to 7.0. Cultivation was performed aerobically at 5°C with rotary shaking at 150 rpm in Erlenmeyer flasks, and growth was followed by measuring the optical density at 660 nm.

Escherichia coli DH5α, which was used for plasmid propagation, was grown at 37°C in 2× tryptone–yeast medium, when necessary, being supplemented with ampicillin (50 μg/ml).

β-Galactosidase assay

β-Galactosidase activity was determined by measuring the rate of hydrolysis of 20 mM *o*-nitrophenyl-β-D-galactopyranoside (ONPG) or 5 mM lactose in 50 mM borate buffer, pH 8.0. The reaction mixture was incubated at 10°C for 10 min, and the enzyme reaction was stopped by using 1 M Na_2CO_3. With the ONPG method, the increase in absorbance at 415 nm was followed, and one unit of activity was defined as the amount of enzyme that produced 1 μmol of ONP per minute. On the other hand, hydrolysis of lactose was measured with a glucose-detection kit (Glucose B-test Wako; Wako Pure Chemical Industries, Osaka, Japan), and one unit of activity was defined as the amount of enzyme that produced 1 μmol of glucose per minute.

Protein was determined by the method of Bradford with a protein assay kit (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as the standard.

Purification of β-galactosidase from *A. psychrolactophilus* strain F2

Lactose-grown cells of *A. psychrolactophilus* strain F2 were harvested and then washed with 5 mM sodium phosphate buffer, pH 7.0. The cells were disrupted with a Sonifier 450 (Branson Ultrasonics, Danbury, CT, USA), and then the cell debris was removed by centrifugation at 20,000×*g*. The extract was fractionated by the addition of solid ammonium sulfate (40% saturated). The resultant supernatant was applied to a DE52 (Whatman International, Maidstone, UK) column (ϕ 33 by 270 mm), and was eluted with a stepwise increase in NaCl from 210 to 390 mM. The β-galactosidase-active fraction was applied to a Butyl Toyopearl 650S (Tosoh, Kanagawa, Japan) column (ϕ33 by 270 mm), and eluted with a linear gradient of 450 mM to 350 mM $(NH4)_2SO_4$. Next, the active fractions were applied to a HW75F (Tosoh) column (ϕ 25 by 1,110 mm). After the HW75F column chromatography, the fraction containing β-galactosidase gave a single band on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970).

Determination of the NH_2-terminal amino acid sequence

The NH_2-terminal amino acid sequence of the purified β-galactosidase was determined by automated Edman degradation using an Applied Biosystems protein sequencer (ABI, Foster City, CA, USA).

Cloning and sequencing of the *bglA* gene from *A. psychrolactophilus* strain F2

According to the NH_2-terminal amino acid sequence of the β-galactosidase from strain F2, an F1 primer, 5′-gCggCCgCTAYATHACNgAYCARggNCCNgg-3′, was designed and synthesized. On the other hand, an R1 primer, 5′-gCggCCgCggCggTTgACgCCgTggAA-3′, was designed from a high homology region in other β-galactosidase genes from *Arthrobacter* strains (Coker et al. 2003; Trimbur et al. 1994). The ca. 1.0-kb PCR fragment that was amplified from the genomic DNA with the F1 and R1 primers was subcloned into the pT7Blue T-vector (Novagen, Madison, WI, USA).

The *bglA* coding and 3′ flanking regions were amplified by means of the homologous PCR method and the cassette PCR method (Isegawa et al. 1992; Nakagawa et al. 2004a), using a Takara LA PCR in vitro cloning kit (Takara, Kyoto, Japan). The strain F2 genomic DNA was digested completely with *Eco*RI and then ligated with an *Eco*RI cassette (Takara). The first PCR was performed with the Cassette C1 Primer (Takara) and an N-S1 primer, 5′-ggCACTCACCCTgCgCTTTg-3′. The second PCR was

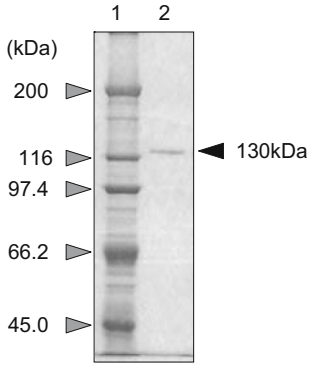

Fig. 1 SDS-PAGE analysis of the purified β-galactosidase from *A. psychrolactophilus* strain F2. Lanes: *1*, molecular standard high-range markers (Bio-Rad, Hercules, CA); *2*, purified β-galactosidase

performed with the Cassette C2 Primer (Takara) and an N-S2 primer, 5′-CACCggATggACggCTgACACC-3′. After the second PCR, the amplified ca. 1.0-kb fragment was subcloned into the pT7Blue T-vector. On the other hand, the next *bglA* coding region was amplified with an F3 primer, 5′-ACgCCATgggCAACggCC-3′, and an R3 primer, 5′-TgCAAAgTCCggCCATACAT-3′, designed from a high homology region in other β-galactosidase genes from *Arthrobacter* strains (Coker et al. 2003; Trimbur et al. 1994). Moreover, the 3′ outside of the cloned *bglA* gene was amplified by means of the cassette PCR method. The strain F2 genomic DNA was digested completely with *Eco*RI and then ligated with an *Eco*RI cassette (Takara). The first PCR was performed using the Cassette C1 Primer (Takara) and a C-S1 primer, 5′-gCACACggCACAg CAggTCA-3′. The second PCR was performed with the Cassette C2 Primer (Takara) and a C-S2 primer, 5′-CgTCAAgCCACAgCTACCTg-3′. After the second PCR, the amplified ca. 0.5-kb fragment was subcloned into the pT7Blue T-vector. The DNA fragments were sequenced with DNA sequencers, models 377 and 3130 (ABI).

The nucleotide sequence of *bglA* has been submitted to GenBank and assigned accession number AB243756.

Results

Characterization of the β-galactosidase from *A. psychrolactophilus* strain F2

In a previous study, we showed that *A. psychrolactophilus* strain F2, which did not have any β-galactosidase isozymes, exhibited high specific β-galactosidase activity at 0°C (Nakagawa et al. 2003). Because we thought that the enzyme from strain F2 may be a novel type of cold-active β-galactosidase, we attempted to purify the β-galactosidase from strain F2. The β-galactosidase was purified until a single band was obtained on SDS-PAGE, which corresponded to a molecular mass of ca. 130 kDa (Fig. 1). On the other hand, the determination of molecular mass of the native protein appeared to be ca. 548 kDa on native PAGE (data not shown). Therefore, it seems that the β-galactosidase from *A. psychrolactophilus* strain F2 is a tetrameric protein. The purification procedure for the β-galactosidase is summarized in Table 1.

At first, we examined the effect of temperature on the activity of the β-galactosidase from *A. psychrolactophilus* strain F2. The optimum temperature of the β-galactosidase was 10°C, and the specific activity of the purified enzyme was 33.3 U/mg for ONPG at 10°C. Moreover, the enzyme exhibited ca. 80% of the maximal activity even at 0°C. On the other hand, the β-galactosidase activity decreased with a further increase in temperature until it was undetectable above 60°C (Fig. 2a). To determine its thermostability, the β-galactosidase was incubated at a particular temperature before measuring the residual activity under standard conditions. Although the enzyme was very stable at 5°C, a drastic decrease in stability was observed above 30°C, and the β-galactosidase was inactivated within 5 min at 50°C (Fig. 2b). Judging from these findings, the β-galactosidase from *A. psychrolactophilus* strain F2 is a cold-active enzyme that is extremely heat-labile. One attribute of cold-active enzymes is their thermolability; i.e., they can be inactivated

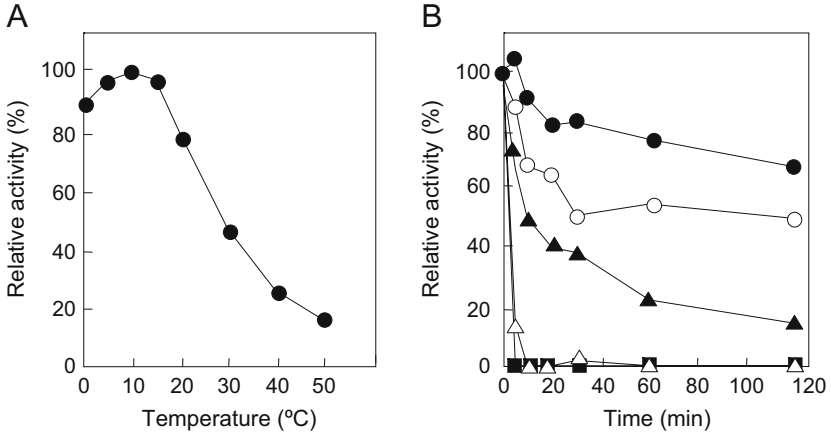

Fig. 2 Effects of temperature on the activity (**a**) and stability (**b**) of the purified β-galactosidase. In the stability test, the enzyme was incubated under the indicated conditions, and remaining activity was assayed under the optimum conditions for β-galactosidase. Symbols for the stability test (**b**): *black circles*, 30°C; *white circles*, 35°C; *black triangles*, 40°C; *white triangles*, 45°C; and *black squares*, 50°C

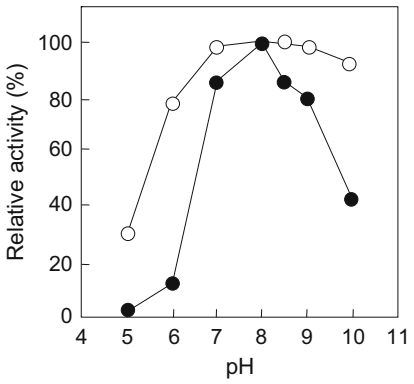

Fig. 3 Effects of pH on the activity and stability of the purified β-galactosidase. Symbols: *black circles*, activity, and *white circles*, stability of the purified β-galactosidase

at moderate temperatures close to those at which the enzymes from mesophiles are functional (Russell 2000).

β-Galactosidase showed optimum activity at pH 8.0, it exhibited above 80% of the optimum activity at pH 7.0 and 9.0, and its pH stability range was 6.0–10.0 (Fig. 3). On the other hand, β-galactosidase substrate specificity was determined by assaying it with several chromogenic substrates (Table 2). The enzyme exhibited about 26.5% of the ONPG activity when *p*-nitrophenyl-β-D-galactopyranoside was used as the substrate, but it showed less than 1% of the ONPG activity with any of the other substrates tested (Table 2). Moreover, the β-galactosidase was able to hydrolyze lactose, as well as ONPG; although the K_m values with ONPG and lactose were calculated to be 2.8 mM and 50 mM, respectively, at 10°C. On the other hand, the K_{cat} values of the enzyme with ONPG and lactose were calculated to be 93.5 S^{-1} and 18.0 S^{-1}, respectively, at 10°C. d

Judging from these results, the β-galactosidase from *A. psychrolactophilus* strain F2 is a cold-active enzyme that exhibits the lowest optimum temperature and is the most heat labile among the β-galactosidases reported so far. Moreover, 1 U of the enzyme was able to hydrolyze about 80% of the lactose in 1 ml of milk at 10°C in 24 h (data not shown). Therefore, it seems that the β-galactosidase from *A. psychrolactophilus* strain F2 exhibits good potential for uses as an industrial cold-active enzyme.

Table 1 Summary of purification of the β-galactosidase from *A. psychrolactophilus* strain F2

	Total protein (mg)	Total activity (units)	Specific activity (U/mg)	Purification (fold)
Crude extract	1,470	75.3	0.0511	1
$(NH_4)_2SO_4$ (40%)	695	297	0.427	8.37
DEAE-cellulose DE52	30.7	63.1	2.05	40.2
Butyl TOYO PEARL 650 S	0.474	10.2	21.6	423
HW-75 F	0.131	4.37	33.3	652

Table 2 Activity of the purified β-galactosidase with various nitrophenyl-derived chromogenic substances

Substrate	Relative activity (%)
o-nitrophenyl-β-D-galactopyranoside	100
p-nitrophenyl-β-D-galactopyranoside	25.6
p-nitrophenyl-α-D-galactopyranoside	<1
p-nitrophenyl-β-D-glucopyranoside	<1
p-nitrophenyl-β-D-xylopyranoside	<1
p-nitrophenyl-β-D-mannopyranoside	<1
p-nitrophenyl-β-D-alabinopyranoside	<1
p-nitrophenyl-β-D-fucopyranoside	<1

Primary structure of the β-galactosidase from *A. psychrolactophilus* strain F2

The NH_2-amino acid sequence of the purified β-galactosidase was NH_2-MTPADVSYITDQGPGSGLRV, which was very similar to those of the β-galactosidases from *Arthrobactor* sp. strain C2-2 (identity of 17/20 amino acids) (Karasova-Lipovova et al. 2003) and *A. psychrolactophilus* strain B7 (identity of 15/20 amino acids) (Loveland et al. 1994).

Using information of the NH_2-amino acid sequence of the purified enzyme, we attempted to clone the *bglA* gene encoding β-galactosidase from *A. psychrolactophilus* strain F2 and determined the complete primary structure of the enzyme. The *bglA* gene consists of a 3,084-bp open reading frame (ORF) corresponding to a protein of 1,028 amino acid residues. This ORF was identified as the gene encoding β-galactosidase from strain F2 based on (1) the identity of the N-terminal amino acid sequence determined with that of the purified β-galactosidase protein, and (2) agreement of the calculated molecular weight of this protein (111,666 Da) with that of the purified β-galactosidase determined by SDS-PAGE (130 kDa). Moreover, the deduced amino acid sequence encoding the *bglA* gene showed high similarity to those of the β-galactosidases from *Arthrobacter* sp. strain C2-2 (79.0% identity) (Karasova-Lipovova et al. 2003), strain B7 (77.5% identity) (Loveland et al. 1994), and strain SB (63.9% identity) (Coker et al. 2003).

BglA had contained glutamic acid residues (446 and 542) involved in catalysis in the *E. coli* LacZ enzyme (Fowler and Smith 1983). Moreover, BglA contained several conserved regions for glycosyl hydrolase family 2, e.g., glycosyl hydrolase 2 (GH2) sugar binding domain (29–218), GH2 acid-base catalyst (432–446), GH2 triose-phosphate isomerase barrel domain (317–618) (Jacobson et al. 1994), GH2 signature 1 (367–392), and several other GH2 conserved regions (Fig. 4). From these facts, it seems that *bglA* is a member of glycosyl hydrolase family 2.

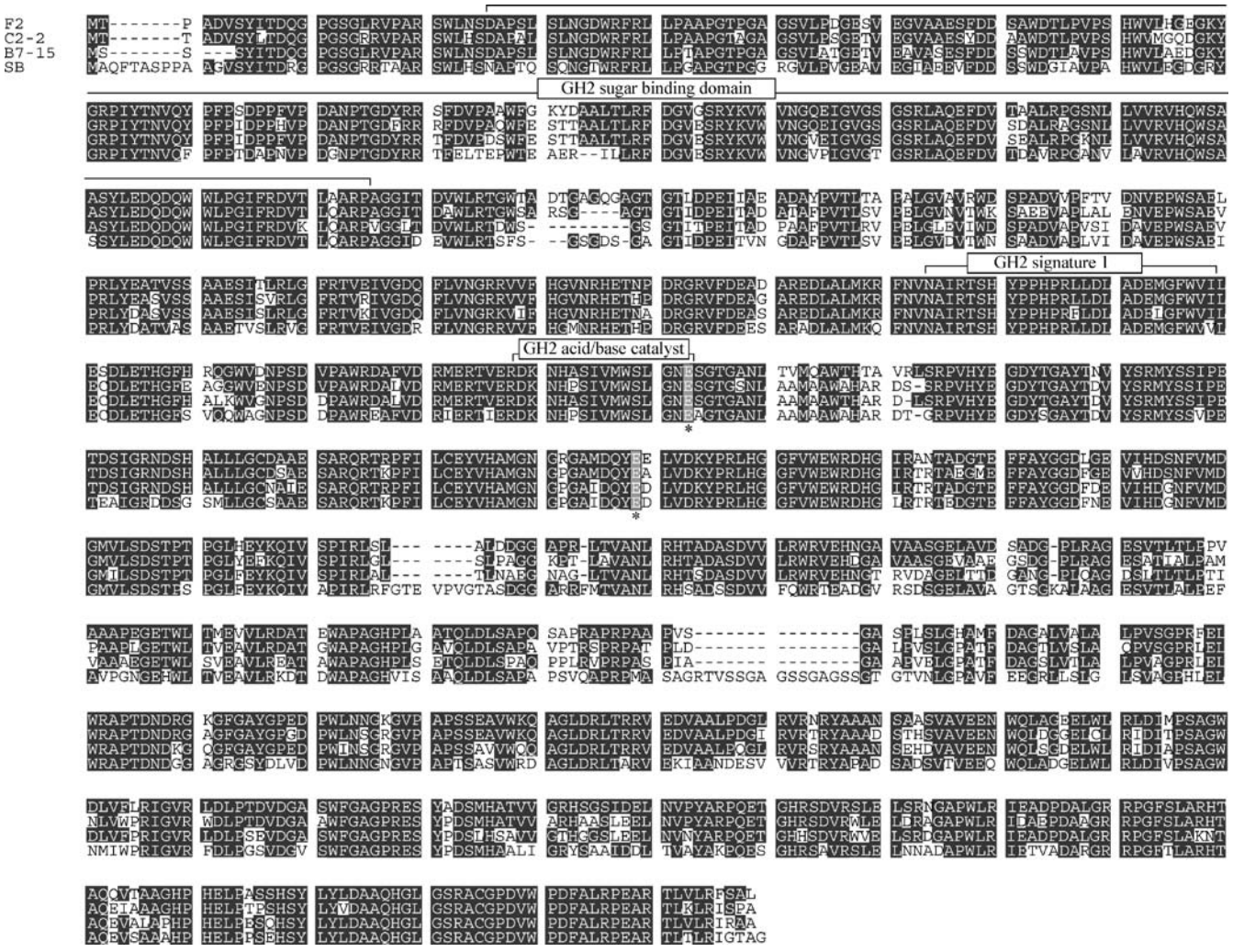

Fig. 4 Alignment of the deduced amino acid sequence of *bglA* from *A. psychrolactophilus* strain F2 with sequences of *Arthrobacter* sp. strain C2-2 (GenBank accession number CAD29775), strain B7 (U12334), and strain SB (AAQ19029). *White letters* indicate identical amino acid residues with BglAp from strain F2. *Gray boxes with asterisks* indicate glutamic acid residues involved in catalysis in the *E. coli* LacZ enzyme

Discussion

In this study, we revealed the enzymatic properties and primary structure of a β-galactosidase from *A. psychrolactophilus* strain F2. It has been reported that several *Arthrobacter* strains produce cold-active β-galactosidase isozymes (Coker et al. 2003; Karasova-Lipovova et al. 2003; Loveland et al. 1994; Nakagawa et al. 2003). However, *A. psychrolactophilus* strain F2 has only one functional β-galactosidase, *bglA*, and its gene product, BglA, is the only type of functional β-galactosidase in strain F2 because (1) Southern analysis of the genomic DNA from strain F2 with the *bglA* gene showed only a one-signal band for genomic DNA digested with *Bgl*II, *Hin*dIII, *Xba*I, and *Xho*I (data not shown), and (2) the intracellular fraction of strain F2 grown on lactose gave only one activity band for β-galactosidase (Nakagawa et al. 2003). On the other hand, the amino acid sequence of BglA exhibits high identity with those of the β-galactosidases from other *Arthrobacter* sp. strains. Moreover, BglA contained several conserved regions for the glycosyl hydrolase family 2. From these facts, we judged that BglA from strain F2 belongs to glycosyl hydrolase family 2, like the β-galactosidases of *Arthrobacter* sp. strains.

The β-galactosidase purified from strain F2 was able to hydrolyze lactose as a substrate, as well as ONPG, and exhibited high specific activity at 0°C; although its optimum temperature was 10°C. One β-galactosidase from strain SB exhibited an optimum temperature of ca. 18°C and showed ca. 50% of the maximal activity at 0°C (Coker et al. 2003). Other cold-active β-galactosidases that have been reported exhibit optimum temperatures of around 30 to 50°C and practically lower activities at 0°C than the activity of strain F2 (Cieslinski et al. 2005; Fernandes et al. 2002; Hoyoux et al. 2001; Karasova-Lipovova et al. 2003; Loveland et al. 1994). Moreover, K_{cat} values of the enzyme at 10°C were about tenfold higher than those of β-galactosidase from mesophilic *E. coli* (Hoyoux et al. 2001); although they had same or slightly lower levels than those from strains C2-2 or SB (Karasova-

Lipovova et al. 2003; Coker et al. 2003). On the other hand, the temperature stability of β-galactosidase from strain F2 is lower than those of the β-galactosidases of other *Arthrobacter* strains. While the activity of β-galactosidase from strain C2-2 decreased to 40% on incubation at 45°C in 10 min, the enzyme from strain F2 lost all its activity under the same conditions. In short, it was possible to inactivate the β-galactosidase rapidly at 45°C in 5 min, which are mild conditions. Moreover, the enzyme was able to hydrolyze about 80% of the lactose in milk at 10°C in 24 h (data not shown).

From these facts, we conclude that the β-galactosidase from *A. psychrolactophilus* strain F2 is a cold-active enzyme that is extremely heat-labile, and that the enzyme could have advantageous applications in the food industry, e.g., the treatment of chilled dairy products while avoiding flavor tainting and the risk of microbial contamination.

Acknowledgements We are indebted to Professor Toshihiro Watanabe, Tokyo University of Agriculture, for his help in the amino acid sequence determination. We are also grateful to Ms. Kumiko Sato, Ms. Takae Tamura, Ms. Riwako Shima, and Mr. Takahito Hayashi for their skillful assistance. This research was supported in part by a grant from the Northern Advancement Center for Science & Technology to T.N.

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