

β -Glucoside Hydrolyzing Enzymes from Ale and Lager Strains of Brewing Yeast

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ABSTRACT

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A study of β -glucosidase in diverse yeast strains was undertaken. The enzyme is present at higher levels in ale and lager strains than in the wine and other yeast strains tested. The enzyme has been purified from both types of brewing yeast and has a molecular weight of approx. 50,000, an ability to function efficiently over the pH range 4 to 6, an ability to hydrolyze both β 1-3 and β 1-4 glucosidic linkages, and a relative intolerance of heat (more than 50% activity loss in 30 min at 50°C). Its location primarily within the cell suggests that it probably does not catalyze the hydrolysis of β -glucosides found in wort.

Keywords: β -Glucosidase, Yeast

RESUMEN

Un estudio de la β -glucosidasa en cepas diversas de levadura se llevó a cabo. La enzima está presente en niveles más altos en las cepas de ale y lager que en el vino y otras cepas de levadura ensayadas. La enzima ha sido purificada a partir de ambos tipos de levadura de cerveza y tiene un peso molecular de aprox. 50,000, una capacidad para funcionar eficazmente entre el intervalo de pH 4-6, una capacidad para hidrolizar tanto β 1-3 y β 1-4 enlaces glucosídicos, y una intolerancia relativa de calor (más de 50% de pérdida de actividad en 30 minutos a 50°C). Su ubicación principalmente dentro de la célula sugiere que es probable que no catalizan la hidrólisis de β -glucósidos encontrados en el mosto.

Palabras claves: β -Glucosidasa, Levadura

Wort and beer contain a range of substances containing β -glucosidic linkages. Prominent among these are the degradation products arising from the hydrolysis of the mixed-linkage β -glucans that compose the primary component of the cell walls of the starchy endosperm of barley (2,3). We have previously shown that, although there is an enzyme portfolio developed in germinating barley that is, in theory, capable of completely converting these polymers to glucose, this does not occur and there are sizable amounts of β -linked oligosaccharides present in wort and beer (14). It was argued that the reason for this is that the enzyme that would ostensibly produce glucose from those oligosaccharides has a low affinity for them (4).

Nevertheless, enzymes have been reported in certain *Saccharomyces* strains that are capable of hydrolyzing β -glucosides (9). These include a β 1-4-glucosidase (10) and an exo- β -glucanase (11). Indeed there are diverse β -glucosidases (EC. 3.2.1.-) in nature and they have a breadth of functions and activities. They have long been reported to have significant roles in the growth of many

plants and microorganisms, including as part of the sporulation process in yeast cells (18). The substrates for β -glucosidases include β -linked oligosaccharides but also β -O-glucosides of alkyl and aryl compounds (17). In the former context, they are important for the degradation of polysaccharides, such as β -glucans. In the latter context, they have been employed in the food and beverage industries for the hydrolysis of bitter compounds (naringin) in citrus juice and the release of flavor from grape juices, including in the production of wine (1,5,11). It has been suggested that some of the components of hops are also glycosidically linked and that β -glucosidases from yeast may have a role in releasing the flavor-active aglycone (12,15).

In this article, we report the screening of a diversity of *Saccharomyces* yeasts for their ability to hydrolyze β -glucosidic linkages and report some of the properties of the enzymes isolated from ale and lager yeast.

EXPERIMENTAL

Yeast Strains and the Detection of β -Glucosidase Activity

Yeasts evaluated were 2206 Bavarian Lager, 2000 Budvar Lager, 2278 Czech Pils, 2007 Pilsen Lager, 2035 American Lager, 2565 Kölsch, and 1056 American Ale (Wyeast Laboratories, Odell, OR); lager yeast S-23, lager yeast W-34/70, and ale yeasts S-33, S-04, and T-58 (Fermentis, Milwaukee, WI); NRIC 0083, NRIC1560, and wine yeast NRIC 1500 (NODAI Culture Collection Center, Tokyo); and wine yeast W-3 and Sake yeasts K-7, K-9, and K-10 (Brewing Society of Japan, Tokyo). Each yeast strain was cultivated in 10 mL of malt extract (Muntions Dry Malt Extract Light was dissolved in deionized water to 12°P) until stationary phase (10^8 cells/mL) at 15°C in 15 mL centrifugation tubes. Yeast growth was monitored by measuring absorbance at 620 nm. Cultures were centrifuged at $4,800 \times g$ for 10 min and 10 mM p-nitro phenyl β -D-glucopyranoside (Wako Pure Chemical Industries, Osaka, Japan) was added to the supernatants in 50 mM citrate-phosphate buffer (pH 5.0) containing 5% ethanol. The mixture stood at 15°C for 7 days prior to measurement of absorbance at 400 nm. The experiment was performed in quadruplicate.

Extraction of β -Glucosidase

Ale yeast (1056 American ale) and lager yeast (2007 Pilsen Lager) were cultivated in 1 L of malt extract (12°P) at 15°C for 7 days. The yeast cells were harvested by centrifugation at $4,800 \times g$ for 10 min. Cells were disrupted in 50 mM citrate-phosphate buffer, pH 5.0, containing 2 mM ethylenediaminetetraacetic acid disodium (Sigma-Aldrich, St. Louis), 2-mercaptoethanol (Wako Pure Chemical Industries), and 0.5% sodium chloride, using glass beads in a spin-down mixer (GM-01; TAITEC Co., Ltd., Koshigaya, Saitama, Japan). The extract was recovered by centrifugation at $10,000 \times g$ for 20 min.

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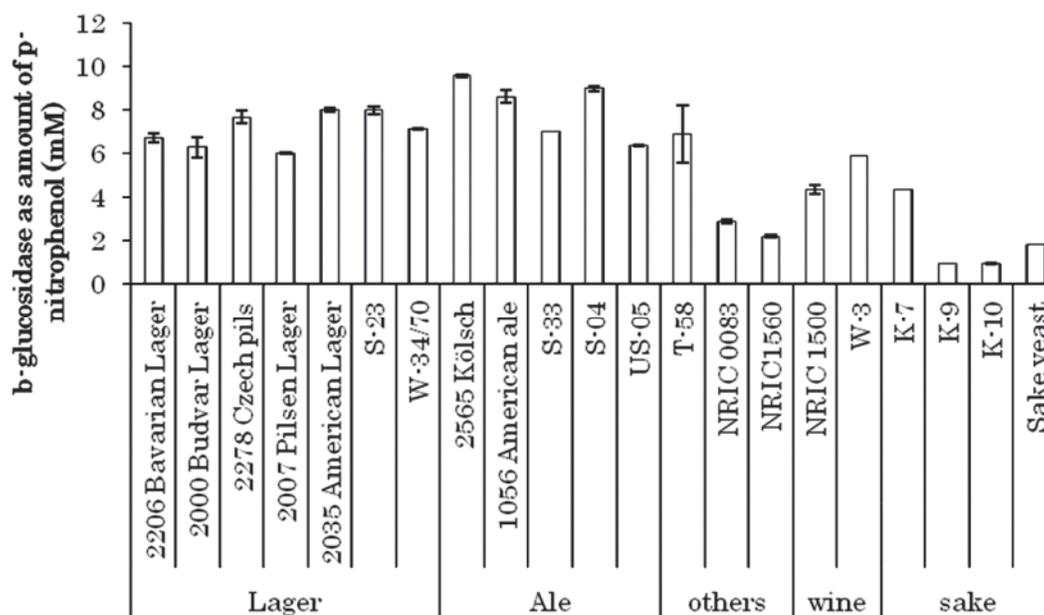


Fig. 1. Levels of extracellular β -glucosidase detected in a diversity of yeasts grown on malt extract.

Assay of β -Glucosidase

The substrate was laminarin at 5 mg/mL dissolved in 50 mM citrate-phosphate buffer, pH 5.0. Enzyme solution (50 μ L) was added to substrate solution (100 μ L) and the mixture was incubated for 30 min at 37°C. The reaction was stopped by adding 1M NaOH (50 μ L) and allowed to stand for 30 min prior to the addition of HCl (1M, 50 μ L). Glucose produced in the reaction was determined by the Glucose CII Test (catalog number 30167002; Wako Pure Chemical Industries). One unit of enzyme is the amount that produces 1 mM glucose from the substrate in 1 min.

Assay of Protein

Protein was assayed according to Bradford (6).

Enzyme Purification

Ammonium sulfate was added to the crude extract to between 50 and 60% saturation and the precipitate redissolved in 5 ml of 50 mM citrate-phosphate buffer, pH 5.0, containing 2 mM EDTA and 2 mM 2-mercaptoethanol. The solution was dialyzed overnight at 4°C against 1 L of the same buffer. Ion exchange chromatography used a column (25 by 300 mm) of Macro-Prep DEAE. The protein was eluted by a 0 to 1 M NaCl linear gradient, flowing at a rate of 2 ml min^{-1} , and 4 ml eluate fractions were collected. Those fractions containing the highest level of activity were pooled and reprecipitated using 80% saturation of ammonium sulfate. The precipitate was redissolved in 1 ml of 50 mM phosphate buffer, pH 5.0, and subjected to gel permeation chromatography (a 10 by 350 mm column of P-100 gel; Bio-Rad Laboratories, Inc., Hercules, CA) using the same buffer as eluent flowing at 0.5 mL min^{-1} with collection of 5 ml fractions.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The samples were separated on a 12.5% uniform gel (e-PAGEL, catalog number E-T12.5L; ATTO, Tokyo). Electrophoresis was carried out as described by Laemmli (16), with the following modifications: the samples (0.01 mL) were added to 0.01 mL of sample buffer and then heated at 100°C for 3 min. Samples were added at 10 μ l per well. The gels were run at 20 mA at a gel thickness of 1.5 mm. Molecular weight standards were from Bio-Rad Laboratories, Inc. The gel was stained with 0.25% Coomassie Brilliant Blue R-250.

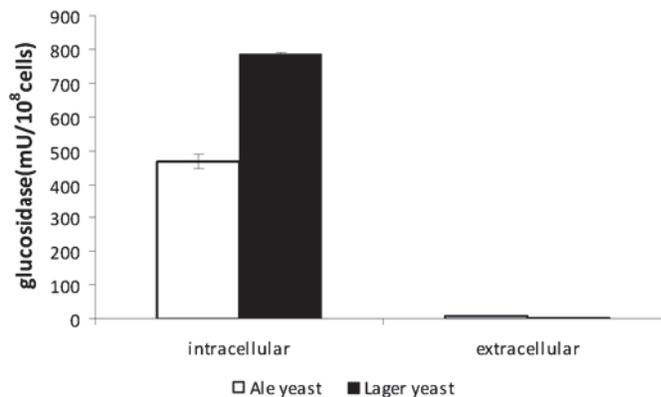


Fig. 2. Levels of β -glucosidase in intracellular and extracellular compartments of yeast.

Determination of Optimum pH and Heat Stability

Activity was determined at various pHs in the range of 2.0 to 9.0 by performing the standard assay using either citrate-phosphate buffer (pH 2.0 to 8.0) or borate buffer (pH 8.0 to 9.0). For determination of heat stability, the enzyme solution was heated at 30 to 100°C for 30 min and cooled in ice prior to the standard assay.

Determination of Kinetic Parameters

Laminarin, cellobiose, or p-nitrophenyl β -D-glucopyranoside concentrations were varied in the standard assay and the values for Michaelis constant (K_m) and maximum velocity (V_{max}) determined by classical Lineweaver-Burk plots.

RESULTS

β -Glucosidase was detected in all the yeast strains examined but at very low levels (Fig. 1). Brewing strains tended to display higher levels than were found in other yeast strains, including wine and sake yeasts. For both ale and lager strains, the level of activity released from the cell during growth was vastly less than that which could be released from the cell by disruption (Fig. 2), with less than 2.5% being extracellular.

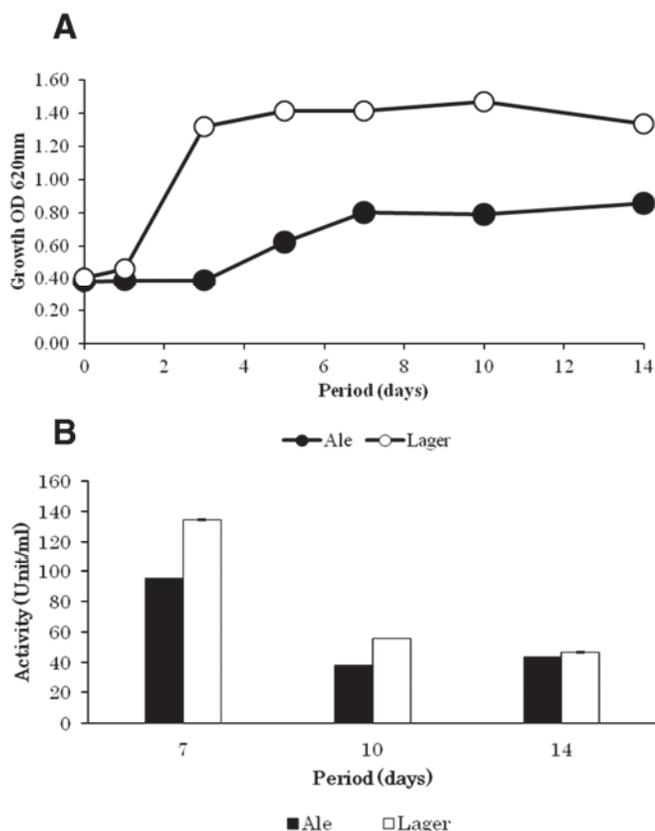


Fig. 3. A, Growth of yeast on malt extract and B, β-glucosidase levels after different growth periods.

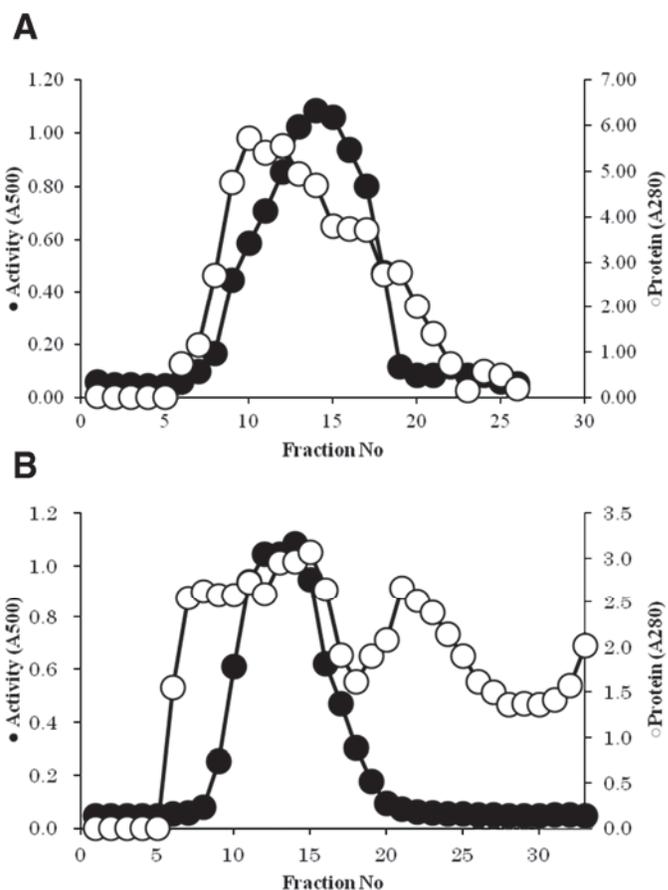


Fig. 5. Gel permeation chromatography of extracts of A, ale yeast and B, lager yeast.

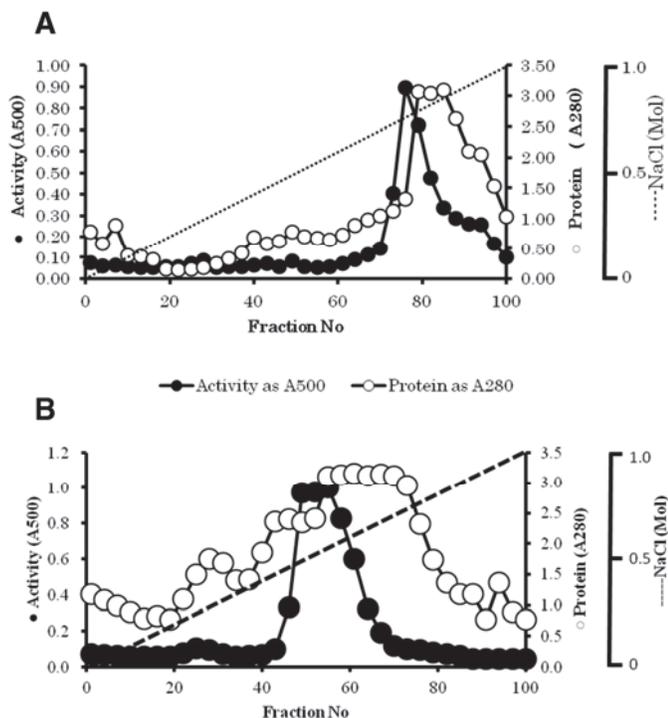


Fig. 4. Ion exchange chromatography of extracts of A, ale yeast and B, lager yeast.

Activity of β-glucosidase was found to decrease on prolonged contact of yeast with wort (Fig. 3). A single β-glucosidase was detected in both ale and lager strains of yeast, as made evident by ion exchange and gel permeation chromatography (Figs. 4a and b and 5a and b). In each case, a single band of protein was detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis of β-glucosidase purified from either ale or lager yeast and this band was of molecular weight of approx. 50,000 (Fig. 6). Other characteristics of the enzymes from both ale and lager strains are very similar. They both displayed activity over a relatively broad spectrum of pH (Fig. 7) and showed comparable heat tolerance (Fig. 8).

β-Glucosidase isolated from both ale and lager yeast displayed a relatively high affinity (low K_m) for both laminarin (β1-3) and cellobiose (β1-4) (Table I).

DISCUSSION

In the present study, we have identified a single β-glucosidase in brewing strains of yeast. By contrast, Daenen (9) could not detect such an enzyme in either ale or lager strains. However, Daenen (9) did discuss the presence of an exo-β1,3-glucoanase (EC 3.2.1.58) which does have the ability to hydrolyze p-nitrophenyl-β-D-glucoside (20), the screening substrate used in the present work. It is likely that the enzyme characterized in this study is, indeed, that enzyme.

Previous reports conflict in that some suggest that β-glucosidase is extracellular (8) while others suggest that it is in the protoplasm and periplasmic space (7). Our observation that the

TABLE I
Kinetic Parameters for β -glucosidase

	Laminarin		<i>p</i> -Nitrophenyl β -glucopyranoside		Cellobiose	
	K_m (mg/mL)	V_{max} (mg/min)	K_m (mM)	V_{max} (mL/min)	K_m (mM)	V_{max} (mM/min)
Ale Yeast	14.0	2.4	12.5	73.5	1.34	172.41
Lager Yeast	3.10	2.65	15.5	135.1	1.5	188.68

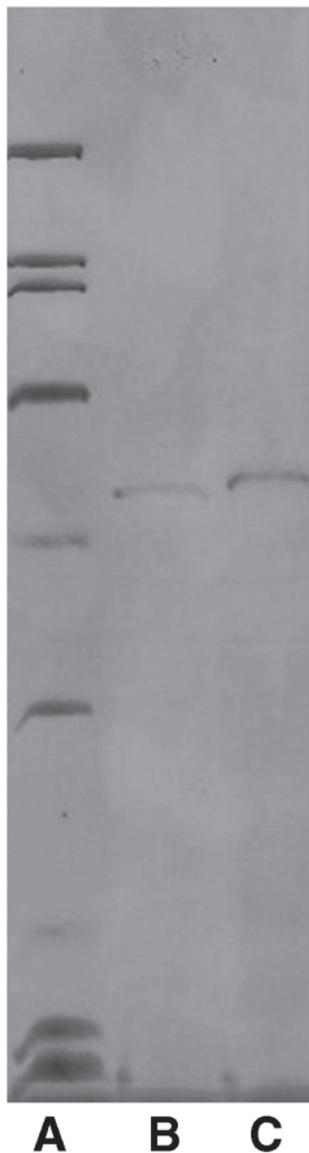


Fig. 6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of purified β -glucosidase from lane A, ale yeast, and lane C, lager yeast. Lane A represents molecular weight standards.

enzyme levels measurable are greatly increased by disruption of the wall are consistent with the latter claim. In terms of substrate specificity (20), molecular weight (19), and thermotolerance (19), the values reported for the present enzyme are comparable with previous reports and confirm that the enzyme is capable of catalyzing the hydrolysis of both β 1-3 and β 1-4 linked saccharides.

The intracellular location of the enzyme makes it unlikely that it has much ability to degrade β -linked oligosaccharides derived in mashing and mashing and which, accordingly, are found in finished beer (13,14). It is as yet unknown whether this enzyme has any capability to hydrolyze hop-derived entities of the type described

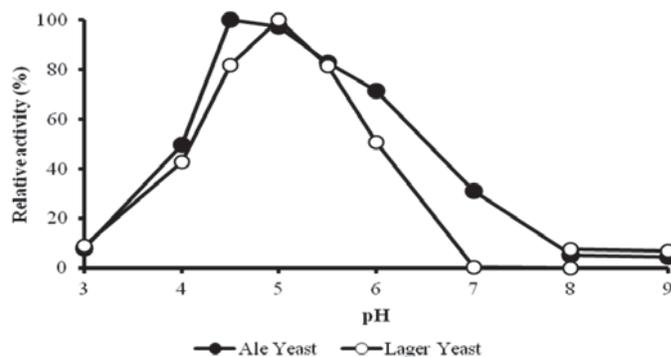


Fig. 7. pH optimum of β -glucosidase.

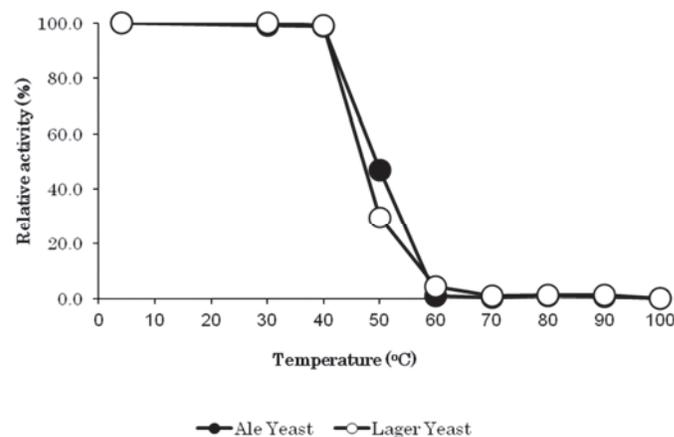


Fig. 8. Temperature stability of β -glucosidase.

above but the work of Daenen et al (9) suggests that it may be so. However, it remains to be seen whether the very low levels of the enzyme that are released from intact cells are sufficient to effect any significant changes in this regard with impact on the aroma of beer.

CONCLUSIONS

A broad pH spectrum β -glucosidase is present in both ale and lager strains of yeast at higher levels than in other *Saccharomyces* spp. and it can hydrolyze both β 1-3 and β 1-4 linkages. Its function is unclear but it may have a role in mobilizing the glucan component of the yeast cell. It is unlikely to impact the β -linked oligosaccharides present in wort.

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