Purification, Characterization, and a Potential Application of β -Glucosidase from *Aspergillus pulverulentus* YM-80

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Abstract: A fungus, strain YM-80, isolated from a soil sample produced β -glucosidase (β -D-glucoside glucosylhydrolase EC 3.2.1.21) in solid culture. The fungus was identified as *Aspergillus pulverulentus* from its taxonomical characteristics. The β -glucosidase was purified 18-fold from the culture extract by $(\text{NH}_4)_2\text{SO}_4$ precipitation, DEAE-Toyopearl 650M, Butyl-Toyopearl 650M and Sephacryl S-200 chromatography. The purified enzyme appeared as a single band on SDS-PAGE. The molecular weight of the purified enzyme was estimated to be 118,000 by SDS-PAGE and 240,000 by gel filtration. The enzyme was an acidic protein with a *pI* of 4.5 and the specific activity was 308 U/mg protein. The optimum temperature and pH for the enzyme activity were 60°C and 4.0, respectively. This enzyme was stable in the pH range 3.0–7.0 and also up to 50°C. The activity of the enzyme was completely inhibited by Hg^{2+} and SDS. The purified enzyme was active with a broad range of β -linked glucosides but not α -linked saccharides. Added during tofu processing, the enzyme was useful for the preparation of aglycon isoflavone-enriched tofu by hydrolysis of the glucoside form. However, isoflavones with 6''-O-acetyl and 6''-O-malonyl glucosides were not converted into the aglycon form.

Key words: β -glucosidase, *Aspergillus*, solid culture, isoflavone, tofu

By definition, β -glucosidase (EC 3.2.1.21; β -D-glucoside glucosylhydrolase) hydrolyzes compounds containing β -glucosidic linkages by splitting off the terminal non-reducing β -D-glucose residues with release of β -D-glucose.¹⁾ Many studies of β -glucosidases have been reported, because of their wide distribution in nature and their usefulness for food processing. A β -glucosidase with wide substrate specificity can catalyze the hydrolysis of not only β -oligoglucosides but also aryl and alkyl β -glucosides.²⁾ In plants used as food, the glycosides of various flavones and anthocyanins are pigments that contain a sugar residue and a non-sugar portion termed the aglycon, which is often a phenolic derivative.³⁾ For example, the β -glucosidases contribute to the formation of flavor, color and anti-oxidants originating in their aglycon in fermentation-produced food such as wine⁴⁾ or in the food industries such as tea.⁵⁾ The bioavailability of these phenolic compounds converted into aglycon form is enhanced and contributes to human health.

β -Glucosidases from fungi such as *Aspergillus niger*,⁶⁾ *Thermomyces langinosus*,⁷⁾ *Cladosporium resinae*⁸⁾ and *Acremonium persicinum*⁹⁾ have been reported (especially *Aspergillus* species) to be good sources of β -glucosidase. The β -glucosidase from *A. niger*,¹⁰⁾ *A. oryzae*,¹¹⁾ *A. fumigatus*¹²⁾ and *A. sojae*¹³⁾ have been purified and their characteristics recorded. Above all, black *Aspergillus* species are one of the best producers of food processing enzymes recognized as food additives, both from the standpoint of historical results and non-pathogenicity. We searched for a β -glucosidase-producing black *Aspergillus* with wide substrate specificity toward phenolic compounds with a β -glucoside form for use as a food additive during food

processing. Our screening discovered *Aspergillus pulverulentus* YM-80, which had been isolated from a soil sample and was found to be very suitable for this purpose.

In this paper we report the purification of β -glucosidase from a solid culture and the general characteristics such as molecular weight, optimum pH and potential application.

MATERIALS AND METHODS

Chemicals. *p*-Nitrophenyl monosaccharide, glycosides, sophorose, and laminaribiose were purchased from Sigma Chemical Co. Cellooligosaccharides were obtained from Wako Pure Chemical Industries, Ltd. All other chemicals were of analytical grade.

Microorganisms. The strain YM-80, isolated from soil in Japan, was used in this study. Strain YM-80 was identified taxonomically as *Aspergillus pulverulentus* based on the procedures of K.B. Raper.¹⁴⁾ The culture was maintained on potato dextrose agar slants.

Cultivation. An aliquot containing hyphae grown on slants was inoculated into 100 mL of culture solution containing 10% (w/v) commercial wheat bran. After incubation at 30°C for 40 h, the culture broth was used as a subculture. One liter Erlenmeyer flasks with cotton stoppers were charged with 360 g of medium prepared by thoroughly mixing 1000 g wheat bran with 800 mL of tap water, then sterilized at 121°C for 45 min. After cooling, the flasks were inoculated with 40 mL of subculture. The contents were mixed thoroughly and incubated at 29°C for 4 days.

Purification of a β -glucosidase. All operations were performed at 5°C.

Step 1. Ammonium sulfate fractionation. About 840 g of the solid culture of *A. pulverulentus* YM-80 grown

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on wheat bran at 29°C for 4 days was immersed in about 4000 mL of distilled water for 16 h. After filtration with filter paper, the filtrate was concentrated to 300 mL by ultrafiltration using AIV-1010 (Asahi Kasei Co.) with 100% recovery of β -glucosidase activity. The enzyme solution (300 mL) was re-filtered to remove insolubles. To 275 mL of the filtrate, solid $(\text{NH}_4)_2\text{SO}_4$ was added up to 70% saturation with stirring and left overnight at 5°C. The resulting precipitates were collected by filtration, and dissolved in 90 mL of 50 mM Tris-HCl buffer (pH 8.5). The enzyme solution was dialyzed against 50 mM Tris-HCl buffer (pH 8.5).

Step 2. DEAE-Toyopearl 650M column chromatography. The enzyme solution in step 1 was applied to a DEAE-Toyopearl 650 M column ($4\phi \times 20$ cm) equilibrated with 50 mM Tris-HCl buffer (pH 8.5) at a flow rate of 6 mL/min. After the column was washed with 50 mL of equilibration buffer, the adsorbed enzyme was eluted with 500 mL of a linear gradient of 0–0.3 M NaCl in the same buffer. Protein with enzymatic activity was eluted with 0.15 M NaCl in the buffer and the active fraction pooled was equilibrated with 50 mL Tris-HCl buffer (pH 7.0) containing 2 M $(\text{NH}_4)_2\text{SO}_4$ by dialysis against the same buffer.

Step 3. Butyl-Toyopearl 650M column chromatography. The enzyme solution in step 2 was applied to Butyl-Toyopearl 650 M column ($3\phi \times 10$ cm) equilibrated with the same buffer with 2 M $(\text{NH}_4)_2\text{SO}_4$. After the column was washed with 50 mL of equilibration buffer, the adsorbed enzyme was eluted with 150 mL of a linear gradient of 2–0 M $(\text{NH}_4)_2\text{SO}_4$ in the same buffer. The active fractions eluted with 1 M $(\text{NH}_4)_2\text{SO}_4$ were pooled and dialyzed against 50 mM Tris-HCl buffer (pH 7.0) with 0.5 M NaCl.

Step 4. Sephacryl S-200 column chromatography. Four mL of enzyme solution obtained from step 3 was subjected to gel filtration on a Sephacryl S-200 column ($3.5\phi \times 100$ cm) using the same buffer at a flow rate of 20 mL/h. After the active fractions were collected and concentrated with ultrafiltration, the purified enzyme solution was dialyzed against 50 mM McIlvaine buffer (pH 4.0) and stored at 4°C.

Enzyme assay. The standard assay mixture for β -glucosidase activity contained 0.5 mL of 10 mM *p*-nitrophenyl β -D-glucoside (*p*NPG), 0.4 mL of 50 mM McIlvaine buffer (pH 4.0) and 0.1 mL (0.007–0.035 U) of enzyme solution. After incubation for 15 min at 50°C, 4 mL of 0.1 M NaHCO_3 was added to the reaction mixture. *p*-Nitrophenol liberated was estimated by measuring the absorbance of the mixture at 420 nm. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the liberation of 1.0 μmol of *p*-nitrophenol/min under the standard assay conditions.

Protein assay. The protein concentration was measured with Bio-Rad protein reagent (Bio-Rad Laboratories, Richmond, CA, USA) using bovine serum albumin as the standard. The absorbance at 280 nm was used for monitoring protein in column effluent.

Electrophoresis. SDS-PAGE was done with the Phast System (Pharmacia Biotech Co., Ltd.) using a Phast Gel Homogeneous 7.5 (Pharmacia Biotech Co., Ltd.). Protein

markers *Mr* 30,000–205,000 (Amersham Biosciences K. K. Tokyo, Japan) and 1 μL of SDS-treated enzyme solution (20 $\mu\text{g}/\text{mL}$) were used for the molecular weight calibration. Protein was stained with Coomassie Brilliant Blue R-250. The *pI* of the purified enzyme was measured using an LKB Ampholine electrofocusing apparatus (110 mL), by the method of Vesterberg and Svensson at 500 V and 4°C for 48 h.¹⁵⁾ The ampholite concentration was 1% with a pH range of 3–6 in a sucrose gradient and 5 mg of enzyme protein was applied.

Gel filtration for molecular weight determination.

The molecular weight was also estimated by gel filtration, with a Sephadex G-200 column ($2\phi \times 95$ cm). After equilibration with 50 mM Tris-HCl buffer (pH 7.0) with 0.5 M NaCl, 1 mL of the purified enzyme (350 U/mL) was applied to the column. The flow rate was 10 mL/h, and 2.5 mL fractions were collected.

Measurement of sugar content. The sugar content in the purified enzyme (100 μg of protein) was measured by the phenol-sulfuric acid method as glucose equivalents.¹⁶⁾

Preparation of tofu enriched isoflavone aglycon form.

One hundred grams of commercial soybean was soaked in 600 mL of tap water at 10°C for 15 h, and ground with a homogenizer (Nihon Seiki Co). Purified β -glucosidase (100 U) was added to the ground soybean suspension and incubated at 50°C for 1 h. The resulting suspension was heated to boiling for 4 min and filtered through cotton cloth to separate soybean milk. The coagulant (2 g of CaSO_4) was added to the soymilk and mixed. After discarding the supernatant (whey), the white coagulated mass was transferred to a box with cloth inside and pressed to remove excess whey. The mass was taken out and the content of isoflavone was measured with HPLC based on Toda's method.¹⁷⁾

RESULTS

Identification of an isolated fungus.

The morphological characteristics of the fungus strain YM-80 are shown in Table 1. Culture tests were done by the procedures of K.B. Raper¹⁴⁾ by using Czapeck-Dox agar and Malt extract agar as test media. The color of its conidial heads was brownish-black to black. The surfaces of conidia were rough-wall and conidiogenous cells were biserial. Conidiophores were 6–8 mm in length. On comparing these characteristics with the literature, the fungus strain YM-80 was identified as *Aspergillus pulverulentus*. The fungus strain YM-80 has been deposited at the Fermentation Research Institute, Japan, as FERM P-15340.

Purification of the enzyme.

The enzyme was purified by $(\text{NH}_4)_2\text{SO}_4$ fractionation and consecutive column chromatography. A summary of the purification is presented in Table 2. A minor active fraction, about 4% of the total activity, was removed with Butyl-Toyopearl column chromatography. The final recovery of enzyme activity was 15% and the specific activity increased 18-fold against the concentrated extract from solid culture. The purified enzyme was used for subsequent characterization.

Table 1. Morphological characteristics of *Aspergillus pulverulentus* YM-80.

Cultural characteristics

Characteristics	Czapek agar	Malt extract agar
Growth rate	Rather restricted	Fairly rapid
Conidiogenesis	Good	Good
Colony color	Conidial areas black	Conidial areas brownish black
Surface texture	Mycelium white	Mycelium white
Reverse	High, flocculent	High, flocculent
	White	White to pale yellow

Microscopic characteristics (grown on Czapek agar)

Conidial heads	Diameter	100–500 μ m
	Shape	Radiate or splitting into columns
	Color	Brownish black
Conidiophores	Length	6–8 mm
	Width	6–14 μ m
	Walls	Smooth, thick
	Color	Pale brown
Vesicles	Diameter	10–80 μ m
	Shape	Globose
Conidia	Diameter	3–5 μ m
	Shape	Globose to subglobose
	Color	Brownish black
	Surface	Rough
Sclertia	Not observed	
Cleistothecia	Not observed	

Table 2. Purification of β -glucosidase from *Aspergillus pulverulentus* YM-80.

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)
Culture filtrate	3110	53100	17.1	100
Ammonium sulfate	1150	40800	35.5	77
DEAE-Toyopearl 650M	198	24400	123	46
Butyl-Toyopearl 650M	55.5	12200	220	23
Sephacryl S-200	25.9	7970	308	15

The enzyme activities were measured by the standard assay method with *p*NP β -glucoside.

Properties of the purified enzyme.

The purified enzyme gave a single band corresponding to a molecular weight of about 118,000 on SDS-PAGE (Fig. 1) and 240,000 by gel filtration (Fig. 2). The purified enzyme was tested with isoelectric focusing to find the isoelectric point and also to check its homogeneity. The enzyme was judged to be homogeneous and the *pI* value was 4.5. The sugar content of the purified enzyme was 28.1% (data not shown).

Effect of pH and temperature on the activity of the enzyme.

The effect of pH and temperature on the activity of the purified enzyme (0.015 U) was studied under the standard assay conditions. The purified enzyme showed maximum activity at pH 4.0 (Fig. 3A) and at 60°C (Fig. 3B).

Stabilities of the enzyme toward pH and temperature.

Enzyme stability at different pH levels was measured by incubating the enzyme solution (3 U/mL) at 40°C and different values of pH for 60 min. After cooling in an ice bath and 20-fold dilution with 50 mM McIlvain buffer

Fig. 1. SDS-PAGE of the purified β -glucosidase.

Lanes 1 and 2 indicate standard proteins and the enzyme, respectively. Standard marker proteins: myosin rabbit muscle (205,000); β -galactosidase *E. coli* (116,000); phosphorylase rabbit muscle (97,000); bovine serum albumin (66,000); ovalbumin chicken egg (45,000); carbonic anhydrase bovine erythrocyte (30,000) as a standard.

Fig. 2. Determination of molecular weight of β -glucosidase by Sephadex G-200 gel filtration.

A, myokinase yeast (32,400); B, enolase yeast (67,000); C, lactic dehydrogenase pig heart (142,000); D, glutamate dehydrogenase yeast (290,000) as a standard. Arrow indicates eluting position of β -glucosidase.

Fig. 3. Optimum pH and pH stability (A), and optimum temperature and thermal stability (B) of the purified β -glucosidase.

(A) Optimum pH (●, ○) and pH stability (■, □) after incubation at 40°C for 60 min were measured by the standard assay method. Buffer systems used were McIlvain buffer, pH 3.0–7.0 (●, ■); 50 mM Tris-HCl buffer, pH 7.0–9.0 (○, □). (B) Optimum temperature (○) at pH 4.0 and thermal stability (■) after incubation at pH 4.0 for 10 min were measured by the standard assay method.

(pH 4.0), the residual activity was measured under the standard assay conditions with 0.015 U of enzyme. The enzyme was stable over the range pH 3–7 (Fig. 3A). Thermostability was measured by incubating the enzyme solution (0.15 U/mL) at pH 4.0 and various temperatures for 10 min. After cooling in an ice bath, the residual activity was measured under the standard assay conditions with 0.015 U of enzyme. The purified enzyme retained its original activity on heating below 50°C (Fig. 3B).

Effect of various metal ions and chemical reagents on the activity of the enzyme.

The enzyme activity was assayed with the standard assay method, using 0.02 U of enzyme and 0.5 mM of metal ions or reagents. The enzyme was completely inactivated by Hg^{2+} and SDS, but Ag^+ , Sn^{2+} , Fe^{3+} and *p*-chloro-mercuribenzoic acid reduced it to 45, 15, 15 and 60% of its original activity, respectively. EDTA and other metal ions (Na^+ , K^+ , Mn^{2+} , Al^{3+} , Fe^{2+} , Mg^{2+} , Zn^{2+} , Ba^{2+} , Cd^{2+} , Cu^{2+} , Ni^{2+} , Co^{2+} , Ca^{2+}) tested had no inhibitory effect on the activity of the enzyme. The effects of ethanol and glucose on the enzyme activity were also measured with the standard assay method with 0.02 U of enzyme. Ethanol was added to the reaction mixture at a final concentration of 5, 10, and 20%. In the case of glucose, 100, 200 and 400 mM of glucose were added to the reaction mixture. The addition of ethanol increased enzyme activity at 5% ethanol (1.1-fold) and 10% ethanol (1.05-fold), but had no effect at 20%. In contrast the enzyme activity decreased with increasing glucose concentration. The relative activity was decreased 12% at 100 mM, 20% at 200 mM and 35% at 400 mM glucose.

Substrate specificity.

The purified enzyme was tested for its substrate specificity under the standard assay conditions with 0.035 U of enzyme and a 5 mM concentration of each substrate in the reaction mixture at pH 4.0 and 50°C for 15 min (Table 3). The tests of various substrates (except *p*NP-compounds) were measured by the Somogyi and Nelson method^{18,19} with glucose as a standard.

The enzyme has a fairly wide substrate specificity. Among substrates tested, cellobiose, cellotriose, gentiobiose and *p*-nitrophenyl β -D-glucoside were the most favorable substrates. The enzyme was shown to hydrolyze efficiently oligosaccharides having β -1,2-, β -1,3-, β -1,4- and β -1,6-glucosidic linkages. The relative rate of hydrolysis of cellooligosaccharides decreased with increasing degree of polymerization. On the other hand, the enzyme had no measurable activity with *p*-nitrophenyl β -xyloside, *p*-nitrophenyl β -galactoside and *p*-nitrophenyl α -D-glucoside and maltose as substrates.

Preparation of tofu.

Results are shown in Table 4. The aglycon form of isoflavone in tofu B with added β -glucosidase was increased 10-fold and formation of glucosides was decreased. Malonyl (6''-O-malonyl β -glucoside) and acetyl (6''-O-acetyl β -glucoside) were formed at almost the same level.

Table 3. Substrate specificity of the purified enzyme from *Aspergillus pulverulentus* YM-80.

Substrate*	Relative activity** (%)
<i>p</i> -Nitrophenyl β -D-glucoside	100
<i>p</i> -Nitrophenyl β -D-xyloside	0
<i>p</i> -Nitrophenyl β -D-galactoside	0
<i>p</i> -Nitrophenyl α -D-glucoside	0
Cellobiose (β -1,4)	125
Cellotriose (β -1,4)	105
Cellotetraose (β -1,4)	92
Cellopentaose (β -1,4)	86
Sophorose (β -1,2)	90
Gentiobiose (β -1,6)	125
Laminaribiose (β -1,3)	84
Maltose (α -1,4)	0
Helicin	91
Salicin	24
Arbutin	75
Phloridin	19
Stevioside	trace
Esculin	21
Genistin	11
Daidzin	10

* Substrate was used at 5 mM in the reaction mixture. ** The activity toward *p*NP-compounds was determined by measuring the release of *p*NP, and the relative activity is expressed as the percentages of the activity toward *p*NPG (Substrate consumption: 10.5%).

Table 4. Isoflavone contents in tofu.

Compound	Soybean	Tofu A* (mg/100g of soybean)	Tofu B**
Glucoside			
Daidzin	51.8	21.2	1.8
Glycitin	28.6	11.4	0.6
Genistin	72.0	30.9	2.3
Malonyl			
Daidzin	33.2	12.8	12.6
Glycitin	15.2	5.9	5.4
Genistin	40.8	17.1	15.3
Acetyl			
Daidzin	0.2	0.1	0.2
Glycitin	0.3	0.2	0.2
Genistin	6.8	0.9	0.7
Aglycon			
Daidzein	5.8	2.9	21.0
Glycitein	—	—	8.8
Genistein	4.8	2.1	27.1

* Untreated with enzyme. ** Treated with enzyme. Isoflavone contents were defined with the value of 100 g soybean equivalent.

DISCUSSION

β -Glucosidase from *Aspergillus pulverulentus* YM-80 isolated from soil was highly purified by consecutive column chromatography. This is the first report of β -glucosidase from *A. pulverulentus*. *A. pulverulentus* is well known as the producer of pectinase that is recognized as a food additive, so this strain is acceptable as a producer of food additive enzymes.

Many filamentous fungi have been reported to produce multiple β -glucosidases,^{20–23} but this fungus produced almost exclusively one single β -glucosidase from solid culture with wheat bran. The purified β -glucosidase had a specific activity of 308 U/mg protein and was homoge-

neous on SDS-PAGE as well as with isoelectric focusing. The molecular weight of the purified enzyme was 118,000 (by SDS-PAGE) and 240,000 by gel filtration, and is most probably a structural dimer. The molecular weight is close to that of β -glucosidase from *A. sojae*.¹³⁾ The *pI* of the purified enzyme is 4.5, which is close to that of *A. oryzae*¹¹⁾ but different from that of *A. fumigatus* (6.0).¹²⁾ The purified enzyme contained 28.1% carbohydrate by weight. The β -glucosidase from a solid culture of *A. sojae*¹³⁾ is also reported to have a high carbohydrate content in its enzyme protein (23.8%).

The optimum pH (4.0) of the purified enzyme is very similar to that of β -glucosidase II from *A. tubingensis* CBS 643.92,²⁴⁾ but lower than that of other fungal β -glucosidases such as that from *A. niger* (5.0)⁶⁾ or *A. japonicus* (5.0).²⁵⁾ The pH-stability (3–7) of the enzyme is within the range of the β -glucosidase of *A. fumigatus* (2–8)¹²⁾ and *A. niger* (2.5–9.0).¹⁰⁾ The optimum temperature (60°C) of the purified enzyme is similar to that of *A. sojae* (60°C)¹³⁾ but higher than that of *A. niger* (50°C).⁶⁾ The thermal stability of this purified β -glucosidase extended up to 50°C at pH 4.0 for 10 min incubation, almost the same as that from *A. sojae*¹³⁾ and *A. niger*.⁶⁾

The β -glucosidases from *A. oryzae*¹¹⁾ and *Botrytis cinerea*²⁶⁾ have been reported to have the ability to hydrolyze both α - and β -glucosides, but this purified enzyme did not hydrolyze α -linked disaccharides like maltose. The β -glucosidases from *A. oryzae*¹¹⁾ and *A. niger* CCRC 31494²⁷⁾ are reported to hydrolyze β -linked glucopyranosides and xylopyranosides, but this enzyme acted specifically on β -linked glucopyranosides. The characteristic of lacking the ability to hydrolyze β -linked xylopyranosides and α -linked saccharides is favorable, because this enzyme will produce fewer effects in foodstuffs with many components.

In general, fungal β -glucosidases have wide substrate acceptance for β -linked glucosides.^{9,11,13,28)} This enzyme was also able to hydrolyze a variety of β -linked disaccharides such as sophorose (1 $\rightarrow$ 2), laminaribiose (1 $\rightarrow$ 3), cellobiose (1 $\rightarrow$ 4) and gentiobiose (1 $\rightarrow$ 6). Thus, the purified enzyme was not limited to only the β -D-(1 $\rightarrow$ 4) linkage, but could easily hydrolyze β -D-(1 $\rightarrow$ 2), β -D-(1 $\rightarrow$ 3) and β -D-(1 $\rightarrow$ 6) linkages.

Many β -glucosidases have been reported to hydrolyze aryl β -glucosides. As a typical example, the β -glucosidase from *A. phoenicis* QM329²⁹⁾ is known to hydrolyze several aryl β -glucosides such as esculin, salicin, phlorizin and arbutin. This purified enzyme also hydrolyzed these aryl β -glucosides, but the rate of hydrolysis was somewhat different. The purified enzyme easily hydrolyzed *p*NPG, hellicin, and arbutin, compared with salicin, esculin, phloridin, genistin and daidzin. The difference of hydrolysis rate toward aryl β -glucosides was caused by inhibition due to the three-dimensional structure or size of aglycons. *A. phoenicis* is a member of the black *Aspergillus* genus and taxonomically close to *A. pulverulentus*.

This enzyme's activity was inhibited by glucose. Competitive inhibition by glucose or substrate is a common characteristic of fungal β -glucosidases.^{2,30)} On the other hand, alcohol enhanced the activity of this enzyme. The activity of β -glucosidase from *A. niger* CCRC31494²⁷⁾

was also reported to be enhanced by alcohol. From these results, this enzyme could be useful in wine making to enhance flavor; however glucose slightly inhibits its β -glucosidase activity.

We have also studied other possible applications of this enzyme. Soybean products have become more and more popular in the world since the U.S. Food and Drug Administration approved the health claim that soy protein reduces the risk of heart disease. In Japan, there are many traditional foods based on soybeans, fermented or not fermented. Recently, isoflavones have been reported to inhibit the growth of cancer, decrease age-related bone loss, and lower blood cholesterol.^{31–33)} Soybeans are also a good source of isoflavones. Isoflavones in soybeans are found mainly as glycosides in unfermented soy foods like tofu.³⁴⁾ Aglycon form isoflavones are absorbed faster than the glycoside form in humans,³⁵⁾ so we have tested the production of aglycon form-rich tofu by this β -glucosidase. By addition of the β -glucosidase, the content of the aglycon form of isoflavone in tofu was increased about 10-fold and the glucoside form was decreased.

Modified glucosides such as 6''-O-acetyl β -glucosides and 6''-O-malonyl β -glucosides were resistant to the enzyme.

We are now studying the kinetic properties of this β -glucosidase, such as its inhibition by glucose or substrate, and also investigating additional applications such as winemaking.

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Aspergillus pulverulentus YM-80 の産生する β -グルコシダーゼの精製と諸性質およびその応用

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食品加工に適した、基質特異性の広い β -グルコシダーゼを産生する菌として、土壌から *Aspergillus pulverulentus* YM-80 を分離した。本菌株の産生する β -グルコシダーゼを、固体培養後の抽出粗酵素液の硫酸塩析、DEAE-Toyopearl 650M, Butyl-Toyopearl 650M および Sephacryl S-200 クロマトグラフィーにより、精製収率15%で電気泳動的に単一にまで精製した。精製酵素は比活性 308 U/mg タンパク質であり、等電点は4.5であった。SDS-PAGEによる分子量測定では118,000、ゲルろ過では240,000であった。最適pHは4.0、最適温度は60°C、pH 3.0–7.0で安定であり、pH 4.0では50°Cまで安定であった。本酵素の活性はSDSや Hg^{2+} により強く阻害された。本酵素はセロビオースやゲンチオビオース、ソホロース等の β 結合を持つオリゴ糖に広い特異性を示したが、マルトースなどの α 結合型には作用しなかった (Table 3)。本酵素は豆腐加工において、大豆イソフラボンのグルコシド配糖体のアグリコン化に有用であったが、アセチル化やマロニル化されたグルコシド配糖体のアグリコン化はほとんど認められなかった。