

Purification and Characterization of β -Glucosidase from *Agaricus bisporus* (White Button Mushroom)

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Abstract β -Glucosidase (β -D-glucoside glucohydrolase, EC 3.2.1.21) is a catalytic enzyme present in both prokaryotes and eukaryotes that selectively catalyzes either the linkage between two glycone residues or between glycone and aryl or alkyl aglycone residue. Growing edible mushrooms in the soil with increased cellulose content can lead to the production of glucose, which is a process dependent on β -glucosidase. In this study, β -glucosidase was isolated from *Agaricus bisporus* (white button mushroom) using ammonium sulfate precipitation and hydrophobic interaction chromatography, giving 10.12-fold purification. Biochemical properties of the enzyme were investigated and complete characterization was performed. The enzyme is a dimer with two subunits of approximately 46 and 62 kDa. Optimum pH for the enzyme is 4.0, while the optimum temperature is 55 °C. The enzyme was found to be exceptionally thermostable. The most suitable commercial substrate for this enzyme is *p*-NPGlu with K_m and V_{max} values of 1.751 mM and 833 U/mg, respectively. Enzyme was inhibited in a competitive manner by both glucose and δ -gluconolactone with IC_{50} values of 19.185 and 0.39 mM, respectively and K_i values of 9.402 mM and 7.2 μ M, respectively. Heavy metal ions that were found to inhibit β -glucosidase activity are I^- , Zn^{2+} , Fe^{3+} , Ag^+ , and Cu^{2+} . This is the first study giving complete biochemical characterization of *A. bisporus* β -glucosidase.

Keywords *Agaricus bisporus* · White button mushroom · β -Glucosidase · Hydrophobic interaction chromatography · Purification · Characterization

Abbreviations

4-MUG	4-Methylumbelliferyl β -D-glucopyranoside
BSA	Bovine serum albumin
<i>o</i> -NP	<i>o</i> -Nitrophenol
<i>o</i> -NPGal	2-Nitrophenyl beta-D-galactopyranoside
<i>o</i> -NPGlu	2-Nitrophenyl beta-D-glucopyranoside
<i>p</i> -NP	<i>p</i> -Nitrophenol
<i>p</i> -NPGal	4-Nitrophenyl beta-D-galactopyranoside
<i>p</i> -NPGlu	4-Nitrophenyl beta-D-glucopyranoside
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

1 Introduction

β -glucosidase (β -D-glucoside glucohydrolase, EC 3.2.1.21) is a catalytic enzyme present in both prokaryotes and eukaryotes. It selectively catalyzes either the linkage between two glycone residues or between glycone and aryl or alkyl aglycone residues. In humans, it is called glucocerebrosidase and its deficiency leads to the development of Gaucher's disease. In plants, β -glucosidase plays different roles, including chemical defense against pests, lignification, regulation of phytohormones, improving the food quality, flavor enhancement, and beverage quality improvement [1–3]. Fungal β -glucosidases are important for pathogenicity of phytopathogenic fungi since they are able to hydrolyze saponin, an anti-fungal molecule secreted by many plants as part of plant defense system, and to

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successfully overcome plant immunity in that way [4]. When it comes to animals, β -glucosidase was most widely studied in insects, where it is usually found in the intestinal tract [5–7].

Apart from being important for physiological processes in all classes of living organisms, β -glucosidase is an industrially and economically important enzyme, being used in food, feed, textile, detergents, pharmaceutical, and bioethanol conversion industries [1, 2, 8]. An important application of fungal β -glucosidases is the production of glucose, a food source and an important metabolite, by the edible mushrooms grown in the forest soil with increased cellulose content [9]. The process is carried out by secreting fungal enzymes from hyphal tips into the soil. There, cellulose gets digested into glucose, which is later used as a fungal metabolite and can also be used as an energy source through bioethanol conversion process [10].

Cellulose is the most abundant renewable biomaterial in the biosphere. In cellulose hydrolysis to glucose, β -glucosidase acts along with other two enzymes, namely endo-1,4- β -glucanase (EC 3.1.2.4) and cellobiohydrolase (or exoglucanase; EC 3.1.2.91). In this process, fungal β -glucosidase converts cellobiose to glucose, but also enhances glucose yield in the process, since cellobiose alone acts as an inhibitor of other two enzymes. Fungal β -glucosidases are especially interesting for industry since they have high yields, high activity, and useful enzymatic properties, such as thermal stability [11].

Agaricus bisporus, also known as the white button mushroom, is one of the most widely used edible mushrooms in human nutrition that is being cultivated for about 350 years [10, 12]. This mushroom is considered a healthy food since it contains high levels of polyphenols, vitamins, minerals, polysaccharides, and especially proteins [13]. Its natural habitat is leaf and needle litter in the forests of Western, Central, and Southern Europe, American continent, and Northern Africa, where it acts as a secondary decomposer. Through sequencing projects, it was shown that *A. bisporus* genome contains genes for cellulose degradation [10].

In this paper, β -glucosidase was isolated and purified from *A. bisporus* flesh using ammonium sulfate precipitation and hydrophobic interaction chromatography. Complete biochemical characterization of the purified enzyme was carried out in order to compare it to the previously characterized fungal and yeast β -glucosidases and to determine whether this enzyme can be utilized in industry.

2 Materials and Methods

2.1 Chemicals

All chemicals used in this research were of the highest available grade and were ordered from Sigma-Aldrich (St.

Louis, MO, USA), except for glycine which was manufactured by Merck KGaA (Darmstadt, Germany) and protein molecular weight marker which was supplied by Thermo Scientific (Waltham, MA, USA).

2.2 Obtaining Crude Extract

Fresh white button mushrooms (*A. bisporus*) were obtained in the local market in Balıkesir, Turkey. In order to isolate the enzyme, 25 g of mushrooms was mixed with 100 ml of Tris-HCl buffer (pH 7.5) for 2 min using kitchen-type blender. Obtained solution was filtered using double sterile cheesecloth and the filtered aliquot was used as a crude extract.

2.3 Enzyme Purification

All purification steps were performed at 4 °C unless otherwise stated. Crude extract was treated with solid ammonium sulfate to obtain 40–80 % saturation fraction, as that fraction was identified as optimal for *A. bisporus* β -glucosidase precipitation (data not shown). After dissolving the salt, samples were centrifuged at 15,000 rpm for 30 min. The precipitated material was dissolved in 50 mM sodium phosphate buffer (pH 6.8) and final saline concentration was adjusted to 1 M with ammonium sulfate.

β -glucosidase was purified from the dissolved pellet by using hydrophobic interaction chromatography column through previously prepared Sepharose-4B-L-tyrosine-1-naphthylamine gel [1]. Column was pre-equilibrated with 50 mM sodium phosphate buffer (pH 6.8) with 1.5 M ammonium sulfate. Samples were loaded and 2 ml fractions were collected in Eppendorf tubes at a flow rate of 200 ml/h. Enzyme was eluted using a linear gradient of 1.5–0 M ammonium sulfate. The tubes with the highest activity were combined together and used as a purified enzyme for subsequent characterization experiments.

2.4 Enzyme Activity Assay

Enzyme activity was measured in order to determine the activity of β -glucosidase when reacting against its substrates. Samples were assayed in a 96-well plate by mixing 70 μ l of 5 mM substrate solution in 50 mM sodium acetate buffer (pH 5.5) and 70 μ l of the purified enzyme. After incubation at 37 °C for 30 min, reactions were terminated by the addition of 70 μ l of 0.5 M sodium carbonate solution and yellow coloration that arose as a consequence of *p*-*o*-NP (*p*-*o*-nitrophenol) liberation was recorded at 405 nm. Enzyme activity was expressed as the μ mol of substrate converted into the reaction product per minute of time (1 U).

2.5 Protein Amount and Concentration Determination

Protein amount was determined at 280 nm by putting 200 μ l of pure enzyme into a quartz 96-well plate reader or using 1 ml of pure enzyme in quartz cuvettes.

Modified Lowry protocol for protein concentration determination was also utilized [14]. Bovine serum albumin (BSA) solution was used as a protein standard for the construction of a standard curve.

2.6 Enzyme Kinetics

Enzyme kinetics of β -glucosidase was studied on two different substrates, namely 4-nitrophenyl beta-D-glucopyranoside (*p*-NPGlu) and 2-nitrophenyl beta-D-glucopyranoside (*o*-NPGlu) and K_m and V_{max} values were determined. Moreover, relative enzyme activity was determined against two other substrates, 4-nitrophenyl beta-D-galactopyranoside (*p*-NPGal) and 2-nitrophenyl beta-D-galactopyranoside (*o*-NPGal). For all these experiments, substrate was present in final concentrations in the range from 0.36 to 2.5 mM.

2.7 Optimum pH Determination

In order to determine optimum pH, the enzyme was incubated with 5 mM *p*-NPGlu solution in buffers of different pH values: sodium acetate buffer (pH 3.0–6.0), phosphate buffer (pH 6.0–8.5), and glycine-NaOH buffer (pH 8.5–12.0).

2.8 Temperature Optimum Determination and Thermal Denaturation Study

In order to determine temperature optimum, the purified enzyme was incubated with 5 mM *p*-NPGlu at seven different temperatures in the range from 25 to 85 °C for 30 min.

Thermal denaturation of the enzyme was investigated at six different temperatures in the range from 25 to 75 °C. Purified enzyme was incubated for 2 h at all temperatures, except for 75 °C, where incubation was terminated after 1 h.

2.9 Inhibition Studies

Inhibition studies were performed in order to find K_i and IC_{50} values, as well as inhibition types, of glucose and δ -gluconolactone (D-glucono-1,5-lactone). Double reciprocal Lineweaver–Burk plot was used to calculate these parameters. Also, relative activity of β -glucosidase in the presence of variable volumes of 0.005 M solutions of six heavy

metal ions was determined, namely I^- , Zn^{2+} , Fe^{3+} , Ag^+ , Cu^{2+} , and Pb^{2+} . Relative activity was determined as a %, where 100 % activity corresponds to the enzymatic activity with no inhibitor present.

2.10 Polyacrylamide Gel Electrophoresis

In order to determine molecular weight of the enzyme, SDS-PAGE and native PAGE were performed, both using the Mini-PROTEAN Tetra Cell system (Bio-Rad Laboratories, Hercules, CA, USA). SDS-PAGE was performed on 10 % separating gel and 3 % stacking gel. Gel was stained using Coomassie Brilliant Blue G-250 solution and destained using standard methods.

Enzyme activity was detected on 8 % resolving and 3 % separating native gels. Gel was incubated on a rotating platform in 50 mM sodium acetate buffer (pH 5.5) for 45 min in three buffer changes and in a 0.1 % 4-MUG solution for 30 min at 45 °C. Enzyme activity band was photographed under the UV light.

3 Results

In order to purify β -glucosidase from mushroom flesh, three steps were performed: obtaining crude extract, ammonium sulfate precipitation of proteins (that is, protein salting-out), and hydrophobic interaction chromatography. The efficiency of this process can be represented through the purification fold and percent yield (Table 1).

The results of hydrophobic interaction chromatography are presented in the figure below (Fig. 1). It can be observed that the tubes 20–35 gave the most active pure enzyme, as well as the highest enzyme amount, so they were used as the purified enzyme for subsequent characterization steps. Enzyme activity and amount were determined at 405 and 280 nm, respectively.

Enzyme kinetics was studied on two substrates, namely *p*-NPGlu and *o*-NPGlu. K_m and V_{max} values for *p*-NPGlu were 1.751 mM and 833 U/mg, respectively, while those values for *o*-NPGlu were 8.547 mM and 556 U/mg, respectively (Fig. 2a, b). These results imply that *A. bisporus* β -glucosidase has much higher affinity for *p*-NPGlu than it is the case with *o*-NPGlu.

Additionally, relative activities against four substrates were compared in triplicate, namely: *p*-NPGlu, *o*-NPGlu, *p*-NPGal, and *o*-NPGal (Fig. 2c). According to the results of the present study, *p*-NPGlu was the most suitable substrate for mushroom β -glucosidase, so enzyme activity with this substrate was taken to be 100 %. When compared to *p*-NPGlu, relative activities towards other three substrates were calculated to be 28.25, 81.07, and 39.84 % for *o*-NPGlu, *p*-NPGal, and *o*-NPGal, respectively. Therefore,

Table 1 Purification table of *A. bisporus* β -glucosidase

Step	Total activity (U/ml)	Total protein (mg)	Specific activity (U/mg)	% Yield	Purification fold
Crude extract	25133.37	4.37	5750.98	100	N/A
Ammonium sulfate precipitation	10360.28	1.27	8181.22	41.22	1.42
Hydrophobic interaction chromatography	1018.65	0.02	58175.56	4.05	10.12

Ammonium sulfate precipitation in 40–80 % saturation fraction was performed, while hydrophobic interaction chromatography was done with already prepared Sepharose-4B-L-tyrosine-1-naphthylamine gel

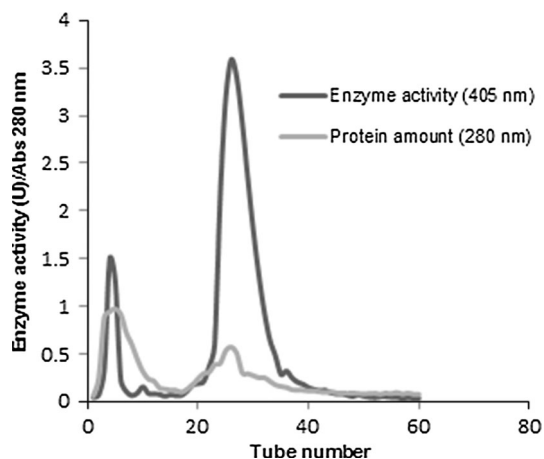


Fig. 1 The results of hydrophobic interaction chromatography reveal the highest enzyme activity and the highest enzyme amount in the same tubes (tube numbers 20–35). Peaks in both enzyme activity and enzyme amount were observed after the gradual decrease of salt concentration in sodium phosphate buffer started. Enzyme activity was determined at 405 nm, while enzyme amount was determined at 280 nm

it can be concluded that *para* isomers of these substrates are more suitable for the activity of *A. bisporus* β -glucosidase when compared to their *ortho* isomers.

Optimum pH and temperature for *A. bisporus* β -glucosidase were determined in the study. Optimum pH was found to be 4.0 (Fig. 3), while optimum temperature for this enzyme equals to 55 °C (Fig. 4, upper panel).

Thermal denaturation study revealed that mushroom β -glucosidase is an exceptionally stable enzyme, since the decrease in activity did not happen at the temperatures below 65 °C. It showed decline in activity at 65 °C after approximately 20 min of incubation, while it got almost instantly deactivated at 75 °C (Fig. 4, bottom panel).

Glucose and δ -gluconolactone were used for inhibition studies in the current research. Glucose inhibited enzyme activity in a competitive manner since the graph of enzyme activity is showing that V_{max} value remains almost the same in different concentrations of inhibitor and in the absence of inhibitor, while K_m value gets higher when the inhibitor is present (Fig. 5a). K_i and IC_{50} values were calculated to be 9.402 mM (Fig. 5a) and 19.185 mM (Fig. 5b), respectively.

In the case of δ -gluconolactone, inhibition effects were much more dramatic, which is demonstrated by K_i and IC_{50} values for this inhibitor that were calculated to be 0.0072 mM (7.2 μ M) and 0.39 mM, respectively (Figs. 5c, d), while enzyme activity was inhibited in a competitive way (Fig. 5c).

Relative enzyme activity was studied in the presence of six heavy metal ions which were potential inhibitors of β -glucosidase activity, namely: I^- , Zn^{2+} , Fe^{3+} , Ag^+ , Cu^{2+} , and Pb^{2+} . Metal ion concentration in these reactions was in the range from 0 to 1.5 mM. All of them, with the exception of lead ion, showed inhibitory effects (Fig. 6) with relative enzyme activity of 57.33, 81.11, 95.65, 64.72, and 49.01 % in the presence of I^- , Zn^{2+} , Fe^{3+} , Ag^+ , and Cu^{2+} , respectively. When it comes to lead, increase in enzyme activity was observed at metal concentrations below 0.5 mM and only slight inhibition at higher concentrations. Since all other metal ions exhibited only inhibitory effects on β -glucosidase activity, lead was excluded from relative enzyme activity calculations.

In order to determine protein molecular weight, both SDS-PAGE and native PAGE were performed. SDS-PAGE gel showed two bands that correspond to molecular weights of approximately 46 and 62 kDa. In order to confirm that *A. bisporus* β -glucosidase is truly a dimer, native PAGE gel was analyzed. Single band corresponding to approximately 110 kDa was observed, which confirmed that this enzyme has two subunits (Fig. 7b, c). Native PAGE coupled to incubation in 4-MUG solution showed a single band of enzymatic activity of mushroom β -glucosidase after exposure to UV light (Fig. 7d).

4 Discussion

Throughout the precipitation and chromatography steps, enzyme was purified 10.12-fold. This purification fold can be considered satisfying when compared to the purification folds of fungal β -glucosidases from the previous studies. Enzyme from *Penicillium piceum* [15] was purified 8.7-fold, while *Rhizomucor miehei* [11] β -glucosidase had 18.2-fold purification. However, the majority of studies brought data about higher purification folds. For example,

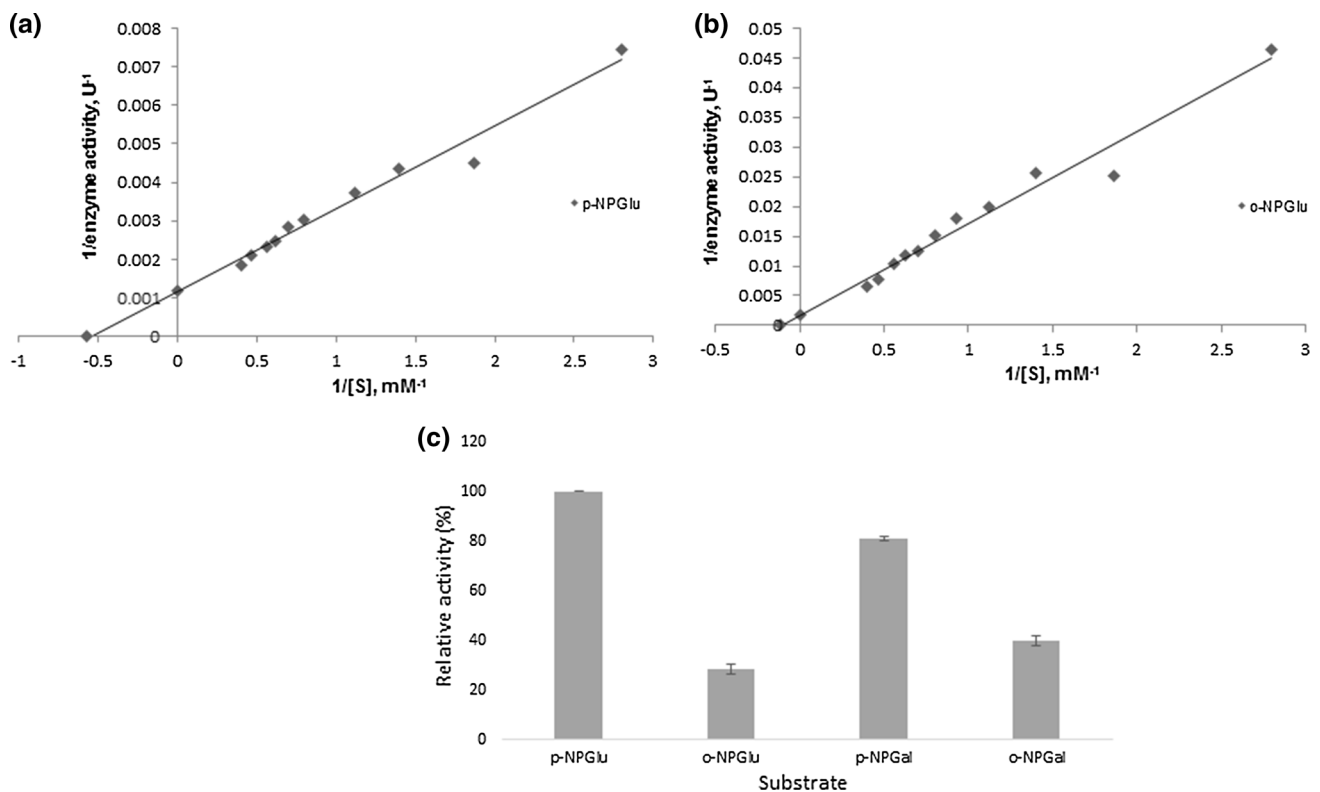


Fig. 2 Enzyme kinetics was studied with two β -glucosidase substrates: *p*-NPGlu (a) and *o*-NPGlu (b). Both substrates were present in concentrations from 0.36 to 2.5 mM and were incubated at 37 °C for 30 min in 50 mM sodium acetate buffer, pH 5.5. c Relative activities

of β -glucosidase against four different substrates: *p*-NPGlu, *o*-NPGlu, *p*-NPGal, and *o*-NPGal. Enzyme activity against *p*-NPGlu was taken to be 100 % and relative activities against other substrates were calculated accordingly

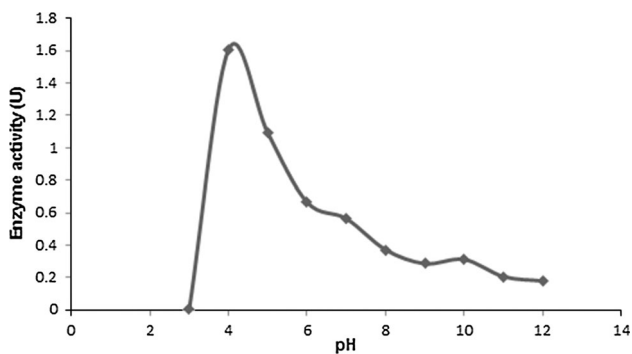


Fig. 3 The graph for optimum pH value determination. Enzyme activity was measured spectrophotometrically at 405 nm following enzyme and *p*-NPGlu incubation for 30 min at 37 °C in three different buffers: sodium acetate buffer (pH 3.0–6.0), phosphate buffer (pH 6.0–8.5), and glycine-NaOH buffer (pH 8.5–12.0)

β -glucosidase from *Pichia pastoris* [3] had 99.5-fold purification, one from *Daldinia eschschdzii* [16] had 50.23-fold, while the enzyme from *Pholiota adiposa* [17] was purified 130.3-fold. These results imply that better purification fold could be obtained if enzyme of interest was purified using more than two purification steps. However, numerous steps usually cause dramatic decrease in enzyme

activity. In the present study, high enzyme activity was one of the most important properties and purification was done in a way that would preserve the majority of that activity.

K_m values for *p*-NPGlu and *o*-NPGlu in this study were 1.751 and 8.547 mM, respectively. K_m value for *p*-NPGlu is comparable to the results obtained for other species. Some examples are *D. eschschdzii* with K_m value of 1.52 mM [16], *Botrytis cinerea* with 1.05 mM [18], and *P. adiposa* with 2.23 mM [17]. On the other hand, V_{max} values for *p*-NPGlu and *o*-NPGlu were 833 and 556 U/mg, respectively. V_{max} values for *p*-NPGlu in fungal β -glucosidases were ranging from 0.077 U/mg in *Termitomyces clypeatus* [19] to 4390 U/mg in *P. adiposa* [17]. Thus, *A. bisporus* β -glucosidase showed high conversion rate of *p*-NPGlu into reaction product *p*-NP and, therefore, is the enzyme with relatively high activity rate.

By comparing *A. bisporus* β -glucosidase activity against four different substrates (*p*-NPGlu, *o*-NPGlu, *p*-NPGal, and *o*-NPGal), it was concluded that *p*-NPGlu gave the highest enzyme activity. Due to that, enzyme activity against *p*-NPGlu was taken to correspond to 100 % relative activity, while relative activities against other substrates were calculated as follows: 28.25 % against *o*-NPGlu, 81.07 % against *p*-NPGal, and 39.84 % against *o*-NPGal. Similar

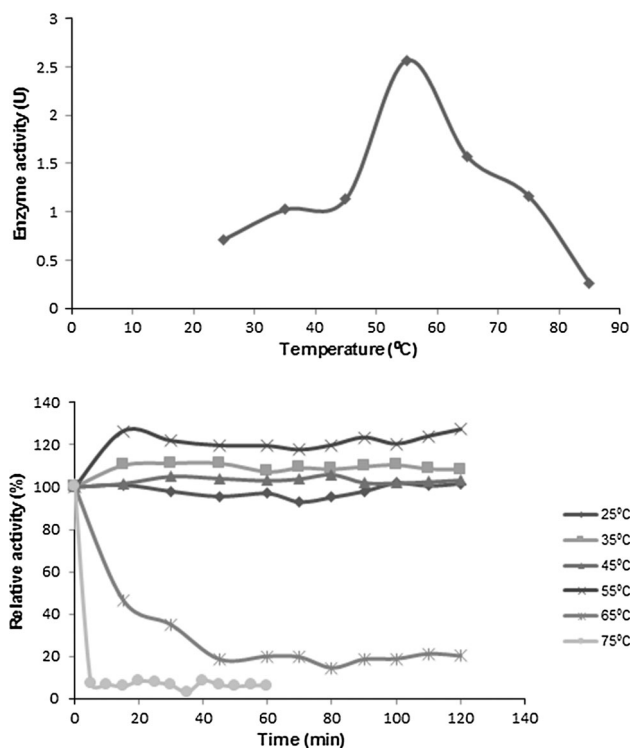


Fig. 4 Upper panel Optimum temperature determination for *A. bisporus* β -glucosidase was done by incubating enzyme with 5 mM *p*-NPGlu solution on seven different temperatures from 25 to 85 °C. Bottom panel Thermal denaturation of mushroom β -glucosidase was done by incubating enzyme for 2 h on five temperatures from 25 to 65 °C and for 1 h at 75 °C. Enzyme activity obtained after usual activity assay was taken to be 100 % and the rest of the results are calculated accordingly

behavior has been observed with other fungal β -glucosidases. Another study of fungal enzyme from *D. eschschdzii* [16] offered similar results, since it was concluded that *p*-NPGlu corresponds to 100 % and *o*-NPGlu to 15.5 % relative enzyme activity. At the same time, *P. adiposa* β -glucosidase [17] had 100 % activity against *p*-NPGlu and 23.7 % against *o*-NPGlu. Enzyme from *R. miehei* was active against three substrates used in the current study in the following manner: 100 % against *p*-NPGlu, 20 % against *o*-NPGlu, and 69 % against *p*-NPGal [20], while *Paecilomyces thermophila* enzyme was active against two substrates: 100 % for *p*-NPGlu and 50.7 % for *p*-NPGal [8]. Many other fungal β -glucosidases did not show any activity against either *p*-NPGal or *o*-NPGal. The most prominent exception from all these results is the study of *P. pastoris*, where *o*-NPGlu gave 116 % relative enzyme activity when compared to *p*-NPGlu, which was taken as 100 %. This enzyme also did not exhibit any activity against galactopyranosides [3].

The optimum pH for *A. bisporus* β -glucosidase was found to be 4.0. This finding was expected since many other fungal β -glucosidases were found to prefer acidic

over neutral or basic pH, many of them having pH optimum of 5.0. Some examples are β -glucosidases from the following fungal species: *D. eschschdzii* [16], *Aspergillus niger* [21], *Tricholoma matsutake* [22], *Periconia* sp. [23], *P. adiposa* [17], *P. piceum* [15], *R. miehei* [11, 20], and *T. clypeatus* [19]. Other species also exhibited pH optima in acidic environment, like *B. cinerea* that had pH optimum 3.0 [18] and *Zygosaccharomyces bailii* [24] with pH optimum of 5.5.

The optimum temperature for *A. bisporus* β -glucosidase is 55 °C, which is the same temperature optimum as found in the study of another edible mushroom, *Volvariella volvacea* [9] and in thermophilic fungus *P. thermophila* [8]. High temperature optima are characteristic for other fungal β -glucosidases, most prominently 60 °C optimum, which is the most suitable temperature for many fungal species, like *P. piceum* [15], *T. matsutake* [22], and *B. cinerea* [18]. Additionally, 50 °C was optimum for enzyme activity of some species, like *D. eschschdzii* [16] and *R. miehei* [11].

The results of thermal denaturation study revealed that β -glucosidase from the present study is a very stable enzyme, since its denaturation and loss of activity did not happen at the temperatures below 65 °C. Similar behavior was observed in *V. volvacea* β -glucosidase, which experienced rapid denaturation at temperatures above 60 °C, but was not affected by lower temperatures [9]. Also, *A. niger* β -glucosidase was another fungal thermostable enzyme [21].

In this study, both glucose and δ -gluconolactone had certain inhibitory effects on β -glucosidase activity, both of them acting as competitive inhibitors and gluconolactone being much more efficient inhibitor. This can be confirmed by comparing K_i values for glucose and gluconolactone, which were calculated to be 9.402 mM and 7.2 μ M, respectively. Similar results were found in the previous studies of yeast and fungal β -glucosidases. All of them identified gluconolactone as more efficient inhibitor when compared to glucose. Many fungal species showed competitive inhibition type for glucose and had similar K_i values. For example, *P. pastoris* β -glucosidase had K_i value of 7.2 mM [3], *R. miehei* 8 mM [20], while *Piromyces* sp. had K_i value for glucose of 9.5 mM [25]. However, many fungal β -glucosidases were completely inhibited by glucose and, thus, had very low K_i values, implying high inhibition potential of glucose. Some of them are: *Talaromyces emersonii* with K_i value of 0.245 mM [26], *Z. bailii* with 0.137 mM for extracellular and 0.105 mM for intracellular enzyme [24], and *D. eschschdzii* with 0.79 mM [16]. The third group of fungal β -glucosidases was almost completely insensitive to inhibitory effects of glucose, having very high K_i values. Two examples are *P. adiposa* with K_i value of 40.6 mM [17] and *Periconia* sp. with 20 mM [23]. Therefore, it can be

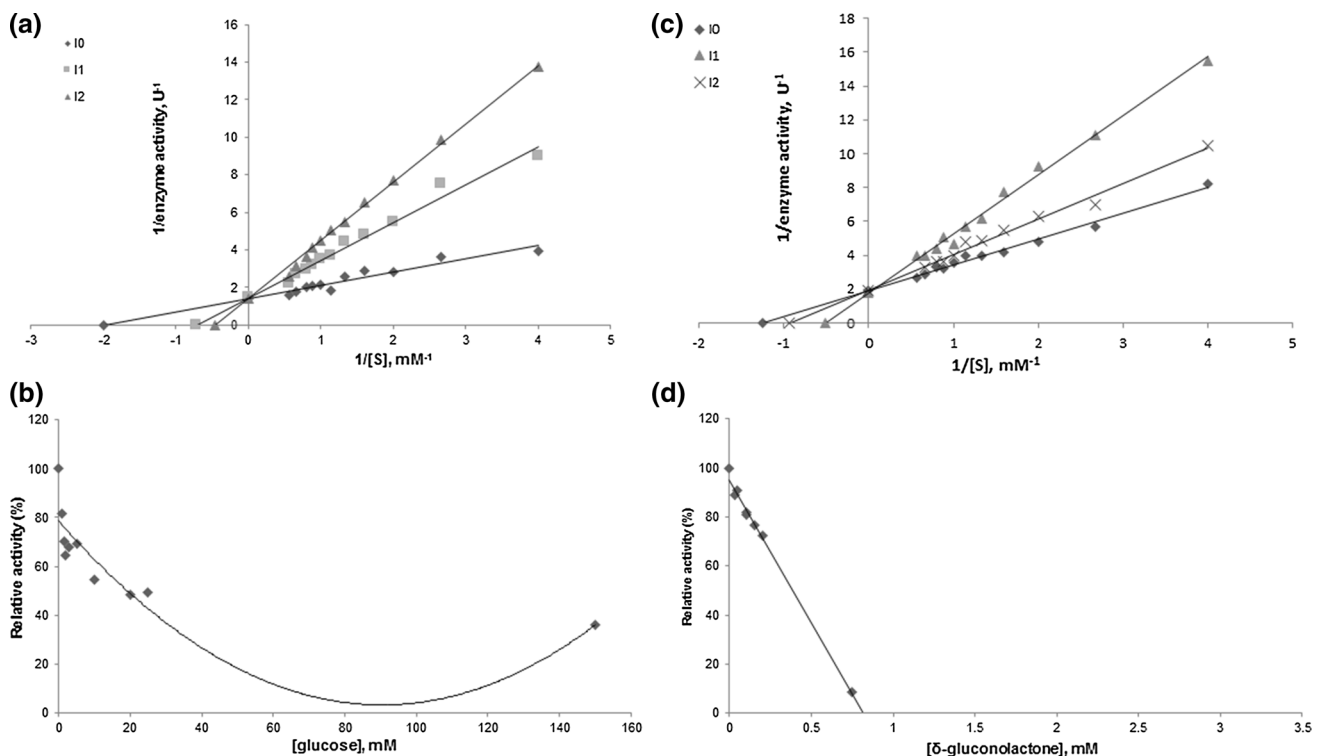


Fig. 5 **a** Glucose inhibition type against *p*-NPGlu as a substrate was found to be competitive. In the graph, I_0 denotes enzyme activity without inhibitor, I_1 with 3 mM of glucose, and I_2 with 25 mM of glucose in total reaction volume. **b** Inhibition of *A. bisporus* β -glucosidase activity by glucose. While enzyme and substrate (*p*-NPGlu) concentrations were kept constant, inhibitor concentration was increased from 0 to 150 mM. Enzyme activity without inhibitor was taken as 100 % and other results were normalized accordingly.

c Inhibition type for δ -gluconolactone was found to be competitive against *p*-NPGlu as a substrate. I_0 represents enzyme activity with no inhibitor present, I_1 with 0.05 mM of δ -gluconolactone, and I_2 with 0.003 mM of δ -gluconolactone present in the reaction. **d** Inhibition curve for δ -gluconolactone. While enzyme and substrate concentrations were constant, inhibitor concentration varied from 0 to 3 mM. Enzyme activity at 0 mM inhibitor concentration was taken as 100 % activity

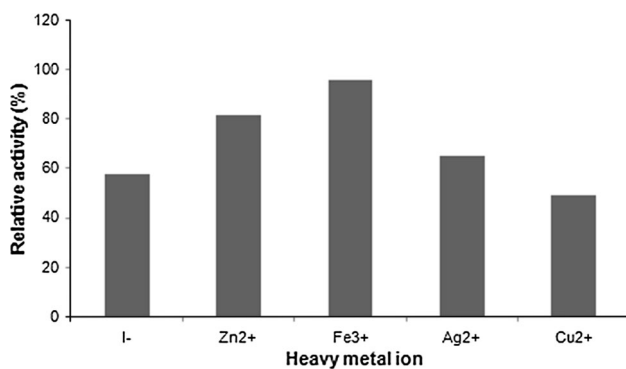


Fig. 6 Relative enzyme activity in the presence of five heavy metal ions: I^- , Zn^{2+} , Fe^{3+} , Ag^{2+} , and Cu^{2+} , in the range from 0 to 1.5 mM of total reaction volume. Enzyme activity without inhibitor was taken to be 100 % and other results are normalized accordingly

concluded that glucose had moderate inhibitory effect on the activity of *A. bisporus* β -glucosidase. On the other hand, all fungal species studied so far experienced strong inhibition by gluconolactone with very low K_i values. Many examples were found in the available literature,

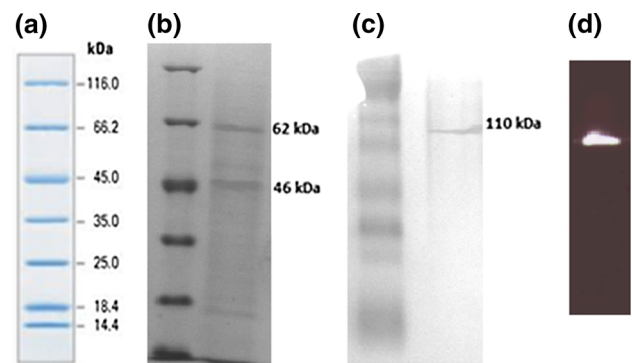


Fig. 7 **a** Protein molecular weight marker (Thermo Scientific, Waltham, MA, USA) with the following bands: 116 kDa (β -galactosidase), 66.2 kDa (bovine serum albumin), 45 kDa (egg albumin), 35 kDa (lactate dehydrogenase), 25 kDa (REase Bsp981, *E. coli*), and 18.4 kDa (β -lactoglobulin). **b** Image of SDS-PAGE gel. Lane 1 (left) contains molecular weight marker, while lane 2 (right) contains *A. bisporus* β -glucosidase with two subunits of approximately 46 and 62 kDa. **c** Image of native gel. Lane 1 (left) contains non-denatured protein molecular weight marker, while lane 2 (right) contains non-denatured β -glucosidase of approximately 110 kDa. **d** Image of native gel coupled to incubation in 4-MUG solution. β -glucosidase activity was recorded under the UV light

some of them being *Piromyces* sp. with K_i value of 22 μM [25] and both extracellular and intracellular β -glucosidase from *Z. bailii* with K_i values of 30 and 1 μM , respectively [24].

According to the results of this study, five heavy metal ions were successful inhibitors of *A. bisporus* β -glucosidase activity, namely I^- , Zn^{2+} , Fe^{3+} , Ag^+ , and Cu^{2+} . These ions were mentioned in literature as the inhibitors of enzyme activity in other fungal species as well. For example, Cu^{2+} and Zn^{2+} were mentioned as inhibitors of β -glucosidases from *D. eschschdzii* [16], *T. clypeatus* [19], and *R. miehei* [20], while Fe^{3+} and Cu^{2+} were effective inhibitors of the activity of β -glucosidase from *P. adiposa* [17]. β -glucosidase from *T. matsutake* was inhibited by Ag^+ ion, while Pb^{2+} was activator of enzyme activity until certain metal concentration, which corresponds to the present results [22].

SDS-PAGE and native PAGE gels showed that *A. bisporus* β -glucosidase is a dimer, consisted of two subunits of approximately 46 and 62, or 110 kDa for non-denatured enzyme. Therefore, this enzyme is relatively small. Through the search of previously published literature, it was found that many other fungal β -glucosidases are indeed small enzymes. As an example, *A. niger* β -glucosidase had an enzyme of identical molecular weight of 110 kDa [21], *T. clypeatus* had an enzyme of 116 kDa [19], *P. piceum* β -glucosidase has molecular weight of 92 kDa [15], while edible mushroom *V. volvacea* had a 158 kDa enzyme [9].

To conclude, β -glucosidase from *A. bisporus* was successfully purified using ammonium sulfate precipitation and hydrophobic interaction chromatography and its biochemical characterization was done. Its high activity and substrate specificity, along with high thermal stability and relative resistance to glucose inhibition make it an excellent choice for a range of industrial applications, most prominently for glucose production by cellulose degradation. To our knowledge, this is the first detailed study of β -glucosidase from the white button mushroom.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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