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Journal of Chromatography B, 770 (2002) 111–119

JOURNAL OF CHROMATOGRAPHY B

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Liquid chromatographic and electrophoretic characterisation of extracellular β-glucosidase of *Pleurotus ostreatus* grown in organic waste

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Abstract

The production of β-glucosidase by the ligninolytic fungus *Pleurotus ostreatus* has been studied in different culture media containing agro-industrial wastes. The enzyme is purified by anion-exchange chromatography, the molecular mass and isoelectric point of purified β-glucosidase are measured by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and isoelectric focusing and the stability and kinetic parameters of the enzyme assessed by spectrophotometry. It has been established that the retention time, molecular mass and isoelectric point of the enzyme depend on the composition of the culture media while the activity and stability of β-glucosidases of different origin were very similar. The combined chromatographic and electrophoretic methods have proved to be suitable techniques for the purification and characterisation of the β-glucosidases produced by the ligninolytic fungus *Pleurotus ostreatus* in different culture media. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Pleurotus ostreatus*; β-Glucosidase; Enzymes

1. Introduction

Excess biomass or wastes from agricultural and agro-industrial residues have always been extensively employed to produce energy, feeds or foods and other useful products. For centuries wood and other agricultural residues have been applied as sources of food, fuel, construction materials and papermaking, as well as for other purposes. Because of their capacity to degrade wood, white rot fungi are of increasing biotechnological interest [1,2]. They produce a wide range of extracellular enzymes that enable them to degrade lignin [3], cellulose [4] and hemicellulose of insoluble lignocellulosic substrates into soluble substances which can be taken up by the mushroom as nutrition [5,6]. Xylanases catalyse the hydrolysis of xylane to xylooligosaccharides [7,8], lignin peroxidase attacks both phenolic and non-phenolic aromatic structures the latter giving rise to cationic radicals that fragment spontaneously [9], laccase, a copper-containing phenoloxidase catalyses the four-electron reduction of oxygen to water and it

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PII: S0378-4347(01)00561-8

is accompanied by the oxidation of the phenolic substrate [10], etc. β-Glucosidases also play a considerable role on the decomposition of lignocellulosic substrates catalysing the hydrolysis of alkyl- and aryl-β-glucosides, diglucosides and oligosaccharides. The main role of β-glucosidase in the saccharification of cellulose is the degradation of cellobiose, an inhibitor of the depolymerizing enzymes, and cello-oligosaccharides to glucose [11].

Because of the high separation capacity and sensitivity various liquid chromatographic (LC) techniques mainly size-exclusion [12] and affinity chromatography [13] have found application in the analysis of a wide variety of enzymes. Glucosidases have also been separated and purified by LC. Thus, the application of affinity chromatography on controlled porosity glass activated by aminopropyltriethoxysilane and oxiranes [14], immobilized liposome chromatography [15], expanded bed chromatography in small diameter columns [16], gel permeation chromatography coupled to hydrophobic interaction chromatography [17], and size-exclusion chromatography combined with isoelectric focusing and sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) [18] have been reported.

The objectives of the study were the determination of the production of β-glycosidases during the complete life cycle of *Pleurotus ostreatus* cultivated in liquid media composed of agro-industrial residues, and the use of various chromatographic and spectrophotometric techniques for the purification and characterization of β-glucosidases.

2. Materials and methods

2.1. Materials

Potato dextrose agar, polyethyleneglycol 6000 (PEG), NaCl, CH_3COOH, CH_3COONa, NaOH, sodium phosphate, sodium citrate, sodium tartarate, glycine (each of pro analysi quality) and Tris-(hydroxymethyl)–aminomethan GR were purchased from Merck KGaA (Darmstadt, Germany), *p*-nitrophenyl-β-D-glucopyranoside (pNPβG), 2,6-dimethoxyphenol (99%, GC), birchwood xylan, xylose, bovine serum albumin, protein standards of relative molecular mass, dialysis membrane (BDH) were purchased from Sigma–Aldrich Chemie (Steinheim, Germany). All chemicals have been employed as received.

2.2. Organisms and culture conditions

Strain of *Pleurotus ostreatus* was taken from the culture collection of the National Agronomical Station (Oeiras, Portugal). They were maintained on potato dextrose agar (PDA) (Merck KGaA) at 4 °C. The fungus was grown in 400 ml of liquid medium (200 ml of distilled water and 200 ml of extract) in Erlenmeyer flasks of 1 l. Extracts were prepared by mixing separately 350 g of dried pepper, 560 g of dried potato or 45 g of dried straw in 1 l of distilled water for 24 h then they were filtered and centrifuged at 20 000 *g* for 20 min. Extracts used for fermentation experiments consisted of (straw–pepper–potato extract, 5:2:3, v/v/v, F_1), (straw–pepper–potato extract, 5:4:1, v/v/v, F_2), and pepper extract (F_3). Liquid medium were sterilised at 121 °C for 20 min, cooled to 26 °C and inoculated by adding a piece of agar (about 10 mm of diameter) with mycelium. Cultures were incubated at 24±2 °C in the dark for 30 days, then they were transferred to the fructification room (18±2 °C) with 12 h light/day for 30 days. Each 7th day samples were taken from the culture medium under sterile conditions and the activity of β-glucosidase was determined.

2.3. Determination of the activity of enzymes

The activity of β-glucosidase has been determined by mixing 250 μl of *p*-nitrophenyl-β-D-glucopyranoside (1.5 mg/ml), 250 μl of acetate buffer (0.2 *M*, pH 4.0) and 500 μl of enzyme solution appropriately diluted. After 60 min of incubation at 37 °C the reaction was stopped by adding 1 ml of 0.4 *M* glycine–NaOH (pH 10.8) and the absorption was measured at 430 nm using a UNICAM 8700 Spectrophotometer (Cambridge, UK). Reaction mixture for the determination of the activity of xylanase contained 250 μl of sample and 125 μl of 2% birchwood xylan dissolved in 50 m*M* of sodium acetate buffer (pH 5.0). The mixture was incubated for 30 min at 50 °C and the concentration of reducing sugars was measured by the dinitrosalicylic acid method and expressed as xylose equivalents

[19]. The laccase activity was determined in a reaction mixture containing 10 mM 2,6-dimethoxyphenol in 100 mM sodium tartarate (pH 5.0) and 300–600 μl of enzyme solution in 1 ml of total volume. The absorbance was measured at 469 nm after 5 min of incubation at 30 °C.

2.4. Preparation of crude β-glucosidase

When the maximal activity of β-glucosidase is reached a sample of supernatants (total volume of 3600 ml) is taken from the Erlenmeyer flasks. The sample is filtered through a Whatman No. 1 paper and centrifuged at 5000 rev./min for 15 min. Supernatants are concentrated with PEG using dialysis membrane of 21 mm diameter and a capacity of 100 ml·30.48 cm^{-1}. Dialysis is carried out for 24 h at 4 °C. The activity of β-glucosidase is determined after dialysis.

2.5. Anion-exchange chromatography

Purification of crude β-glucosidase is performed with an ISCO LPLC system (UA-5 Detector, WIZ canal peristaltic pump, Retriever 2 fraction collector, and fraction programmer 3100, ISCO, Lincoln, Nebraska) on an anion-exchange column (50×10 mm I.D.) filled with Fractogel EMD-TMAE 650 (S) (particle size, 25–40 μm) (Merck KGaA). The column was equilibrated with 20 mM of Tris–HCl buffer (pH 8.0) for 15 min at 25 °C. An aliquot of 200 μl of the crude enzyme preparation was applied to the column. Proteins were separated with an NaCl stepwise gradient (15 ml of 0.2 M; 15 ml of 0.6 M, and 10 ml of 1.0 M of NaCl) at a flow-rate of 1 ml/min. The protein content was monitored at 280 nm. The individual fractions were collected manually and the xylanase, lactase and β-glucosidase activity of each fraction was determined.

Fraction 4 containing the purified β-glucosidase was collected, concentrated, dialysed and stored at −20 °C.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out in an Multiphor II electrophoresis instrument (Pharmacia Biotech., Uppsala, Sweden) using ExcelGel SDS gradient 8–18%. Fraction 4 of the chromatogram containing the purified β-glucosidase (about 0.022 μg of enzyme) was employed for SDS–PAGE. Separations were performed at limiting values 600 V, 50 mA and 30 W. The separation time was 45 min. Molecular mass standards of 7 μg have been added to each run. After separation proteins were detected by staining the gels with silver as described previously [20]. The relative molecular masses of β-glucosidases produced by *P. ostreatus* grown in various culture media were determined by plotting the relative mobilities of standard markers vs. logarithm of their relative molecular masses. The relative molecular masses of β-glucosidases have been determined using linear regression analysis.

2.6. Isoelectric focusing

IEF focusing was carried out in the same electrophoresis instrument as SDS–PAGE using PreCast gels in the pH range 3.0–10.0. The same protein fraction was investigated as by SDS–PAGE. Separations were performed at limiting values 1500 V, 30 mA and 30 W. The separation time was 90 min. Standards of 10 μg have been employed for each run. After separation proteins have been detected by staining the gels with silver as for SDS–PAGE. The relative mobilities of the standard markers are plotted vs. their isoelectric point and the pI value of β-glucosidase is assessed using linear regression relationship.

2.7. Determination of the effect of pH on the activity and stability of purified β-glucosidase

The effect of pH on the activity of purified β-glucosidase has been assessed between pH 2.0 and 12.5. The following media have been employed at a concentration of 50 mM: sodium citrate (pH 3.0–6.0), sodium phosphate (pH 7.0–8.0) Tris–HCl (pH 9.0), and glycine–NaOH (pH 10.5–12.5). The enzyme was incubated in the presence of substrate in the various media and after 1 h incubation the residual activity was measured as described above. The effect of pH on the stability of purified β-glucosidase was assessed by incubating the enzyme solution at the various pH values for 20 h at 25 °C. After incubation the residual activity was determined as described above the pH being restored before incubation with the substrate.

2.8. Determination of the effect of temperature on the activity and stability of purified β-glucosidase

The dependence of the activity of the purified β-glucosidase on temperature has been assessed between 0 and 100 °C at pH 4.0 using the method described above. After 1 h incubation with the substrate at the given temperature the residual activity was measured. The thermal stability of the purified enzyme was determined by incubating the samples for 1 h at the corresponding temperature in the absence of substrate then the activity of β-glucosidase was determined at 37 °C as described above.

2.9. Determination of the kinetic parameters of purified β-glucosidase

The V_{max} values related to the maximum velocity of the reaction and Michaelis–Menten (K_m) parameter representing the affinity of the enzyme for the substrate, have been determined by measuring the activity of the same amount of enzyme at various concentrations of substrate between 0.5 and 2.5 mg/ml as described above. The V_{max} value was calculated from the dependence of the activity on the concentration of the substrate and the K_m value was calculated after the Lineweaver–Burk linearization.

2.10. Data evaluation by linear and nonlinear regression analysis

In order to model the dependence of β-glucosidase activity on the fermentation time and on the composition of culture media, linear relationships have been calculated between the activity of β-glucosidase and the fermentation time separately for each culture media. The regression coefficients characterizing enzyme production in the various culture media have been compared by the Student's *t*-test. In order to calculate the influence of pH and temperature on the activity of β-glucosidase and the stability of β-glucosidase at different pH's and temperatures the highest activity has been taken as 100% and the relative activities have been calculated accordingly. As the effect of pH and temperature on the activity and stability of β-glucosidase was markedly nonlinear quadratic equations have been fitted to the data and the coefficients of regression have been compared as desribed above.

3. Results and discussion

3.1. Dependence of β-glucosidase activity on fermentation time and composition of culture media

The activities of β-glucosidase measured at different fermentation times and culture medium are compiled in Table 1. The data in Table 1 clearly show that the activity of β-glucosidase increases during fermentation, and the highest activities can be observed in culture media F_1. Significant linear relationships have been found between the activity of β-glucosidase and the fermentation time (days).

Culture media F_1:

$$\text{Activity} = -6.69 \times 10^{-2} + 6.59 \times 10^{-3}\ \text{days} \quad (1)$$

$$n = 9 \quad r_{\text{calc.}} = 0.9217 \quad r_{99.9\%} = 0.8982$$

Culture media F_2:

$$\text{Activity} = -5.18 \times 10^{-3} + 2.10 \times 10^{-3}\ \text{days} \quad (2)$$

$$n = 9 \quad r_{\text{calc.}} = 0.9791 \quad r_{99.9\%} = 0.8982$$

Culture media F_3

$$\text{Activity} = 3.76 \times 10^{-2} + 1.32 \times 10^{-3}\ \text{days} \quad (3)$$

$$n = 9 \quad r_{\text{calc.}} = 0.6667 \quad r_{95\%} = 0.6664$$

The *t*-test proved that the regression coefficient of Eq. (1) significantly differed from those of Eq. (2)

Table 1
Activities of β-glucosidase (μmol min^{-1} ml^{-1}) measured at different fermentation times and culture medium

Fermentation time (days)	Culture media		
	F_1	F_2	F_3
7	0.050	0.030	0.050
14	0.065	0.043	0.079
21	0.104	0.061	0.133
28	0.097	0.061	0.133
35	0.097	0.061	0.094
42	0.147	0.079	0.097
49	0.334	0.101	0.097
58	0.385	0.129	0.108
63	0.298	0.115	0.097

($t_{calc.}$ = 4.24; $t_{99.9\%}$ = 4.14) and Eq. (3) ($t_{calc.}$ = 4.45; $t_{99.9\%}$ = 4.14) while no significant difference was found between the corresponding values of Eqs. (2) and (3) ($t_{calc.}$ = 1.35). This finding indicates that the highest activity of β-glucosidase can be obtained by using culture medium F_1 and the capacities of culture media F_2 and F_3 to produce β-glucosidase are significantly lower and are similar to each other.

3.2. Anion-exchange chromatography

Typical chromatograms of crude enzyme preparations are shown in Fig. 1. The chromatograms indicate that the proteins in the crude enzyme preparation can be separated into four distinct peaks obtaining baseline separation. The retention time and relative area of peaks are compiled in Table 2. The data show that the chromatograms are similar but not identical, the retention time and relative area of peaks being slightly different. This result indicates that the composition of culture media influences the enzyme profile produced by *P. ostreatus*. Measurement of the enzyme activities of the separated peaks established that the first peak shows only xylanase activity, peaks 2 and 3 expose mixed xylanase, laccase and β-glucosidase activity and only peak 4 shows only β-glucosidase activity. This finding draws attention to the fact that the individual peaks well separated by the liquid chromatographic method contain probably more than one protein showing

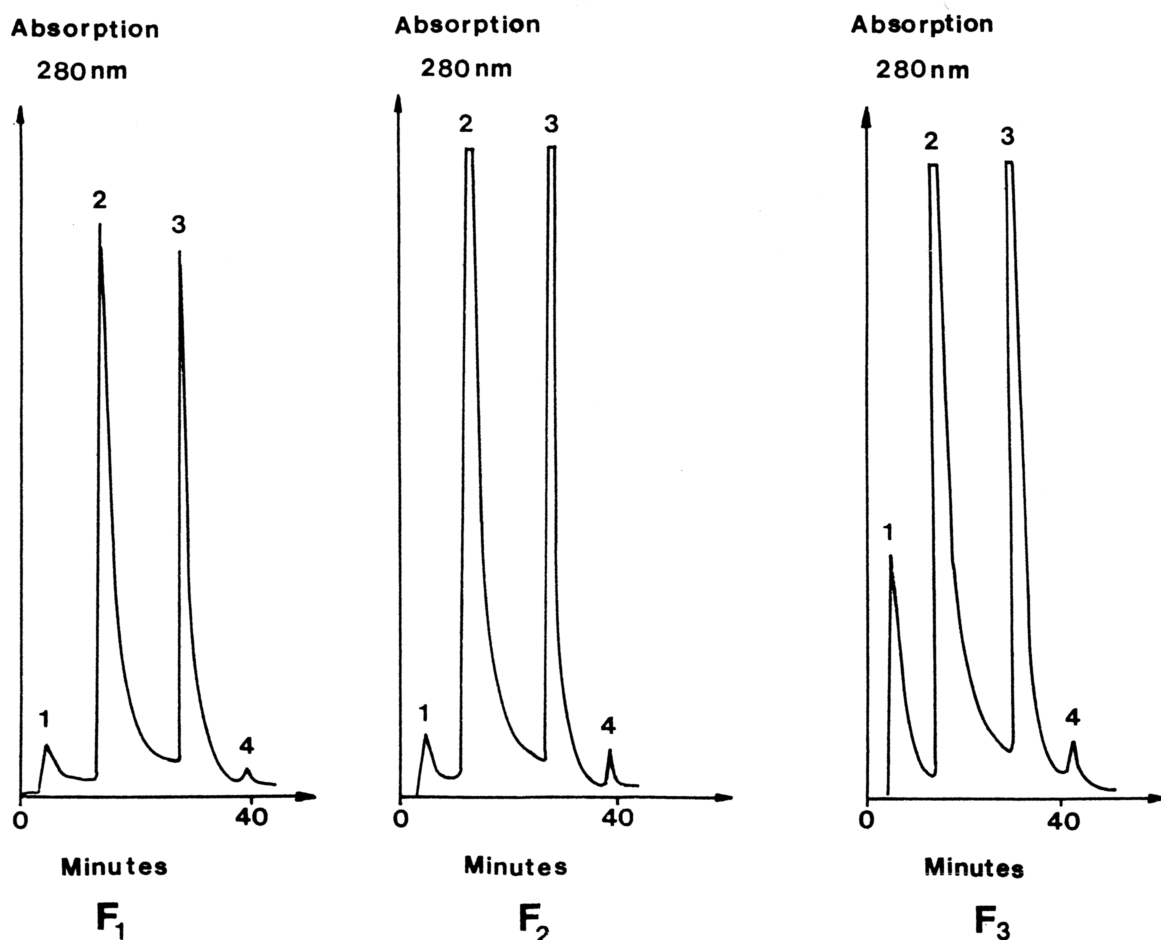

Fig. 1. Typical chromatograms of crude enzyme preparations. For symbols and chromatographic conditions see Experimental.

Table 2
Retention time and relative area of enzyme peaks in the crude enzyme preparation of *P. ostreatus* grown in different culture media

Culture media	No. of peak	Retention time (min)	Peak area (relative %)
F_1	1	12	2.47
	2	20	56.08
	3	30	40.22
	4	38	1.23
F_2	1	12	4.23
	2	18	49.64
	3	30	44.03
	4	38	2.00
F_3	1	10	11.03
	2	18	53.12
	3	34	34.54
	4	44	1.31

For symbols see Experimental.

various enzymatic activity, however, they are co-eluted in this chromatographic system. It has been concluded from the data that only fraction 4 can be used for further study of the enzyme kinetics of β-glucosidase.

3.3. SDS–PAGE and IEF

The relative molecular masses and isoelectric points of purified β-glucosidases produced by *P. ostreatus* grown on different culture media are compiled in Table 3. The data in Table 3 entirely support our previous conclusions drawn from the results of anion-exchange chromatography and from the determination of the enzyme activity of peaks. Both the relative molecular mass and isoelectric point of the β-glucosidases show considerable differences emphasizing again the marked influence on the composition of culture media on the character of β-glucosidase.

Table 3
Relative molecular masses and isoelectric points (p*I*) of purified β-glucosides produced by *P. ostreatus* grown on different culture media

Culture media	Relative molecular mass	p*I*
F_1	35	7.5
F_2	50	7.3
F_3	66	8.5

For symbols see Experimental.

3.4. Effect of pH on the activity and stability of purified β-glucosidase

The data (each in relative % of the highest activity) showing the effect of pH on activity and stability of purified β-glucosidase are compiled in Table 4. It can be seen from the data in Table 4 that not only the retention time, relative molecular mass and p*I* value of the purified β-glucosidase show considerable differences but also their activity at various pH values and their stability at different pH's prove again the different character of the enzyme produced in various culture media by the same fungus. The data further prove the good pH stability of the enzyme suggesting that it can be used even at extreme pH occurring in polluted environments. The

Table 4
Effect of pH on the activity and stability of purified β-glucosidases (in relative %)

pH	Culture medium		
	F_1	F_2	F_3
Activity of β-glucosidase			
2.0	0	0	10.1
3.0	77.2	62.4	85.2
4.0	100	100	97.5
5.0	86.2	95.8	100
6.0	84.7	90.2	85.7
7.0	84.7	87.9	79.5
8.0	69.7	75.7	78.6
9.0	69.7	52.3	63.2
10.5	0	0	0
Stability of β-glucosidase			
2.0	0	0	0
3.0	88.5	79.5	66.5
4.0	100	100	79.8
5.0	98.5	95.4	100
6.0	93.9	90.2	85.7
7.0	93.9	90.2	78.2
8.0	92.3	85.2	85.3
9.0	84.6	75.4	53.2
10.5	76.9	66.5	53.2
12.5	69.7	51.2	45.0

For symbols see Experimental.

parameters of quadratic equations describing the relationship between the pH and the activity and stability of β-glucosidase are compiled in Table 5. The quadratic equation fits well to the experimental data the significance level being always over 95% (compare calculated and tabulated F values). The equations explain 57.22–91.81% of the total variance present in the original data (see r^2 % values). The normalized slope values (b_i' %) indicate that the linear and quadratic forms of the independent variable exert a similar impact on the activity and stability of β-glucosidase justifying the application of quadratic function. No significant differences have been found among the coefficients of correlation of the quadratic equations indicating that the activity and pH stability of the enzymes produced in different culture media did not show significant deviations.

Table 5
Parameters of quadratic equations describing the relationship between the pH and the activity and stability of β-glucosidase

Parameters	Culture medium		
	F_1	F_2	F_3
Activity			
a	−58.63	−88.86	−72.66
b_1	54.19	65.90	56.77
s_{b1}	10.54	8.13	11.61
b_2	−4.63	−5.23	−4.74
s_{b2}	0.84	0.64	0.92
b_1' (%)	48.12	48.79	48.72
b_2' (%)	51.88	51.21	51.28
r^2 (%)	84.59	91.81	81.89
$F_{calc.}$	16.47	33.63	13.56
$F_{99\%}$	10.92	10.92	10.92
Stability			
a	−19.81	−17.34	−12.03
b_1	29.44	31.05	29.62
s_{b1}	9.74	9.91	10.55
b_2	−2.05	−2.13	−1.93
s_{b2}	0.67	0.68	0.72
b_1' (%)	49.64	49.98	51.20
b_2' (%)	50.36	50.02	48.80
r^2 (%)	57.38	58.71	57.22
$F_{calc.}$	5.17	5.98	5.16
$F_{95\%}$	5.14	5.14	5.14

For symbols see Experimental.
Activity $= a + b_1 \cdot \text{pH} + b_2 \cdot (\text{pH})^2$; Stability $= a + b_1 \cdot \text{pH} + b_2 \cdot (\text{pH})^2$.

3.5. *Effect of temperature on the activity and stability of purified β-glucosidase*

Data (each in relative % of the highest activity) demonstrating the influence of temperature on the activity and stability of purified β-glucosidase are compiled in Table 6. Similarly to data in Table 4 β-glucosidase show marked activities and high stability at markedly low and high temperatures. This finding suggests again that the enzyme can be successfully employed between a wide temperature interval in both industrial processess and environmental protection. The characteristics of quadratic equations describing the correlation between the temperature and the activity and stability of β-glucosidase are compiled in Table 7. The quadratic equation fits well to the data; the significance level was in each instance higher than 95% (compare calculated and tabulated F values). The equations

Table 6
Effect of temperature on the activity and stability of purified β-glucosidases (in relative %)

Temperature (°C)	Culture medium		
	F_1	F_2	F_3
Activity of β-glucosidase			
0	9.5	0	0
10	52.3	67.8	65.7
20	71.3	72.9	70.2
30	80.2	85.9	83.4
40	100	97.6	98.5
50	97.5	100	100
60	90.2	98.7	86.4
70	80.3	85.4	83.4
80	75.2	80.2	79.0
90	52.1	60.3	82.8
100	0	0	0
Stability of β-glucosidase			
0	0	0	0
10	75.6	82.5	79.2
20	85.4	100	100
30	98.2	95.8	93.5
40	100	86.3	86.3
50	94.5	80.4	83.5
60	85.2	75.3	76.3
70	72.3	61.3	85.2
80	0	0	0

For symbols see Experimental.

Table 7
Parameters of quadratic equations describing the relationship between the temperature (T °C) and the activity and stability of β-glucosidase

Parameters	Culture medium		
	F_1	F_2	F_3
Activity			
a	13.59	12.83	12.97
b_1	3.49	3.70	3.55
s_{b1}	0.27	0.43	0.46
b_2	-3.53×10^{-2}	-3.71×10^{-2}	-3.55×10^{-2}
s_{b2}	2.57×10^{-3}	4.12×10^{-3}	4.43×10^{-3}
b_1' (%)	48.76	49.01	49.08
b_2' (%)	51.26	50.99	50.92
r^2 (%)	95.94	90.99	88.89
$F_{calc.}$	94.50	40.38	32.01
$F_{99.9\%}$	21.69	21.69	21.69
Stability			
a	13.50	21.50	19.29
b_1	4.67	4.22	4.26
s_{b1}	0.74	0.87	1.05
b_2	-5.88×10^{-2}	-5.54×10^{-2}	-5.40×10^{-2}
s_{b2}	8.86×10^{-3}	10.45×10^{-3}	12.65×10^{-3}
b_1' (%)	48.82	47.80	48.65
b_2' (%)	51.51	52.20	51.35
r^2 (%)	88.09	82.81	75.32
$F_{calc.}$	22.06	14.45	9.15
$F_{99\%}$	13.27	13.27	–
$F_{95\%}$	–	–	5.79

For symbols see Experimental.
Activity $= a + b_1 \cdot T + b_2 \cdot (T)^2$; Stability $= a + b_1 T + b_2 \cdot (T)^2$.

explain 75.32–95.94% of the total variance (see r^2 % values). The similarity of the normalized slope values (b_i' %) indicate that the linear and quadratic forms of the independent variable exert a comparable impact on the activity and stability of β-glucosidase, therefore, the use of quadratic function is recommended. No significant differences have been found among the coefficients of correlation of the quadratic equations showing that the activity and thermal stability of the enzymes produced in different culture media did not deviate significantly from each other.

3.6. Kinetic parameters of the purified β-glucosidase

The kinetic parameters of β-glucosidases are compiled in Table 8. Both kinetic parameters are fairly similar to each other indicating that the kinetic behaviour of the various β-glucosidases does not show marked differences and they cannot be differentiated according to their kinetic parameters.

Table 8
Kinetic parameters of purified β-glucosidases produced by *P. ostreatus* grown in different culture medium

Culture media	V_{max} (Unit ml^{-1})	K_m (mg ml^{-1})
F_1	95	0.695
F_2	98	0.713
F_3	102	0.733

For symbols see Experimental.

4. Conclusion

The combination of anion-exchange chromatography, SDS–PAGE, isoelectric focusing and the measurement of enzyme activity by spectrophotometry allowed the chemical and biochemical characterisation of β-glucosidases produced by *Pleurotus ostreatus* in culture media composed of agro-industrial wastes. These data can be used for the production of β-glucosidases with special biochemical characteristics on an industrial scale.

Acknowledgements

This work was supported by the Portugese–Hungarian cooperation project ‘‘Development of new methods and their application for the assessment of the effect of environmental conditions on the stability of colour pigments in foods’’.

References

[1] Y.J. Cai, J.A. Buswell, S.T. Chang, Mycol. Res. 98 (1994) 1019.
[2] C.S. Evans, M.V. Dutton, F. Guillen, R.G. Veness, FEMS Microbiol. Rev. 13 (1994) 235.
[3] R. Bourbonnais, M.G. Paice, I.D. Reid, P. Lanthier, M. Yaguchi, Appl. Environ. Microbiol. 61 (1995) 1876.
[4] T.M. Wood, Biochem. J. 109 (1968) 217.
[5] T.K. Kirk, R.L. Farrell, Annu. Rev. Microbiol. 41 (1987) 65.
[6] R. Bourbonnais, M.G. Paice, FEBS Lett. 267 (1990) 99.
[7] P. Biely, Trends Biotechnol. 3 (1985) 286.

[8] A. Ruiz-Arribas, J.M. Fernandez-Abalos, P. Sánchez, A.L. Garda, R.I. Santamaria, Appl. Environ. Microbiol. 61 (1995) 2414.
[9] P.J. Kersten, M. Tien, B. Kalyanamaran, T.K. Kirk, J. Biol. Chem. 260 (1985) 2609.
[10] C.F. Thurston, Microbiology 140 (1994) 19.
[11] E.S. Lymar, B. Li, V. Renganathan, Appl. Environ. Microbiol. 61 (1995) 2976.
[12] M. Wrobel, J. Czubak, J. Chromatogr. B 746 (2000) 315.
[13] H. Arnostová, Z. Kučerová, I. Tislerova, T. Trnka, M. Tichá, J. Chromatogr. A 911 (2001) 211.
[14] J. Rogalski, M. Wojtas-Wasilevska, A. Leonowicz, Acta Biotechnol. 11 (1991) 485.
[15] T. Shimanouchi, S. Morita, H. Umakoshi, R. Kuboi, J. Chromatogr. B 743 (2000) 85.
[16] S. Ghose, H. Chase, Bioseparation 9 (2000) 29.
[17] C.T. Tomaz, J.A. Queiroz, J. Chromatogr. A 865 (1999) 123.
[18] N.J. Parry, D.E. Beever, E. Owen, I. Vandenbeghe, J. van Beeuman, M.K. Bhat, Biochem. J. 353 (2001) 117.
[19] G.L. Miller, Anal. Chem. 31 (1959) 426.
[20] H. Blum, H. Beier, H.J. Gross, Electrophoresis 8 (1987) 93.