Biochemical Characterization and Kinetic Properties of White Rot Fungal β- Glucosidase

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Introduction

White rot fungi (WRF) secrete extracellular enzymes to digest food needed for their growth and development. WRF produce β-glucosidase, a component of a suite of enzymes used in degradation of lignocellulose. β-glucosidase enzyme due to its versatile nature relative to substrate specificity is very important in biomass degradation or conversion; and different WRF differ in their ability to produce this enzyme. This study focused on evaluating β-glucosidase activities among selected WRF. WRF showing the best activities can be used individually or in combination with other organisms, for bioconversion of biomass to fermentable sugars and other bio-products.

Materials & Methods

Seventeen WRF from six genera (*Pleurotus, Grifola, Auricularia, Polyporus, Trametes, and Lentinula*) were evaluated for β-glucosidase activity. β-glucosidase activity in the extract from cultivation medium was assayed at 37°C for 30 minutes, using *p*-nitrophenyl β-D-glucopyranoside as substrate (prepared in 50 m*M* sodium acetate buffer, pH 5.0). Total carbohydrate and protein were estimated using phenol-sulfuric acid method and Better Bradford assay kit respectively.

Acknowledgments

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Results

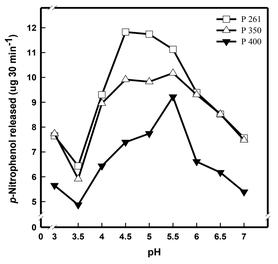

Fig. 1. Relationship between pH and β-glucosidase activity of *Pleurotus ostreatus*.

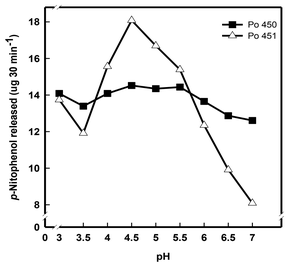

Fig. 4. Relationship between pH and β-glucosidase activity of *Polyporus squamosus*.

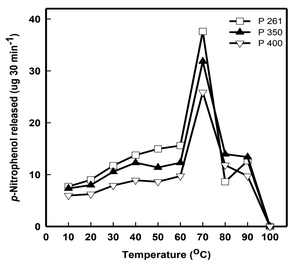

Fig. 7. Relationship between temperature and β-glucosidase activity of *Pleurotus ostreatus*.

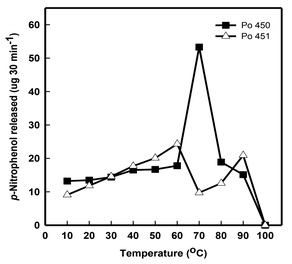

Fig. 10. Relationship between temperature and β-glucosidase activity of *Polyporus squamosus*.

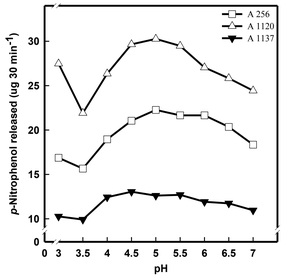

Fig. 2. Relationship between pH and β-glucosidase activity of *Auricularia auricula*.

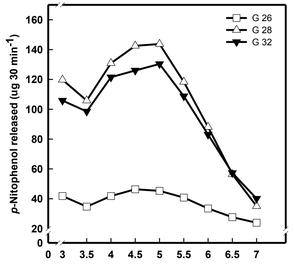

Fig. 5. Relationship between pH and β-glucosidase activity of *Grifola frondosa*.

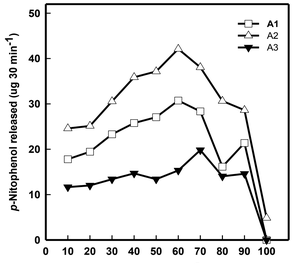

Fig. 8. Relatationship between temperature and β-glucosidase activity of *Auricularia auricula*.

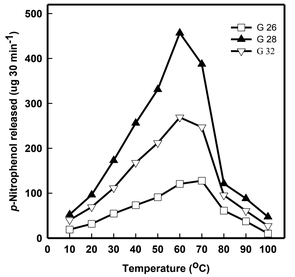

Fig. 11. Relationship between temperature and β-glucosidase activity of *Grifola frondosa*.

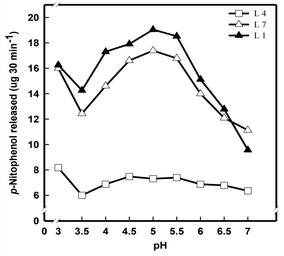

Fig. 3. Relationship between pH and β-glucosidase activity of *Lentinus edodes*.

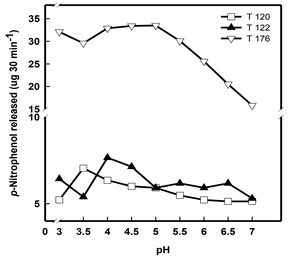

Fig. 6. Relationship between pH and β-glucosidase activity of *Trametes versicolor*.

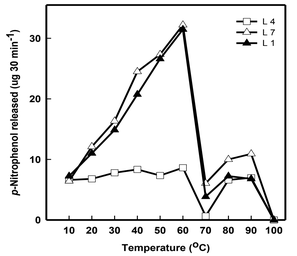

Fig. 9. Relationship between temperature and β-glucosidase activity of *Lentinula edodes*.

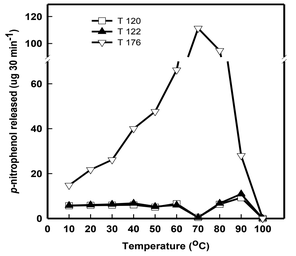

Fig. 12. Relationship between temperature and β-glucosidase activity of *Trametes versicolor*.

Table 1. Total protein and carbohydrate contents in secretions of various white rot fungi.

Samples	Protein (ug mL^{-1})		Carbohydrate (ug mL^{-1})	
Pleurotus 261	68 ± 12.5[†]	(I)[§]	65 ± 10.0[†]	(DE)[§]
Pleurotus 350	77 ± 1.4	(IJ)	46 ± 6.4	(G)
Pleurotus 400	82 ± 6.9	(HIJFGHIJ)	44 ± 10.0	(G)
Grifola 26	92 ± 6.0	(FGHIJ)	59 ± 2.5	(EF)
Grifola 28	121 ± 0.3	(DEFGH)	14 ± 9.6	(H)
Grifola 32	98 ± 4.7	(EFJHI)	53 ± 8.4	(GF)
Lentinula 1	112 ± 5.7	(DEFGH)	79 ± 10.9	(C)
Lentinula 4	71 ± 9.3	(IJ)	53 ± 11.2	(GF)
Lentinula 7	123 ± 5.8	(DEFGH)	70 ± 8.9	(D)
Auricularia 265	228 ± 74.7	(A)	91 ± 0.2	(B)
Auricularia 1120	200 ± 45.4	(AB)	53 ± 6.8	(GF)
Auricularia 1137	163 ± 12.2	(BCD)	48 ± 4.6	(G)
Polyporus 450	172 ± 9.1	(BC)	64 ± 4.6	(DE)
Polyporus 451	144 ± 10.6	(CDE)	108 ± 4.6	(A)
Trametes 120	132 ± 19.1	(CDEFG)	79 ± 5.0	(C)
Trametes 122	135 ± 13.2	(CDEFG)	83 ± 1.8	(BC)
Trametes 176	141 ± 9.6	(CDEF)	68 ± 8.7	(DE)

[†] Standard deviation.

[§] ANOVA (Means with the same letters within the same columns are not significantly different).

Summary

- Extracts showed significant differences at $p < 0.05$ in total protein and carbohydrate contents (Table 1).
- Most of the secretions exhibited pH optima between 4.5 and 5.0 (Figs. 1 - 6), and temperature optima at either 60 or 70°C (Figs. 7 - 12).
- V_{max} values ranged from 6.4 - 291 µg 30 min^{-1}, while K_m values ranged from 0.51 to 660 µ*M* (using non-linear regression fit analysis).
- β-glucosidase activity in extracts was significantly but negatively correlated with carbohydrate content.
- There was no significant correlation between protein content and β-glucosidase activity.