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Regular paper

Effective and complex stimulation of the biodegradation system of fungus *Cerrena unicolor* by rapeseed meal fermentation

Magdalena Jaszek[1]✉, Justyna Miłek[1], Jerzy Żuchowski[2], Dawid Stefaniuk[1] and Monika Prendecka[3]

[1]Department of Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland; [2]Department of Biochemistry, Institute of Soil Science and Plant Cultivation, Puławy, Poland; [3]Chair and Department of Human Physiology, Medical University of Lublin, Lublin, Poland

The effect of supplementation of medium with rapeseed meal (RM) on production of biotechnologically important enzymes was investigated in submerged cultures of the white rot fungus *Cerrena unicolor*. The addition of RM (3.5% w/v) distinctly stimulated the activities of laccase, chitinase, and β-glucosidase. As compared to the control, the activities of chitinase, β-glucosidase, and laccase in the RM supplemented cultures were up to 4.1, 8.4, and 3.9 times higher, respectively. The results of the spectrophotometric and spectrofluorometric measurements were additionally confirmed by zymographic analysis of the samples. The level of sugars and phenolic compounds as well as the antioxidative ability of fungal preparations were also determined. The results obtained indicate that the submerged liquid fermentation of rapeseed meal can be proposed as an inexpensive and very effective method for biotechnological production of chitinase, β-glucosidase, and laccase by *C. unicolor*.

Key words: *Cerrena unicolor*, laccase, chitinase, β-glucosidase, white rot fungi, rapeseed meal

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INTRODUCTION

The growing world demand for rapeseed oil, a major raw material in the production of biodiesel, results in increasing production of rapeseed meal (RM), a by-product of oil extraction. For this reason, development of new methods for utilization of this waste material becomes increasingly important. Being a rich source of nutrients, rapeseed meal can be used as a valuable feed additive. However, its high nutritional value is significantly decreased by high levels of fiber, glucosinolates, phytic acid, and phenolic compounds (Vioque *et al.*, 2000; Vuorela *et al.*, 2004). Biotransformation of RM with white rot fungi *Trametes versicolor* and *Pleurotus ostreatus* resulted in fast reduction of its sinapic acid content. Moreover, the presence of rapeseed meal in the cultivation medium strongly induced the extracellular laccase activity in both investigated species (Hu & Duvnjak, 2004; Żuchowski *et al.*, 2013). This observation suggests that RM may be applied in production of biotechnologically relevant enzymes.

White rot fungi are equipped by nature with the most effective lignin-degrading system among living organisms. They are known as efficient producers of two groups of extracellular biocatalysts. One of them comprises a number of hydrolases enabling degradation of polysaccharides (cellulases, hemicellulases). The other group of enzymes (laccase, lignin peroxidase, manganese peroxidase) is involved in special interaction with low molecular weight mediators and responsible for effective lignin depolymerization (Sanchez, 2009). The biotechnological application of both groups of enzymes has been broadly investigated for many years. *C. unicolor* is a white rot fungus belonging to the phylum *Basidiomycetes*, known as a very efficient laccase or hydrolase producer (Janusz *et al.*, 2007; Belova *et al.*, 2014). It can also be used to produce dietary nutraceuticals or supplements having antioxidant and antibacterial properties (Jaszek *et al.*, 2013).

Laccase (benzenediol: oxygen oxidoreductase EC 1.10.3.2), described as a multicopper oxidase, is widely applied in modern biotechnology. The enzyme is able to oxidize numerous aminophenols, polyphenols, metoxyphenols, lignin, aryl diamines, and other compounds (Polak & Jarosz-Wilkolazka, 2012; Majeau *et al.*, 2010; Arora & Sharma, 2009). White rot *Basidiomycetes* are the most intensively investigated group of laccase producers. The broad substrate specificity, simple mechanism of the catalyzed reaction, and high stability of this enzyme are the reasons for its very wide industrial and biotechnological potential (Majeau *et al.*, 2010). The delignification of lignocelluloses, biopulping and biobleaching, textile dye decolorization, degradation of pollutants, construction of biosensors, or medical use may be mentioned as the most important applications of laccase (Arora & Sharma, 2009).

Chitinase (EC 3.2.1.14) is an enzyme widely distributed among fungi, plants, and bacteria. Since chitinase-mediated hydrolytic degradation of chitin results in production of biologically active chitooligosaccharide derivatives, development of new sources of the enzyme and methods for stimulation of its activity seems to be important and beneficial for industrial applications. Furthermore, chitinases play an important role in fungal nutrition processes, morphogenesis, defense against chitin-containing pathogens, as well as in inter-specific interactions between different fungal strains in nature (Duo-Chuan, 2006; Hiscox *et al.*, 2010).

β-glucosidase (1,4-β-D-glucosidase EC 3.2.1.21) belongs to a group of synergistically acting cellulases that catalyze depolymerization of cellulose, forming cellobiose and other derivatives (Gao *et al.*, 2008). This enzyme is responsible for decomposition of cellobiose to glucose,

✉e-mail: magdalena.jaszek@poczta.umcs.lublin.pl

Abbreviations: *C. unicolor*, *Cerrena unicolor*; RM, rapeseed meal

which abolishes the inhibitory effect of cellobiose on the other cellulases (Sternberg *et al.*, 1977). Although most β-glucosidases used in industry are of bacterial origin, it is known that many species of fungi, such as *Pholiota adiposa* or *Penicillum pinopholium,* are efficient producers of this enzyme (Joo *et al.*, 2010; Jagtap *et al.*, 2013). The search for new, more efficient biological sources of β-glucosidase is important from the point of view of different branches of industry related to saccharification of industrial and agricultural cellulosic derivatives. The main objective of the present work was to investigate the possibility of effective stimulation of the natural biodegradation system of *C. unicolor* by rapeseed meal supplementation of the culture medium. Activities of intra- and extracellular hydrolases (chitinase, β-glucosidase) and laccase were compared during the growth of *C. unicolor* in liquid media with or without the RM addition. Levels of reducing sugars, superoxide anion radicals, and phenolic compounds were also determined.

MATERIALS AND METHODS

Fungal strain, media, fermentation conditions, and preparation of biological samples. The fungal strain *C. unicolor* (Bull. ex Fr.) Murr. was obtained from the Regensburg University culture collection and deposited in the fungal collection of the Department of Biochemistry Maria Curie-Skłodowska University, Lublin, Poland. This strain was identified by its ITS sequence deposited in Genbank database (Accession number: DQ056858). Fungal mycelia were stored on 2% (w/v) malt agar slants at 4°C. The experimental cultures were conducted in 250 ml Erlenmeyer flasks containing 75 ml of liquid medium prepared according to Fahreus and Reinhammar (Fahreus & Reinhammar, 1967) with glucose (20 g L^{-1}) and L-asparagine (2.5 g L^{-1}) as a carbon and nitrogen source, respectively. Half of the experimental flasks were enriched with 3.5% of RM. The other half, without the RM addition, was used as control cultures. All cultivation media were autoclaved for 30 min. at 121°C. To prepare the inoculum, *C. unicolor* was grown for 14 days in Erlenmeyer flasks containing 75 ml of Fahreus and Reinhammar medium, at 25°C. The mycelium was subsequently collected and homogenized. After inoculation of the experimental flasks with 1.25% (v/v) of the homogenized mycelium, 15-day submerged liquid fermentation (SLF) (Gregori *et al.*, 2007) of the experimental cultures was carried out at 30°C in a Multitron incubator (Infors) (agitation rate 100 rpm). One cycle of cultivation included three repetitions of each experimental variant. Rapeseed meal obtained from a local feed shop contained 34.8% of crude protein (N×6.25) and 13% of crude fiber. Its sinapic acid content was 6.15 mg×g^{-1} (Żuchowski *et al.*, 2013). Fungal samples were collected periodically from the 7th to 15th day of cultivation at 4-day intervals. All harvested mycelia were separated from the culture fluid by filtration through Miracloth (Calbiochem), washed twice with distilled water, and homogenized in phosphate buffer (pH 7.4) using a glass Potter homogenizer at 4°C. After centrifugation (15 min, 10 000 × *g*), crude supernatants were divided into smaller portions, frozen (–20°C), and used in the assays. Extracellular fluids of the experimental fungal cultures obtained after separation of the mycelium biomass were prepared as described above.

Enzyme activity assays. Laccase (LAC) activity assay. Extracellular and intracellular LAC activities were determined using syringaldazine (4-hydroxy-3,5-dimetoxybenzaldehyde) as a substrate (Leonowicz & Grzywnowicz, 1981). The catalytic activity of laccase was calculated based on the extinction coefficient for the reaction product obtained (65 000 $M^{-1}cm^{-1}$) and expressed in nanokatals per milligram of protein (intracellular activity) or in nanokatals per liter (extracellular activity).

Chitinase (CHT) activity assay. In order to determine the CHT activity, 4-methylumbelliferyl-β-N,N',N''-triacetylchitotrioside [MUF-3] was used as a substrate (McCreath and Gooday, 1992). The reaction mixture comprised the following components: 50 µl of the enzyme sample, 0.4 ml of 100 mM citrate-phosphate buffer (pH 6.5), and 12.5 µl of the substrate solution (5 µM). The addition of the substrate solution started the enzymatic reaction. After 10-minute incubation at 20°C, the release of the 4-MU derivative was monitored using the FluoroMax-2 spectrofluorometer (Horiba, Japan) (excitation 390 nm, emission 460 nm). The intracellular CHT activity was calculated as the amount of the 4-MU formed per min per mg of protein and expressed as percentage of the control samples collected from the 7th day of the cultivation period.

β-Glucosidase (β-GLU) activity detection. The β-GLU activity was determined using 4-methylumbelliferyl β-D-glucopyranoside [MUG] as the reaction substrate, according to Kim and coworkers (Kim *et al.*, 2007), with own modifications. The following reaction mixture was used: 10 µl of the enzyme sample, 0.4 ml of 100 mM citrate-phosphate buffer (pH 4.8), and 12.5 µl of the substrate solution (5 µM). The addition of the substrate solution started the enzymatic reaction. The samples were incubated for 10 minutes at 20°C and next the release of the 4-MU derivative was estimated using the FluoroMax-2 spectrofluorometer (Horiba, Japan) (excitation 390 nm, emission 460 nm). The intracellular β-GLU activity was calculated as the amount of the 4-MU formed per min per mg of protein and expressed as percentage of the control samples collected on the 7th day of the cultivation period.

The control from the first measurement day of the experiment was chosen as a basis on which the dynamics of CHT and β-GLU activity can be traced on subsequent measurement days, both in controls and in cultures stimulated with RM.

Assay of the level of superoxide anion radicals (SOR), phenolic compounds (PHC), proteins, and reducing sugars (RS). The relative level of SOR was estimated according to the method for rapid detection of superoxide anion generation in fungal material. The reaction is based on spectrophotometric detection of superoxide-dependent formation of formazan from nitrotetrazolium blue (NBT) under alkaline conditions (Paździoch-Czochra *et al.*, 2003). The reaction mixture used for SOR detection comprised the following components: 1.55 ml of distilled water 25 µl of 1 M NaOH, 50 µl of 5 mM NBT solution, and 50 µl of the sample. The samples were incubated for 30 min. at 20°C and the absorbance was measured at 560 nm. The level of SOR (after consideration of the protein concentration in the case of mycelia) was expressed as the percentage of the control samples obtained from the 7-day cultures (100%). In the case of the fungal samples, the alkaline conditions applied prevented precipitation of dark-blue formazan for about 40 min (Paździoch-Czochra *et al.*, 2003). The concentration of reducing sugars was estimated according to the Somogyi-Nelson method based on the colored arsenomolybdate complex formed by the reaction of ammonium molybdate $(NH_4)_6Mo_7O_{24}$ with sodium arsenate Na_2HAsO_7 (a procedure described

Table 1. The concentration of intracellular and extracellular reducing sugars in RM supplemented *C. unicolor* cultures grown in submerged conditions

Time of cultivation (days)						
	7		11		15	
	CControl	RM	Control	RM	Control	RM
Intracellular[1]	362.2 ±3.2	219±1.8	261±2.1	125±1.2	436.3±3.9	9.9±0.1
IntraExtracellular[2]	144.9±1.5	113.18±0.9	118.2±1.1	28.44±0.4	94.8±0.8	12.9±0.3

[1]Intracellular concentration of reducing sugars was expressed in μg/mg of protein. [2]Concentration of extracellular reducing sugars was expressed in μg/ml. The given values (± standard deviation) are averages of three independent experiments performed in triplicate. The values within the lines (for a particular day of cultivation) are significantly different ($p \leq 0.05$)

by Hope and Burns, with own modifications) (Hope & Burns, 1987). The concentration of phenolic compounds (PHC) (-hydroxyl, -metoxy phenolic acids) was detected with diazosulfanilamide (SA) as a reaction substrate according to the DASA test. The reaction sample was a mixture of the following ingredients: 0.1 ml of SA (1% SA in 10% HCl), 0.1 ml of 5% $NaNO_2$ solution, and 0.1 ml of the biological sample. The samples were intensively mixed and neutralized by addition of 1 ml of 20% Na_2CO_3 solution, and absorbance at 500 nm was determined. The PHC concentration was calculated using a calibration curve (y=6.85x-0.0218, R^2=0.999) and expressed in μmol of vanillic acid equivalent per ml for culture liquids and per mg of protein for mycelia (Malarczyk, 1989). The Coomassie brilliant blue (G-250) dye-binding method was used to determine the protein concentration (Bradford, 1976). Bovine serum albumin was used as the standard protein.

Analysis of LAC, CHT, and β-GLU activities by native polyacrylamide gel electrophoresis. Both the supernatant of homogenized mycelia and the samples of the extracellular culture fluid were concentrated and separated by ultrafiltration using Microcon Centrifugal Filter Units 3000 NMWL designed by Millipore. Subsequently, 15 μg of protein was deposited per lane. The 10% native PAGE prepared according to the Laemmli method was used for detection of LAC activity (Laemmli *et al.*, 1970). Electrophoretic separations were conducted in the cold (4°C) at 145 V. Laccase activity was visualized by the reaction with guaiacol (Sigma Chemical Co. St. Luis, USA) in 100 mM citrate-phosphate buffer (pH 4.6) at 25°C. The gels were then documented using the Syngene G-Box system. The 7.5% native gels, prepared as described above, were used for detection of CHT and β-GLU activities. Electrophoretic separations were conducted in the cold (4°C) at 145 V. In order to visualize CHT activities, the gels were at first incubated in the 100 mM citrate-phosphate buffer (pH 6.5) (step I) for 30 minutes and next in the 25 μM solution of 4-methylumbelliferyl-β-N,N',N''-triacetylochitotrioside [MUF-3] at 25°C in the same buffer for 30 min (step II) (the method of McCreath and Gooday, with own modifications) (McCreath and Gooday, 1992). Then, the gels were washed with the buffer and the released 4-methylumbelliferone (MU-4) was visualized under ultraviolet light. A similar three-step procedure was used in visualization of β-GLU activity bands. In this case, 4-methylumbelliferyl β-D-glucopyranoside (MUG) in 100 mM citrate-phosphate buffer (pH 4.8) was used as the reaction substrate (the method of Kim and coworkers (Kim *et al.*, 2007), with own modifications). Gel images were captured using the Syngene G-Box system.

Statistical analysis. The experiments were performed in triplicate; the results are express as means ±S.D. The ANOVA analysis (Statgraphics Online) was conducted for all results and means were compared using post hoc Tukey's test. A level of significance of $p<0.05$ was used.

RESULTS AND DISCUSSION

Many lignin-containing agricultural byproducts can stimulate white rotting fungi to produce biotechnologically applicable ligninolytic and hydrolytic enzymes (Żuchowski *et al.*, 2013; Belova *et al.*, 2014). The results obtained clearly indicate that SLF of the RM-containing medium is a very good growth environment for *C. unicolor*. After 7 days, the surface of the medium was completely covered with growing fungal mycelia. The results are similar to data obtained earlier for *T. versicolor* and *P. ostreatus* (Żuchowski *et al.*, 2013). The development of the mycelia corresponded with the decrease in the concentration of extracellular reducing sugars in the control as well as in the RM supplemented cultures (Table 1).

LAC activities

Given the wide application potential of laccase, methods for economical production of highly active and stable enzymes are being very intensively searched (Lisova *et al.*, 2010; Couto & Toca-Herrera, 2007). Until now, many natural agricultural or food-processing byproducts e.g. olive mill wastes, corn stover, mandarin peel, or residues of ethanol production have been used as efficient substrates for induction of laccase activity by solid-state fermentation (Majeau *et al.*, 2010; Ruiz-Rodriguez *et al.*, 2010; Wan & Li, 2010). The submerged fermentation of RM by *C. unicolor* was accompanied by a very significant increase in extracellular Lac activity, compared to the control cultures containing only glucose as a source of carbon and asparagine as a source of nitrogen. Laccase activity developed gradually over the cultivation period and reached its maximum (up to 3.9 times higher than the control) on day 11 (Fig 1A). In the case of intracellular LAC activities, significant ($p \leq 0.05$) stimulation by the RM addition was detected only on day 7 (Fig 1C). The results of spectrophotometric measurements were confirmed by native-PAGE analysis of the samples. Multiple isoenzymes (5 activity bands) of *C. unicolor* laccase were detected on the zymograms (Fig 1B, D). There are very distinct qualitative and quantitative differences between the control and RM stimulated preparations. This situation may arise from the fact that, according to the available literature, 10 genes encoding laccase have been identified in *C. unicolor* (http://genome.jgi.doe.gov/). It is probable that the expression of these genes can be stimulated by growth conditions resulting in appearance of new active forms of the enzyme in the cultures. Moreover, dif-

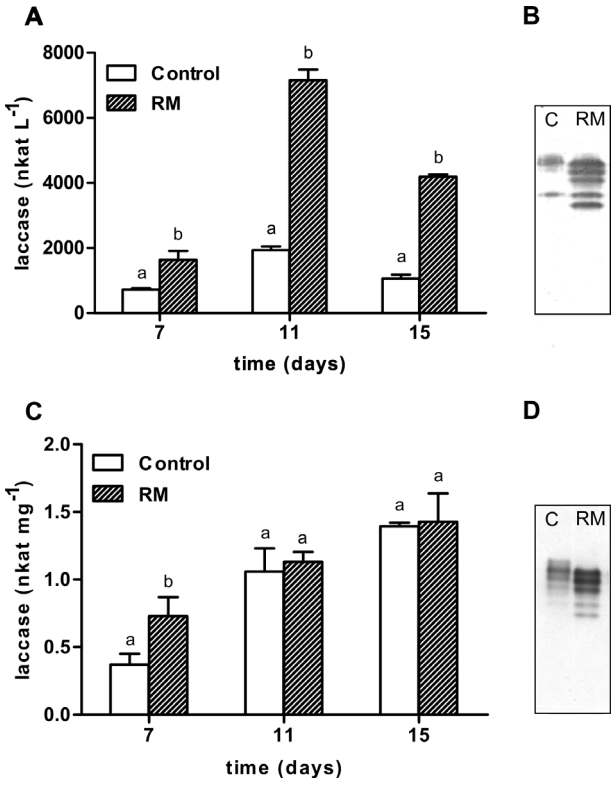

Figure 1. The effect of RM supplementation on LAC activities in culture fluids (A) and mycelia (C) of *C. unicolor* grown in submerged conditions.
Data are means ± S.D. (n=3); pairs of means marked with different letters are significantly different ($p \leq 0.05$). Native PAGE of culture fluid (**B**) and intracellular preparation (**D**) from 11-day culture of *C. unicolor* (C, control culture; RM, rapeseed meal supplemented culture)

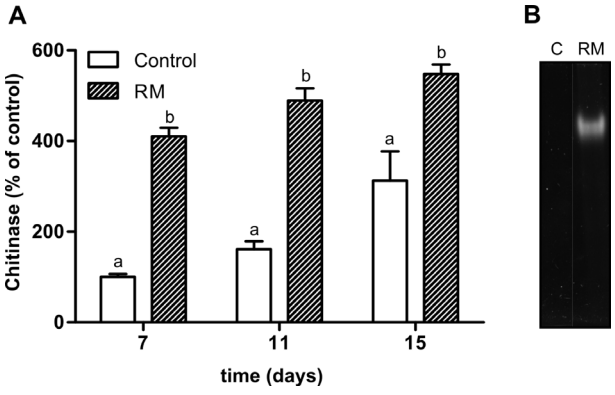

Figure 2. The effect of RM supplementation of culture medium on CHT activities in mycelia of *C. unicolor* grown in submerged conditions (A).
The results are expressed as % of the control; data are means ± S.D. (n=3); pairs of means marked with different letters are significantly different ($p \leq 0.05$). Native PAGE of intracellular preparation (**B**) from 11-day culture of *C. unicolor* (C, control culture; RM, rapeseed meal supplemented culture)

ferences in the molecular weight of the laccase in the cultures stimulated with RM compared with the control may result from quantitative and qualitative differences in the carbohydrate content of the enzyme molecule. The characterization of glycosylation of *C. unicolor* laccase isoenzymes was described in the previous paper (Kucharzyk *et al.*, 2012; Lisova *et al.*, 2010). The fact that the enzyme is secreted mostly in the form of isoenzymes was confirmed by the presented studies (Majeau *et al.*, 2010). It is a well-known fact that the production of laccase can be considerably increased by the addition of different inducers belonging mainly to aromatic and phenolic compounds e.g. lignin derivatives (Lisova *et al.*, 2010; Farnet *et al.*, 1999). An evident correlation was observed between extracellular laccase activity and the elevated level of phenolic compounds in the culture medium. The reverse correlation appearing on the 11th day of culture suggests consumption of a part of PHC as a substrate by the laccase present in the culture fluid. The obtained results correspond also very well with data obtained for *P. ostreatus* and *T. versicolor* by Żuchowski and coworkers (Żuchowski *et al.*, 2013) who showed stimulation of LAC activity by solid-state fermentation (SF) of RM. Increased production of LAC was also detected by Winquist *et al.*, who cultivated *C. unicolor* on different industrial waste materials (Winquist *et al.*, 2008). The comparison of laccase produced by *C. unicolor* with laccases reported earlier showed that SF of RM could be proposed as an effective way for enzyme production.

CHT activities

Fungal chitinases are inducible enzymes. Earlier reports described glucose repression as a common mechanism of regulation of fungal chitinase genes. As shown, the activity of chitinase increased along with the decreasing concentration of glucose in the medium (Duo-Chuan, 2006). This study demonstrates that the RM supplementation significantly increased the intracellular chitinase activity in submerged cultures of *C. unicolor*. The CHT activity grew progressively throughout the experimental period, showing a maximum on day 15 (Fig 2A). However, it should be noted that after the initial significant enzyme activity induction observed on the 7th day of the culture, the growth rate of CHT activity decreased compared with the controls, which may be connected with changes in the availability of the proper substrate with the aging of the mycelia. The zymographic analysis showed the presence of a strong CHT activity band in the mycelial homogenates from the RM supplemented cultures, which was not visible in the control preparations (Fig 2B). It should be emphasized that the significant growth in CHT activity corresponds well with the decrease in the level of reducing sugars in the culture medium. To the best of our knowledge, this is the first description of the stimulation of chitinase activity by addition of RM to the culture medium of a white rot fungus. Results presented previously indicate that the activity of CHT can be induced by e.g. colloidal chitin, carbon starvation, or stress conditions (Duo-Chuan, 2006). The oxidative stress-caused induction of CHT activity has been described earlier for the white rot fungus *Phellinus pini* (Jaszek *et al.*, 2014). Given the vast possibilities of practical application of CHT, the proposal of production intensification presented in this work fits well with the needs of the modern market.

β-GLU activities

C. unicolor has been reported to produce cellulases and xylanase, especially when it is grown in a medium containing microcrystalline cellulose (Belova *et al.*, 2014; Elisashvili *et al.*, 2002). The submerged fermentation of rapeseed meal by *C. unicolor* was correlated with a very distinct increase in β-glucosidase activities in the medium and in mycelia (Fig 3A, C). The

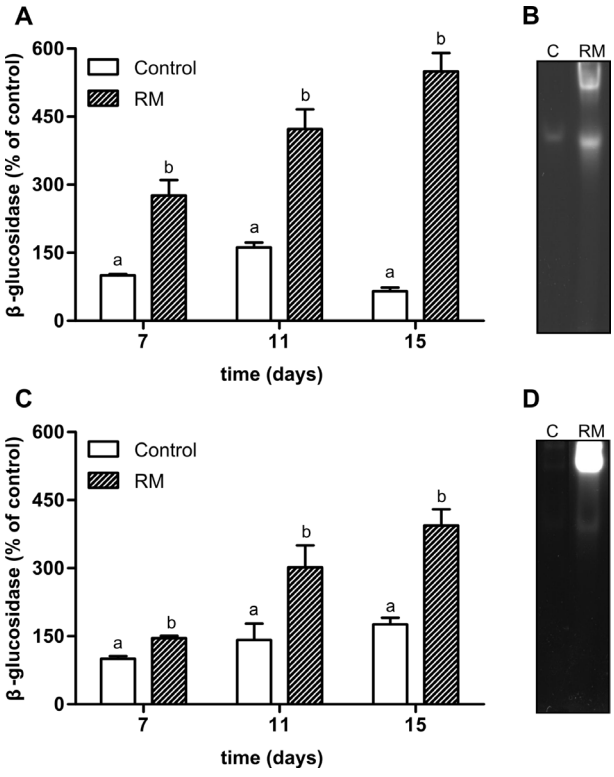

Figure 3. The effect of RM supplementation on β-GLU activities in culture fluids (A) and mycelia (C) of *C. unicolor* grown in submerged conditions.
The results are expressed as % of the control; data are means ±S.D. (n=3); pairs of means marked with different letters are significantly different ($p \leq 0.05$). Native PAGE of culture fluid (**B**) and intracellular preparation (**D**) from 11-day culture of *C. unicolor* (C, control culture; RM, rapeseed meal supplemented culture)

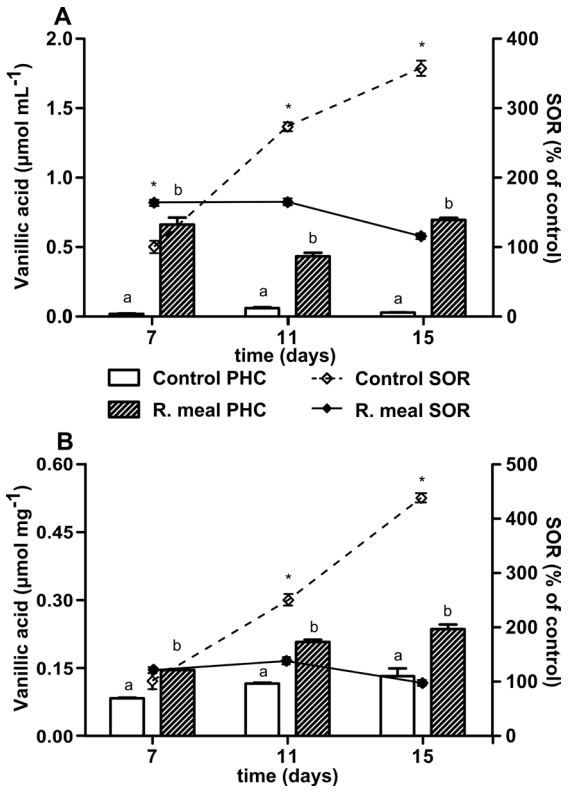

Figure 4. Alteration in PHC and SOR levels in *C. unicolor* (A) culture fluids and mycelia (B) after addition of RM to the culture medium.
Data are mean ±S.D. for three measurements (n=3); pairs of means marked with different letters or an asterisk are significantly different ($p \leq 0.05$)

β-GLU activity increased gradually over the entire period of cultivation, showing the maximum on day 15 (8.4 and 2.2 times above the control for extracellular and intracellular activities, respectively). As in the case of CHT, a similar correlation between the growing β-GLU production and the decreasing concentration of reducing sugars was observed. As shown in the previous reports, the activity of β-GLU produced by the fungus *C. unicolor* is closely related to the carbon source present in the culture medium. The highest activities of this parameter were observed in the case of cultures containing polysaccharides (Elisashvili *et al.*, 2002). Two bands of β-GLU activity were detected by native-PAGE in extra- and intracellular samples in the RM supplemented cultures and intracellular control preparation (Fig 3B, D). In the case of extracellular control samples only one clearly visible band of β-GLU activity was noted. It seems that the presence of suitable inducers such as RM, besides stimulation of the constitutive activity, can probably also cause the occurrence of an induced extracellular form of this enzyme**.** β- glucosidases have a broad application potential in food, pharmaceutical, and cosmetic industry (Saibi *et al.*, 2012). Recently, examples have been published of the use of different natural byproducts, e.g. wheat and rice straw, corn cobs, wheat bran, oat bran, and oil palm residues, as substrates for production of fungal β- glucosidases (Kalogeris *et al.*, 2003; Saritha *et al.*, 2012; Teoh and Mashitah, 2010). Our results indicate that the RM-enriched cultures of *C. unicolor* are a promising source of active and easily isolated β-GLU.

Intra- and extracellular SOR and PHC levels

Many phenolic compounds are classified as bioactive nutritional constituents (Martins *et al.*, 2011). Rapeseed meal is rich in sinapic acid esters and condensed tannins. As could be expected, the concentrations of phenolic compounds (presented as the vanillic acid level) were much higher in the RM-enriched cultures, which was particularly visible in the case of the culture fluid (Fig 4). The changing amount of PHC (high in the medium and gradually growing in the mycelia) suggests that they were partly derived from the RM and partly produced by the growing fungal biomass. A connection between the high PHC level and β-GLU activity is also visible. This enzyme is able to catalyze hydrolysis of phenolic glycosides resulting in release of free phenolic acids. Hence, the described antioxidative activity of fermented products, e.g. rice, is associated with high β-GLU activity (Martins *et al.*, 2011). The antioxidative properties of the detected PHC were indirectly confirmed by the level of superoxide anion radicals. There was a very clear reverse correlation between the PHC and SOR levels (Fig 4).

CONCLUSIONS

The implementation of the experimental program presented in this study has resulted in proposal of a new effective method for laccase, chitinase, and β-glucosidase production. It may be concluded from the presented results that rapeseed meal can be used as a promising sup-

plement for submerged production of fungal ligninolytic and hydrolytic enzymes. The data obtained can be applied in two ways: biotechnological production of natural biocatalysts and bioactive compounds or microbial pretreatment of RM in order to increase the possibility of using thereof as a dietary supplement in animal feeding. The results have also confirmed the possible use of the white rot fungus *C. unicolor* as a modern biotechnological tool.

AcknowledgE0ments

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