# The agricultural waste utilization for exploration of the extracellular enzyme potential of three novel white-rot Basidiomycetes: *Fomes fomentarius* TMF2, *Schizophyllum commune* TMF3 and *Bjerkandera adusta* TMF1

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# Abstract

**Purpose** This study aimed in exploitation of lignocellulosic wastes for the evaluation of the newly isolated white-rot fungal strains enzymatic potential, covered by the circular economy frame.

**Methods** A standard microbiological methods for the isolation of the white-rot fungal mycelia were used, followed by DNA extraction, PCR amplification, and the API ZYM test. The determination of laccase activity was based on the oxidation of guaiacol while the DNS method was used for the hydrolase determination.

**Results** The isolates, belonged to Basidiomycetes, *Fomes fomentraius* TMF2, *Schizophyllum commune* TMF3, and *Bjerkandera adusta* TMF1, could synthesize extracellular laccase and various hydrolase while growing on lignocellulosic waste materials. More specifically, for the first time, *F. fomentarius* TMF2 synthesized laccase using sunflower meal as a substrate. This substrate could stimulate *B. adusta* TMF1 for Carboxymethyl cellulase and Avicelase production. The strain TMF1 was able to produce amylase during its growth on brewer's spent grain, which is up to now the best result reported for this activity of any *B. adusta* strain. Soybean meal was the most potent substrate for stimulating pectinase production by TMF1 and *S. commune* TMF3. While growing on brewer's spent grain, TMF1 and TMF3 strains produced high levels of xylanase. Spent coffee residues were for the first time tested as a substrate for hydrolase production by selected fungal species.

**Conclusion** The obtained results showed that newly isolates of white-rot fungi can grow on unexploited lignocellulosic waste materials to produce different enzymes as a value-added products suitable for various biotechnological applications.


# Statement Of Novelty

This research was undertaken to identify a novel white-rot fungal strains, with a very potent enzymatic cocktail suitable for various biotechnological applications. Keeping in mind that white-rot fungal enzymes are widely used in many industries, especially for lignocellulose degradation and biofuel production, our main goal was to reveal an advanced wood decay fungi enabled to produce an enzyme mixture, including laccase, cellulase – Carboxymethyl cellulase, Avicelase, pectinase, xylanase, amylase. For this purpose, an unexploited and easy available waste substrates, some of them still unexplored for high enzyme titer production, without harmful effects on the environment were used. The produced enzyme cocktails could have a great impact on future lignocellulose hydrolysis, especially in the biorefinery approach.

# Introduction

Fungal diversity in the natural environment is enormous. They are widely distributed, while Basidiomycetes, a phylum within the fungal kingdom, have an essential role in ecosystem balance. They

are widely distributed in almost all ecosystems, while Basidiomycetes, a phylum within the fungal kingdom, have an essential role in wood-decay.

Basidiomycetes are the major decomposers of plant biomass and are also called wood-decaying fungi. These fungi can utilize different natural carbon sources because of their various plant-polysaccharide-degrading capabilities [1, 2]. Particularly, basidiomycetes from the white-rot fungi (WRF) group are among the most efficient extracellular hydrolytic and oxidative enzyme producers [3, 4]. Their hydrolytic system mainly consists of cellulases and xylanases, while the oxidative-ligninolytic system is composed of laccases, ligninases and peroxidase, which degrade lignin and phenyl components. This is of fundamental importance for the hydrolysis of the recalcitrant lignocellulosic biomass to simple sugars and further biotechnological applications [5, 6].

Lignocellulose, which consists of cellulose, hemicellulose and lignin, is the major renewable source of organic matter in the world. There are different agricultural types of waste with predominant lignocellulose components including brewer's spent grain (BSG), the main by-product of the brewing industry. BSG contains mostly arabinoxylan, lignin (12-21%) and cellulose [7, 8], while starch content varies, depending on the mashing efficiency [9]. Sunflower meal (SFM) has a high content of raw fiber, lignin (9-12%), phenols and proteins [10]. Soybean meal (SBM) is rich in proteins, pectin and lignin (1-4%) (Liu & Li, 2017), while spent coffee residues (SCR) have a high cellulose content (8.6 %), but very low levels of the pectin (only 0.01%) and lignin (0.05%) [11]. Although some of the mentioned waste materials can be used as raw materials or find direct application, such as SBM, which is used as feed, when accumulated in large quantities, agricultural wastes cause environmental and pollution problems [12]. To overcome these problems, following the principles of the circular economy, the effective exploitation of these waste materials, by their reuse for the production of biotechnologically added value products is highly desirable.

The utilization of lignocellulose waste materials is often based on WRF, which, owing to their unique and potent enzymatic system gained great interest for application in biotechnology, in various industrial processes, especially in bio-refinery for biofuels production. It is well known that many fungi and bacteria are capable of lignocellulosic biomass degradation in a different manner and extent. Also, many various commercial enzymatic preparations for this purpose already exist on the market. Nevertheless, there is a constant need for screening the new strains, particularly fungi, that are suitable for developing the more efficient enzyme cocktails for complete and enhanced lignocellulose hydrolysis [13].

Thus, the aim of this study was to explore the enzymatic potential of tree newly isolated white-rot fungi during the growth on four different and abundant agricultural waste materials and to evaluate the most suitable substrate for the production of different classes of enzymes.

# Experimental Section (Or Materials And Methods)

### Chemicals and sample materials

Carboxymethyl cellulose (CMC), pectin and xylan, were purchased from Sigma-Aldrich, while soluble starch and microcrystalline cellulose (Avicel) were purchased from Merck. The malt extract broth was purchased from Torlak, Serbia. Guaiacol was purchased from Fischer Scientific Acro. Other chemicals were the highest commercial grades purchased from Merck and Lach-Ner. SFM and SBM were kindly donated from a local factory Bioprotein, Serbia, while BSG was donated by the local brewery from Serbia. The SCR were collected from the local café.

### Isolation of fungi

White-rot fungi were collected from the deciduous forest of mountain Avala, Serbia, from a beech (*Fagus sylvatica*) tree. Fruiting bodies were sterilized in 70% ethanol and inner parts were cut into pieces (3-5mm) and transferred into fresh malt extract agar plates (malt extract 17g/l and agar 15 g/l). Incubation was carried out at 30 °C in a dark. After a few days, mycelium growth has occurred. Small pieces (1x1cm) of mycelium were cut from the Petri dish (malt extract agar) and transferred into a fresh malt extract agar plate until the pure culture was obtained. The pure mycelium culture was stored at + 4 °C on malt extract agar plates.

### Molecular identification of fungal isolates

DNA extraction and PCR amplification were carried out according to the procedure described by Jović and coworkers with some modifications [14]. For PCR amplification of the internal transcribed spacer (ITS) region of the ribosomal RNA coding DNA, a primer set ITS1/ITS4 was used. The extracted total DNA served as a template. The 50-μL reaction mixture contained: 1 μL of DNA template, 1 μL10 μmol/L forward primer (ITS1: 5’-TCCGTAGGTGAACCTGCGG-3’), 1 μL 10 μmol/L reverse primer (ITS4: 5’-TCCTCCGCTTATTGATATGC-3’), 25 μL 2× PCR LongNova-RED Master Mix(DNA GDAŃSK, Blirt S.A., Gdańsk, Poland) and 22 μL ultrapure distilled water. The PCR amplification was performed in QB24 Thermal Cycler (Quanta Biotech Ltd., Byfleet, UK). The temperature parameters were set as follows: 15 min at 95 °C forinitial denaturation, 30 cycles of denaturation step (30 s at 95°C), annealing step (30 s at 55 °C) and elongation step (90 s at72 °C), and 5 min at 72 °C for final extension [14].

Amplified PCR products were double side sequenced by Macrogen, quality checked and aligned. The resulting sequences representing the partial sequence of ITS region of approximately 500bp in length were deposited in the NCBI Gen-Bank database.

### API ZYM test for determination of enzymatic extracellular activities

For the API ZYM test, the fungal mycelia were grown on malt extract agar plates. After 4 days of incubation, a mycelium from agar plates was used to prepare a suspension in a saline solution. Biochemical tests were performed using the API ZYM kit (bioMérieux, USA) according to the manufacturer’s instructions.

The incubation of the strips was done at 30 °C. The color readings were performed using the API ZYM color scale, supplied by the manufacturer ranging from 0 (negative)–5(maximum).

### Inoculum preparation

The inoculum was prepared by growing the mycelia on malt extract agar plates. Mycelia discs (2x2cm) were cut out of the mycelia growing on malt extract agar and aseptically transferred into the center of different agar plate which contained selective substrate for testing enzymatic activity. For the inoculation of SFM, SBM, BSG and SCR, four mycelia discs (1x1cm) were cut from the same Petri dish with malt extract agar and transferred into Erlenmeyer flasks that contained a wetted substrate.

### Qualitative test for detection of enzyme activity

Selective agar medium plates (malt extract 17g/l and agar 15 g/l) supplemented with selective substrates (1.0 g/l) were used for the detection of enzyme activities. For cellulases activity, CMC–carboxymethylcellulose, and Avicel (microcrystalline cellulose) were added separately, for amylase soluble starch, for pectinase pectin from apple and for xylanase *beechwood* xylan were added.  he plates were incubated at 30 °C in dark for 4-6 days. After incubation, agar plates were flooded with Gram's iodine (2.0 g KI and 1.0 g $I_2$ in 300 ml distilled water) for5 minutes. Clear zones around mycelium indicated enzyme activity [15].

For the determination of laccase production, 0.05% guaiacol was added to malt extract broth with 1.5% agar-agar. The plates were incubated in dark at 30 °C for 2-4 days. The presence of dark/brown color under and/or around mycelia was considered as a positive result for guaiacol oxidation and laccase production.

### Solid-state fermentation and enzymes production

For solid-state fermentation, SFM, SBM, BSG and SCR were used as waste substrates for mycelia growth and enzyme production. The substrates were wetted to obtain final moisture content of 70%, and each substrate was weighed and placed in 300 ml Erlenmeyer flasks. After autoclaving (121 °C, 20 min) and cooling, the flasks were inoculated with mycelium. Incubation was carried out at 30 °C in a dark for 6 days. Extraction of enzymes was performed in a rotary shaker (190 rpm, 30 min, 25 °C) by mixing the fermented media with 50 ml of 0.1 M acetate buffer pH 5.0. After centrifugation (6000 rpm, 15 min, 4 °C), the obtained supernatant was used as a crude enzyme extract and was further analyzed for laccase, amylase, pectinase, xylanase and cellulases (CMCase and Avicelase) activities.

### Quantitative test for enzyme activity

The determination of laccase activity was based on the oxidation of guaiacol. This assay was performed according to the procedure described by Jović et al.[14].

Cellulases (CMCase and Avicelase), amylase, xylanase and pectinase activities were measured by a reduction of 3,5-dinitrosalicylic acid in the presence of reducing sugar released by enzymatic hydrolysis of cellulose, starch, xylose and pectin, respectively, according to the method of Miller [16]. The calculations were performed based on the standard curves prepared using appropriate reducing sugar

(glucose, xylose) and D-galacturonic acid. The assays for hydrolytic enzyme activity determination was performed as described earlier [17].

Enzyme activity (E.A.), IU/g, of solid substrate for each enzyme, was calculated according to the equation:

$$E.A.\ (IU/g) = E.A.\ (U/ml) \times V/m_s \quad (1)$$

where E.A. is enzyme activity (U/ml), V is buffer volume used for enzyme extraction (ml) and $m_s$ is a mass of solid substrate (g).

### Statistical analysis

The results of enzyme activities are mean values of three replicates ± standard errors. The means were compared by analysis of variance (ANOVA). One-way ANOVA followed by Tukey test was applied to evaluate the effect of each substrate for the particular enzyme production by specified WRF. Differences were considered significant at $p<0.05$. Data analysis was performed using OriginPro8.5.

## Results And Discussion

### Identification of isolated white-rot fungi

WRF were preliminary identified based on morphological characteristics. All tested white-rot fungal isolates belonged to the Basidiomycetes phylum. The WRF strains, designed as *Fomes fomentarius* TMF2, *Bjerkandera adusta* TMF1 and *Schizophyllum commune* TMF3 were identified as *Fomes fomentarius*, *Bjerkandera adusta*, and *Schizophyllum commune* based on the internal transcribed spacer region (ITS) sequence, located between the 18S and 5.8S rRNA coding genes. This region is widely used for analyzing fungal diversity in environmental samples [18]. The obtained sequences were deposited in the NCBI-GenBank database under the following accession numbers: MW327505 for *B. adusta*, MW327506 for *S. commune* and MW327504 for *F. fomentarius*.

### Detection of enzyme activity using the API ZYM

The examination of the hydrolytic enzyme activities of all tested isolates was done using the API ZYM test (Table 1). This test is simple, rapid and reliable and is widely used for identifying various hydrolytic enzymes present in novel strains [19].

All tested WRF showed a broad range of extracellular enzyme activities. Beta glucosidase activity was very high for these isolates, indicating that these fungi have a good potential for cellulose degradation [20]. Acid phosphatase was negative for *F.fomentarius* TMF2 and *S. commune* TMF3 but strong for *B. adusta* TMF1*. F.fomentarius* TMF2 and *S. commune* TMF3 produced α- and β-galactosidase, and mannosidase, suggesting potential for enzymatic hydrolysis of different carbohydrates.EsteraseC4, esterase lipase C8, leucinearyl amidase, valinearyl amidase were present at medium levels at *B. adusta* TMF1 while cystinearyl amidase was found at a low level at *F.fomentarius* TMF2 and *B. adusta* TMF1.

Very high levels of lipase C14 were detected in *F. fomentarius* TMF2 and *S. commune* TMF3.

The API ZYM test confirmed that the enzymatic system of the isolated WRF is diverse.

### Detection of enzyme activity using a qualitative test for hydrolases and laccase

*F.fomentarius* TMF2, *B.adusta* TMF1 and *S.commune* TMF3 were able to grow on CMC, Avicel, pectin, starch, xylan and guiacol agar plates. For *F.fomentarius* TMF2 and *S. commune* TMF3, extracellular hydrolytic enzyme activities were observed already after 2 days of mycelia growth.

The occurrence of a halo zone around mycelium indicated the area of the substrate hydrolysis and thus the presence of the specific enzyme (**Fig. 1**). These strains were able to utilize both types of cellulose, CMC as a soluble form of cellulose and Avicel as microcrystalline cellulose, because of their synergistic endoglucanases and exoglucanases systems, which is typical for WRF [21]. *F. fomentarius* TMF2 and *B. adusta* TMF1 mycelia were very dense and within the mycelia growth was the inner clearance zone that indicated amylase, pectinase and xylanase activity. A similar zone of clearance for *Fomes sp*. was reported by Hadda and coworkers [21]. The very dense mycelium of *B. adusta* TMF1 on starch, pectin and xylan substrates made it impossible to identify the zone of clearance (**Fig. 1**), but after removing the mycelium, the amylase, pectinase and xylanase activities could be detected as very clear zone throw the entire growth surface (**Fig. 1**).

Laccase activity was noticed after 24h of mycelia incubation for *F. fomentarius* TMF2 and *B. adusta* TMF1 and after 48 h of incubation for *S. commune* TMF3 using guaiacol as an oxidizing reagent. The brown color under mycelia indicated the presence of laccase (**Fig. 1**).

### Solid-state fermentation (SSF) and enzymes production

In SSF, the most important step is the selection of appropriate lignocellulosic substrate for WRF growth and enzyme synthesis [3]. Literature data showed that various lignocellulosic waste substrates, such as sugarcane bagasse, wheat straw, wheat bran, or corn stover could be applied for the enzyme production by WRF [12]. Among them, wheat bran is the most used substrate for WRF enzyme induction. For example, wheat bran was used for cellulase, xylanase and laccase production by *Fomes sp*. *[22, 23],* and for cellulase production by *S. commune* [24], while *wheat straw was used for cellulase synthesis by B. adusta* [25].

The literature data about the simultaneous production of laccase and hydrolytic enzymes on BSG, SCR, SFM and SBM by WRF is still very scarce. In this study, the production of cellulases (CMCase and Avicelase), amylase, pectinase, xylanase and laccase by three tested isolates on these lignocellulosic waste substrates was evaluated. The results showed that all used substrates could support WRF growth and enzyme synthesis. However, some differences were observed in terms of the suitability of the substrates to stimulate the synthesis of specific enzymes by each WRF tested (Tables 2-4).

For *F. fomentarius* TMF2, SFM seemed to be the most potent lignocellulosic waste substrate for induction of both laccase and hydrolases production (Table 2). This substrate is usually used for microbial amylase production by genus *Bacillus* [26], but it was also used as a substrate for the simultaneous production of various hydrolases (cellulase, amylase, pectinase and xylanase) by actinomycetes *Streptomyces fulvisimuss* CKS7, isolated in our group [17]. Thermophilic fungus *Humicola lanuginose* was used for cellulase production during SSF of SFM [27]. It has not been shown until this study, that a strain of *F.fomentarius* could grow on sunflower meal and synthesize extracellular laccase. Compared with *Trametes versicolor* laccase, produced in submerged fermentation using 2% (w/v) sunflower stems [28], higher laccase activity was reported in our study (35 IU/L vs. 2.45 IU/g). Slightly lower *F. fomentarius* TMF2 laccase activity was obtained on BSG (2.28 IU/g), while activities on SBM and SCR were significantly lower.

*F. fomentarius* TMF2 showed also the highest amylase activity (27.29 IU/g) on SFM, compared to other tested substrates. Pectinase and xylanase produced by *F. fomentarius* TMF2 on SFM reached activity of 10.86 IU/g and 16.84 IU/g, respectively, and these values were also the highest among all tested substrates. However, these values were not significantly higher than the pectinase activity on SBM (9.34 IU/g) and xylanase activity obtained on BSG (15.88 IU/g) suggesting these three substrates were suitable for pectinase and xylanase production by *F. fomentarius* TMF2.

Spent coffee waste, as lignocellulosic material, induced a very low enzyme activity, especially for laccase and pectinase (0.21 IU/g and 1.02 IU/g, respectively). However, SCR showed to be a suitable substrate for CMCase production by *F. fomentarius* TMF2 with 1.32 IU/g, which was not significantly lower than values obtained on SFM and BSG (1.42 IU/g and 1.49 IU/g, respectively). Keeping in mind its composition, these results were expected.

Literature data showed that coffee pulp, coffee husk and spent coffee grounds were mainly used as substrates for WRF growth [8, 29, 30]. The different chemical composition of these coffee by-products affects the fungal growth and the absorption of nutrients necessary for enzyme synthesis [11]. The most studied WRF, with the potential to grow on different coffee waste substrates, belong to genus *Pleurotus* [29, 30]. However, there is a lack of literature data about Basidiomycetes growth on coffee waste substrates.

*B. adusta* TMF1 and *S. commune* TMF3 showed a similar pattern of response to substrates used for stimulation of different enzyme synthesis (Tables 3 and 4). The laccase activities of 1.44 IU/g and 2.79 IU/g produced by *B. adusta* TMF1 and *S. commune* TMF3, respectively, obtained on BSG as a substrate were significantly higher than laccase activities on other waste substrates. The literature data generally reports lower laccase activities produced by these WRF species. For example, testing the ligninolytic potential of *B. adusta* under different growth conditions, Tripathi and coworkers [31] found laccase activity of 64 U/L only in nutrient-rich medium, after 20 days of growth under static conditions. These authors suggested that laccase activity was inducible in this WRF. There are studies that reported no laccase activity in *B. adusta*. [32, 33].

Pariatamby and Nithiya reported laccase activity ~ 1.0 IU/g for *S. commune* after 7 days growth on BSG [34]. In the study of Zhu and coworkers no laccase activity was detected for *S. commune* during 30 days SSF on Jerusalim artichoke stalk [13]. However, literature data also reported higher values of laccase activity under optimized conditions [35].

The most suitable substrate for amylase production by *B. adusta* TMF1 and *S. commune* TMF3 was BSG with measured activities of 11.47 IU/g and 10.98 IU/g, respectively. Moderate amylase activities were obtained on SFM and SBM, while SCR was the least appropriate substrate for inducing amylase production. It is interesting to note that the enzymatic potential of *B. adusta* was mainly associated with the decomposition of lignocellulose biomass, while in this study we showed, for the first time, that amylases produced by this species could degrade starch to simple sugars. Shimazaki and coworkers [36] reported amylase activities after 9 days of cultivation *S. commune* under shaking conditions from 0.69 IU/mL to 30.0 IU/mL, depending on the carbon source. The highest activity was obtained with 5% wheat bran as a carbon source and it was higher than the highest activity obtained in our study (30.0 IU/mL vs. 10.98 IU/g). Starch content in wheat bran varies from 9-26% [37], and this is higher content than present in all different substrates used in our study. Therefore, even higher amylase activities produced by *S. commune* TMF3 could be expected after cultivation on a richer substrate.

The most suitable substrate for pectinase production by *B. adusta* TMF1 and *S. commune* TMF3 was SBM (Tables 3 and 4). This could be expected given that the pectin content in SBM is higher than in the other tested substrates [38, 39].

Ganbarow and coworkers [40] studied pectinase production of five fungi belonging to the genus *Bjerkandera* on wheat bran during SSF. Two of these five fungi (*B. adusta* 41 and *B. fumosa* 97) had a lower pectinase activity, then pectinase activity obtained in our study (22.1 IU/g and 10.3 vs. 23.73 IU/g), while higher pectinase activities were reported at *B. adusta* 1 (25.4 IU/g), *B. adusta* 40 (54.1 IU /g) and *B. fumosa* 22 (44.7 IU/g) strains.

Within this study, 9.25 IU/g for pectinase activity of *S. commune* TMF3 was noted on the SBM. Mehmood et al. [41] reported a higher value of produced pectinase (480.45 IU/g) than in our study, by growing *S. commune* on optimized solid medium citrus waste - mosambi peels (sweet limetta). Interestingly, during SSF on Jerusalim artichoke stalk, pectinase activity for *S. commune* was not detectable [13]. It is important to note that enzyme activity in our study was determined only after 6 days of growth and no optimization has been performed, and that could contribute to their latively lower observed pectinase activity.

The highest levels of xylanase activities of 18.39 IU/g and 17.47 IU/g produced by *B. adusta* TMF1 and *S. commune* TMF3 respectively, were measured on BSG as substrate (Tables 3 and 4). A high level of xylanase activity by *S. commune* was also reported in the work of Gautam and coworkers [42]. Another study showed that *S. commune* could produce xylanase in submerged fermentation, while growing on bamboo, sugarcane bagasse and banana stem, for 15 days. The obtained xylanase activity was very low on bamboo and banana stem, while on sugarcane bagasse this activity reached the value of ~1.2 IU/mL.

A literature survey showed that studies about *Bjerkandera sp*. xylanase production on waste substrates are extremely scarce. Only Qirouz-Castaneda and coworkers [25] reported xylanase production by this WRF on several waste substrates with maximum xylanase specific activity of 0.4 IU/mg of proteins obtained using oak dust for *B. adusta* growth.

*S. commune* TMF3 and *B. adusta* TMF1 produced a maximum of cellulase activity (2.51 IU/g and 3.71 IU/g respectively) during the growth on SFM. Literature data about cellulase production by *S. commune* during SSF are very rare with exception of the work of Zhu and coworkers [13]. In their study, *S. commune* was grown on a Jerusalem artichoke stalk (mixed with Mandels' salt solution) during 30 days and produced cellulases. Maximum of endoglucanase activity 15.2 IU/g was reached after 20 days of incubation. In the study of Qirouz-Castaneda [22] *B. adusta* was grown on a solid agar plate with 2% wheat straw and produced maximum CMCase activity of 2.4 IU/mg proteins. It is important to note that all of these results are difficult to compare due to the different methods for fungal growth and due to the different reported expression of cellulase activities.

# Conclusions

In this study, the enzymatic potential of three newly isolated white-rot fungi strains was examined during the growth on different agricultural waste substrates. According to obtained results, sunflower meal, soybean meal, spent coffee residues, and brewer's spent grain were found to be potent substrates for the production of industrially important enzymes by tested fungal isolates. However, their potency varied in specific enzymes induction, mainly caused by substrates' composition, but was also strain dependent.

The newly isolated *F. fomentarius* TMF2 was able to grow and produce various hydrolase and laccase with maximum activities obtained on sunflower meal. The brewer's spent grain was a very potent substrate for amylase and pectinase that were produced by *B. adusta* TMF1 and *S. commune* TMF3, while on soybean meal maximum of pectinase activity for *B. adus*ta TMF1 was reported. The spent coffee residues were the least potent substrate for all tested white-rot fungi isolates.

The obtained results showed that three novel strains of white-rot fungi have a great enzymatic potential that could be used for the conversion of low-cost waste lignocellulosic biomass to valuable biotechnological products.

# Declarations

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## Conflicts of interest

No conflict of interest to declare.

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# Tables

Table 1. The API ZYM test for white-rot fungal isolates *F. fomentarius* TMF2, *S. commune* TMF3 and *B. adusta* TMF1

| Enzymes (API ZYM) | *F. fomentarius* TMF2 | *S. commune* TMF3 | *B. adusta* TMF1 |
|---|---|---|---|
| Control | 0 | 0 | 0 |
| alkaline phosphatase | 2 | 2 | 3 |
| acid phosphatase | 0 | 0 | 4 |
| esterase C4 | 0 | 1 | 3 |
| esterase lipase C8 | 0 | 3 | 3 |
| leucine arylamidase | 3 | 0 | 4 |
| valinearylamidase | 0 | 4 | 4 |
| cystinearylamidase | 0 | 4 | 1 |
| naphthol-AS-BIphosphohydrolase | 2 | 2 | 2 |
| N-acetyl-*β*-glucosaminidase | 0 | 2 | 3 |
| lipase C14 | 5 | 5 | 0 |
| trypsin | 3 | 2 | 4 |
| chymotrypsin | 5 | 0 | 1 |
| *β*-galactosidase | 3 | 3 | 2 |
| *β*-glucuronidase | 2 | 2 | 1 |
| *α*-glucosidase | 2 | 1 | 2 |
| *β*-glucosidase | 5 | 4 | 4 |
| *α*-galactosidase | 4 | 4 | 0 |
| *α*-mannosidase | 3 | 4 | 1 |
| *α*-fucosidase | 3 | 0 | 0 |

Table 2. Enzymes production (IU/g) on waste substrates (SSF, 6 days, 30 °C) by *F.fomentarius* TMF2

| substrate | Laccase | Amylase | Pectinase | Xylanase | CMCase | Avicelase |
|---|---|---|---|---|---|---|
| | (IU/g) | (IU/g) | (IU/g) | (IU/g) | (IU/g) | (IU/g) |
| SFM | 2.45±0.04[a] | 27.29±0.42[a] | 10.86±0.62[a] | 16.84±0.45[a] | 1.49±0.10[a] | 1.02±0.07[a] |
| BSG | 2.28±0.06[a] | 11.11±0.18[b] | 1.02±0.08[b] | 15.88±0.24[a] | 1.42±0.11[a,c] | 1.43±0.08[b] |
| SBM | 0.91±0.08[b] | 10.08±0.18[b] | 9.34±0.60[a] | 5.70±0.11[b] | 1.00±0.04[b,c] | 0.98±0.05[a] |
| SCR | 0.21±0.02[c] | 0.55±0.03[c] | 1.63±0.04[b] | 0.89±0.04[c] | 1.32±0.12[a,c] | 0.66±0.03[c] |

[a,b,c]Symbols having the same letters are not significantly different from each other (Tukey test, $p < 0.05$)

Table 3. Enzymes production (IU/g) on waste substrates (SSF, 6 days, 30 °C) by *B.adusta* TMF1

| substrate | Laccase | Amylase | Pectinase | Xylanase | CMCase | Avicelase |
|---|---|---|---|---|---|---|
| | (IU/g) | (IU/g) | (IU/g) | (IU/g) | (IU/g) | (IU/g) |
| SFM | 1.13±0.05[a] | 7.25±0.16[a] | 10.61±0.07[a] | 12.88±0.17[a] | 3.71±0.18[a] | 1.06±0.11[b,c] |
| BSG | 1.44±0.04[b] | 11.47±0.19[b] | 4.96±0.15[b] | 18.39±0.13[b] | 2.76±0.16[b] | 1.26±0.19[b] |
| SBM | 0.47±0.02[c] | 5.35±0.05[c] | 23.73±0.27[c] | 5.06±0.13[c] | 0.44±0.04[c] | 0.18±0.04[a] |
| SCR | 0.24±0.03[d] | 1.19±0.06[d] | 1.57±0.04[d] | 1.21±0.11[d] | 0.95±0.06[c] | 0.53±0.07[a,c] |

[a,b,c,d]Symbols having the same letters are not significantly different from each other (Tukey test, $p < 0.05$)

Table 4. Enzymes production (IU/g) on waste substrates (SSF, 6 days, 30 °C) by *S. commune* TMF3

| substrate | Laccase | Amylase | Pectinase | Xylanase | CMCase | Avicelase |
|---|---|---|---|---|---|---|
| | (IU/g) | (IU/g) | (IU/g) | (IU/g) | (IU/g) | (IU/g) |
| SFM | 1.22±0.04[a] | 6.88±0.07[a] | 5.22±0.11[a] | 13.21±0.18[a] | 2.51±0.13[a] | 1.60±0.09[a] |
| BSG | 2.79±0.07[b] | 10.98±0.19[b] | 4.23±0.06[b] | 17.47±0.09[b] | 1.21±0.11[b] | 0.55±0.04[b] |
| SBM | 0.52±0.04[c] | 4.73±0.10[c] | 9.25±0.14[c] | 1.86±0.22[c] | 0.68±0.10[c] | 0.56±0.04[b] |
| SCR | 0.38±0.04[c] | 1.72±0.05[d] | 1.90±0.08[d] | 6.23±0.29[d] | 0.78±0.09[b,c] | 0.89±0.12[b] |

[a,b,c,d] Symbols having the same letters are not significantly different from each other (Tukey test, $p < 0$)

# Figures


**Figure 1**

The occurrence of a halo zone around mycelium indicated the area of the substrate hydrolysis and thus the presence of the specific enzyme.

## Supplementary Files

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