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Review

Review of Recent Advances in the Physiology of the Regulation of Cellulase and Xylanase Production by Basidiomycetes

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Abstract: The potential of wood-rotting and litter-deconstructing basidiomycetes to convert lignocellulose into a wide variety of products has been extensively studied. In particular, wood-rotting basidiomycete secretomes are attracting much attention from researchers and biotechnology companies due to their ability to produce extracellular hydrolytic and oxidative enzymes that effectively degrade cellulose, hemicellulose, and lignin of plant biomass. An analysis of the available literature data shows that Basidiomycota fungi, which are most adapted to the depolymerization of plant polysaccharides, are promising but so far unexploited sources of new hydrolytic enzymes. The review summarizes the latest data on the great variety, common features, and unique properties of individual fungi and the production of cellulases and xylanases by various physiological and ecological groups of basidiomycetes. The most important microbial cellulase-producing strains for submerged and solid-phase fermentation, as well as the main substrates, including the use of agro-industrial waste, are considered. It highlights ways to increase both cellulase and xylanase expression levels and the cost-effectiveness of producing these enzymes for various biotechnological applications. It is anticipated that this review will be particularly useful to novice scientists working in the lignocellulose biorefinery, as it describes current knowledge and issues related to the production and regulation of polysaccharide hydrolyzing enzyme synthesis.

Keywords: basidiomycetes; lignocellulose; fermentation; cellulase; xylanase; production; cultivation conditions

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1. Introduction

Polysaccharides of lignocellulosic biomass are cheap, abundant, and renewable resources used for their microbial or enzymatic conversion in biofuels and a wide range of chemicals. Polysaccharide-hydrolyzing enzymes (PHEs) are produced by a wide range of saprophytic microbes that grow on dead and decaying plant materials. Complete hydrolysis of cellulose to glucose monomers provides a synergistic action of endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) [1,2]. Enzymatic hydrolysis of hemicelluloses, consisting mainly of xylan, galactoglucomannan, and xyloglucan, occurs under the action of a wide range of enzymes: endo-1,4-β-xylanase, β-xylosidase, α-l-arabinofuranosidase, α-D-glucuronidase, acetyl xylan esterase, feruloyl and coumaroyl esterase, endo-1,4-β-mannanase, β-mannosidase, α-galactosidase, acetyl mannan esterase, and xyloglucanase [3,4]. Biochemical characterization of cellulases and hemicellulases is presented in several comprehensive reviews [1,3,5].

The demand for the application of PHEs in biofuel production as well as in food, textile, brewery, wine, pulp and paper, and laundry industries and agriculture is growing [6,7]. The bottleneck in the widespread commercialization of ethanol 2G is thought to be the production cost of the PHEs. Low-cost enzymes with high catalytic activity, stability at elevated temperatures and a certain pH, and high tolerances to end-product inhibition

are highly desired for use in these applications. Therefore, the identification of new overproducers of PHEs with complete cellulase and hemicellulases systems, the elucidation of the physiological features of the regulation of these enzyme syntheses, and, on this basis, the development of new approaches to bioprocessing for the maximum expression of the enzymatic activity of microorganisms remain a paramount task. Currently, filamentous fungi belonging to the division *Ascomycota*, such as *Trichoderma*, *Aspergillus*, and *Penicillium*, are sources of commercial PHEs. Analysis of the existing literature data shows that several species of *Basidiomycota* fungi are promising but still unexploited as producers of novel and potent PHEs. Indeed, wood-rotting fungi are one of the best decomposers of lignocellulosic materials owing to their ability to secrete a variety of hydrolytic and oxidative enzymes suited for the depolymerization of cellulose, hemicelluloses, and lignin, and provide fungal mycelium with energy and nutrients. However, the enzymes of basidiomycetes involved in the degradation of polysaccharides have received less attention than those of ascomycetes. In particular, few researchers have focused on systematic studies of the environmental factors that modulate the expression of PHEs during submerged and solid-state fermentation (SSF) of plant materials.

The main purpose of this review was to summarize the latest literature data and results of authors' work on the physiology of cellulases and xylanase synthesis by the Basidiomycota species since the literature on genomic studies and approaches has been comprehensively discussed [5,8–10]. The review focuses on the diversity and common features of wood-rotting and litter-decomposing basidiomycetes producing PHEs as well as on strategies and approaches to increase their yield and reduce the cost. We anticipate that this review will be especially useful for aspiring academic and industrial researchers working in lignocellulose biorefinery, as it describes current knowledge and issues related to the production and regulation of synthesis of individual fungal cellulases and xylanases or enzyme cocktails with high yields.

2. Diversity and Distinctions of Basidiomycetes Producing PHEs

Basidiomycota is one of two large divisions that, together with the Ascomycota, constitute the subkingdom Dikarya within the kingdom Fungi. Depending on the mode of feeding, Basidiomycota can be divided into saprobic, symbiotic, and parasitic groups. Saprobic fungi decomposing wood and leaf litter have received much attention for applications in biocatalysis and biorefinery. In nature, Basidiomycota species inhabit diverse ecological niches, and colonize and use a wide range of lignocellulosic materials to grow, including dead and living trees, forest litter, grasses, plant debris in the soil, and plant roots. More than 90% of wood-destroying basidiomycetes are white rot fungi [11], which are capable of destroying all polymers in plant cell walls due to the presence of a hydrolytic enzymatic system for the degradation of polysaccharides and an oxidative enzymatic system for the deconstruction of lignin. Unlike the white-rot basidiomycetes (WRBs), brown rot fungi (BRBs) use PHEs and highly reactive oxidizing agents to depolymerize plant polysaccharides [8,12]. Although a very limited amount of BRB has been evaluated for cellulase production to date, it has been found that these fungi produce endoglucanase but rarely secrete exoglucanase activity. Only some of them, in particular, *Glaeophyllum trabeum* [13] and *Fomitopsis palustris* [14], are able to decompose crystalline cellulose. Interestingly, *G. trabeum* produces processive endoglucanase, which hydrolyzes microcrystalline cellulose, compensating for the absence of cellobiohydrolase during cellulose degradation.

The production of cellulases and xylanase is reported for a dozen genera and hundreds of species and strains belonging to different taxonomic groups and isolated from a wide variety of ecological niches. It is rather difficult to compare the activity of PHEs obtained from different fungal cultures due to differences in the composition of the medium, especially due to the content of different lignocellulosic growth substrates, and sometimes due to different methods for determining enzyme activity. Moreover, some authors have expressed enzymatic activity in SSF experiments in U/g biomass, others in U/mL. Nevertheless, the selected results presented in Tables 1 and 2 make it possible to draw several important

conclusions. First, these results clearly show both interspecific and intraspecific differences in the ability and potential of basidiomycetes to express cellulase and xylanase activities. For example, in the submerged fermentation of cellulosic materials, basidiomycetes CMCase activity varied from 0.2 U/mL in cultures of *Coniophora puteana* [15] and *Pycnoporus sanguineus* [16] to 122 U/mL in *Pseudotrametes gibbosa* [17]. Moreover, in the SSF of wheat straw, different strains of *Pleurotus ostreatus* expressed CMCase and xylanase activities from 0.7 U/g [18] to 166.9 U/g and 47.6 U/g [19], respectively. On the contrary, no differences in CMCase activity were found when plant substrates were fermented with strains of *Pleurotus eryngii* [20] (Table 2). This leads to the second conclusion that, along with the geographical and ecological origin of the fungal strain, the growth substrate plays a critical role in maximizing its biosynthetic potential, as will be discussed in the next section. It is clear that for a correct assessment and comparison of the cellulase activity of fungi belonging to different taxonomic and physiological groups, it is necessary to cultivate them using the same substrate and fermentation method. During screening programs, we usually use mandarin peels or mandarin pomace obtained after the extraction of juice. These materials are appropriate growth substrates containing basic compounds required for the rapid initial growth and development of microbial culture and production of PHEs by basidiomycetes belonging to different taxonomic and ecological groups. Besides cellulose (21%), hemicellulose (13%), and nitrogen (1.2%), mandarin peels contain free sugars and organic acids providing an abundant growth of all tested fungi under submerged fermentation conditions, but the enzymatic activity of the individual strains differed greatly [17]. Endoglucanase and xylanase activities of basidiomycetes varied from 2 to 122 U/mL and from 3 to 63 U/mL, respectively. *Pseudotrametes gibbosa* IBB 122 followed by *Trametes pubescens* IBB 11 secreted especially high CMCase and xylanase activities (Table 1), providing fungal cultures with nutrients essential for their biosynthetic activity.

Table 1. Cellulase and xylanase activity of individual basidiomycetes under the submerged fermentation of lignocellulosic materials.

Fungi	Growth Substrate	Enzyme Activity (U/mL) *			References
		CMCase	Xylanase	FPA	
Agaricus arvensis	Rice straw	0.3	ND	0.5	[21]
Agaricus brunnescens	Crop straw	19.9	ND	ND **	[22]
Bjerkandera adusta	Corn stover	8.8	25.5	ND	[23]
Coniophora puteana	Wheat bran	0.2	1.2	0.02	[15]
Coprinus comatus	Crop straw	29.4	ND	ND	[21]
Cotylidia pannosa	Wheat bran	20.0	17.0	ND	[24]
Dichomitus squalens	Avicel	3.2	6.5	ND	[12]
Fomes fomentarius IBB 9	Mandarin peels	4.0	7.0	ND	[17]
F. fomentarius IBB 38	Mandarin peels	52.0	78.0	ND	[17]
	Vinasse	88.0	195.0	ND	
	Apple pomace	4.0	7.0	ND	
	Grape pomace	5.0	4.0	ND	
	Maple leaves	19.0	26.0	ND	
Ganoderma applanatum	Cellulose	3.3	8.2	ND	[25]
Irpex lacteus	Avicel	53.7	66.8	5.1	[26]
	Mandarin pomace (MP)	18.1	20.3	1.9	
	Wheat straw	23.0	29.1	2.3	
	Avicel + MP	76.2	106.2	6.8	
	Wheat straw + MP	40.3	34.2	2.5	
Inonotus obliquus	Rice straw	1.4	307.0	0.4	[27]
	Wheat straw	1.3	283.0	0.5	
	Corn stover	1.2	288.0	0.3	

Table 1. *Cont.*

Fungi	Growth Substrate	Enzyme Activity (U/mL) *			References
		CMCase	Xylanase	FPA	
Peniophora sp.	Rice straw 0.2%	6.0	ND	ND	[28]
	Rice straw 0.6%	17.4	ND	ND	
	Potato infusion 10%	24.0	ND	ND	
	Potato infusion 80%	100.0	ND	ND	
Pholiota adiposa	Rice straw	26.0	ND	1.2	[29]
Pleurotus ostreatus	Crop straw	28.2	ND	ND	[22]
Pleurotus ostreatus	Cottonseeds	1.7	1.6	ND	[30]
Postia placenta	Wheat bran	0.4	1.0	0.1	[15]
Pseudotrametes gibbosa	Mandarin peels	120.0	37.0	ND	[17]
	Vinasse	112.0	48.0	ND	
	Apple pomace	39.0	32.0	ND	
Pycnoporus coccineus	Avicel	63.0	31.0	4.6	[26]
	Mandarin pomace (MP)	19.0	14.0	2.2	
	Wheat straw	22.0	18.0	1.9	
	Avicel + MP	82.0	65.0	5.7	
	Wheat straw + MP	27.0	19.0	2.2	
Pycnoporus sanguineus	Orange peels	0.2	0.1	ND	[16]
Pycnoporus sanguineus	Palm oil mill effluent and oil palm frond	18.2	ND	13.4	[31]
Schizophyllum commune	Rice straw	44.4	1.1	0.6	[32]
S. commune	Avicel	39.0	626.0	2.1	[26]
	Mandarin pomace (MP)	23.0	531.0	3.0	
	Wheat straw	8.0	120.0	1.3	
	Avicel + MP	39.0	740.0	4.2	
	Wheat straw + MP	14.0	528.0	2.2	
Sistotrema brinkmannii	Rice straw	9.8	ND	0.3	[21]
Sporotrichum pulverulentum	Corn stover	6.6	33.5	ND	[23]
Trametes hirsuta	Avicel	34.3	48.8	2.5	[33]
	Avicel + mandarin peels	64.6	73.0	5.3	
T. hirsuta	Orange peel	0.5	45.0	ND	[34]
Trametes pubescens	Mandarin peels	74.0	32.0	ND	[17]
T. pubescens	Wheat bran	1.6	4.8	ND	[35]
Trametes spp.	Avicel	0.4	ND	ND	[36]
T. trogii	Corn stover	6.5	45.4	ND	[23]
T. versicolor	Carboxymethyl cellulose	1.6	4.5	ND	[37]
Tricholoma giganteum	Pithecellobium Dulce wood	ND	338.0	0.3	[38]
	Tamarindus Indica wood	ND	375.0	0.3	

* In Tables and Figures one unit of enzyme activity is defined as the amount of enzyme, releasing 1 µmol of reducing sugars per minute. ** ND - not determined.

Thirdly, not all WRBs and BRBs are efficient producers of cellulase or xylanase, only a few of them can accumulate significant enzymatic activity, and some Basidiomycota strains have shown exceptional potential for the production of certain groups of hydrolytic enzymes under appropriate cultivation conditions. Thus, *Coprinellus disseminatus* produced 469 U/mL of alkali-thermotolerant xylanase along with negligible cellulase activity [39] while *Armillaria gemina* secreted up to 146 U endoglucanase/mL, 15 U β-glucosidase/mL, and 1.72 U FPA/mL [40]. Further, Jagtap et al. [29] achieved very high β-glucosidase activity (45.2 U/mL) in the submerged cultivation of *Pholiota adiposa* in a medium containing rice straw and corn steep powder. During the extensive screening of wood and litter-deconstructing basidiomycetes for lignocellulolytic enzyme production, *Fomes fomentarius*, *Panus lecometei*, *Pseudotrametes gibbosa*, *Trametes versicolor* [17,41], *Irpex lacteus*, and *Schizophyllum commune* [33] were revealed as especially promising cellulases and xylanases producers in the submerged fermentation of cellulose or plant raw materials (Table 1). Among these fungi, *S. commune* appeared to be an outstanding producer of xylanase and β-glucosidase, producing 740 U/mL and 18.6 U/mL, respectively [26]. Interestingly, un-

der SSF conditions, as high as 512 U/g endoglucanase activity was achieved utilizing poplar wood as the growth substrate for *Trametes trogii* [42], and 10196 U/g xylanase was achieved under SSF conditions using rice straw as the growth substrate for *Schizophyllum commune* [43]. In contrast to WRBs, a very limited number of BRBs strains have been evaluated for cellulase and xylanase activities, although their ability to produce these enzymes has been well documented and some of them may be excellent producers of PHEs. For example, *Fomitopsis* sp. secreted 75 U/g of CMCase and 4.2 U/g FPA during SSF of soybean meal [44], while *Piptoporus betulinus* accumulated 58.4 U/g CMCase and 7.4 U/g FPA during SSF of rice straw [45] (Table 2). Our newly discovered strain *Gloeophyllum abietinum* produced 64.2 U/mL of CMCase and 15.8 U/mL xylanase in submerged fermentation of an ethanol production residue (Table in Section 8).

Table 2. Cellulase and xylanase activities (U/g) of individual basidiomycetes during SSF of lignocellulosic materials.

Fungal Species	Growth Substrate	Enzyme Activity (U/g)			References
		CMCase	Xylanase	FPA	
Bjerkandera adusta	Sunflower meal	12.9	3.7	ND	[46]
	Brewer's spent grain	18.4	2.8	ND	
	Soybean meal	5.1	0.4	ND	
	Spent coffee residue	1.2	1.0	ND	
Calocybe indica	Wheat bran	1.7	0.8	ND	[47]
Coprinus cinereus	Sisal leaf biomass	2.1	1.2	ND	[48]
Coriolus versicolor	Sweet sorghum bagasse	2.2	11.9	6.7	[49]
Coriolus versicolor	Oak sawdust, coconut husks, soybean oil, corn bran, and coffee husks	78.8	124.2	ND	[50]
Fomes fomentarius	Sunflower meal	1.5	16.8	ND	[46]
	Brewer's spent grain	1.4	15.9	ND	
	Soybean meal	1.0	5.7	ND	
	Spent coffee residues	1.3	0.9	ND	
Fomitopsis sp.	Corn cob	2.2	ND	0.2	[44]
	Corn stover	3.7	ND	0.2	
	Wheat straw	2.4	ND	0.2	
	Prosopis juliflora	1.1	ND	0.7	
	Wheat bran	71.5	ND	3.3	
	Soybean meal	75.0	ND	4.2	
Ganoderma lucidum	Sugarcane bagasse and wheat bran	17.6	16.3	4.9	[51]
Gloeophyllum trabeum	Pinus taeda wood chips	0.1	1.2	0.1	[52]
G. trabeum	Jerusalem artichoke stalk	6.3	37.2	ND	[53]
Inonotus obliquus	Wheat bran	27.2	ND	3.2	[54]
Lentinula edodes	Oak sawdust, coconut husks, soybean oil, corn bran, and coffee husks	66.9	107.5	ND	[50]
Lentinula edodes	Wheat straw	0.8	ND	ND	[55]
	Reed grass	1.0	ND	ND	
	Beanstalk	0.9	ND	ND	
Microporus xanthopus	Green tea waste	81.8	ND	ND	[56]
Phanerochaete chrysosporium	Sugarcane bagasse	63.8	220.0	13.1	[57]
	Sugarcane Barbojo	126.3	227.6	19.3	
	Grass powder	188.7	427.0	30.2	
	Corn straw	114.0	32.0	11.2	
	Rice straw	33.6	112.0	5.3	

Table 2. *Cont.*

Fungal Species	Growth Substrate	Enzyme Activity (U/g)			References
		CMCase	Xylanase	FPA	
Phanerochaete chrysosporium	Rice bran	182.2	293.0	11.1	[19]
	Paddy straw	34.3	81.9	16.7	
	Wheat straw	97.8	124.3	13.3	
	Sorghum straw	132.2	302.2	16.6	
	Bajra straw	77.8	113.5	19.3	
	Sorghum hay	95.5	84.7	28.2	
	Maize straw	113.3	118.4	16.7	
Phlebia radiata	Spruce wood	0.1	ND	ND	[58]
Piptoporus betulinus	Rice straw	58.4	ND	7.4	[45]
Pleurotus eryngii 160	Cotton sake	0.2	ND	ND	[20]
	Barley oats straw	0.1	ND	ND	
Pleurotus eryngii 163	Poplar wood sawdust	0.2	ND	ND	[20]
	Barley oats straw	0.2	ND	ND	[20]
Pleurotus eryngii 166	Barley oats straw	0.1	ND	ND	[20]
Pleurotus eryngii 173	Barley oats straw	0.1	ND	ND	[20]
Pleurotus florida	Typha weed	307.0	358.0	ND	[59]
Pleurotus ostreatus	Wheat straw	0.7	0.7	ND	[18]
Pleurotus ostreatus	Sugarcane bagasse and wheat bran	19.9	8.7	6.7	[51]
Pleurotus ostreatus	Oak sawdust, coconut husks, soybean oil, corn bran, and coffee husks	47.3	80.5	ND	[50]
Pleurotus ostreatus	Rice bran	79.5	56.3	30.1	[19]
	Paddy straw	39.4	39.9	19.1	
	Wheat straw	148.5	13.9	19.8	
	Sorghum straw	137.2	47.6	30.7	
	Bajra straw	166.9	7.5	14.8	
	Sorghum hay	140.8	44.5	11.4	
	Maize straw	109.4	29.5	15.8	
Pleurotus ostreatus	Grape pomace	0.1	ND	ND	[60]
Pleurotuspulmonarius	Grape pomace	0.1	ND	ND	[60]
Pleurotus sajor caju,	Rice bran	36.9	66.5	27.2	[19]
	Paddy straw	46.5	24.1	16.9	
	Wheat straw	59.1	87.0	14.8	
	Sorghum straw	45.3	45.0	15.5	
	Bajra straw	25.3	65.9	19.1	
	Sorghum hay	44.2	27.6	15.5	
	Maize straw	14.8	63.1	12.7	
Porodaedalea pini	Picea jezoensis wood samples	4.2	5.4	ND	[61]
Schizophyllum commune	Rice straw	ND	10196	ND	[43]
S. commune	Jerusalem artichoke stalk	15.2	106.5	ND	[53]
S. commune	Sunflower meal	13.2	2.5	ND	[46]
	Brewer's spent grain	17.5	1.2	ND	
	Soybean meal	1.9	0.7	ND	
	Spent coffee residue	6.2	0.8	ND	
Trametes trogii	Poplar wood	512.2	ND	ND	[42]
T. versicolor	Sugarcane bagasse and wheat bran	56.5	36.7	9.5	[51]
T. versicolor	Oak sawdust, coffee husk, and corn bran	ND	9.1	11.6	[62]

Fourthly, the ratio of cellulase and xylanase activities varies greatly depending on the fungus species. Although most fungal species are capable of producing approximately equal amounts of cellulase and xylanase activity, *Pseudotrametes gibbosa* [17] and especially *Schizophyllum commune* [32] predominantly produce endoglucanase in submerged fermentation of lignocellulosic materials whereas *Inonotus obliquus* [27], *T. hirsuta* [34], and *T. trogii* [23] secrete many times higher xylanase activity compared to endoglucanase (Table 1). It should be noted that the ratio of cellulase and xylanase activities largely depends on the type and chemical composition of the lignocellulosic growth substrate, as will

be discussed below. Finally, some fungal strains, such as *Pycnoporus sanguineus* [31], *Irpex lacteus* [33], *Phanerochaete chrysosporium* [57], and *Pleurotus ostreatus* [19] probably possess a well-balanced cellulolytic system, exhibit high filter paper activity (FPA), and, therefore, are promising candidates for use in biorefining processes.

3. Effect of Carbon Source on Cellulase and Xylanase Production

Developing a highly productive fermentation process to enhance the ability of fungi to produce a complete cellulase system is challenging [63]. PHEs production by Basidiomycota fungi is strongly influenced by the availability of nutrients such as carbon and nitrogen sources, growth factors, and microelements, as well as medium pH, fermentation temperature, aeration, and other factors. Various approaches have been used to enhance the production of cellulases and xylanase in submerged and SSF fermentation of lignocellulosic materials. It is clear that optimizing the medium composition that provides nutrients and energy for the growing organism and establishing favorable environmental factors are necessary to achieve both maximum enzymatic activity and productivity. In this case, careful selection of the carbon source (and potential inducer of PHEs synthesis) in the presence of which the enzyme producer grows is of paramount importance.

For PHEs production by basidiomycetes, the growth medium usually includes pure cellulose, which serves as a source of carbon and as an inducer of the synthesis of enzymes that decompose biomass polysaccharides. To gain insight into the peculiarities of PHEs production by individual wood- and litter-degrading basidiomycetes, several research groups tested the effects of various mono-, di-, and polysaccharides along with lignocellulosic substrates. For example, cellulose had the most significant inducing effect on cellulase and xylanase production by *Ganoderma applanatum* LPB MR-56, but CMC and xylose were also effective in inducing these enzymatic activities [25]. Altaf et al. [64] reported that the values of xylanase activity of the basidiomycetes *Flammulina velutipes* and *Pleurotus eryngii* using xylose as a carbon source were higher than those produced with xylan. Among four chemically pure carbon sources, Avicel provided the highest FPA and β-glucosidase activity of *Agaricus arvensis* 0.18 U/mL and 7.2 U/mg protein, respectively, while in the presence of CMC, xylan, and cellobiose FPA was equal to 0.06–0.08 U/mL and β-glucosidase activity achieved 5.5, 2.3, and 0.5 U/mg protein, respectively [65]. Kumar et al. [66] compared CMCase and FPA of *Schizophyllum* CMCase and FPA activities of the fungal culture were as high as in the medium with wheat bran, but many times higher than those observed in media containing rice straw, rice husk, wheat straw, and sugarcane bagasse. It is interesting that this fungus secreted significant CMCase activity in the cultivation of this strain in a medium containing sucrose.

In our experiments, we compared promising producers of cellulase and xylanase (two WRBs: *Trametes gibbosa* 17 and *Trametes versicolor* 13, and three BRBs: *Gloeophyllum abietinum* 89, *Pholiota aurivella* 437, *Piptoporus betulinus* 327) for their growth and enzyme activity depending on various forms of chemically pure carbon sources. Fungi were cultivated on a shaker at 160 rpm and 27 °C in a medium containing (g/L): carbon source—10.0, peptone—3.0, KH_2PO_4—0.8; K_2HPO_4—0.6; $MgSO_4 \bullet 7H_2O$—0.5, and yeast extract—3.0. Results in Table 3 indicate that basidiomycetes were able to metabolize all tested carbon sources; however, the yield of fungal biomass varied significantly. The highest mycelial biomass production occurred when *P. aurivella* 437, *P. betulinus* 327, and *T. versicolor* 13 were cultivated in the presence of glucose; glycerol ensured the highest yield of *G. abietinum* 89 biomass accumulation, while cellobiose and glycerol were favorable for the growth of *P. gibbosa*. Carboxymethyl cellulose followed by xylan proved to be poor carbon sources for fungal growth, providing the lowest biomass yield of all basidiomycetes.

Table 3. Effect of carbon source on the basidiomycete's growth and enzyme production.

Carbon Sources	Biomass	CMCase		Xylanase	
	(mg/mL)	(U/mL)	(U/mg)	(U/mL)	(U/mg)
		Gloeophyllum abietinum 89			
Avicel	5.1 ± 0.2 [a]	50.7 ± 6.7	9.9	9.6 ± 1.1	1.9
CMC	2.2 ± 0.1	44.1 ± 6.9	20.0	10.9 ± 0.9	5.0
Xylan	4.6 ± 0.2	33.4 ± 4.5	7.2	8.2 ± 1.1	1.8
Glucose	5.8 ± 0.2	26.2 ± 2.8	4.5	8.9 ± 0.9	1.5
Cellobiose	6.2 ± 0.3	56.2 ± 7.6	9.1	13.3 ± 1.0	2.1
Glycerol	6.4 ± 0.2	20.1 ± 2.7	3.1	8.2 ± 1.0	1.3
		Pholiota aurivella 437			
Avicel	4.3 ± 0.2	16.9 ± 2.1	3.9	15.6 ± 1.3	3.6
CMC	2.5 ± 0.1	7.1 ± 0.9	2.8	10.3 ± 0.9	4.1
Xylan	5.1 ± 0.2	4.9 ± 0.5	1.0	6.2 ± 0.5	1.6
Glucose	6.6 ± 0.2	10.4 ± 1.3	1.6	5.9 ± 0.5	1.2
Cellobiose	6.0 ± 0.2	4.0 ± 0.6	0.7	8.1 ± 1.0	1.4
Glycerol	4.3 ± 0.1	4.6 ± 0.4	1.1	3.6 ± 0.2	0.8
		Piptoporus betulinus 327			
Avicel	2.7 ± 0.1	1.3 ± 0.1	0.5	4.0 ± 0.5	1.1
CMC	2.8 ± 0.1	6.8 ± 1.0	2.4	6.3 ± 0.6	2.3
Xylan	4.3 ± 0.1	4.0 ± 0.5	0.9	7.5 ± 0.9	1.7
Glucose	5.7 ± 0.2	0.3 ± 0.1	0.05	0.5 ± 0.1	0.09
Cellobiose	5.6 ± 0.2	12.7 ± 1.6	2.3	10.6 ± 1.3	1.9
Glycerol	4.0 ± 0.1	0.3 ± 0.1	0.07	0.3 ± 0.1	0.08
		Trametes gibbosa 17			
Avicel	4.0 ± 0.3	34.2 ± 5.0	8.6	29.5 ± 3.9	7.4
CMC	1.2 ± 0.1	1.3 ± 0.2	1.1	0.8 ± 0.1	0.7
Xylan	2.6 ± 0.1	1.0 ± 0.1	0.4	0.6 ± 0.1	0.2
Glucose	4.7 ± 0.2	0.1 ± 0.02	0.02	0.1 ± 0.02	0.02
Cellobiose	5.1 ± 0.2	0.1 ± 0.01	0.02	0.4 ± 0.07	0.1
Glycerol	5.0 ± 0.2	0.1 ± 0.02	0.02	0.1 ± 0.02	0.02
		Trametes versicolor 13			
Avicel	3.6 ± 0.2	10.2 ± 1.4	2.8	11.5 ± 1.1	3.2
CMC	1.1 ± 0.1	1.0 ± 0.1	0.9	1.2 ± 0.1	1.1
Xylan	2.5 ± 0.2	0.3 ± 0.04	0.1	0.7 ± 0.1	0.3
Glucose	4.9 ± 0.3	0.1 ± 0.02	0.02	0.1 ± 0.01	0.02
Cellobiose	4.5 ± 0.2	0.1 ± 0.01	0.02	0.2 ± 0.03	0.04
Glycerol	4.3 ± 0.1	0.1 ± 0.02	0.02	0.1 ± 0.01	0.02

Samples were taken after 5, 7, 10, and 14 days of submerged cultivation. Values shown are the mean maximum activity ± SD of two experiments with three replicates. [a] In the Avicel-containing media, biomass was calculated from the protein content.

The data obtained show that the production of CMCase and xylanase strongly depended on the nature of the fungus and the carbon source (Table 3). When growing WRB in the presence of low molecular weight compounds (glucose, cellobiose, or glycerol), very low enzyme activity was detected throughout the entire period of fungal cultivation, including when the carbon source was completely consumed from the nutrient medium. Among polysaccharides, neither CMC nor xylan appeared to be appropriate carbon sources for the significant production of both enzymes. In experiments by Haltrich and Steiner (1994), neither xylan nor galactomannan induced xylanase or mannanase activity when used as the sole carbon source for *S. commune*. Instead, cellulose, cellobiose, lactose, and l-sorbose induced xylanase, cellulase, and mannanase activities indicating a common regulatory control in this fungus [67].

Results presented in Table 3 show that the Avicel in the concentration of 1% induced the highest cellulase and xylanase activities in WRBs. This observation is consistent with the results of other authors showing an inducible production of cellulases and xylanases by white-rot *Basidiomycota* species [25,32,68]. Our calculations showed that the specific

enzymatic activities of *P. gibbosa* 17 and *T. versicolor* 13 in media with easily metabolizable carbon sources do not exceed 0.02 U/g of biomass, which can probably be taken as the basal level of both enzymes. At the same time, in media with Avicel, the specific CMCase activities of these fungi were 8.6 and 2.8 U/g, respectively; therefore, the induction ratio for *P. gibbosa* 17 and *T. versicolor* 13 CMCase is 430 and 140, respectively. Similar calculations showed that the induction ratio in the synthesis of xylanase by these fungi is 369 and 160, respectively.

When cultivating BRBs, a completely different picture of the fungal response to the presence of various carbon sources in the nutrient medium was revealed. In this case, active secretion of cellulase and xylanase was observed during the cultivation of *G. abietinum* 89 and *P. aurivella* 437 in the presence of both polymeric compounds and easily metabolizable carbon sources indicating that these fungi produce cellulase and xylanase constitutively (Table 3). Cellobiose ensured the highest volumetric enzyme activity of *G. abietinum* 89 while even glucose was a suitable carbon source for cellulase and xylanase production by *P. aurivella* 437 although crystalline cellulose was the best source of carbon for the production of volumetric cellulase and xylanase activity by this fungus. The most interesting finding is that carboxymethyl cellulose, which provided very poor growth of all fungi, promoted the expression of the activity of both enzymes by BRBs so that the specific cellulase and xylanase activities of *P. aurivella* 437 in media with Avicel and CMC were comparable, while in the cultivation of *G. abietinum* 89, the fungus enzymatic activities in medium with CMC turned out to be two times higher than those in the medium with crystalline cellulose. These results and the literature data [69,70] indicate that the characteristic feature of brown-rot fungi is a constitutive synthesis of cellulase and xylanase, even in the presence of glucose as the only source of carbon and energy. However, no formation of cellulolytic enzymes was observed during the growth of *Coniophora puteana* on glucose alone, although this fungus secreted four endocellulases and two exo-cellobiohydrolases in the presence of amorphous cellulose as the sole carbon source [71]. Likewise, in our study, *P. betulinus* 327 expressed very low CMCase and xylanase activities in submerged cultivation in the presence of glucose or glycerol with a specific activity of 0.07–0.09 U/g biomass (Table 3). At the same time, this fungus was able to secrete high activities of both endoglucanase and xylanase during growth in media containing not only polysaccharides but also cellobiose. Moreover, in the presence of a disaccharide, the fungus accumulated the highest volumetric enzymatic activity. Nevertheless, the maximum specific activity of CMCase and xylanase was recorded during the cultivation of the fungus in a medium with carboxymethyl cellulose, so the induction ratios for these enzymes were 34 and 29, respectively.

4. Induction and Catabolite Repression of Cellulase and Xylanase Synthesis in Basidiomycetes

Transcriptional regulation of PHEs synthesis is well analyzed in several recent comprehensive reviews [5,9,10,12]. Therefore, this paper considers mainly the physiological features of the production of cellulase and xylanase activity by white and brown rot basidiomycetes in response to several nutritional factors. The study of the physiological regulation of cellulase synthesis is necessary for the development of reliable strategies and approaches to enhance the production of PHEs.

Usually, WRBs demonstrate similar inducible mechanisms of cellulases and xylanase synthesis while brown-rot fungi produce these enzymes constitutively. The induction of cellulases and xylanases in WRBs is a highly regulated process that occurs only under conditions that require fungi to use specific plant polymers, such as cellulose, as a source of carbon and energy [9,10,70,72–75]. However, these polymers are insoluble and cannot directly enter fungal cells. Therefore, it is considered that fungi secrete a basal level of hydrolases, the catalytic activity of which leads to the formation of trace amounts of soluble inducers that trigger the transcription of cellulase and hemicellulases genes [5,9,10]. This means that the basal level of cellulase/xylanase activity is provided by the synthesis of small amounts of specific mRNAs in the absence of inducers.

To gain insight into the induction and catabolite repression of endoglucanase and xylanase production, *T. gibbosa* and *P. betulinus* were selected in our study using the above-mentioned nutrient medium. Glycerol can be considered as a source of carbon providing abundant growth of fungi and a non-inducing, constitutive level of cellulase activity (Figure 1A,B). When growing *T. gibbosa* on a medium containing 1% Avicel, and *P. betulinus* on a medium with cellobiose, noticeable CMCase activities were detected after two and one day of fungi cultivation, respectively, and they gradually increased until the end of experiments, reaching 38 and 12 U/mL, respectively. Cultivation of *T. gibbosa* and *P. betulinus* in media containing a mixture of 0.5% glycerol with, respectively, 1% crystalline cellulose and 1% cellobiose from the beginning of cultivation, a delay in the formation of cellulases was observed during three and two days of cultivation of fungi, respectively. It is important to note that only traces of reducing sugars or their absence could be found in the culture media at this time. When growing in a medium with two carbon sources, glycerol was used first and after its depletion during the specified time, the induction of enzyme synthesis occurred, accompanied by its rapid secretion. Finally, 1% Avicel and 1% cellobiose were added to the cultures of *T. gibbosa* and *P. betulinus* growing in the presence of 0.5% glycerol for four and three days, respectively. In this case, cellulase secretion started within 24 h. It is worth noting that a similar response to the media composition and the same regularities were revealed in the monitoring of xylanase induction indicating that the induction of cellulases and xylanases in WRB is regulated in a coordinated manner.

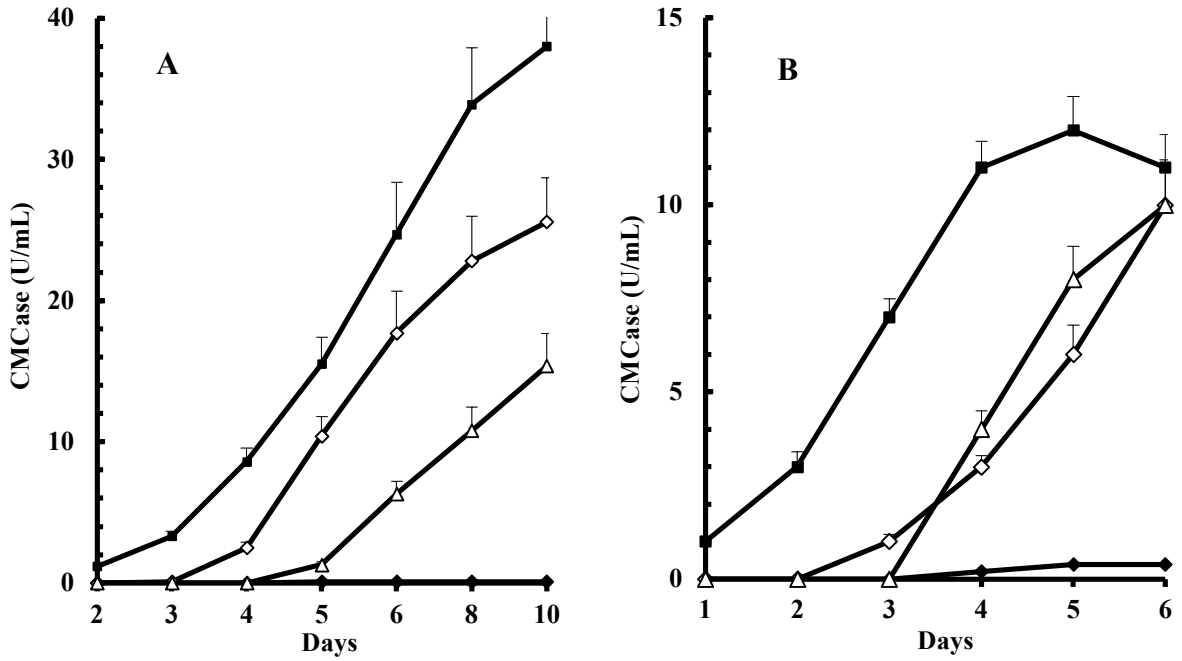

Figure 1. Induction of *Trametes gibbosa* (**A**) and *Piptoporus betulinus* (**B**) endoglucanase synthesis. *T. gibbosa* was grown in media containing 1% Avicel (■), 0.5% glycerol (♦), 0.5% glycerol + 1% Avicel (◊), 0.5% glycerol + 1% Avicel on day 4 (Δ). *P. betulinus* was grown in media containing 1% cellobiose (■), 0.5% glycerol (♦), 0.5% glycerol + 1% cellobiose (◊), 0.5% glycerol + 1% cellobiose on day 3 (Δ).

In WRB, another cellular economy mechanism is widespread, namely catabolite repression of cellulases and xylanase synthesis. To confirm this fact, glucose and glycerol at a final concentration of 0.4% were added to a three-day culture of *T. gibbosa* growing in the presence of 1% Avicel. Additional carbon sources stimulated the growth of fungi and increased the biomass yield by 19–29% compared to the medium containing Avicel. The production profiles shown in Figure 2A indicate that in the cellulose-based medium, a comparatively high CMCase activity was detected already after three days of *T. gibbosa* cultivation, and the maximum enzyme activity was achieved by day 10–11. Supplemen-

tation of the induced *T. gibbosa* culture with glucose or glycerol accelerated the growth of the fungus but resulted in repression of endoglucanase synthesis and partial inactivation of already secreted enzymes. However, after one and two days of cultivation after the addition of glucose and glycerol, respectively, the secretion of the enzyme resumed, which indicates the reversibility of the mechanism of repression of hydrolase synthesis by easily metabolized compounds. Interestingly, after the depletion of glycerol or glucose, the rate of accumulation of CMCase and xylanase was significantly higher than in the culture containing only Avicel and after 11 days of submerged cultivation of *T. gibbosa* in media with two carbon sources, the volumetric enzymatic activity of the fungus exceeded that in the medium with Avicel.

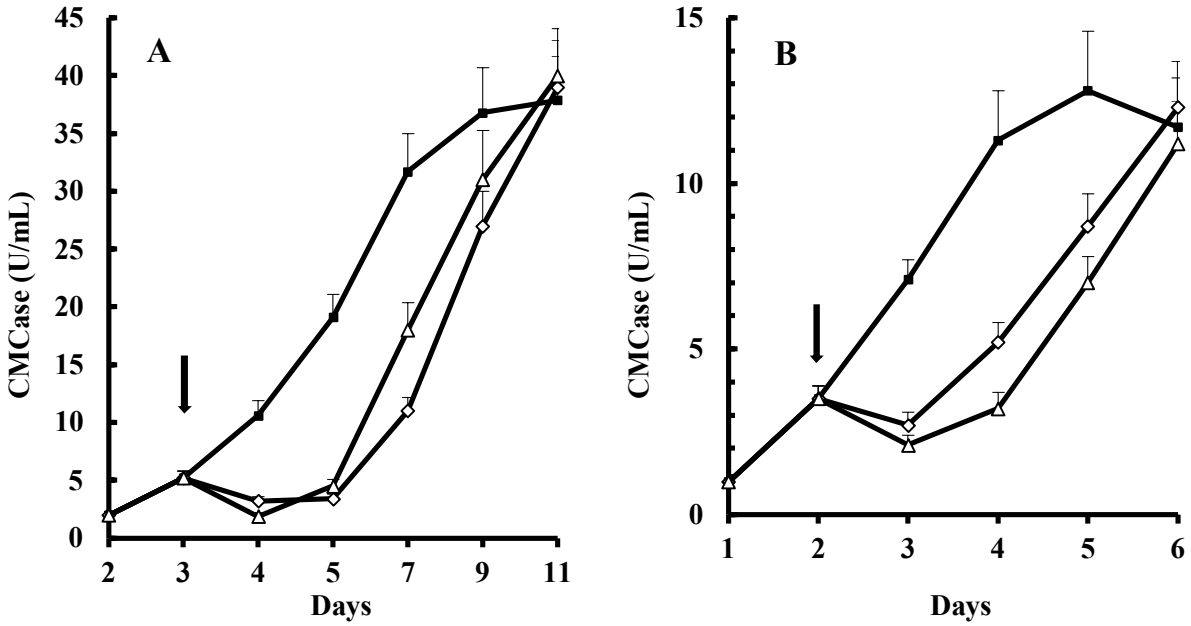

Figure 2. Catabolite repression of *T. gibbosa* (**A**) and *P. betulinus* (**B**) endoglucanase synthesis. *T. gibbosa* was grown in media containing 1% Avicel (■), 1% Avicel + 0.4% glycerol on day 3 (◊), and 1% Avicel + 0.4% glucose (Δ) on day 3. Arrow indicates the day of glucose or glycerol addition. *P. betulinus* was grown in media containing 1% cellobiose (■), 1% cellobiose + 0.2% glycerol on day 2 (◊), and 1% cellobiose + 0.4% glycerol (Δ) on day 2. Arrow indicates the day of glucose or glycerol addition.

Similarly, when cultivating *P. betulinus* in a cellobiose-based medium, it was observed that the addition of glycerol to the induced culture strongly suppressed the production of endoglucanase, and the higher the concentration of glycerol in the culture medium, the longer the duration of repression (Figure 2B). In addition to catabolite repression, a decrease in the activity of already synthesized enzymes was observed, apparently due to the acidification of nutrient media. In addition to the catabolite repression, a reduction of already synthesized enzyme activity was observed, obviously because of inactivation during the acidification of the nutrient media. Subsequently, when the more beneficial carbon source was completely utilized, the secretion of cellulase resumed, and by the end of cultivation, the enzyme activity in these media achieved that in the cellobiose-containing medium. It should be noted that the measurement of xylanase activity revealed a similar response of both fungi to the addition of glucose and glycerol to the induced cultures. Hence, it can be speculated that endoglucanase and xylanase activities are coordinately expressed and are under a common regulatory control mechanism. Moreover, not only glucose, but other readily metabolizable carbohydrates repress the synthesis of enzymes related to the catabolism of polysaccharides. Overall, both the presence of a suitable inducer and the absence of an easily metabolizable carbon source is essential for cellulase

and xylanase production in most fungi, where their synthesis is regulated by induction and catabolite repression.

In recent years, several studies have been carried out to acquire an understanding of the mechanisms regulating the synthesis of carbohydrate-active enzymes (CAZymes) by Basidiomycota fungi [74,76–80]. These studies proved that the expression of the genes encoding PHEs and the synthesis of these enzymes by WRB are regulated at the mRNA level by mechanisms of induction and repression; however, several transcriptional activators and repressors have been characterized mainly from ascomycete fungi.

Cellobiose, cellotriose, cellotetraose, cellopentaose, sophorose, gentiobiose, lactose, and xylose have been identified as inducers of fungal cellulase and hemicellulase synthesis. Early studies showed that the transcripts of cel2 and cel3 from *Agaricus bisporus* [81,82], and *cel7A* and *cel6B* from *Lentinula edodes* [83] are strongly induced when the fungi are grown in a medium containing crystalline cellulose, and they are not expressed in a medium containing glucose. In *Polyporus arcularius*, the expression of the cellobiohydrolase encoding genes *cel1* and *cel2* is induced by microcrystalline cellulose and cellopentaose but repressed by glucose, cellobiose, cellotriose, and cellotetraose [76]. Minimal transcription was observed using a combination of Avicel and either glucose or cellobiose. In the cellulase-producing medium, the transcription of *cel1* and *cel2* increased, starting the first day and achieved the maximum amounts two days after the mycelium was transferred to the cellulase-producing medium. It is interesting that in a medium containing Avicel as the sole carbon source, the maximum concentration of cellopentaose was detected after 12 h while the induction of *cel1* and *cel2* transcription occurred after another 12 h. These results indicate that *P. arcularius* may constitutively produce a very low level of endoglucanases that can degrade insoluble crystalline cellulose, after which the reaction products such as cellooligosaccharides induce transcription of *cel1* and *cel2*. Likewise, Suzuki et al. quantitatively compared the transcript levels of the genes encoding cellobiohydrolases (*cel6A*, and *cel7A* to *cel7F/G*) in cultures of *P. chrysosporium* containing glucose, cellulose, and cellooligosaccharides [78]. They showed that *P. arcularius* cellotriose and cellotetraose derived from cellulose, but not cellobiose, are possible natural inducers of cellobiohydrolase gene transcription. In particular, the highest level of *cel7C* transcripts (2.7×10^6) was observed in the presence of cellotetraose, whereas the highest level of *cel7D* transcripts (1.7×10^6 per 10^5 actin gene transcripts) was found in the presence of cellotriose. Interestingly, cellooligosaccharides did not affect the transcription of *cel7A*, *cel7B*, and *cel7E*; therefore, the authors suggested that *Cel7A*, *Cel7B*, and *Cel7E* do not participate in cellulose degradation, but perform a different function than other *Cel7s*. However, findings from these studies raise many questions, to which the answers can only be obtained through deeper genetic studies that will allow us to understand the mechanisms that cause differences in the regulation of the synthesis of cellulases, especially their isoenzymes when using different carbon sources.

5. Role of the Lignocellulosic Growth Substrate

Despite numerous studies of the synthesis of PHEs by basidiomycetes, the general and distinctive features of the production of these enzymes during the cultivation of taxonomically, ecologically, and physiologically different fungi in the presence of chemically different lignocellulosic materials remain insufficiently clear. Microcrystalline cellulose is commonly used for the production of cellulase by filamentous fungi [25,26,33,65,68,73,75,84]. Undoubtedly, it is the most appropriate growth substrate for the production of PHEs. Nevertheless, cellulose or cellulose derivatives, such as carboxymethyl cellulose, are quite expensive; therefore, more attention has been paid to the use of cellulose-rich biomass instead of expensive cellulose for the production of these enzymes. Lignocellulosic materials are cheap, renewable, and abundant, and their use as growth substrates rich in required nutrients provides the production of hydrolases and, if necessary, other complementary enzymes involved in the degradation of plant biomass. However, it is not clear how the production of enzymes depends on the content of cellulose, hemicellulose, and lignin in the plant substrate. In particular, if any plant material contains cellulose and hemicellulose, then

why is the range of cellulase activity so varied (Tables 1 and 2) when the same fungus is cultivated on different substrates?

The structure and chemical composition of the lignocellulosic substrates is critical; they must contain sufficient readily available nutrients and microelements to ensure abundant growth and biosynthetic activity of the fungus to provide enhanced production of PHEs. Likewise, Brijwani and Vadlani [85] showed that the physicochemical properties of the substrate, such as porosity and crystallinity, significantly affect the production of cellulase and xylanase. Clearly, a high cellulose content and a low lignin content in the growth substrate are preferable for the active production of PHEs. It should be noted that in materials such as woody residues, the accessibility to cellulose for microbes is poor due to a higher percentage of lignin content; therefore, their pre-treatment is necessary to improve the accessibility of cellulose to microbial enzymes. This probably explains why mainly herbaceous substrates and food industry wastes have been exploited for cellulase production and not woody substrates. Although, this may also be because food industry wastes are produced in significant quantities in many countries and are cheap. It is important to note that when WRBs are used to ferment lignocellulosic growth substrates such as straw and sawdust, there is no particular need for their pretreatment since these fungi can produce both hydrolases and lignin-modifying enzymes. However, in this case, a wide range of extracellular enzymes belonging to different classes are involved in the degradation of lignocellulosic polymers and their synthesis requires significant energy and material resources, while the fermentation of cellulose or xylan requires only a few specific glycoside hydrolases for their hydrolysis.

Literature data indicate that some of the substrates significantly stimulate the synthesis of individual PHEs without supplementation of the culture medium with specific inducers [17,27,31]. Some substrates, especially food industry by-products that are low in lignin and contain free sugars and organic acids can stimulate cellulase production. It was found that among the tested lignocellulosic materials, wheat bran provides the maximum production of CMCase (71.5 U/g), FPA (3.3 U/g), and b-glucosidase (50.7 U/g) of *Fomitopsis* sp. RCK2010 while corn cob was not suitable for enzyme secretion [44]. Results in Tables 1 and 2 show that Basidiomycota strains cultivated on the same substrate express significant differences in cellulase and xylanase activities. However, the same strain of fungus that expresses exceptionally high cellulase activity in the presence of a particular substrate may produce low cellulase activity when cultivated on a different substrate, i.e., fungal growth and enzyme activity expression might be substrate-specific. Thus, Ilić et al. [46] showed that the SSF of brewer's spent grain by *F. fomentarius* provided the formation of only 1.4 U/g of CMCase activity, whereas in the fermentation of the same substrate by *Bjerkandera adusta* and *S. commune*, the enzyme activities achieved 18.4 and 17.5 U/g, respectively. In the same work, the xylanase activity of *F. fomentarius* varied from 0.9 U/g in the medium with spent coffee residues to 16.8 U/g in the medium containing sunflower meal. In our study, in the submerged fermentation of different lignocellulosic materials, the CMCase and xylanase activities of *P. coccineus* 310 varied from 5.1 to 67.5 U/mL and from 8.9 to 47.0 U/mL, respectively (Table 3). More significant changes were observed in the cultivation of *I. lacteus* 104 in the presence of the same substrates. Undoubtedly, crystalline cellulose was the best growth substrate for both enzyme synthesis by all fungi. Mandarin peels ensured the secretion of significant levels of CMCase and xylanase, while wheat bran favored xylanase production in the cultivation of *I. lacteus* 104. Likewise, wheat bran provided the highest enzyme activity of *T. versicolor* 13. It is worth noting that among the lignocellulosic materials, lignified wheat straw appeared to be the best substrate for enzyme production by *P. coccineus* 310 while *T. versicolor* 13 failed to actively produce cellulase and xylanase in the same medium. Interestingly, the addition of mandarin pomace to Avicel or wheat straw led to a significant increase in the yield of enzymes [26,33], (Table 1). This strategy promoted rapid initial growth and accumulation of fungal biomass due to the presence of free sugars, organic acids, and other essential nutrients for microbial growth.

The results obtained and the literature data [86] (Tables 1 and 2) evidence that the lignocellulosic substrate appears to determine the type and yield of enzymes produced by a given wood-rotting fungus in a species and strain-specific manner. Moreover, using lignocellulosic substrates with different chemical compositions, it is possible to obtain enzyme complexes with different levels and ratios of individual enzymes. Thus, Shradhdha and Murty [19] successfully implemented SSF of rice bran and sorghum straw for the maximum production of both cellulases and xylanase using *P. chrysosporium* and demonstrated the suitability of paddy straw and sorghum hay for the predominant production of xylanase or cellulase, respectively. Furthermore, Bentil et al. [86] summarized the results on the rate of cellulase production and reported that in particular for SSF, the recorded rates of enzyme production range from 0.001 to 72 U/g/day for various fungi and lignocellulosic substrates. Probably the decisive role in the stimulation of PHEs expression belongs to the presence of significant amounts of readily available cellulose in the medium. In any case, it is important to search for a suitable combination of fungal strain and lignocellulosic substrate for maximum production of PHEs.

6. Cultivation Methods for Cellulases Production

Both submerged fermentation (Table 1) and SSF (Table 2) of different lignocellulosic materials can be successfully used for PHEs production by Basidiomycota fungi [10,86,87]. Currently, submerged fermentation is the main industrial process; it ensures controlled cultivation conditions, uniform availability of nutrients and oxygen, the formation of proper fungal pellets, and easy product recovery and reproducibility. Many studies proved that compared to the SSF method, submerged fermentation of plant raw materials provides fast production and a higher yield of cellulase [30,49,88]. However, significantly higher amylase, endoglucanase, and xylanase activities were recorded during the SSF of cassava peel by *Trametes polyzona* BKW001 than during submerged fermentation [89]. The activity of β-glucosidase and exoglucanase was also slightly higher with SSF than with submerged fermentation. In recent years, interest in SSF has increased because, in the cultivation of basidiomycetes, it provides a growth environment similar to their natural habitat and ensures major advantages: (1) high volumetric productivity and product yield, (2) relatively higher concentration of the products, (3) simpler downstream processing, (4) less effluent generation, (5) requirement for simpler fermentation equipment, and (6) opportunities to organize on-site tailor-made enzyme production without requiring large capital investments. It can be assumed that during colonization of the growth substrate under SSF conditions, the rate of substrate uptake is sufficiently high and the accumulation of easily metabolizable products of polysaccharide hydrolysis does not occur, which provides a high rate of PHEs synthesis due to the absence of catabolic repression.

Compared to submerged fermentation, in SSF, the fungal mycelium is in direct contact with the lignocellulosic material, it grows on the surface and then penetrates the substrate. The fungal growth rate, biomass, and PHEs yields are directly related to the lignocellulosic substrate chemical composition and structure [87,90]. Of particular importance is also the porosity and specific surface of the particles of plant material, which determine the efficiency of air diffusion and the water-holding capacity of the substrate. The substrate's porosity must be sufficient to not limit the supply of necessary oxygen and the removal of carbon dioxide and the resulting metabolic heat [91]. In this regard, it is necessary to take into account the thickness and particle size of the substrate. The moisture content in the growth substrate in the range of 65–75% of the total mass is considered the most optimal both for the growth of basidiomycetes and the production of PHEs. A medium containing appropriate nitrogen and mineral salts is usually used to moisten the substrate.

It should be noted that a comparative assessment of the effectiveness and benefits of both cultivation methods is difficult since only a few studies used the same fungal species and cellulose substrate for comparison. Okal et al. [10] indicated that the majority of WRB produce higher yields of cellulases in submerged fermentation of lignocellulosic biomass than in SSF. Moreover, Tengerdy [92] compared the production of cellulase in

submerged and SSF systems and indicated that when using SSF, the cost of production is reduced by about 10 times. Undoubtedly, the influence of the cultivation method on the production of cellulases varies depending on the individual peculiarities of the fungal strain and the nature of biomass residues used as a growth substrate. Highly lignified residues like straw and tree leaves are suitable for SSF while nutrient-rich fruit residues are appropriate for submerged fermentation [86]. In this regard, it is worth noting that in many studies media of the same composition and concentration were used for both SSF and submerged fermentation of plant materials. However, the volume of the medium used to wet the substrate to a moisture content of 70–75% is limited, and the amount of additional necessary elements may not be enough for both abundant fungal growth and maximum cellulase production. Therefore, it is desirable to increase the concentration of the medium components by 5–10 times for SSF.

Undoubtedly, the production of cellulases in the SSF of plant raw materials is promising for scaling up to an industrial level, but innovative knowledge and design solutions are required to create effective systems. This method of basidiomycetes cultivation is especially promising for the organization of on-site enzyme production using, for example, food industry by-products as biomass feedstock. In this case, the entire fermented product enriched with fungal biomass and enzymes can be directly used as a feed additive. This approach is cost-effective, energy-efficient, and more environment-friendly as compared to the off-site production of enzymes [7]. In this regard, a promising and feasible approach to improve the yield of cellulase production approach may be the co-cultivation of two or more strains of WRB fungi. Compared to monocultures, co-cultivation provides a number of advantages, such as better substrate degradation and nutrient supply to the producer as well as higher productivity [6,93]. In addition, this is a way not only to enhance the expression of the target enzyme but also to supplement enzyme systems with complementary enzymes. However, in order to achieve maximum results, it is necessary to search for compatible fungal strains. Thus, when *Schizophyllum commune* was co-cultivated with *Irpex lacteus*, an increase in cellulase activity occurred compared with individual cultures, while co-cultivation of *S. commune* with *Pycnoporus coccineus* or *Trametes hirsuta* had a negative effect on cellulase production [33].

7. Influence of Nitrogen Source on the Cellulase and Xylanase Production

The growth rate of basidiomycetes, the yield of fungal biomass, and, accordingly, the production and yield of PHEs are largely dependent on the nitrogen sources present in the fermentation medium. In a synthetic medium containing cellulose, a source of nitrogen is an essential component for optimal fungal growth and enzyme production. Lignocellulosic materials used as a growth substrate, to a certain extent, serve as sources of nitrogen and provide the accumulation of fungal biomass and target enzymes. However, to accelerate both processes, it is necessary to introduce an additional source of nitrogen into the medium, especially when the yield of cellulases correlates with the amount of fungal biomass. Both the nature and concentration of nitrogen sources affect cellulase and xylanase activity in Basidiomycota strains. For example, among various sources of organic nitrogen, urea caused the maximum production of CMCase (81.8 U/g) by *Fomitopsis* sp. RCK2010 during SSF of wheat bran, while casein and soy flour resulted in the maximum production of FPA (4.7 U/g) and β-glucosidase (69.1 U/g), respectively, while inorganic sources of nitrogen had no significant effect on the increase in enzyme activity [44].

An analysis of the literature data shows that peptone is probably the most suitable source of nitrogen, providing a significant growth of most fungi and the production of PHEs. For example, in the SSF of tree leaves, the addition of peptone to the medium as an additional source of nitrogen provided a twofold increase in the *P. ostreatus* protein content compared to the control medium and an increase in the activity of CMCase and xylanase from 20 U/mL to 28 and 35 U/mL, respectively [88]. Replacing peptone with ammonium sulfate reduced the activity of enzymes to 13 and 17 U/mL, respectively, although the increase in fungal biomass protein was comparable to that in the peptone medium. Am-

monium sulfate is a physiologically acidic salt, and in its presence, nitrogen consumption is accompanied by the acidification of the medium. It is possible that this circumstance affected both the secretion of enzymes and the stability of the already synthesized enzyme during long-term cultivation. Nevertheless, Coniglio et al. [36] found that both peptone and ammonium sulfate as nitrogen sources favored the secretion of cellobiohydrolase activity by *Trametes villosa* LBM 033. Unfortunately, in most studies of the effect of nitrogen and its concentration on the cellulase activity of fungi, there are no data on the accumulated biomass. As a result, some authors make an incorrect conclusion about the stimulation or inhibition of enzyme production by one or another source of nitrogen.

Interestingly, the effect of an additional source of nitrogen on the fungal cellulase activity may depend on the nature and chemical composition of the lignocellulosic substrate. For example, in SSF of beech leaf by *P. dryings*, peptone was the most suitable source of nitrogen for CMCase production, while in the presence of wheat straw, the maximum activity of the enzyme was detected when $(NH_4)_2SO_4$ was added to the medium [94]. Therefore, to maximize cellulase production, it is necessary to find the right combination of nitrogen source and lignocellulosic substrate for each particular fungal strain. Moreover, Salmon et al. [25] showed that the effect of nitrogen source on the activity of *Ganoderma applanatum* LPB MR-56 xylanase and cellulase depends on the concentration of cellulose used. Yeast extract was found to be the best nitrogen source when using 1% cellulose, while peptone was the best nitrogen source when using 0.5% cellulose.

8. Other Factors Modulating Fungal PHEs Activity

Physicochemical factors such as the medium pH, the cultivation temperature, aeration, and agitation may play an important role in the production of PHEs by wood-rotting basidiomycetes. The pH of the medium is usually adjusted at the beginning of the submerged fermentation or SSF to ensure the maximum growth rate of the microbial culture and it is not controlled throughout the culture. In both submerged fermentation and SSF, the initial pH first decreases due to the metabolism of carbohydrates and the formation of organic acids; then it gradually increases. Usually, changes in the pH of the medium depend mainly on the form of the carbon source and the physiological peculiarities and metabolic activity of the fungus, although some lignocellulosic materials and nitrogen sources can also contribute to the change in pH during the fermentation process. It is worth noting that compared to WRB, the fermentation of lignocellulose by BRB is accompanied by faster and more significant acidification of the nutrient medium.

The pH of the medium is one of the most important environmental parameters influencing the growth of fungal mycelium and the production of enzymes. For example, *Fomitopsis* sp. RCK2010 produced maximum CMCase (72.7 U/g), FPA (3.3 U/g), and β-glucosidase (50.9 U/g) in the medium with an initial pH of 5.5 [44]. An increase in the initial pH of the medium from 5.5 to 10.0 resulted in a decrease in the cellulase activity of the fungus by more than 50%. A decrease in the initial pH of the medium from 5.5 to 3.0 also caused a slight decrease in the production of CMCase and FPA. Sharma et al. [95] observed that during the fermentation of wheat bran with *Cotylidia pannosa*, when the initial pH was increased from 3 to 4, there was a gradual increase in endocellulase activity. With an increase in the initial pH of the medium to 5, the enzymatic activity of the culture increased significantly, reaching a maximum value (8.1 U/mL). However, with a further increase in pH to 5.5, a significant decrease in endocellulase activity was observed.

pH control for cellulase production is considered to be a major concern of this process, since different cellulase components may require different pH ranges for synthesis and secretion, depending on the characteristics of the strain used. Moreover, changes in the medium pH during microbial growth also affect the stability of the synthesized enzyme. Supplementation of calcium carbonate in a nutrient medium at a certain concentration can be beneficial both for a growing culture of the fungus and for increasing the yield of the target enzyme. For example, in our experiments, mandarin pomace is often used for the production of lignocellulolytic enzymes. This by-product from mandarin juice

production provides abundant fungal growth and high yields of target enzymes but has a low pH due to the high content of organic acids. Compared to submerged culture, it is almost impossible to set the right pH for SSF. The results presented in Table 4 show that the addition of $CaCO_3$ to the medium solves this problem. In these experiments, SSF was carried out in 100 mL flasks containing 5 g of ground mandarin pomace moistened with 10 mL of the medium (g/L): KH_2PO_4—4.0, K_2HPO_4—3.0; $MgSO_4$—2.5; yeast extracts—25; peptone—25. Although there were no signs of growth of *G. abietinum* 89 after inoculation of a $CaCO_3$-free substrate, the introduction of calcium carbonate in the medium, even at a concentration of 500 mg/5 g of the substrate, promoted the growth of the fungus and the production of significant cellulase and xylanase activities. Nevertheless, the pH of the medium remained extremely low even at the end of cultivation. However, a further increase in the concentration of calcium carbonate led to an increase in the pH of the medium and created more favorable conditions for the rapid colonization of the substrate and the production of enzymes. The higher the salt concentration added, the higher the cellulase activity of the culture, although no effect on the xylanase activity of *G. abietinum* 89 was found. On the contrary, when *S. commune* 632 was cultivated, the concentration of $CaCO_3$ affected both the cellulase and xylanase activity of the fungus. Namely, an increase in the amount of calcium carbonate in the medium from 0 to 1000 mg led to an almost six- and four-fold increase in CMCase and xylanase activities, respectively. The total cellulase activity of the fungal culture grown in the presence of 1500 mg $CaCO_3$ was two times higher than that in the control medium.

Table 4. Effect of calcium carbonate on basidiomycetes enzyme activity in the SSF of mandarin pomace (MP).

$CaCO_3$ (mg/5 g MP)	Final pH	CMCase (U/g)	Xylanase (U/g)	FPA (U/g)
		G. abietinum 89		
0		No growth signs		
500	3.2 ± 0	81 ± 11 ^{10} *	84 ± 9 ^{14}	8 ± 1.0 ^{14}
1000	5.1 ± 0.1	102 ± 10 ^{14}	76 ± 7 ^{14}	13 ± 1.6 ^{14}
1500	6.3 ± 0.1	124 ± 13 ^{14}	80 ± 9^{14}	13 ± 1.4 ^{10}
		S. commune 632		
0	5.2 ± 0.1	36 ± 4 ^{14}	156 ± 11 ^{14}	21 ± 2.2 ^{14}
500	6.1 ± 0.1	94 ± 9 ^{14}	453 ± 55 ^{14}	27 ± 3.2 ^{10}
1000	6.6 ± 0.1	213 ± 15 ^{14}	581 ± 42 ^{14}	32 ± 3.0 ^{10}
1500	6.7 ± 0.2	210 ± 18 ^{14}	529 ± 47 ^{14}	47 ± 5.1 ^{14}

* The numbers indicate the days of peak activity.

Cultivation temperature influences both fungal growth and cellulase production. In most basidiomycetes, the optimum temperature for fungal growth and cellulase production is around 27 °C. However, cultivation of *P. chrysosporium* and enzyme production is performed at 37 °C [76] while *P. sanguineus* expresses the highest CMCase activity at 28 °C [96] but grows better at 37 °C [97]. Optimization of the SSF temperature for the production of *Fomitopsis* sp RCK2010 cellulases showed that the enzymatic activity of the culture increased with an increase in temperature from 25 to 30 °C [44]. However, an increase in temperature above 30 °C had a significantly negative effect on the production of the enzyme. Likewise, the ability of *C. pannosa* to produce endocellulase increased as the temperature increased from 25 to 30 °C when it reached its maximum production capacity [95]. A further increase in the cultivation temperature of the fungus to 37 and 42 °C led to a decrease in the potential for endocellulase production. Temperature change played a significant role in the production of hydrolases by *T. polyzona*, except for exoglucanase [89]. A temperature range of 30–40 °C has been found to be optimal for endoglucanase production, while a wider range of 20–40 °C is optimal for amylase production. The optimal temperature for the production of betaglucosidase and xylanase was 40 and 30 °C, respectively. This suggests

that a desirable fermentation temperature should be a compromise between the optimum temperature for cellulase production as well as fungal growth.

The agitation of fungal culture ensures an even distribution of oxygen and nutrients in the medium during fermentation. Typically, a stirring speed in the range of 120–160 rpm promotes optimal fungal growth and enzyme secretion [98]. Increasing the agitation speed increases the shear force and can even destroy fungal cells, resulting in reduced enzyme production. Nevertheless, Teoh et al. [31] achieved the highest CMCase, FPA, and β-glucosidase activities in the fermentation of palm oil mill effluent by *P. sanguineus* at an agitation speed of 350 rpm in a stirred tank bioreactor. Of course, the agitation rate must be optimized for each fungus growing in the presence of one or another substrate that differs not only in chemical composition but also in particle size and rheological properties.

9. Conclusions & Future Perspectives

Cellulases are one of the most produced and used enzymes. However, their use in biotechnological processes, especially for the enzymatic hydrolysis of lignocellulosic biomass to produce bioethanol, requires the development of new technologies and potent enzymatic systems with reduced production costs. This review showed that many researchers around the world are focusing their efforts on screening for new cellulase-producing microorganisms, as well as optimizing media composition and fermentation parameters. Available data indicate that production rates and cellulase yields are generally higher with submerged fermentation than with SSF and it is currently applied in commercial enzyme production. Given the number of significant advantages of SSF, additional efforts must be made in the design of appropriate bioreactors and to find a suitable combination of fungal strain, lignocellulosic substrate, and SSF conditions to maximize cellulase yield. It may be more advantageous and promising to use SSF for small-scale and on-site production of cellulases or enzyme complexes using a strategy of mixed cultivation of compatible fungi with complementary enzymatic systems.

Genome sequencing of basidiomycetes has revealed a large repertoire of existing and putative new glycoside hydrolases. Nevertheless, the number of basidiomycete secretomes examined is still small and future efforts should be directed toward revealing the catalytic potential of basidiomycete species belonging to diverse taxonomic and ecological groups to discover enzymes with biotechnologically desirable characteristics, such as high catalytic activity towards crystalline cellulose, resistance to end-product inhibition, higher thermostability, and more complete hydrolysis of celluloses to reducing sugars. Then, in order to use the biosynthetic potential of these fungi more effectively and optimize the fermentation process, the most important fundamental and technological task is to elucidate the crucial physiological factors that regulate the expression and secretion of cellulases by basidiomycetes. Therefore, besides genetic manipulations, protein, and metabolic engineering, more in-depth studies are needed to understand the response of transcriptional regulators of cellulase synthesis to specific environmental stimuli to produce target enzymes in high yield and low cost. The synthesis of fungal cellulases is controlled by the mechanisms of induction and repression, and the design of the fermentation process and the composition of the medium for the production of cellulase should take into account these aspects. Of decisive importance are the presence of cellulose in the medium and the concentration of easily metabolizable carbon sources.

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