ABS

Original Research

Cellulase, xylanase, lipase, and protease activities of selected wild mushrooms from Slovenia

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Abstract

There are more than 3000 species of wild mushrooms in Slovenia. These wild mushrooms represent a promising source of bioactive compounds that can also be used as a source of novel enzymes, thus offering potential economic benefits for a wide range of industries and medicine. In this work, the cellulase, xylanase, protease, and lipase activities were measured in aqueous extracts of 64 Slovenian wild mushrooms of different lifestyles (symbiotic, saprobic, parasitic). We have shown that many of the mushroom extracts tested contain a variety of specific hydrolases. Of the 64 wild mushroom extracts, 16 showed cellulase, 24 xylanase, 30 lipase, and five protease activities. These are the first reports of these enzymatic activities for some of the mushrooms tested.

Keywords

mushroom; enzyme; cellulase; xylanase; lipase; protease

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Celulazne, ksilanazne, lipazne in proteazne aktivnosti nekaterih samoniklih slovenskih gob

Izvleček

V Sloveniji je opisanih več kot 3000 gob, ki predstavljajo potencialni vir bioaktivnih molekul kot so biotehnološko uporabni encimi, ki bi se jih lahko uporabilo v različnih industrijskih procesih ali v medicini. V sklopu raziskave smo pripravili vodne izvlečke 64 različnih slovenskih samoniklih gob, ki smo jim določili celulazno, ksilanazno, proteazno in lipazno aktivnost. Pokazali smo, da ti vodni izvlečki vsebujejo različne kombinacije encimskih aktivnosti. V vodnih izvlečkih teh 64 različnih slovenskih gob smo pokazali, da ima 16 izvlečkov celulazno, 24 ksilanazno, 30 lipazno in pet proteazno aktivnost. Mnoge od teh aktivnosti v literaturi še niso bile opisane.

Ključne besede

gobe, encim, celulaze, ksilanaze, lipaze, proteaze

Introduction

The global market for industrial enzymes was estimated at USD 60.5 billion in 2023 and is expected to grow by 4.9 % annually from 2024 to 2030 (Enzymes Market Size, Share, Trends and Growth Report, 2030). Approximately 60 % of industrial enzymes are produced by fungi (Raveendran et al., 2018). Many of these enzymes are used in various industries, e.g. in the food, detergent, paper, and textile industries, as well as in medicine (Pandey et al., 2000). Proteases, which account for 60 % of industrial enzymes, catalyse the depolymerization of proteins to amino acids and are used in the detergent, pharmaceutical, and food industries (Singh et al., 2016; Raveendran et al., 2018). Lipases are enzymes that hydrolyse triglycerides into fatty acids and glycerol. They are indispensable for the production of food, biofuels, detergents, and animal feed (Raveendran et al., 2018). Cellulases are a group of hydrolases that act on the β-1,4-glycoside bond in cellulose to release glucose units (Raveendran et al., 2018). This class of enzymes is widely used in the production of food as well as in the textile, paper, and detergent industries (Sukumaran et al., 2005). Xylanases are a group of enzymes that cleave xylenes, which are a major component of the plant cell wall and are mainly used in the food industry (Raveendran et al., 2018). Although cellulases and xylanases have no pharmacological potential, this is not the case for lipases and proteases. Lipases are being actively investigated for the treatment of modern diseases such as obesity or even cancer (Jawed et al., 2019). Proteases, on the other hand, can be used to combat cardiovascular diseases, digestive disorders, and inflammatory diseases and to promote tissue repair in burns, bone fractures, or surgical trauma (Solanki et al., 2021).

Basidiomycota and Ascomycota are the major phyla of the fungal kingdom, and many of them produce spore-bearing fruiting bodies known as mushrooms (Schmidt-Dannert, 2016). As mushrooms can grow on a variety of substrates and have versatile lifestyles (symbiotic, saprobic, parasitic), they have evolved a powerful enzymatic mechanism. In this regard, mushrooms represent a promising source of novel and undiscovered enzymes that could offer great economic benefits to the industry. We, therefore, collected 65 wild mushrooms and determined their cellulase, xylanase, protease, and lipase activities.

Materials and Methods

Mushroom extract preparation

The mushrooms were collected in Slovenia (Table 1) between December 2019 and February 2021, identified to species level, cut into small pieces and frozen at -80 °C. To obtain aqueous mushroom extracts, the thawed fruiting bodies were homogenized in 50 mM TRIS-HCl buffer, pH 7.4. For 1 g of wet biomass, 1 mL of the pre-cooled buffer was used, except for mushrooms growing on wood, which absorbed more buffer. We mixed 2 mL of the pre-cooled buffer per 1 g of such wet mushrooms. Pieces of the thawed mushroom fruiting bodies were then homogenized in the

Waring Blender (Ika, Germany) for 10 seconds at maximal speed. Mushroom samples weighing less than 1 g were first frozen with liquid N2 and then homogenized in a mortar. Fruiting bodies that were homogenized using either a blender or a mortar were additionally homogenized using the Potter-Elvehjem homogenizer (Ika, Germany). Ten passages at 180 rpm were required to ensure complete homogenization of the mushroom biomass. The homogenized fungal biomass was then centrifuged at 4 °C and 11,900 × g for 30 min. 1 mL of the clear supernatant was then pipetted into a 1.5 mL centrifuge tube and frozen at -20 °C.

Commercial kits

The BCA assay (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify total proteins, the Megazyme endocellulase assay and the Megazyme endoxylanase assay (Megazyme, Bray, Ireland) were used to measure cellulase and xylanase activities, and the Protease Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure total protease activity. Cellulase, xylanase, and protease activities were determined by the protein concentration in the sample, which was the enzyme unit (EU)/mg protein.

Lipase activity

To determine the lipase activity, 50 mM 4-nitrophenyl butyrate (p-NPB) was prepared in acetonitrile as a substrate. After cleavage of the ester bonds in the substrate, a yellow product 4-nitrophenol is formed, which absorbs at 450 nm. For the lipase reaction, a lipase buffer containing 1 ml Triton X-100, 100 mM sodium phosphate and 150 mM NaCl, pH 7.2, was prepared. The aqueous mushroom extracts were thawed and incubated on ice before the experiment. First, 5 µl p-NPB and 450 µl lipase buffer were mixed and incubated at 37 °C for 10 min. Aqueous mushroom extract (45 µl) was then added, and after 10 min of incubation at 37 °C, the reaction was stopped by adding acetone (750 µl). The Eppendorf tubes were then centrifuged for 5 min at room temperature at 15,000 × g, and 200 µl of the supernatant was pipetted into the wells of a 96-well microtiter plate. For the negative control, 5 µl of lipase buffer was added instead of 5 µl of p-NPB. An additional negative control, which was prepared due to the strong absorption of p-NPB, consisted of 495 µl lipase buffer and 5 µl p-NPB. After incubation of negative control samples at 37 °C for 10 min, 750 µl acetone was added, and the mixture was centrifuged at room temperature for 5 min at 15,000 × g. 200 µl of the supernatant was pipetted into microtiter plates. The absorbance was measured using the VIS microplate reader (Dynex Technologies, Chantilly, VA, USA) at a wavelength of 450 nm. The test was repeated three times for each positive sample. Lipase activity was determined by the protein concentration in the sample as enzyme unit (EU)/mg protein. An EU is defined as the amount of sample that causes a change in absorbance at 450 nm of 1 mOD in one minute.

Table 1. Wild mushrooms were collected within this study.

Tabela 1. Divje gobe, zbrane v tej študiji.

Order	Family	Species	Bionomial name	Lifestyle[1]	Edibility[2]
Agaricales	Hygrophoraceae	*Hygrocybe conica*	*Hygrocybe conica* (Schaeff.) P. Kumm. 1871	a*	f
		Hygrocybe coccinea	*Hygrocybe coccinea* (Schaeff.) P. Kumm. 1871	a*	f
		Hygrocybe ovina	*Neohygrocybe ovina* (Bull.) Herink 1958	a*	d
		Hygrocybe citrinovirens	*Hygrocybe citrinovirens* (J.E. Lange) Jul. Schäff. 1947	a*	f
		Cuphophyllus pratensis	*Cuphophyllus pratensis* (Pers.) Bon 1985	a*	e
		Cuphophyllus fornicatus	*Cuphophyllus fornicatus* (Fr.) Lodge, Padamsee & Vizzini 2013	a*	f
		Gliophorus laetus	*Gliophorus laetus* (Pers.) Herink 1958	a*	f
		Porpolomopsis calyptriformis	*Porpolomopsis calyptriformis* (Berk.) Bresinsky 2008	a*	e

	Tricholomataceae	*Tricholoma stiparophyllum*	*Tricholoma stiparophyllum* (N. Lund) P. Karst. 1879	b	f
		Lepista nuda	*Lepista nuda* (Bull.) Cooke 1871	c	g
	Physalacriaceae	*Flammulina velutipes*	*Flammulina velutipes* (Curtis) Singer 1951	c	e
	Pluteaceae	*Volvopluteus gloiocephalus*	*Volvopluteus gloiocephalus* (DC.) Vizzini, Contu & Justo 2011	c	e
	Pleurotaceae	*Pleurotus ostreatus*	*Pleurotus ostreatus* (Jacq.) P. Kumm. 1871	a, c	e
		Pleurotus pulmonarius	*Pleurotus pulmonarius* (Fr.) Quél. 1872	a, c	e
	Schizophyllaceae	*Schizophyllum commune*	*Schizophyllum commune* Fr. 1815	a, c	f
	Marasmiaceae	*Megacollybia platyphylla*	*Megacollybia platyphylla* (Pers.) Kotl. & Pouzar 1972	c	e
	Amanitaceae	*Amanita eliae*	*Amanita eliae* Quél. 1872	b	d
		Amanita rubescens	*Amanita rubescens* Pers. 1797	b	g
		Amanita excelsa	*Amanita excelsa* (Fr.) Bertill. 1866	b	e
	Strophariaceae	*Stropharia eximia*	*Stropharia eximia* Benedix 1961	a	e
		Hypholoma fasciculare	*Hypholoma fasciculare* (Huds.) P. Kumm. 1871	a	d
	Omphalotaceae	*Gymnopus dryophilus*	*Gymnopus dryophilus* (Bull.) Murrill 1916	a	f
	Cortinariaceae	*Cortinarius bolaris*	*Cortinarius bolaris* (Pers.) Zawadzki 1835	b	f
	Nidulariaceae	*Cyathus striatus*	*Cyathus striatus* Willd. 1787	c	f
Russulales	Russulaceae	*Russula queletii*	*Russula queletii* Fr. 1872	b	f
		Russula virescens	*Russula virescens* (Schaeff.) Fr. 1836	b	e
		Russula nigricans	*Russula adusta* (Pers.) Fr. 1838	b	e
		Russula amoenolens	*Russula amoenolens* Romagn. 1952	b	f
		Russula cyanoxantha	*Russula cyanoxantha* (Schaeff.) Fr. 1863	b	e
		Lactarius torminosus	*Lactarius torminosus* (Schaeff.) Pers. 1797	b	f
		Lactarius volemus	*Lactifluus volemus* (Fr.) Kuntze 1891	b	e
		Lactarius vellereus	*Lactifluus vellereus* (Fr.) Kuntze 1891	b	f
Cantharellales	Hydnaceae	*Hydnum repandum*	*Hydnum repandum* L. 1753	b	e
	Cantharellaceae	*Cantharelus friesii*	*Cantharellus friesii* Quél. 1872	b	e
		Cantharellus cibarius	*Cantharellus cibarius* Fr. 1812	b	e
		Cantharellus amethysteus	*Cantharellus amethysteus* (Quél.) Sacc. 1887	b	e
Polyporales	Fomitopsidaceae	*Fomitopsis betulina*	*Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han & Y.C. Dai 2016	a, c	f

		Daedalea quercina	*Daedalea quercina* (L.) Pers. 1801	a, c	f
	Laetiporaceae	*Laetiporus sulfureus*	*Laetiporus sulphureus* (Bull.) Murrill 1920	a, c	g
	Polyporaceae	*Daedaleopsis confragosa*	*Daedaleopsis confragosa* (Bolton) J. Schröt. 1888	c	f
		Trametes hirsuta	*Trametes hirsuta* (Wulfen) Lloyd 1924	c	f
		Trametes versicolor	*Trametes versicolor* (L.) Lloyd 1921	c	f
		Trametes suaveolens	*Trametes suaveolens* (L.) Fr. 1838	c	f
		Trametes gibbosa	*Trametes gibbosa* (Pers.) Fr. 1838	c	f
		Fomes fomentarius	*Fomes fomentarius* (L.) Fr. 1849	a, c	f
		Neofavolus alveolaris	*Neofavolus alveolaris* (DC.) Sotome & T. Hatt. 2012	c	f
		Polyporus ciliatus	*Lentinus substrictus* (Bolton) Zmitr. & Kovalenko 2016	c	f
		Cerioporus leptocephalus	*Cerioporus leptocephalus* (Jacq.) Zmitr. 2016	c	f
	Meruliaceae	*Phlebia tremellosa*	*Phlebia tremellosa* (Schrad.) Nakasone & Burds. 1984	c	f
	Irpicaceae	*Gloeoporus taxicola*	*Meruliopsis taxicola* (Pers.) Bondartsev 1959	c	f
Auriculariales	Auriculariaceae	*Auricularia mesenterica*	*Auricularia mesenterica* (Dicks.) Pers. 1822	c	f
		Auricularia auricula-judae	*Auricularia auricula-judae* (Bull.) Quél. 1886	c	e
	Melanommataceae	*Phragmotrichum chailletii*	*Phragmotrichum chailletii* Kunze 1823	c	f
Hymenochaetales	Hymenochaetaceae	*Phylloporia ribis*	*Phylloporia ribis* (Schumach.) Ryvarden 1978	a, c	f
Boletales	Boletaceae	*Boletus reticulatus*	*Boletus reticulatus* Schaeff. 1774	b	e
		Strobilomyces strobilaceus	*Strobilomyces strobilaceus* (Scop.) Berk. 1851	b	f
		Tylopilus felleus	*Tylopilus felleus* (Bull.) P. Karst. 1881	b	f
	Tapinellaceae	*Tapinella atrotomentosa*	*Tapinella atrotomentosa* (Batsch) Šutara 1992	c	f
	Sclerodermataceae	*Scleroderma citrinum*	*Scleroderma citrinum* Pers. 1801	b	d
	Suillaceae	*Suillus luridiformis*	*Neoboletus luridiformis* (Rostk.) Gelardi, Simonini & Vizzini 2014	b	e
Gloeophyllales	Gloeophyllaceae	*Gloeophyllum odoratum*	*Gloeophyllum odoratum* (Wulfen) Imazeki 1943	c	f
Helotiales	Sclerotiniaceae	*Mitrula paludosa*	*Mitrula paludosa* Fr. 1816	c	f
	Chlorociboriaceae	*Chlorociboria aeruginascens*	*Chlorociboria aeruginascens* (Nyl.) Kanouse 1948	c	f
Pezizales	Tuberaceae	*Tuber aestivum*	*Tuber aestivum* (Wulfen) Spreng. 1827	b	e

[1] a*, parasitic biotrophs; a, parasitic; b, symbiotic; c, saprobic.

[2] d, poisonous; e, edible; f, inedible; g, conditionally edible.

Results

Of the 64 wild mushrooms examined, 23 were symbiotic fungi, and 41 had a saprobic or/and parasitic lifestyle. None of the examined mushrooms showed all four enzymatic activities, and 17 mushrooms had no enzymatic activities. In 23 mushroom samples, we could detect one or two enzymatic activities. The mushroom extracts from *Tricholoma stiparophyllum* and *Trametes* suaveolens showed three different enzymatic activities. We also investigated the correlations between the edibility (for humans) of the mushrooms and their enzymatic activities and found no correlations.

Cellulase activity

Sixteen of 64 wild mushrooms (25 %) showed cellulase activity (Figure 1), and 12 of these 16 mushrooms were not symbiotic. The highest cellulase activities were found in aqueous extracts of Fomitopsis betulina and Cyathus striatus. The mushrooms for which cellulase activity was already determined were *Pleurotus ostreatus, Pleurotus pulmonarius, Cyathus striatus, Fomitopsis betulina, Trametes versicolor, Trametes suaveolens, Trametes gibbosa* and *Auricularia auricula-judae* (Yerkes, 1967; Bhattacharjee et al., 1992; Valášková and Baldrian, 2006; Wang et al., 2014; Araújo et al., 2021; Beyisa Benti Diro et al., 2021; Montoya et al., 2021; Lu et al., 2022). Cellulase activity was determined for the first time for eight of these 16 wild mushrooms. These mushrooms were *Gliophorus laetus, Porpolomopsis calyptriformis, Tricholoma stiparophyllum, Volvopluteus gloiocephalus, Cantharelus friesii, Cantharellus amethysteus, Phragmotrichum chailletii* and *Suillus luridiformis*.

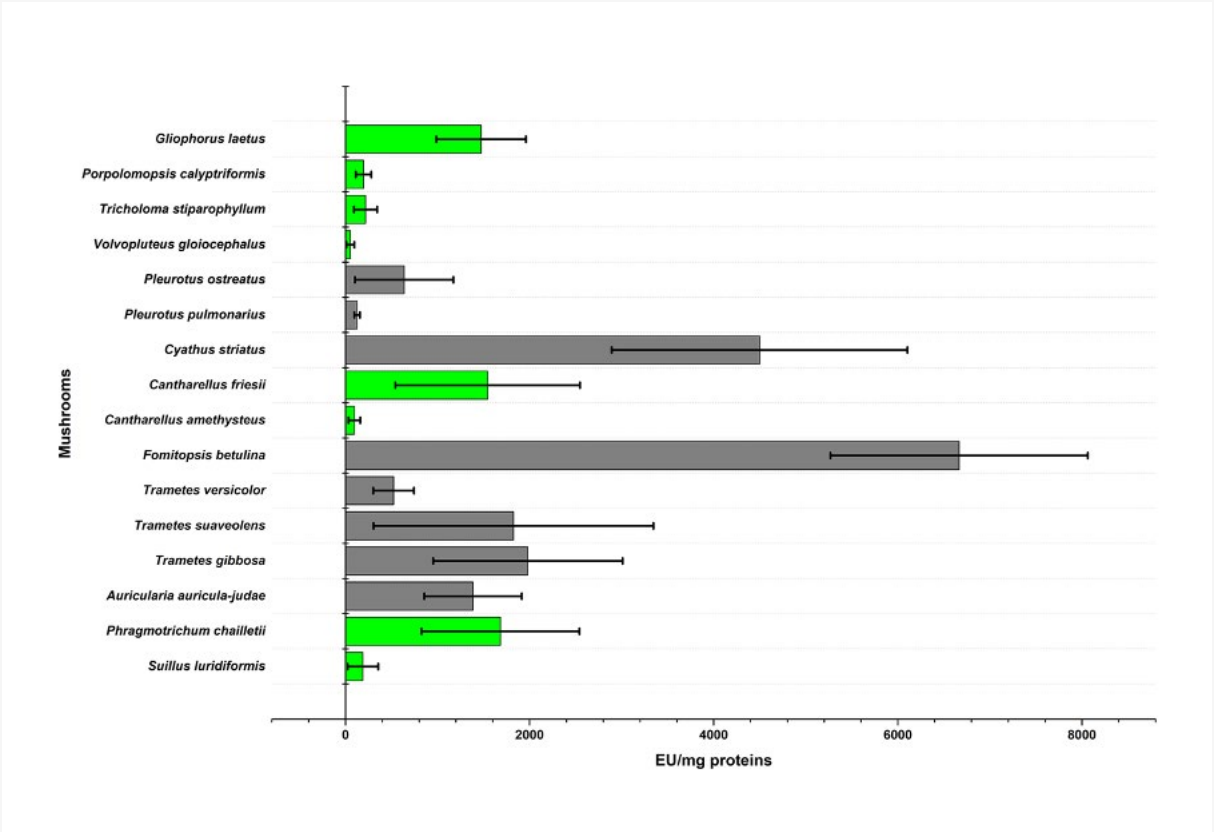

Figure 1. Aqueous extracts from wild mushrooms with detected cellulase activity. The mean values of triplicate samples with standard errors are shown. In green: the mushrooms for which cellulase activities are reported for the first time.

Slika 1. Vodni izvlečki divjih gob z ugotovljeno aktivnostjo celulaze. Prikazane so povprečne vrednosti trikratnih vzorcev s standardnimi napakami. V zeleni barvi: gobe, za katere so bile celulazne aktivnosti navedene prvič.

Xylanase activity

Twenty-four of 64 wild mushrooms (37.5 %) exhibited xylanase activity (Figure 2), of which 19 were non-symbiotic. The highest xylanase activities were found in aqueous extracts of *Phragmotrichum chailletii* and *Stropharia eximia*. The mushrooms for which xylanase activity has already been determined were *Pleurotus pulmonarius, Schizophyllum commune, Hydnum repandum, Fomitopsis betulina, Trametes versicolor* and *Trametes gibbosa* (Paice et al., 1978; Valášková and Baldrian, 2006; Inácio et al., 2015; Megersa and Alemu, 2019; Sergentani et al., 2016; Tišma et al., 2021). For 19 of these 24 wild mushrooms, we have demonstrated their xylanase activity for the first time. These mushrooms were *Hygrocybe ovina, Hygrocybe citrinovirens, Lepista nuda, Volvopluteus gloiocephalus, Amanita eliae, Stropharia eximia, Gymnopus dryophilus, Cyathus striatus, Russula nigricans, Cantharelus friesii, Trametes suaveolens, Neofavolus alveolaris, Polyporus ciliatus, Phlebia tremellosa, Gloeoporus taxicola, Phragmotrichum chailletii, Tylopilus felleus*, and *Chlorociboria aeruginascens*. The combined results of cellulase and xylanase activities of these wild mushrooms indicate that these two activities are more pronounced in non-symbiotic mushrooms.

Lipase activity

Our results show that of the four enzymatic activities tested, lipase activity is the most prevalent in the mushroom samples. Thirty of 64 wild mushrooms (46.9%) exhibited lipase activity (Figure 3), and it was somehow more pronounced in non-symbiotic mushrooms, as 19 of 30 mushrooms were non-symbiotic. *Flammulina velutipes, Daedaleopsis*

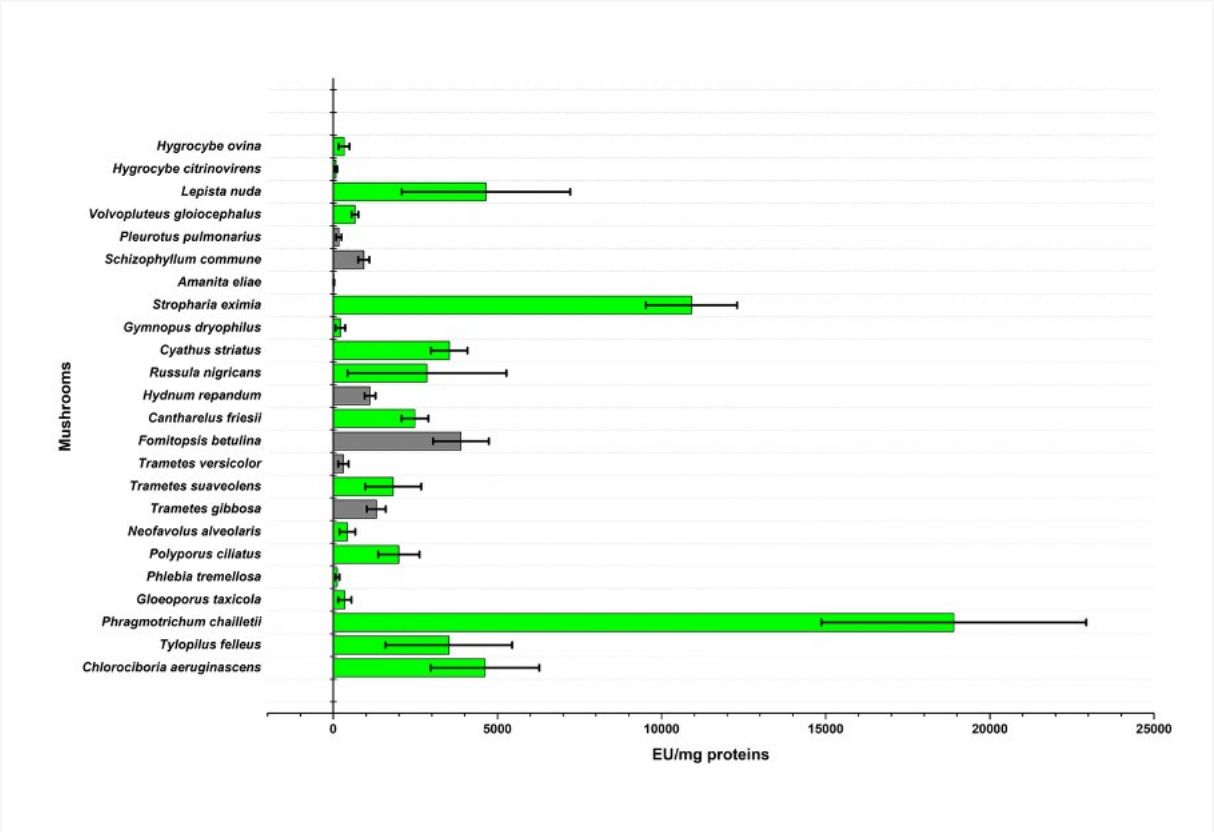

Figure 2. Aqueous extracts from wild mushrooms with detected xylanase activity. The mean values of triplicate samples with standard errors are shown. In green: the mushrooms for which xylanase activities were reported for the first time.

Slika 2. Vodni izvlečki divjih gob z ugotovljeno ksilanazno aktivnostjo. Prikazane so povprečne vrednosti trikratnih vzorcev s standardnimi napakami. V zeleni barvi: gobe, za katere so bile ksilanazne aktivnosti navedene prvič.

confragosa, Cuphophyllus fornicatus, Polyporus ciliates and *Lactarius torminosus* had the highest lipase activities. The mushrooms for which lipase activity had already been determined were *Schizophyllum commune, Amanita rubescens, Lactarius torminosus, Laetiporus sulfureus* and *Daedaleopsis confragosa* (Jaya et al., 2009; Krupodorova et al., 2014; Singh et al., 2015; Sepčić et al., 2019). We have demonstrated their lipase activity for 24 of these 30 wild mushrooms for the first time. These mushrooms were *Hygrocybe conica, Hygrocybe coccinea, Hygrocybe ovina, Cuphophyllus pratensis, Cuphophyllus fornicatus, Porpolomopsis calyptriformis, Tricholoma stiparophyllum, Lepista nuda, Flammulina velutipes, Megacollybia platyphylla, Amanita eliae, Stropharia eximia, Gymnopus dryophilus, Russula queletii, Russula virescens, Russula amoenolens, Lactarius volemus, Trametes suaveolens, Neofavolus alveolaris, Polyporus ciliatus, Phlebia tremellosa, Boletus reticulatus, Tylopilus felleus* and *Suillus luridiformis*. In addition, the five mushrooms that exhibited protease activity also showed lipase activity.

Protease activity

Five out of 64 wild mushrooms (7.8 %) showed protease activity (Figure 4). Four of the five mushrooms that showed protease activity were non-symbiotic fungi. The highest protease activity was found in the aqueous extract of *Daedaleopsis confragosa*. For all these five wild mushrooms, we are the first to detect their protease activity. These mushrooms were *Hygrocybe conica, Hygrocybe coccinea, Cuphophyllus pratensis, Tricholoma stiparophyllum* and *Daedaleopsis confragosa*.

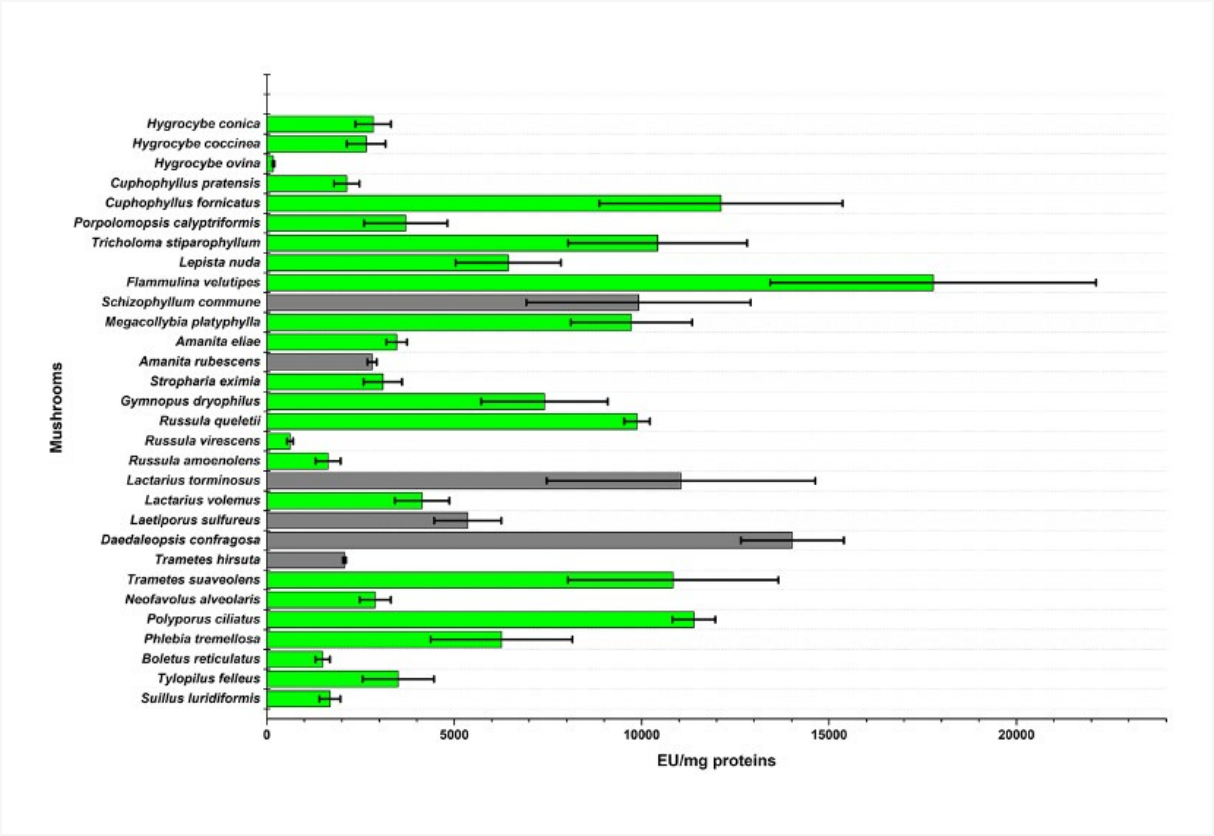

Figure 3. Aqueous extracts from wild mushrooms with detected lipase activity. The mean values of triplicate samples with standard errors are shown. In green: the mushrooms for which lipase activities are reported for the first time.

Slika 3. Vodni izvlečki divjih gob z ugotovljeno aktivnostjo lipaze. Prikazane so povprečne vrednosti trikratnih vzorcev s standardnimi napakami. V zeleni barvi: gobe, za katere so prvič poročali o aktivnosti lipaz.

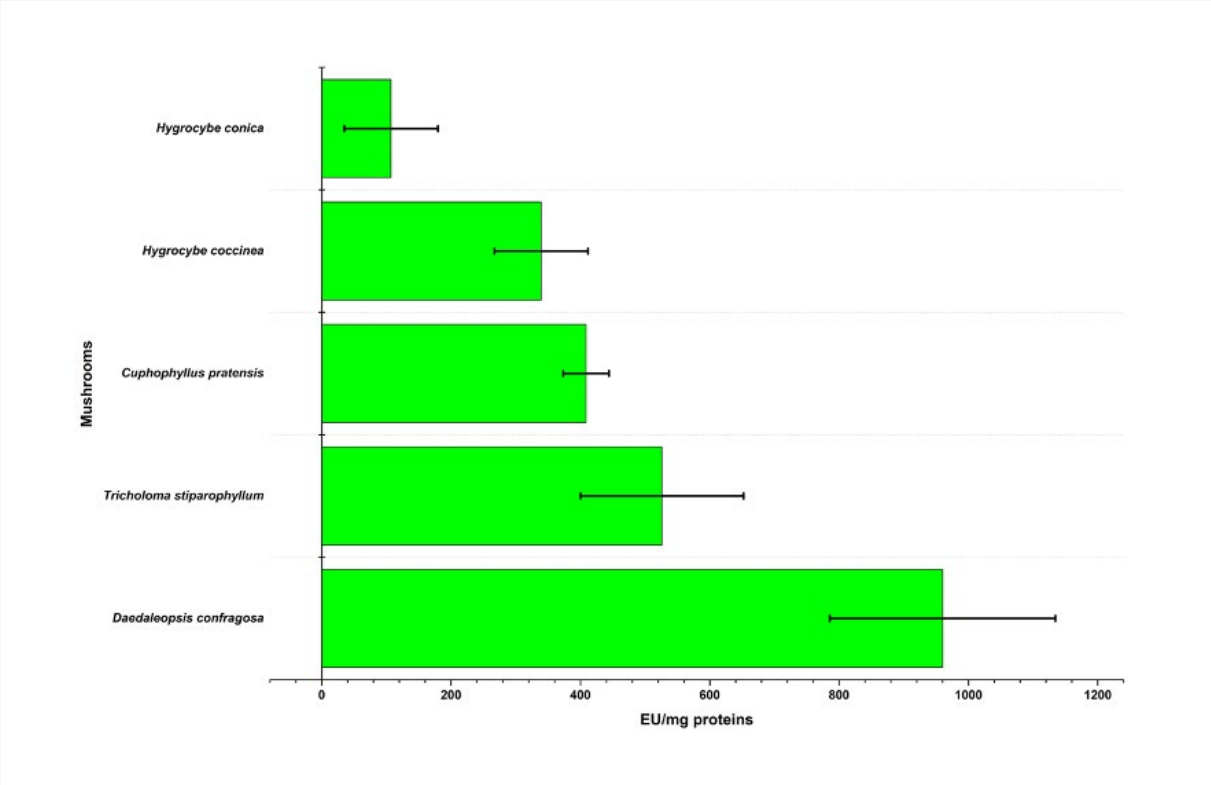

Figure 4. Aqueous extracts from wild mushrooms with detected protease activity. The mean values of triplicate samples with standard errors are shown. In green: the mushrooms for which protease activities are reported for the first time.

Slika 4. Vodni izvlečki divjih gob z ugotovljeno proteazno aktivnostjo. Prikazane so povprečne vrednosti trikratnih vzorcev s standardnimi napakami. V zeleni barvi: gobe, za katere so proteazne aktivnosti navedene prvič.

Discussion

Microbial hydrolases, including those from fungi, are the most important source of industrial enzymes. Fungal-derived enzymes account for more than 50% of the total enzyme market, and most of these enzymes are derived from moulds (El-Gendi et al., 2021). Almost 50% of these enzymes are produced by recombinant host strains, and most of them are also moulds (Arnau et al., 2019). Moulds are preferred enzyme factories because they can be grown in relatively inexpensive substrates under submerged conditions (Arnau et al., 2019). Since these enzymes are secreted extracellularly by moulds, they are relatively easy to separate from the liquid culture media. However, this does not apply to saprobic mushrooms, which have to be cultivated on solid culture media. The extraction of industrial enzymes from moulds is, therefore, less time-consuming, labour-intensive and more economically efficient than the extraction of enzymes from substrates overgrown with mushroom mycelia. While saprobic mushrooms can at least be cultivated on solid culture media, this is not always possible with symbiotic mushrooms, as only a few of them can be cultivated under laboratory conditions.

Alternative solutions for the production of mushroom enzymes should, therefore, be explored. One of the possible solutions is the cloning of genes from the producing mushrooms into suitable host(s). For this purpose, genome sequencing of mushrooms with high enzymatic activity should be performed. The target genes should be annotated, cloned and expressed in suitable microbial or fungal hosts, and the activity of these recombinant enzymes should be evaluated. By using recombinant technology to produce these enzymes in a host organism, it is possible to obtain large quantities of enzymes with the desired properties, which could lead to more efficient and sustainable industrial processes. In this context, Phragmotrichum chail-

letii and Stropharia eximia are proving to be a source of potent xylanases, Flammulina velutipes a source of potent lipase and Daedaleopsis confragosa a source of potent lipase and protease.

Although new fungal species have been identified in this work as producers of important industrial enzymes, it is not possible to compare these activities with the activities of pure enzymes derived from moulds grown under laboratory conditions. This is because these industrial mould strains grow in inducible substrates that trigger higher production of the enzymes. While the industrial enzymes produced by moulds are extracted directly from the liquid medium in which the moulds are grown, the extracts used in our study were derived directly from the producing organism. Since most symbiotic mushrooms cannot grow under laboratory conditions, recombinant expression of the target genes is the only solution for the evaluation and possible production of these enzymes on an industrial scale.

Conclusions

A growing number of industrial processes are replacing chemical-based solutions with biological-based solutions, which require new and effective enzymes. Our study, therefore, focused on the search for enzymes in higher fungi growing in nature, namely those that form fruiting bodies, which we believe represent an underestimated source of previously unexplored industrially important enzymes. The purpose of our study was to determine the cellulase, xylanase, lipase, and protease activities of 64 mushrooms collected in nature. This is the first report on the ability of many of these mushrooms to produce these hydrolytic enzymes. The findings of this study provide valuable insight into the occurrence of cellulases, xylanases, lipases, and proteases in wild mushrooms. They highlight, in particular, the diversity of mushrooms that can produce these enzymes. The use of recombinant technology to introduce genes encoding cellulase, xylanase, lipase and protease from mushrooms into a host organism may have various applications, particularly in industries such as biotechnology, agriculture, biofuel production and medicine.

Author Contributions

Conceptualization, M.S.; methodology, M.S. and K.S; validation, M.S.; formal analysis, I.P. and L.L.P; investigation, L.Š., L.L.P, I.P., M.H. and D.F.; resources, M.S. and K.S.; data curation, M.S.; writing—original draft preparation, M.S.; writing—review and editing, M.S., L.Š., I.P., M.H., D.F. and K.S.; visualization, L.P.P.; supervision, M.S.; project administration, M.S.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability

All raw or analysed data are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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