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Original Research

Further Studies of Extracellular Enzyme Profiles of Xerophilic Fungi Isolates from Dried Medicinal Plants

K. Janda[1], K. Ulfig[2]*, A. Markowska-Szczupak[3]

[1]Department of Microbiology and Environmental Biotechnology, Agriculture University of Szczecin, Słowackiego 17, 71-434 Szczecin, Poland
[2]Division of Biomaterials and Microbiological Technologies, Polymer Institute, Szczecin University of Technology, Pułaskiego 10, 70-322 Szczecin, Poland
[3]Institute of Chemical and Environmental Engineering, Department of Biotechnology, Szczecin University of Technology, Pułaskiego 10, 70-322 Szczecin, Poland

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Abstract

The contamination of dried medicinal plants with microscopic fungi has been the subject of many studies. However, no data on extracellular enzyme activities of xerophilic fungi contaminating the plants have been found in the literature. Therefore, the objective of our study was to determine extracellular enzyme profiles of fast-growing xerophilic fungi, i.e. *Aspergillus flavus, A. fumigatus, A. melleus, A. nidulans, A. niger, A. parasiticus* and *Trichothecium roseum* isolated from dried medicinal plants from herbal shops in Szczecin, Poland. Solid media and the API ZYM® test were used for measuring enzyme activities. Among the fungi, *A. melleus* had the highest hydrolytic activity on milk, gelatin, starch, tributyrin, rapeseed oil and biodiesel oil agars, while *A. fumigatus* showed extremely high stimulation index values on rapeseed oil and biodiesel oil agars. The stimulation index increased during a 5-day incubation period. In the API ZYM® test *A. nidulans* showed the highest hydrolase activity. Among the hydrolases, β-glucosidase activity was the highest, followed by acid phosphatase, N-acetyl-β-glucosaminidase and naphthol-AS-BI-phosphohydrolase activities. The fungi contaminating dried medicinal plants are able to utilize a number of substrates and, therefore, possess high biodeterioration potential. Due to the ability to degrade hydrocarbons, fungal isolates from dried medicinal plants can be used for biotechnological purposes, e.g. in air biofiltration and waste or soil bioremediation.

Keywords: xerophilic fungi, *Aspergillus flavus*, *A. fumigatus*, *A. melleus*, *A. nidulans*, *A. niger*, *A. parasiticus*, *Trichothecium roseum*, extracellular enzymes, solid media, API ZYM®, dried medicinal plants

Introduction

Dried medicinal plants are widely used for prophylaxis or treatment of many diseases. About 100,000 tons of herbs are processed throughout the world annually. Poland, with 15,000-20,000 tons of herbs processed per year, is the leader in the medicinal plant market [1]. However, the problem of dried medicinal plant contamination with microscopic fungi is still poorly understood. This contamination may result in: (1) biodeterioration of plant material; (2) production of secondary metabolites, including mycotoxins; and (3) propagation of isolates

*e-mail: k_ulfig@zut.edu.pl

with potential pathogenic properties to humans and animals. Low water activity favors the growth of xerophilic fungi in dried medicinal plants. Although plant contamination with these fungi has been the subject of many studies [2-10], no data on extracellular enzyme activities of these organisms have been found in the literature. The extracellular enzymes of xerophilic fungi may degrade medically important components of dried medicinal plants and contribute to the propagation of toxigenic and pathogenic strains. In a previous study [11], extracellular enzyme activity profiles of slow-growing fungi isolated from dried medicinal plants from herbal shops in Szczecin, Poland, were presented. The goal of the present study was to examine extracellular enzyme profiles of fast-growing fungi, i.e. *Aspergillus flavus, A. fumigatus, A. melleus, A. nidulans, A. niger, A. parasiticus* and *Trichothecium roseum* isolated from the same material using solid media and API ZYM® test.

Material and Methods

Dried medicinal plant samples were taken from herbal shops of Szczecin, Poland. The samples were chamomile, peppermint, lemon balm, St. John's wort and two herbal mixtures. The herbal mixture samples were obtained in one portion, while the other samples were prepared by mixing many infusion bags. The samples consisted of small plant pieces. The amount of each sample was ca. 0.5 kg. The samples were kept in hermetically closed glass jars at 4ºC for 24 hours and then examined for microscopic fungi using MEA, MEA20, MEA40, SGA, DG18 and YpSs agars. The dilution plating method along with direct inoculation of herb pieces on the media were applied. The mycological characteristics of the dried medicinal plant samples were given in previous papers [2, 5]. The fungal species selected for this investigation were *Aspergillus flavus* (2 isolates), *A. fumigatus* (5 isolates), *A. melleus* (1 isolate), *A. nidulans* (2 isolates), *A. niger* (5 isolates), *A. parasiticus* (4 isolates) and *Trichothecium roseum* (2 isolates).

Amylase production was tested on Bacto nutrient agar (Difco) containing 0.2% soluble starch [12]. Casein hydrolysis was detected on the medium containing defatted and powdered milk [13]. Subsequently, the Frazier medium was used for gelatinase [14]. Cellulolytic activity was tested on Bravery's mineral salts medium supplemented with crystalline cellulose [15]. Media for testing fatty substrates (tributyrin, rapeseed oil, biodiesel oil and diesel oil) were those of Janda-Ulfig et al. [16].

The method for catalase was that of Bordner and Winter [17]. Urease production was tested on Christensen's urea agar [18]. Deoxyribonuclease activity was detected on DNA-se test medium (Difco). Finally, pectate lyase and polygalacturonase activities were tested on the medium by Hankin and Anagnostakis [12].

Ten-day fungal cultures on MEA slants at 25ºC were used for preparing spore suspensions. Five ml of sterile physiological saline was added to each slant. The slants were then vigorously shaken with a Vortex for three minutes. The plates were each centrally inoculated with 5 µl of spore suspension and incubated at 25ºC for 5 days. After 2, 3, 4 and 5 days of incubation, colony and hydrolysis zone diameters were measured on starch, milk, gelatin, cellulose, tributyrin, rapeseed oil and biodiesel agars. Only colony diameter was measured on diesel agar. The indices calculated were: (1) hydrolytic activity index on starch, milk, gelatin, cellulose, tributyrin, rapeseed oil and biodiesel oil agars (hydrolysis zone diameter/colony diameter ratio); and (2) stimulation/inhibition index for fatty substrata (colony diameter on fatty substrate agar/colony diameter on control agar ratio). Control agars were devoid of fatty substrata. The experiment was verified in triplicate. Statistical analysis of the data obtained was performed with one-way ANOVA test at $p \leq 0.05$.

Catalase activity was evaluated using the scale:
(+) – weak reaction (single bubbles);
(++) – moderate reaction;
(+++) – strong reaction (abundant bubbles).

The scale for urease, DNA-se, pectate lyase and polygalacturonase activities was the following:
(-) – no activity;
(+) – weak activity (hydrolysis zone width <2 mm);
(++) – moderate activity (hydrolysis zone width 2-5 mm);
(+++) – strong activity (hydrolysis zone width >5 mm).

Activities of 19 hydrolases were measured with the API ZYM® test (bioMèrieux, France); following the instruction of the producer.

Results

After a 5-day incubation period the highest colony diameters were observed in *A. niger* on starch, rapeseed oil, biodiesel oil and diesel oil agars, and in *A. fumigatus* and *T. roseum* on milk and gelatin agars, respectively (Table 1). *A. flavus* and *A. parasiticus* had the highest diameters on tributyrin and cellulose agars, respectively. Except for *A. fumigatus* on milk agar and *A. parasiticus* on cellulose, the other differences in the colony diameter were statistically significant at $p \leq 0.05$. The differences in the colony diameter between *A. fumigatus* and *A. flavus* and *T. roseum* on milk agar and between *A. parasiticus* and *A. flavus* on cellulose agar were not statistically significant.

No hydrolysis zones were observed in *A. fumigatus* and *A. nidulans* on milk agar. In *A. melleus* the casein hydrolysis zone diameter was found to be slightly wider than the colony diameter (index >1; Table 2). In the other species casein hydrolysis zones were observed underneath the colonies (index ≤1). The differences in the caseinase activity index between *A. melleus* and the other species (except for *A. nidulans*) were statistically significant at $p \leq 0.05$. Except for *A. niger*, the other species had indices >1 on gelatin agar. In *A. niger*, no gelatin hydrolysis zone was observed, while *A. melleus* had the highest index (2.1) on this medium. The differences in the gelatinase activity index between *A. melleus* and the other species were statistically significant at $p \leq 0.05$. *A. flavus*, *A. melleus* and *A. parasiticus* showed the index =1 on starch agar. The other species had indices slightly <1 on this medium.

Table 1. Colony diameters of fungal species isolated from dried medicinal plants after a 5-day incubation at 25ºC on agar media with different substrates.

Fungal species	Colony diameter [mm] on agar media with:							
	Milk	Gelatin	Starch	Cellulose	Tributyrin	Rapeseed oil	Biodiesel oil	Diesel oil
A. flavus	42.8 ± 1.3*	32.5 ± 2.9	39.8 ± 0.5	36.3 ± 3	20 ± 0.8	42 ± 1.4	42.8 ± 3.2	16.8 ± 1
A. fumigatus	44 ± 1.3	32.5 ± 1.2	40.5 ± 2.5	34 ± 2.8	14.5 ± 1.1	29.4 ± 1.7	28.2 ± 0.9	7.5 ± 0.5
A. melleus	25 ± 0	21 ± 0	29 ± 1.4	20.5 ± 0.7	15 ± 1.4	30 ± 0	25 ± 0	16.5 ± 0.7
A. nidulans	35 ± 0	26 ± 1.4	29.5 ± 0.7	30.5 ± 0.7	16 ± 0	24 ± 0	28 ± 0	10 ± 0
A. niger	38.4 ± 3	31.7 ± 3.5	48.6 ± 3.2	30.3 ± 4.4	13.1 ± 1.1	57 ± 7.6	53.8 ± 8.3	36.5 ± 8.1
A. parasiticus	37.9 ± 3.2	34.3 ± 2.4	38.5 ± 1.8	37.8 ± 2.9	17.9 ± 1.5	45 ± 9.6	41.9 ± 7.4	14.6 ± 1.5
T. roseum	41 ± 1.4	38.8 ± 4.3	41.3 ± 3.8	30 ± 4.1	12.3 ± 2.2	21.5 ± 2.4	24.5 ± 0.6	5.5 ± 0.6

* - mean ± standard deviation

Table 2. Hydrolytic activity indices of fungal species isolated from dried medicinal plants after a 5-day incubation at 25ºC on agar media containing different substrates.

Fungal species	Hydrolytic activity indices on agar media containing:					
	Milk	Gelatin	Starch	Tributyrin	Rapeseed oil	Biodiesel oil
A. flavus	0.8 ± 0.1*	1.3 ± 0.1	1 ± 0.1	1.1 ± 0	1.5 ± 0.2	1.1 ± 0.1
A. fumigatus	0	1.1 ± 0	0.9 ± 0.1	0	1 ± 0	1 ± 0
A. melleus	1.1 ± 0	2.1 ± 0.1	1 ± 0	1.3 ± 0	1.6 ± 0.1	1.5 ± 0.1
A. nidulans	0	1.5 ± 0	0.9 ± 0.1	1.3 ± 0.1	1 ± 0	1 ± 0
A. niger	0.9 ± 0.1	0	0.9 ± 0	0	1 ± 0	0.8 ± 0.1
A. parasiticus	0.8 ± 0	1.3 ± 0.1	1 ± 0	1.1 ± 0.1	1.4 ± 0.3	1.1 ± 0.1
T. roseum	1 ± 0	1.3 ± 0.1	0.9 ± 0.1	1.1 ± 0	1 ± 0	1 ± 0

* - mean ± standard deviation

The differences in the amylase activity index between *A. flavus*, *A. melleus* and *A. parasiticus* and the other species were statistically significant at $p \leq 0.05$.

No hydrolytic activity was observed in *A. fumigatus* and *A. niger* on tributyrin agar. *A. nidulans* and *A. melleus* had the highest tributyrin hydrolysis index (1.3). The differences in this index between *A. nidulans* and *A. melleus* and the other species were statistically significant at $p \leq 0.05$. In *A. melleus*, *A. flavus* and *A. parasiticus* the lipolytic activity index on rapeseed oil agar ranged from 1.4 to 1.6., while *A. fumigatus*, *A. nidulans*, *A. niger* and *T. roseum* showed the index =1 on the medium. The differences in the lipolytic activity index between the first three species and the others were statistically significant at $p \leq 0.05$. The highest hydrolytic activity index on biodiesel agar was observed in *A. melleus* (1.5). *A. fumigatus*, *A. nidulans* and *T. roseum* had the index =1, while *A. niger* was the only species with the index <1 on the medium. The differences in the biodiesel hydrolysis index between *A. melleus* and the other fungi were statistically significant at $p \leq 0.05$. The isolates did not show enzymatic activity zones on cellulose and diesel agars. Hydrolytic activity indices did not considerably change during incubation.

Tributyrin inhibited the growth of all isolates (inhibition/stimulation index <1; Table 3) and *T. roseum* presented the lowest inhibition index. The differences in the inhibition index between *T. roseum* and the other species were statistically significant at $p \leq 0.05$. Diesel oil slightly stimulated the growth of *A. fumigatus*, *A. flavus* and *A. parasiticus* (index >1), and inhibited the growth of the other species. The differences in the inhibition/stimulation index between the three species and the others were statistically significant at $p \leq 0.05$. In contrast, rapeseed and biodiesel oils stimulated the growth of all species (index >1). The highest stimulation indices on rapeseed oil and biodiesel oil agars were observed in *A. fumigatus* (4.6 and 4.4). The differences in the index between the species and the others were statistically significant at $p \leq 0.05$. Additionally, the increase of the index was observed on all fatty substrates during a 5-day incubation period. *A. flavus*, *A. fumigatus*, and *A. parasiticus* showed the highest index increases. On diesel agar the indices of the three species were negative up to the 2nd or 3rd day to become positive during further incubation.

A. flavus, *A. melleus* and *A. parasiticus* showed high catalase activity (Table 4). In *A. niger* the activity varied

Table 3. Inhibition/stimulation indices on fatty substrates for fungal species isolated from dried medicinal plants.

Substrates	Incubation day	Inhibition/stimulation index for species:						
		A. flavus	*A. fumigatus*	*A. melleus*	*A. nidulans*	*A. niger*	*A. parasiticus*	*T. roseum*
Tributyrin	2	0.6 ± 0.1*	0.3 ± 0.1	0.5 ± 0	0.6 ± 0	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.1
	3	0.7 ± 0	0.4 ± 0	0.5 ± 0	0.6 ± 0.1	0.4 ± 0	0.8 ± 0.2	0.3 ± 0
	4	0.7 ± 0.1	0.5 ± 0	0.5 ± 0.1	0.6 ± 0	0.5 ± 0.1	0.7 ± 0.1	0.3 ± 0
	5	0.8 ± 0	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.4 ± 0	0.8 ± 0.2	0.3 ± 0.1
Rapeseed oil	2	1.9 ± 0.7	2 ± 0.6	1.2 ± 0.1	1.1 ± 0.2	1 ± 0.1	1.6 ± 0.2	2.2 ± 0.3
	3	2 ± 0.2	2.7 ± 0.5	1.1 ± 0.2	1.2 ± 0	1.2 ± 0.1	2.2 ± 0.4	2.3 ± 0.5
	4	2.5 ± 0.3	3.7 ± 0.7	1.4 ± 0	1.5 ± 0	1.2 ± 0.1	2.6 ± 0.6	2.1 ± 0.8
	5	3 ± 0.3	4.6 ± 0.6	1.7 ± 0	1.9 ± 0	1.3 ± 0.1	3.4 ± 0.6	2.3 ± 0.9
Biodiesel oil	2	1.6 ± 0.2	2 ± 0.6	1.1 ± 0.1	0.9 ± 0	0.9 ± 0.1	1.7 ± 0.2	2.5 ± 0.6
	3	2.2 ± 0.2	2.8 ± 0.5	1 ± 0.1	1.4 ± 0	1.3 ± 0.2	2.1 ± 0.4	2.2 ± 0.6
	4	2.6 ± 0.3	3.8 ± 0.6	1.3 ± 0.1	1.8 ± 0.1	1.3 ± 0.2	2.9 ± 0.5	2.3 ± 0.9
	5	3.1 ± 0	4.4 ± 0.5	1.4 ± 0	2.2 ± 0	1.2 ± 0.1	3.1 ± 0.4	2.7 ± 1.3
Diesel oil	2	0.8 ± 0.1	0.9 ± 0.2	0.6 ± 0	0.4 ± 0	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.1
	3	1 ± 0.1	1 ± 0.1	0.8 ± 0	0.5 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	0.6 ± 0.3
	4	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0	0.7 ± 0	0.9 ± 0.2	1.1 ± 0.1	0.5 ± 0.3
	5	1.2 ± 0	1.2 ± 0.1	0.9 ± 0	0.8 ± 0	0.8 ± 0.1	1.1 ± 0.1	0.6 ± 0.3

* - mean ± standard deviation

from weak to strong, while in *A. fumigatus*, *A. nidulans* and *T. roseum* no or weak catalase activity was observed. *A. flavus* and *A. parasiticus* had the strongest urease activity, while *A. melleus* and *A. nidulans* displayed the moderate activity of this enzyme. *A. niger* showed no urease activity, while in *A. fumigatus* the activity varied from none to weak. All species displayed DNA-se activity, which varied from weak (in *A. fumigatus*, *A. melleus*, *A. nidulans*, *A. niger* and *T. roseum*) to moderate (in *A. flavus* and *A. parasiticus*). The strongest pectate lyase activity was observed in *A. melleus*. In *A. niger* the activity varied from moderate to strong, while in *T. roseum* it was moderate. The other species showed weak pectate lyase activity. The fungi showed no polygalacturonase activity.

Naphthol-AS-BI-phosphohydrolase, β-glucosidase and N-acetyl-β-glucosaminidase activities were observed in all species (Table 5). *T. roseum* did not show acid phosphatase activity, whereas alkaline phosphatase activity was not observed in *A. niger*. *A. flavus*, *A. parasiticus* and *T. roseum* did not display esterase (C_4) and esterase lipase (C_8) activities. Leucine arylamidase and valine arylamidase activities were not detected in *A. niger* and *T. roseum*. *A. nidulans* was the only species showing cystine arylamidase activity. *A. nidulans* and *A. niger* were the two species with α-galactosidase activity. *A. flavus*, *A. parasiticus* and *T. roseum* had no α-glucosidase activity, while *A. fumigatus*, *A. nidulans* and *T. roseum* did not display β-galactosidase activity. Finally, *A. flavus* was the only species with α-mannosidase, α-fucosidase and trypsin-like activities.

Discussion

In general, the isolates from dried medicinal plants showed proteolytic activity. However, these isolates showed no or weak caseinase activity (index from 0 to 1.1) compared to *A. flavus*, *A. niger* and *A. parasiticus* isolates from peanuts and coconuts [18]. Subsequently, the *A. melleus* gelatinase activity index was the highest (2.1), while the index for the other species ranged from 0 to 1.5. Similar gelatinase activities were observed by Gobinath et al. [20] for *A. flavus*, *A. fumigatus*, *A. nidulans* and *A niger* isolates from oil-rich environments.

In comparison with *A. flavus*, *A. nidulans* and *A. niger* isolates from oil-rich environments [20], the isolates from dried medicinal plants showed low amylolytic activity (index from 0.9 to 1). In the quoted study, however, no amylolytic activity of *A. fumigatus* was detected, while in the present study the activity of this fungus was found to be low but clear. The results have confirmed the production of α-amylases, the enzymes crucial in starch hydrolysis, by *A. flavus, A. fumigatus, A. niger* and other fungi [21].

The fungi grew on crystalline cellulose agar but no hydrolysis zones were formed by all isolates examined. To the contrary, other workers observed clear hydrolysis zones on cellulose agars formed by *A. flavus, A. fumigatus, A. nidulans, A. niger* and other species [20, 23]. This may be explained by rather low cellulolytic activity of isolates from dried medicinal plants.

The esterase activity index ranged from 0 to 1.3 and from 0.8 to 1.5 on tributyrin and biodiesel oil agars, respectively. The highest esterase activity indices were observed in *A. melleus* and *A. nidulans* (index 1.3 for both species) and in *A. melleus* (index 1.5) on tributyrin and biodiesel oil agars, respectively. The esterase activity of the fungi should be assessed as low to moderate compared with the index values found in the literature [20].

The lipolytic activity of *A. melleus*, *A. flavus* and *A. parasiticus* on rapeseed oil was found to be moderate (index from 1.4 to 1.6), while *A. fumigatus*, *A. nidulans*, *A. niger* and *T. roseum* showed low activity (index =1) on this medium. In general, the results have confirmed the lipolytic activity of the above-mentioned species [24, 25].

There is a vast body of papers on the degradation of both aliphatic and aromatic hydrocarbons by microscopic fungi.

Table 4. Catalase, urease, DNA-se, pectate lyase and polygalacturonase activities of fungal species isolated from dried medicinal plants.

Fungal species	Catalase	Urease	DNA-se	Pectate lyase	Polygalacturonase
A. flavus	+++[1]	+++[2]	++[2]	+[2]	-[2]
A. fumigatus	-/+	-/+	+	+	-
A. melleus	+++	++	+	+++	-
A. nidulans	+	++	+	+	-
A. niger	+/+++	-	+	++/+++	-
A. parasiticus	+++	+++	++	+	-
T. roseum	+	+	+	++	-

[1] (+) – weak reaction; (++) – moderate reaction; (+++) – strong reaction;
[2] (-) - no activity; (+) - weak activity (hydrolysis zone width <2 mm); (++) – moderate activity (hydrolysis zone width 2-5 mm); (+++) – strong activity (hydrolysis zone width >5 mm).

Table 5. Production of 19 hydrolases by fungal species isolated from dried medicinal plants in the APIZYM® test (bioMèrieux). Rounded up means in the test color scale.

Hydrolases	*A. flavus*	*A. fumigatus*	*A. melleus*	*A. nidulans*	*A. niger*	*A. parasiticus*	*T. roseum*
Phosphatase alkaline	3	1	3	2	-	1	1
Esterase (C_4)	-	2	1	2	1	-	-
Esterase Lipase (C_8)	-	1	1	1	1	-	-
Lipase (C_{14})	-	-	-	-	-	-	-
Leucine arylamidase	1	1	1	2	-	1	-
Valine arylamidase	1	1	1	3	-	1	-
Cystine arylamidase	-	-	-	1	-	-	-
Trypsin	1	-	-	-	-	-	-
Chymotrypsin	-	-	-	-	-	-	-
Phosphatase acid	3	3	2	3	3	1	-
Naphthol-AS-BI-phosphohydrolase	2	2	2	3	2	1	1
α-galactosidase	-	-	-	1	2	-	-
β-galactosidase	2	-	1	-	1	1	-
β-glucuronidase	-	-	-	-	-	-	-
α-glucosidase	-	1	2	1	1	-	-
β-glucosidase	3	2	3	5	3	1	2
N-acetyl-β-glucosaminidase	3	2	3	1	2	2	2
α-mannosidase	1	-	-	-	-	-	-
α-fucosidase	1	-	-	-	-	-	-

Among the fungi, *A. niger* has been frequently recorded as a hydrocarbon degrader [26, 27]. In the present study, the *A. niger* isolates had the highest colony diameter on diesel oil agar. This finding indicates that the isolates were presumably able to utilize aliphatic hydrocarbons of diesel oil.

Tributyrin and/or the products of its biodegradation inhibited the growth of all fungi, while diesel oil slightly stimulated the growth of *A. fumigatus*, *A. flavus* and *A. parasiticus*. In contrast, rapeseed and biodiesel oils stimulated the growth of all species. In addition, the index increased during a 5-day incubation period on all fatty substrates. It is noteworthy that *A. fumigatus* showed low hydrolysis index values on rapeseed oil and biodiesel oil but had extremely high stimulation indices on the oils (4.6 and 4.4). This finding indicates that both hydrolytic activities and inhibition/stimulation index values should be considered while evaluating the ability of fungal isolates to use fatty substrata for growth.

The isolates were found to produce catalase, urease and DNA-se. The highest activities of these enzymes were observed in *A. flavus* and *A. parasiticus*. The fungi were also found to produce pectate lyases but no polygalacturonase activities were detected on the medium used [12, 28]. The highest pectate lyase activity was found in *A. melleus* and *A. niger*.

The API ZYM® test complemented the results obtained with two other methods. *A. nidulans* showed the highest hydrolase activity in the test. The fungi were characterized by the production of most enzymes taking part in carbohydrate hydrolysis (α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase). The *A. flavus* and *A. fumigatus* isolates from dried medicinal plants showed lower hydrolase activities compared to the isolates from clinical sources [29]. Interestingly, higher hydrolytic activities were also observed in dermatophyte isolates from clinical sources compared with activities of keratinolytic fungi from soil [30].

A. fumigatus is the species of special epidemiological concern [31]. *A. flavus* and *A. parasiticus* have the ability to produce aflatoxins [32-36]. *T. roseum* is one of the trichothecene-producing fungi, producing crotocin [25] but the fungus has been rarely isolated from dried medicinal plants. Finally, *A. niger* widely occurs in these plants but it is generally considered safe [37].

In conclusion, different environmental conditions provided by isolation sources might be responsible for differences in fungal hydrolytic activities. Differences in extracellular enzyme profiles between fungal species from the plants were also observed. Among the fungi examined, *A. melleus* showed the highest hydrolytic activity. However, this species has rarely been recorded from plant material [2, 3]. In contrast, *A. fumigatus, A. flavus, A. parasiticus*, and *A. niger* have all been found to be widely distributed in this material. Although no or low activities were demonstrated for some hydrolases and species, entire fungal consortia produce a number of extracellular hydrolases; being able to utilize all major components of plant material (proteins, lipids, deoxyribonucleic acid, and different carbohydrates). The literature data indicate that these organisms maintain their activities under depressed water activity [38]. Therefore, the biodeterioration potential of the fungi in relation to dried medicinal plants should be assessed as high. The results described here also indicate that due to the ability to degrade hydrocarbons, the fungi can be used for biotechnological purposes, e.g. in air biofiltration and waste or soil bioremediation. Finally, some fungi contaminating dried medicinal plants also possess pathogenic potential, being mycotoxin producers and agents responsible for mycoses.

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