Original paper

Enzymatic activity of fungi isolated from crops

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Adv Dermatol Allergol 2016; XXXIII (6): 457–463
DOI: 10.5114/ada.2016.63885

Abstract

Aim: To detect and assess the activity of extracellular hydrolytic enzymes and to find differences in enzymograms between fungi isolated from wheat and rye samples and grown on Czapek-Dox Broth and Sabouraud Dextrose Broth enriched with cereal (wheat or rye). Isolated strains were also classified in the scale of biosafety levels (BSL).
Material and methods: The study used 23 strains of fungi cultured from samples of wheat and rye (grain, grain dust obtained during threshing and soil) collected in the Lublin region (eastern Poland). API ZYM test (bioMérieux) was carried out according to the manufacturer's instructions. Classification of BSL (Biosafety levels) was based on the current literature.
Results: High enzymatic activity was found in strains cultured in media containing 1% of wheat grain (*Bipolaris holmi, Penicillium decumbens*) and with an addition of 1% of rye grain (*Cladosporium herbarum, Aspergillus versicolor, Alternaria alternata*). The total number of enzymes varied depending on the type of media, and in most cases it was higher in the culture where an addition of cereal grains was used.
Conclusions: Isolated strains of fungi reveal differences in the profiles of the enzyme assay. It can be assumed that the substrate enriched in grains stimulate the higher activity of mold enzymes.

Key words: enzymatic activity, mold fungi, zymogram, biohazards.

Introduction

API ZYM test (bioMérieux) is a semiquantitative assay allowing to assess the presence and activity of 19 hydrolytic enzymes. The release of hydrolytic enzymes into the environment by dermatophytes, yeasts, and molds is an important component in the pathogenesis of infection. Hydrolases (esterases, sulfatases, glycosidases and peptidases) facilitate the degradation of keratin, and thus the penetration of the mycelium into tissues [1–3].

The isolated and identified strains were also assigned to specific classes of biosafety. The Classification of Biosafety Level (BSL) determines the safety scale of potentially pathogenic fungi for humans and animals by highlighting four hazard classes. The class BSL-1 represents saprophytes or plant pathogens causing a coincidental, superficial, non-invasive or benign threat [4]. However, this may cause non-infectious respiratory diseases of an allergic or immunotoxic nature [5]. Class BSL-2 species are characterized by a relatively high ability to survive in the tissues of vertebrates, and in patients with severe immune disorders can cause a deep, opportunistic infection. Pathogens belonging to the class of BSL-3 are potentially capable of causing severe and deep fungal infections in apparently healthy subjects [4].

Aim

The aim of the study was to detect and assess the activity of extracellular hydrolytic enzymes and to find differences in enzymograms between fungi isolated from wheat and rye samples and grown on Czapek-Dox Broth

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Received: 26.09.2015, **accepted:** 20.10.2015.

Table 1. Activity of mold exoenzyme isolated from wheat on media with and without addition of wheat

Isolate	Medium	Enzyme activity*,**																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Alternaria alternata	Czapek-Dox	0	2	3	0	2	1	0	4	0	1	1	2	0	0	1	5	5	0	0
	Czapek-Dox + wheat	4	2	3	0	5	5	1	2	2	4	5	0	0	2	3	5	5	0	0
	Sabouraud	0	0	0	0	1	1	0	1	0	0	1	2	0	0	1	5	2	0	0
	Sabouraud + wheat	5	4	4	0	4	3	1	0	1	5	5	0	3	3	4	5	5	1	1
Aspergillus fumigatus	Czapek-Dox	1	2	3	1	1	1	1	1	1	5	4	5	3	0	2	3	5	1	0
	Czapek-Dox + wheat	0	1	0	0	3	4	0	0	0	3	4	5	3	0	4	5	3	0	0
	Sabouraud	3	2	1	0	5	1	2	1	2	5	4	1	3	0	0	3	4	4	0
	Sabouraud + wheat	1	1	2	0	3	2	2	2	2	5	2	3	3	0	3	3	4	3	0
Aspergillus glaucus	Czapek-Dox	3	2	3	1	0	0	0	0	0	5	5	5	2	0	1	5	4	1	0
	Czapek-Dox + wheat	2	2	2	0	0	1	0	0	0	2	2	4	1	0	0	2	3	2	0
	Sabouraud	5	3	4	0	4	3	3	3	3	5	5	5	4	4	0	4	4	4	0
	Sabouraud + wheat	3	2	2	0	1	2	0	0	1	2	3	3	5	0	3	5	5	2	0
Aspergillus sydowii	Czapek-Dox	2	1	2	1	0	0	0	3	2	3	5	5	1	0	1	3	3	3	2
	Czapek-Dox + wheat	5	2	2	0	5	2	2	2	2	5	5	5	0	0	0	3	3	3	0
	Sabouraud	2	1	3	1	1	2	2	3	2	4	5	1	1	0	0	2	3	1	1
	Sabouraud + wheat	5	1	2	0	3	2	2	1	0	5	3	2	0	2	0	3	3	3	0
Aspergillus tamarii	Czapek-Dox	0	2	1	1	0	1	1	1	0	2	1	0	0	0	1	3	4	0	0
	Czapek-Dox + wheat	1	2	1	0	1	0	0	1	1	2	2	0	0	0	2	4	4	0	0
	Sabouraud	2	2	2	0	2	0	0	0	0	1	2	2	1	1	2	3	3	0	0
	Sabouraud + wheat	3	2	0	0	3	2	0	1	0	2	2	2	3	3	3	4	3	0	0
Aspergillus terreus	Czapek-Dox	0	1	0	0	0	0	0	0	0	3	3	2	0	1	0	2	2	0	0
	Czapek-Dox + wheat	3	2	0	0	4	1	0	0	0	4	4	5	1	3	2	4	3	4	0
	Sabouraud	1	1	0	0	2	2	1	0	0	1	1	2	0	0	0	4	4	2	0
	Sabouraud + wheat	4	1	0	0	4	2	1	1	0	4	3	4	0	3	1	3	4	3	0
Aspergillus versicolor	Czapek-Dox	3	0	0	0	0	0	0	0	0	4	1	1	0	0	0	5	2	0	0
	Czapek-Dox + wheat	1	0	0	0	0	0	0	0	0	2	1	2	1	0	0	4	0	0	0
	Sabouraud	3	1	1	0	0	0	0	0	0	4	1	4	0	0	2	4	4	3	0
	Sabouraud + wheat	2	1	0	0	0	0	0	0	0	2	5	1	1	0	0	2	0	0	0
Bipolaris holmi	Czapek-Dox	1	0	2	0	1	0	0	0	0	2	2	4	3	0	3	5	5	1	1
	Czapek-Dox + wheat	3	2	2	2	5	2	2	3	0	2	3	2	2	2	3	5	5	2	2
	Sabouraud	4	3	3	1	4	2	3	5	1	5	5	0	4	0	3	5	5	1	0
	Sabouraud + wheat	5	3	2	2	5	2	0	3	0	3	3	2	5	5	2	5	5	2	2
Exserohilum sp.	Czapek-Dox	4	3	3	1	5	5	5	1	1	4	5	2	1	1	3	5	4	1	0
	Czapek-Dox + wheat	1	1	0	1	2	1	0	4	1	0	2	4	4	0	2	5	4	1	0
	Sabouraud	3	2	1	0	4	2	2	3	2	5	5	5	3	3	5	5	3	3	2
	Sabouraud + wheat	3	2	2	0	2	0	0	2	2	3	3	3	2	2	5	3	5	2	0
Fusarium proliferatum	Czapek-Dox	4	1	1	0	2	0	0	1	0	2	2	2	1	0	0	3	3	1	0
	Czapek-Dox + wheat	3	1	2	1	5	0	1	0	1	4	3	5	4	0	1	5	5	3	0
	Sabouraud	4	1	2	0	4	1	1	0	0	3	3	0	0	0	0	3	2	0	0
	Sabouraud + wheat	3	1	3	0	5	1	0	0	1	3	4	3	2	0	0	3	3	0	0
Fusarium tricinctum	Czapek-Dox	1	1	2	0	0	0	0	0	0	3	1	2	0	0	0	2	3	0	0
	Czapek-Dox + wheat	4	2	2	0	4	0	0	2	0	5	2	2	2	0	0	5	4	1	0
	Sabouraud	4	2	3	1	5	2	0	0	0	3	3	1	0	0	0	1	4	0	0
	Sabouraud + wheat	5	3	4	0	3	0	0	0	0	5	5	5	0	0	1	5	5	2	0
Penicillium decumbens	Czapek-Dox	0	2	1	0	3	0	0	0	0	5	4	4	5	5	4	5	4	1	0
	Czapek-Dox + wheat	1	2	2	0	2	0	0	0	0	4	3	4	5	5	4	5	4	2	0
	Sabouraud	0	0	0	0	1	0	0	0	0	4	2	0	5	1	0	5	5	0	0
	Sabouraud + wheat	3	2	2	2	3	2	0	0	0	5	5	5	5	5	5	5	4	4	0
Penicillium expansum	Czapek-Dox	0	1	0	0	2	0	1	0	0	2	3	2	4	1	1	3	3	0	0
	Czapek-Dox + wheat	0	1	2	0	2	0	1	0	0	3	3	3	5	4	3	4	4	0	0
	Sabouraud	0	0	0	0	0	0	1	0	0	1	2	0	0	0	0	1	3	0	0
	Sabouraud + wheat	1	2	3	1	4	2	0	0	0	5	5	5	4	5	4	4	4	2	0
Scopulariopsis brevicaulis	Czapek-Dox	0	2	5	2	3	5	2	0	0	0	1	0	1	0	0	1	0	0	0
	Czapek-Dox + wheat	1	2	4	3	4	5	1	0	0	1	1	0	1	0	0	5	4	2	0
	Sabouraud	0	3	3	0	1	3	0	0	0	0	1	0	0	0	0	1	1	3	0
	Sabouraud + wheat	1	4	4	2	3	4	1	0	0	0	1	1	0	0	1	5	5	3	0
Scopulariopsis brumptii	Czapek-Dox	0	3	3	1	1	3	0	0	0	0	1	0	1	0	0	1	1	1	0
	Czapek-Dox + wheat	1	3	4	2	5	5	0	0	0	0	1	0	4	0	0	5	4	1	0
	Sabouraud	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	1	2	0	0
	Sabouraud + wheat	2	3	4	1	5	3	0	0	0	0	2	0	5	0	2	5	4	2	0

Table 1. Cont.

Isolate	Medium	Enzyme activity*,**																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Stemphylium sp.	Czapek-Dox	0	2	4	1	2	1	0	1	0	1	1	1	0	0	1	5	5	0	0
	Czapek-Dox + wheat	1	0	1	0	1	0	1	1	0	3	1	0	1	0	2	5	5	0	0
	Sabouraud	5	2	1	0	2	2	0	1	0	5	3	1	0	1	0	5	4	0	0
	Sabouraud + wheat	4	2	1	0	3	2	0	2	1	3	2	0	3	1	3	5	5	0	0

**Enzymes: 1) alkaline phosphatase, 2) esterase (C4), 3) esterase lipase (C8), 4) lipase (C14), 5) leucine arylamidase, 6) valine arylamidase, 7) cystine arylamidase, 8) trypsin, 9) α-chymotrypsin, 10) acid phosphatase, 11) naphthol-as-bi-phosphohydrolase, 12) α-galactosidase, 13) β-galactosidase, 14) β-glucuronidase, 15) α-glucosidase, 16) β-glucosidase, 17) N-acetyl-β-glucosaminidase, 18) α-mannosidase, 19) α-fucosidase. **0 – no activity, 1–5 – activity increases with color intensity (1 low activity, 5 high activity).*

Table 2. Enzyme activity in isolates from wheat crops

Isolate	Total enzyme activity summed up				Number of active enzymes			
	Czapek-Dox	Czapek-Dox + wheat	Sabouraud	Sabouraud + wheat	Czapek-Dox	Czapek-Dox + wheat	Sabouraud	Sabouraud + wheat
Alternaria alternata	28	49**	23	50***	12	13	13	14
Aspergillus fumigatus	40	35	41	41	17	10	15	16
Aspergillus glaucus	37*	23	63*^^	39^	12	11	16	14
Aspergillus sydowii	37	46	35	37	15	14	17	14
Aspergillus tamarii	18	21	23	33^	11	11	12	13
Aspergillus terreus	14	40**	21	38*	7	13	11	14
Aspergillus versicolor	16	11	27^	14	6	6	10	7
Bipolaris holmi	30	49*	54^	56	12	18	16	17
Exserohilum sp.	54	33	58*	41	18	14	18	15
Fusarium proliferatum	23	44**^	24	32	12	15	10	12
Fusarium tricinctum	15	35**	29^	43	8	12	11	11
Penicillium decumbens	43^	43	23	57**^^	12	13	7	15
Penicillium expansum	23^^	35*	8	51***^^	11	12	5	15
Scopulariopsis brevicaulis	22	34	16	35**	9	13	8	13
Scopulariopsis brumptii	16	35*	6	38**	10	11	4	12
Stemphylium sp.	25	22	32	37^^	12	11	12	14
Mean	27.6	34.7	30.2	40.1	11.5	12.3	11.6	13.5

*Mann-Whitney-Wilcoxon test for the difference of enzyme activity was performed. Asterisks (*) are stated at the higher number to show the significant difference between pure and supplemented medium and carets (^) are stated at the higher number to show the difference between Czapek-Dox and Sabouraud media (pure and supplemented). The significance of differences: *(^) $p < 0.05$; **(^^) $p < 0.01$; ***(^^^) $p < 0.001$.*

Table 3. Activity of mold exoenzyme isolated from rye on media with and without addition of rye

Isolate	Medium	Enzyme activity*,**																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Alternaria alternata	Czapek-Dox	0	2	4	0	2	2	0	3	0	1	1	1	1	0	2	4	5	0	0
	Czapek-Dox + rye	5	3	4	0	5	3	1	4	0	3	3	4	0	0	4	5	5	0	0
	Sabouraud	2	2	1	0	2	1	0	0	0	1	2	2	1	0	2	3	3	1	0
	Sabouraud + rye	4	3	3	2	5	3	0	2	0	3	3	3	5	0	4	5	5	0	0
Aspergillus versicolor	Czapek-Dox	4	1	2	0	1	1	0	0	0	4	2	5	3	0	0	3	3	0	0
	Czapek-Dox + rye	4	3	4	0	4	5	1	0	1	5	4	5	5	0	1	5	5	1	0
	Sabouraud	4	2	2	0	1	2	1	0	0	1	2	1	1	0	1	5	2	2	0
	Sabouraud + rye	5	4	4	1	4	5	1	0	1	4	2	5	5	0	4	5	4	4	0
Cladosporium herbarum	Czapek-Dox	2	2	1	0	0	0	0	0	0	1	1	3	0	0	0	4	4	0	0
	Czapek-Dox + rye	5	2	4	0	5	4	0	0	0	5	4	5	0	4	2	5	5	4	3
	Sabouraud	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	3	0	0
	Sabouraud + rye	5	3	4	0	3	3	1	1	1	5	5	5	0	5	1	5	4	5	5
Fusarium cerealis	Czapek-Dox	1	2	2	0	1	0	0	4	0	1	1	0	1	0	0	5	0	0	0
	Czapek-Dox + rye	5	3	2	0	4	3	0	1	1	5	4	3	2	0	1	5	0	1	0
	Sabouraud	2	1	0	0	0	0	0	0	0	1	2	0	0	0	0	3	4	0	0
	Sabouraud + rye	4	2	1	0	3	2	0	0	0	5	3	1	0	0	2	4	4	3	0
Fusarium oxysporum	Czapek-Dox	0	1	2	1	3	0	0	1	0	0	0	0	1	0	0	2	2	0	0
	Czapek-Dox + rye	3	3	2	0	4	4	1	4	1	5	5	1	3	0	0	5	0	2	0
	Sabouraud	3	1	1	1	1	0	0	1	0	3	2	0	0	0	0	5	5	0	0
	Sabouraud + rye	4	2	3	0	4	3	0	4	0	5	3	0	4	0	1	5	0	1	0
Fusarium tricinctum	Czapek-Dox	2	2	2	0	1	0	0	1	0	4	0	0	0	0	0	4	0	0	0
	Czapek-Dox + rye	4	1	1	0	3	2	0	1	0	4	4	0	2	0	0	3	0	0	0
	Sabouraud	3	0	1	0	1	0	0	0	0	2	4	1	0	0	0	3	3	0	0
	Sabouraud + rye	5	3	2	0	3	1	0	0	0	5	5	3	2	0	2	4	5	2	0
Penicillium chrysogenum	Czapek-Dox	0	1	1	0	5	2	0	0	0	0	1	0	2	0	3	4	4	0	2
	Czapek-Dox + rye	5	4	2	0	5	4	1	0	5	5	5	5	4	0	5	5	4	3	3
	Sabouraud	0	0	0	0	0	0	0	0	0	0	2	0	2	0	1	3	1	0	0
	Sabouraud + rye	4	2	3	0	5	3	0	0	3	5	5	5	4	0	5	5	4	4	4
Penicillium diverse	Czapek-Dox	4	0	0	0	1	4	0	0	0	3	2	0	0	0	0	5	4	0	0
	Czapek-Dox + rye	5	3	4	0	5	4	1	0	1	5	5	4	3	2	3	3	4	4	0
	Sabouraud	2	0	0	0	3	0	0	0	0	1	2	0	0	0	0	3	3	0	0
	Sabouraud + rye	4	2	1	0	5	1	0	0	0	5	5	5	3	0	4	5	4	5	0
Rhizopus oryzae	Czapek-Dox	2	4	3	0	0	0	0	0	0	0	1	1	1	0	0	1	2	1	0
	Czapek-Dox + rye	5	2	3	0	3	3	0	0	0	5	5	4	5	0	0	5	1	2	0
	Sabouraud	4	3	3	0	2	3	2	0	0	3	3	2	0	0	0	4	0	2	0
	Sabouraud + rye	5	3	3	0	1	0	0	0	0	1	2	5	3	0	0	5	5	3	0
Ulocladium chartarum	Czapek-Dox	1	3	4	0	0	0	0	0	0	2	3	0	0	0	1	4	4	0	0
	Czapek-Dox + rye	2	2	2	0	3	3	1	1	0	1	1	2	4	3	5	5	5	0	0
	Sabouraud	3	3	1	0	4	3	0	0	0	2	1	3	1	1	3	5	5	0	0
	Sabouraud + rye	5	4	0	0	5	4	1	0	0	4	3	3	5	3	4	5	5	1	0

**Enzymes: 1) alkaline phosphatase, 2) esterase (C4), 3) esterase lipase (C8), 4) lipase (C14), 5) leucine arylamidase, 6) valine arylamidase, 7) cystine arylamidase, 8) trypsin, 9) α-chymotrypsin, 10) acid phosphatase, 11) naphthol-as-bi-phosphohydrolase, 12) α-galactosidase, 13) β-galactosidase, 14) β-glucuronidase, 15) α-glucosidase, 16) β-glucosidase, 17) N-acetyl-β-glucosaminidase, 18) α-mannosidase, 19) α-fucosidase; **0 – no activity, 1–5 – activity increases with color intensity (1 low activity, 5 high activity).*

and Sabouraud Dextrose Broth enriched with cereal (wheat or rye). Isolated strains were also classified in the scale of biosafety levels (BSL).

Material and methods

The study used 23 strains of fungi isolated from samples of grain, grain dust obtained during threshing and soil from the crops of wheat and rye collected in the Lublin area. In order to prepare the isolates for the API ZYM test, the strains were grown initially on two solid media: Malt Agar (Becton, Dickinson and Co.) intended for all kinds of fungi and Potato Dextrose Agar (Becton, Dickinson and Co.) which is a selective medium for *Fusarium* spp. Samples were incubated at 24°C or 30°C for 72 h depending on the medium, and then at room temperature until all have been producing spores. Then the biopsy was taken (5 mm in diameter) from the margin of

Table 4. Enzyme activity in isolates from rye crops

Isolate	Total enzyme activity summed up				Number of active enzymes			
	Czapek-Dox	Czapek-Dox + rye	Sabouraud	Sabouraud + rye	Czapek-Dox	Czapek-Dox + rye	Sabouraud	Sabouraud + rye
Alternaria alternata	27^	48*	14	54**	11	14	8	16
Aspergillus versicolor	29	53**	27	58**	11	15	14	16
Cladosporium herbarum	18^	57**	6	61***	8	14	4	17
Fusarium cerealis	18	40*	13	34**	9	14	6	12
Fusarium oxysporum	13	43**	23	39*	8	14	10	12
Fusarium tricinctum	16	25	18	42**^	7	10	8	13
Penicillium chrysogenum	25^	65**	9	61***	10	16	5	15
Penicillium diverse	23	56**	14	49**	7	16	6	13
Rhizopus oryzae	16	43*	31^	36	9	12	11	11
Ulocladium chartarum	22	40	35	52**	8	15	13	14
Mean	20.7	47.0	19.0	48.6	8.8	14.0	8.5	13.9

*Mann-Whitney-Wilcoxon test for the difference of enzyme activity was performed. Asterisks (*) are stated at the higher number to show the significant difference between pure and supplemented medium and carets (^) are stated at the higher number to show the difference between Czapek-Dox and Sabouraud media (pure and supplemented). The significance of differences: *(^) p < 0.05; **(^^) p < 0.01; ***(^^^) p < 0.001.*

the culture and transferred to four liquid media: Czapek-Dox Broth (Becton, Dickinson and Co.) pure and supplemented with 1% of grain (wheat or rye), and Sabouraud Dextrose Broth (Becton, Dickinson and Co.) pure and supplemented with 1% of grain. Media without the tested isolates were used as controls. The isolates were then incubated for 24 days at 24°C. Supernatants obtained from culture (after centrifugation or sedimentation) were tested. Supernatants from control cultures were used as a control. API ZYM tests were performed according to the manufacturer's instructions by placing 65 µl of the supernatant at appropriate points of the test strip. Reading was made visually. Enzyme activity was determined using a scale of 0 to 5, with 0 indicating a negative reaction. Differences in enzyme activity were compared in cultures with and without addition of the grain extract and statistically tested with Mann-Whitney-Wilcoxon test (*R* statistical software environment version 3.0.2) [6].

With the help of available literature the tested isolates were classified into classes of biosafety (BSL) [7].

Results

API ZYM test performed on all the control substrates tested showed no activity of hydrolytic enzymes. The following strains isolated from cultures were characterized by the greatest number of active hydrolytic enzymes: *Exserohilum* sp., *Bipolaris holmi* (Tables 1 and 2) in strains isolated from samples of wheat and *Aspergillus versicolor, Penicillium chrysogenum, Alternaria alternata* isolated from samples of rye (Tables 3 and 4). The highest activity of hydrolytic enzymes (4 or 5 in the adopted scale of activity) was found in the strains of *Bipolaris holmi* and *Penicillium decumbens* which were grown on media containing 1% of wheat grains (Tables 1 and 2); and in strains of *Cladosporium herbarum, Aspergillus versicolor,* and *Alternaria alternata* grown on media containing 1% of rye grains (Tables 3 and 4). In isolates from wheat samples the most active enzymes were N-acetyl-β-glucosyloamidase, β-glucosidase, acid phosphatase (Table 1) while in isolates from rye samples, β-glucosidase, N-acetyl-β-glucosyloamidase, and alkaline phosphatase exhibited the highest activity (Table 3).

Comparing the activity of extracellular enzymes in the medium with and without the addition of grain, it was found that supplementation with cereal grain (wheat, rye) resulted in increased activity of the enzymes produced by the isolates in most cases. From 16 isolates of wheat crops in Czapek-Dox medium with an addition of wheat, in 7 cases statistically significant higher enzyme activity was found, and in one case statistically significant lower enzyme activity was found as

compared to pure Czapek-Dox medium. In the Sabouraud medium with an addition of wheat in 6 cases statistically significant higher activity was found, and in 2 cases statistically significant lower activity was detected comparing to pure Sabouraud medium (Tables 1 and 3). The differences in isolates from rye were more distinct than in Czapek-Dox medium with an addition of rye: 8 from 10 isolates showed statistically significant higher activity, and in Sabouraud medium significant higher activity was found in 9 isolates (Tables 3 and 4). When differences between media were compared the differences were much smaller. In wheat isolates higher activity in pure media was found in 4 Sabouraud cultures and in two Czapek-Dox cultures, and in supplemented media 5 Sabouraud cultures showed higher enzyme activity and one Czapek-Dox culture. The differences between media in rye isolates were smaller.

The vast amount of isolates was assigned to BSL-1 (Table 5), i.e. to the class which represents saprophytes or plant pathogens causing a non-invasive or mild threat.

Table 5. Biosafety levels of isolated molds [7]

No.	Isolate	BSL
1	*Alternaria alternata*	I
2	*Aspergillus fumigatus*	II
3	*Aspergillus glaucus*	I
4	*Aspergillus sydowii*	I
5	*Aspergillus tamarii*	I
6	*Aspergillus terreus*	II
7	*Aspergillus versicolor*	I
8	*Bipolaris holmi*	ND
9	*Cladosporium herbarum*	I
10	*Exserohilum* sp.	ND
11	*Fusarium cerealis*	ND
12	*Fusarium oxysporum*	II
13	*Fusarium proliferatum*	I
14	*Fusarium tricinctum*	ND
15	*Penicillium chrysogenum*	I
16	*Penicillium decumbens*	I
17	*Penicillium diverse*	ND
18	*Penicillium expansum*	I
19	*Rhizopus oryzae*	I
20	*Scopulariopsis brevicaulis*	II
21	*Scopulariopsis brumptii*	II
22	*Stemphylium* sp.	ND
23	*Ulocladium chartarum*	I

ND – not defined.

Discussion

One of molds, which had high enzymatic activity, belonged to the genus *Exserohilum*, which consists of more than 30 species. These saprophytes are commonly found on the remains of plants, some of which can cause diseases of plants, animals and humans (immunocompromised persons are particularly at risk) [8]. Another mold showing high activity of the enzymes was species from *Bipolaris* genus. This type includes more than 100 species, most of them are saprobiotic species found in the soil and some of them have the potential pathogenicity to animals and humans [9]. A significant amount of hydrolytic enzymes was also found in an isolate from the genus *Alternaria*, the causative agent of phaeohyphomycosis or onychomycosis, sinusitis, ulcerative skin infections, corneal inflammation, and deep mycosis. Infections caused by this genus are a growing problem in patients who have been treated with immunosuppressants. It is also a common allergen [10]. *Aspergillus versicolor* is described as a rare cause of various mycoses in humans. It is often isolated from fungal diseases of domestic animals and habitats of various soil [3]. The genus *Cladosporium* is very rarely a cause of opportunistic infections in humans. It is a common allergen [10]. *Cladosporium herbarum* is the most common species in this genus, inhabiting dead organic remains of plants in soil habitats. Molds from the genus *Penicillium* occur commonly in the fields and meadows, and is also found in flats and basements. It is often present in fruit juices, on citrus and moldy bread. This can cause infections particularly in people with immunodeficiency. High concentration of spores of this fungus in the air can act as an allergen. *P. chrysogenum* species has a worldwide range. It was isolated many times from soil in different climatic zones, including the polar zone [3].

Hydrolytic enzymes that are released into the environment by dermatophytes, yeasts, and molds are an important element in the pathogenesis of infection [11]. The enzymatic activity of fungi can colonize most surfaces dead or living. The enzymes produced by fungi allow the degradation and the use of most of the naturally occurring compounds and synthetic substances. Decomposition processes are initiated by adaptive enzymes, produced in response to the "signal" from the environment. Changing the proportions of trophic components in the substrate or appearance of trace amounts of a new substrate, stimulates production of specific enzymes [12]. The hydrolases which are synthesized by pathogenic fungi include esterases, and they include the carboxyl group ester hydrolases (triacylglycerol lipase, phospholipase A2), the phosphoric monoester hydrolases (alkaline phosphatase, acid phosphatase) and sulfuric ester hydrolases (sulfatases); glycosidases (α-glucosidase, β-glucosidase, α-mannosidase); peptidases – exopeptidases (proteinases, e.g. aspartic proteinase, aminopepti-

dases, e.g. arylamidase) and endopeptidase (proteinase hydrolyzing C-N bond, e.g. urease). Using a commercial standardized test (API ZYM, bioMérieux), one can create a different fungal species enzymogram illustrating the characteristic enzymatic activity [3, 13–15].

Obtained results tend to reflect methods of storage and processing of grain; whether the technology completely eliminates molds from food. As the results show, some molds (e.g. *Exserohilum* sp., *Bipolaris holmi, Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus versicolor*, *Penicillium chrysogenum*) isolated from samples of grain, grain dust, and soil from crops of wheat and rye, probably will also be able to show significant enzyme activity in natural environment, which may help to adapt to new conditions, and to colonize various locations and affect human health (especially in immunocompromised persons) exposed to inhalation of large amounts of dust generated during harvesting grain cereals (i.e. threshing with a combine harvester or pouring grain into barns).

Conclusions

Hydrolytic exoenzyme activity in tested fungi depends on the type of the medium and the addition of the grain extract. Particular grains can stimulate activity of extracellular enzymes in tested fungi. Activation of enzymes was influenced by an addition of wheat and rye to the medium. The relationship between high enzymatic activity of examined fungi and BSL scale was not found.

Acknowledgments

This study was funded by the National Science Centre (Poland), grant No. 2011/01/B/NZ7/04300.

Conflict of interest

The authors declare no conflict of interest.

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