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ENZYMATIC ACTIVITIES OF *ASPERGILLUS FUMIGATUS* STRAINS ISOLATED FROM THE AIR AT WASTE LANDFILLS

Arūnas Krikštaponis[1], Albinas Lugauskas[1], Ewa Krysińska-Traczyk[2], Zofia Prażmo[2], Jacek Dutkiewicz[2]

[1]Department of Biodeterioration Research, Institute of Botany, Vilnius, Lithuania
[2]Department of Occupational Biohazards, Institute of Agricultural Medicine, Lublin, Poland

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Abstract: Out of 15 *Aspergillus fumigatus* strains isolated from the air at waste landfills, all revealed cellulolytic and lipolytic activities. Eleven out of 15 strains showed very strong cellulolytic properties, as assessed by the viscosimetric test for endo-1,4-β-glucanase activity (EGA). None of the examined strains revealed a well-expressed proteolytic activity. The results suggest that *Aspergillus fumigatus* strains developing in stored wastes produce strong cellulotytic enzymes which need further studies for the potential allergenic and/or immunotoxic effects of these proteins on exposed workers.

Address for correspondence: Dr. Arūnas Krikštaponis, Department of Biodeterioration Research, Institute of Botany, Žaliųjų ežerų 49, LT-2021 Vilnius, Lithuania.
E-mail: arunas.k@botanika.lt

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INTRODUCTION

Aspergillus fumigatus is the most prevalent airborne fungal pathogen in developed countries [9, 2l]. Physiological peculiarities such as enzymatic activities and toxicity enable the fungus to colonize and assimilate various organic substrates, and to suppress development of other microorganisms. High sporulation capacity not only promotes distribution of the fungus but leads to high concentrations of conidia in the air indoors and outdoors, ranging within 10^1-10^7 cfu/m^3 [8, 12, 18, 21].

Aspergillus fumigatus may cause in man infection of the lung (bronchopulmonary aspergillosis, aspergilloma), allergic alveolitis, asthma, organic dust toxic syndrome and allergic rhinitis [12, 15, 16, 17, 21]. Moreover, it is able to produce tremorgenic mycotoxins that could be hazardous for exposed individuals [9, 19]. *A. fumigatus* conidia are continuously inhaled by humans but rarely cause an infection because usually they are effectively eliminated by the immune response. However, due to the increasing number of immunocompromised individuals such as AIDS patients, diabetics, and patients undertaking chemotherapy, the risk of aspergillosis has increased over the past two decades [6, 20].

Aspergillus fumigatus is widely known as an agent of occupational hazard and has been described as the cause of cases of occupational disease in many industries, including primary aspergillosis and allergic alveolitis in workers in the tobacco industry [10, 22], primary aspergillosis in a worker in a animal feed production plant [22], allergic alveolitis in workers exposed to infected wood chips [21, 23], and allergic alveolitis due to exposure to infected compost [39]. The abundant development of *A. fumigatus* in waste and compost is a matter of particular concern [12, 17, 18, 21]. The concentrations of spores and hyphal fragments of this fungus in the air of waste handling and

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Table 1. Source of isolation of 15 strains of *Aspergillus fumigatus* examined in this study.

Strain	Landfill locality	Sampling date	Sampling site	
			No.	Description
1	F	06-06-2000	I-A	Weighing site
2	F	« «	IV-A	Operator/driver
3	C	14-06-2000	I-B	Sweeping machine
4	C	« «	II-B	Operator of compactor (pressing machine)
5	C	« «	III-B	Operator of compactor (inside cabin)
6	C	« «	IV-B	Weighing site
7	C	« «	« «	« «
8	C	« «	« «	« «
9	C	« «	« «	« «
10	C	« «	V-B	Compactor (pressing machine)
11	C	« «	VI-B	Weighing site
12	C	« «	« «	« «
13	C	« «	VII-B	Edge of the area (enclosure)
14	C	« «	« «	« «
15	C	« «	« «	« «

composting facilities may reach the levels of 10^3-10^6 cfu/m^3 [3, 4, 12, 14, 17, 18, 21, 25]. Though a direct dose/response relationship has not been established as yet [21], the consistent exposure to the inhalation of fungal particles poses a risk of the disease, mostly of the allergic and/or immunotoxic character, among the workers of these facilities.

Results of recent investigations show that extracellular enzymes, released into the environment by *A. fumigatus* are putatively involved in the mechanisms of pathogenicity [13, 14, 20, 23]. Accordingly, the aim of this study was to examine cellulolytic, proteolytic and lipolytic enzymatic activities of 15 strains of *Aspergillus fumigatus* isolated from the air at waste landfills for better assessment of the risk caused by exposure of the workers to airborne particles of this fungus.

MATERIALS AND METHODS

Isolation of strains. Fifteen strains of *Aspergillus fumigatus* were isolated from the air of two waste landfills in the localities "F" and "C" in central Poland [27]. The air samples were collected in June 2000 on glass fibre filters with the use of personal samplers AP-2a (TWOMET, Zgierz, Poland) worn by workers while performing various activities at landfills, such as weighing of waste and operating pressing and sweeping machines (Tab. 1). Samples were collected for 4-6 hours at the airflow of 1.9 l/min.

After transporting to laboratory, filters were extracted for 1 hour in 3-5 ml of saline (0.85% NaCl) containing 0.05% ml (v/v) of Tween 80 and after vigorous shaking, serial 10-fold dilution in saline were made up to 10^{-8}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of malt agar (*Difco*) plates for isolation of fungi. After subsequent incubation for 4 days at 30^{o}C and four days at 37^{o}C, the grown colonies were counted and differentiated for determination of the concentration of fungi in the air (cfu/m^3) and the species composition of the air mycobiota. Fifteen strains of *Aspergillus fumigatus* were subcultured on malt agar slants for further examination. The strains originated from the air samples taken at two sites in locality "F" and at seven sites in locality "C" (Tab. 1). All the strains were isolated outdoors, except for strain No. 5 which was isolated inside the cabin of compactor's operator.

The concentrations of *Aspergillus fumigatus* in the air of the examined landfills in the localities "F" and "C" ranged within 1.1×10^2 - 2.2×10^3 cfu/m^3 and 1.7×10^2 - 9.9×10^4 cfu/m^3, respectively. *Aspergillus fumigatus* strains formed on average 30.4% and 86.9% of the fungal strains isolated from the air of the landfills in the localities "F" and "C", respectively (79.4% of the total strains for both localities).

Determination of enzymatic activity

Qualitative tests for capability of 15 strains of *Aspergillus fumigatus* to produce cellulases, proteases and lipases were performed by inoculating Czapek medium containing respectively cellulose, collagen and fatty acids as the sole sources of carbon. Cultures were incubated for

Table 2. Ability of 15 *Aspergillus fumigatus* strains to develop on Czapek agar and assimilate cellulose as the sole source of carbon.

Strain No.	Evaluation of fungal growth and sporulation intensity, visible changes of filter paper				
	5 days	10 days	15 days	21 days	28 days
1	++	++	+++++	+++++	+++++
2	++	+++	+++++	+++++	+++++
3	+	+++	++++	++++	++++
4	++	+++	++++	+++++	+++++
5	++	+++	++++	++++	++++
6	++	++	+++++	+++++	+++++
7	++	+++	++++	+++++	+++++
8	++	+++	+++	+++++	+++++
9	+	+++	++++	+++++	+++++
10	++	+++	+++++	+++++	+++++
11	++	++	++	++	+++
12	++	++	++++	+++++	+++++
13	++	+++	++++	+++++	+++++
14	++	+++	++++	+++++	+++++
15	++	++	+++	+++++	+++++

+: single non-sporulating hyphae; ++: poorly developed and sporulating mycelium; +++: moderately developed and sporulating mycelium; ++++: good developed, abundantly sporulating mycelium; +++++: very good developed, abundantly sporulating mycelium, evident changes of filter paper.

28 days at 25°C. The ability of fungi to assimilate these substances for their development was assessed by direct observation of growth and sporulation of cultures and changes of the substrates. Classification of the intensity of growth and substrate changes in the scale + - +++++ enabled evaluation of the enzymatic activity.

In addition, a quantitative EGA test for measurement of the activity of endo-1,4-β-glucanase, an important enzyme engaged in mid-stage cellulose degradation, was applied. According to Reese *et al.* [29] and Reese [30], at least three groups of enzymes are involved in cellulose degradation processes. The breaking of hydrogen linkages between fibres of native cellulose is catalysed by C_1 enzyme – exo-1,4-β-glucanase. Then, reactivated cellulose is further decomposed by hydrolytic enzymes C_x, such as endo-β-1,4 glucanase, which break separate cellulose polymers into disaccharide cellobiose. Cellobiose is converting to glucose by enzyme β-glucosidase.

In this study, quantitative analysis of endo-1,4-β-glucanase activity (EGA) was carried out based on the measurement of the decrease of viscosity of the water soluble cellulose derivative – sodium salt of carboxymethylcellulose (Na-CMC) exposed to broth culture of *A. fumigatus* in a water bath. Na-CMC is the most suitable substrate for the evaluation of EGA activity because of its availability only to C_x enzymes.

Qualitative determination of cellulase production. For the detection of cellulase producers, *A. fumigatus* strains were cultivated on filter paper (Filtrak No. 88) immersed in Czapek agar without carbohydrates. The intensity of fungal growth and changes of filter paper were recorded after 5, 10, 15, 21, and 28 days of incubation at 25°C [34].

Quantitative analysis of endo-1,4-β-glucanase activity. The test was carried out by measurement of the decrease of viscosity of Na-CMC (Sigma C4888) after the hydrolysis reaction according to the viscosimetric method described by Sivers *et al.* [34]. All-glass viscosimeter VPZ-2 (produced in the former Soviet Union) was applied for the test. The reaction mixture constituted: 5 ml of aqueous 0.3 % Na-CMC solution; 0.7 ml of the filtrate of *A. fumigatus* culture in liquid Czapek medium with 0,7% cellulose as the sole carbon source, incubated for 10, 15 and 21 days at 25°C; and 0.3 ml of citrate phosphate buffer (pH 5.0). The mixture was incubated for 10 min at 25°C and flowed immediately through the viscosimeter at 25°C. *A. fumigatus* culture inactivated by heating was used as a control. Decrease of viscosity in percent was calculated from the formula:

$$EGA = \frac{(t - t_0) \times 100}{t};$$

where: t = time of control solution flow, s;
t_0 = time of enzyme solution flow, s.

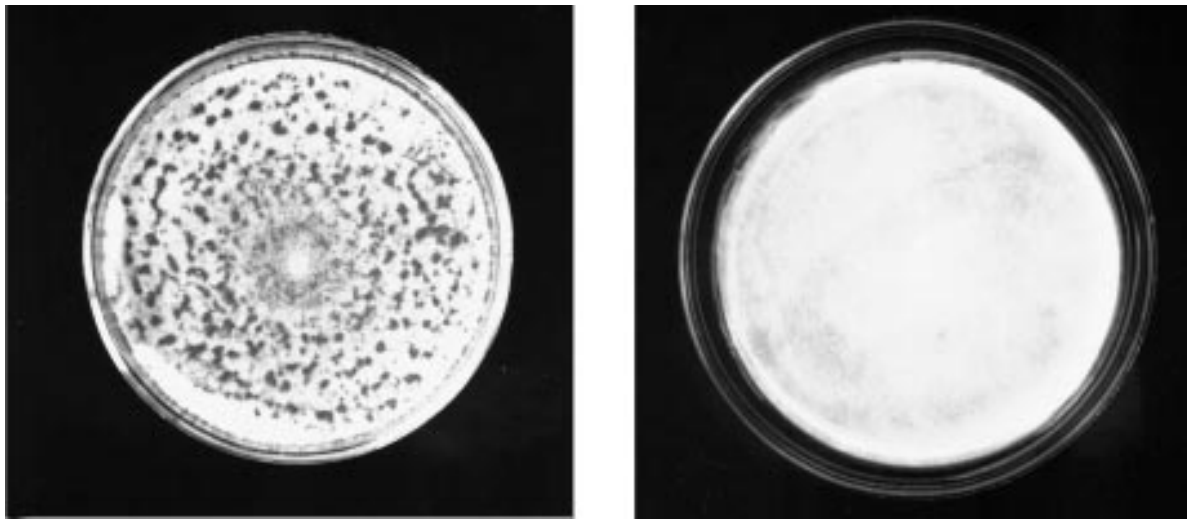

Figure. 1. Growth of the *A. fumigatus* strain No. 12 on filter paper immersed in Czapek's agar after 28 days (A), compared to non-inoculated (control) plate (B).

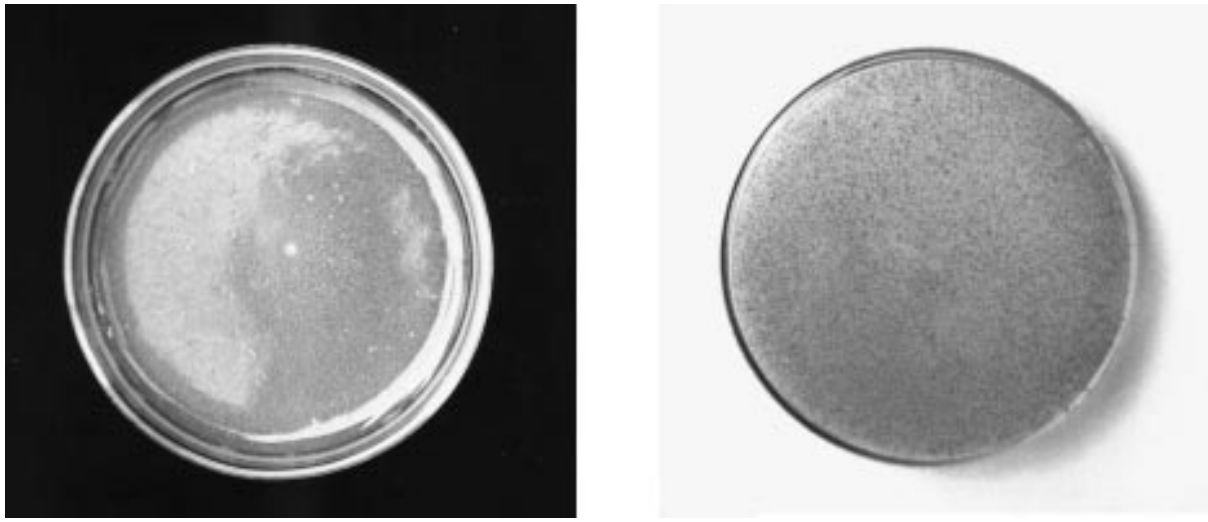

Figure 2. Growth of the *A. fumigatus* strain No. 9 on Czapek's agar without a carbon source supplemented with 0.3% of stained leather powder after 28 days (A), compared with non-inoculated (control) plate (B).

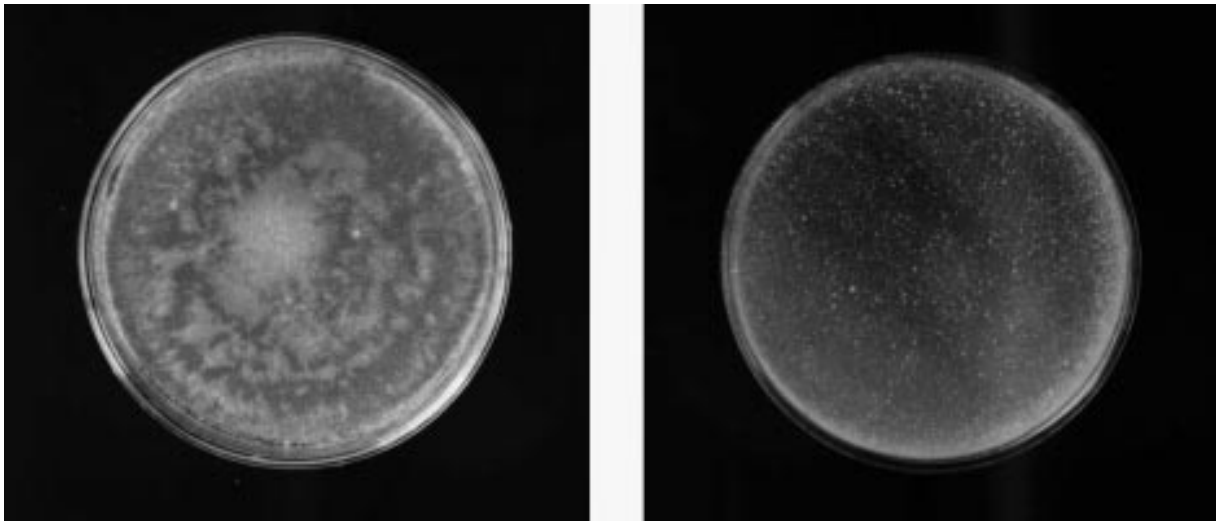

Figure 3. Growth of the *A. fumigatus* strain No. 12 on Czapek's agar without a carbon source supplemented with 2.0% of olive oil after 28 days (A), compared to non-inoculated (control) plate (B).

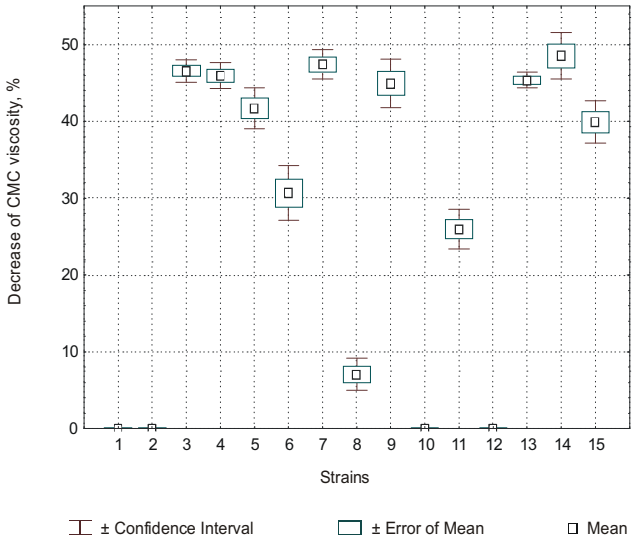

Figure 4. Endo-1,4-β-glucanase activity of *A. fumigatus* strains after10-day development at 25°C.

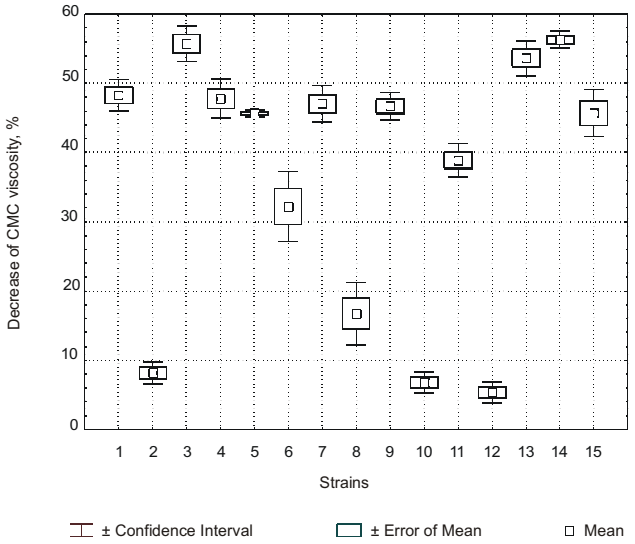

Figure 5. Endo-1,4-β-glucanase activity of *A. fumigatus* strains after15-day development at 25°C.

Analysis of endo-1,4-β-glucanase activity were performed using ANOVA. In the graphs the 95% confidential interval $[\bar{x} \pm 1.96\sigma\bar{x}]$ was presented. Homogeneity of variances was determined by Levene test, and post-hoc procedure for the detection of a homogenous group of means was carried out by Scheffe test. All statistic procedures were performed using SPSS software [2, 11].

Qualitative determination of protease production. *A. fumigatus* strains producing proteases were identified by the assessment of ability to grow on Czapek agar supplemented with 0.3% of leather powder stained with a chromium salt (obtained from the Institute of Biotechnology, Vilnius, Lithuania) as the sole carbon source. The intensity of fungal growth and changes of medium were recorded after 5, 10, 15, 21, and 28 days of incubation at 25°C [1].

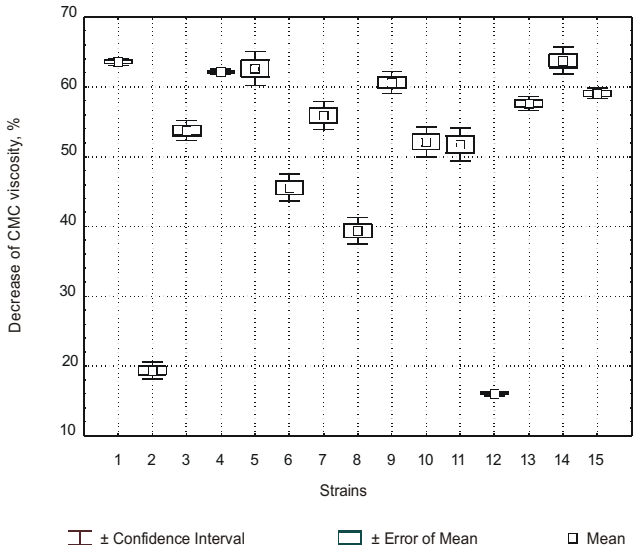

Figure 6. Endo-1,4-β-glucanase activity of *A. fumigatus* strains after 21-day development at 25°C.

Qualitative determination of lipase production. *A. fumigatus* strains producing lipases were detected by cultivating on Czapek agar supplemented with 2.0% of olive oil as the sole carbon source. The intensity of fungal growth and changes of medium were recorded after 5, 10, 15, 21, and 28 days of incubation at 25°C [32, 40].

RESULTS

Production of cellulases on Czapek medium. As seen in Table 2, all the examined strains of *Aspergillus fumigatus* were able to develop on the filter paper. After five days of incubation, slow growth and conidiogenesis beginning in the centre of colonies were observed in all cultures except strains No. 3 and 9 which showed a delayed development. Moderately developed mycelium and limited sporulation were observed in most of the cultures at the age of 10 days but no changes of the substrate were recorded. First pigmented spots on paper filters caused by developing strains No. 1, 2, 6, and 10 were recorded after 15 days. At this time, well developed mycelium and abundant sporulation were seen in most of the cultures, except strain No. 11 which showed a delayed development.

Well developed mycelium and expressed conidiogenesis characterized the vast majority of tested cultures at the age of 21 day. At this time, changes of paper filters such as pigmented spots, and intertwinement of cellulose fibres with fungal hyphae were recorded. The abundant growth of tested cultures and changes of the substrate were also recorded after 28 days (Fig. 1).

Table 3. Ability of 15 *Aspergillus fumigatus* strains to develop on Czapek agar and assimilate collagen as the sole source of carbon.

Strain No.	Evaluation of fungal growth and sporulation intensity, visible changes of stained leather powder				
	5 days	10 days	15 days	21 days	28 days
1	++	++	++	++	++
2	++	++	++	+++	+++
3	++	++	++	++	++
4	++	++	++	++	++
5	++	++	++	++	++
6	++	++	++	+++	+++
7	++	++	++	++	++
8	++	++	++	++	++
9	++	++	++	++	++
10	++	++	++	++	++
11	++	++	++	++	++
12	++	++	++	++	++
13	++	++	++	++	++
14	++	++	++	++	++
15	++	++	++	++	++

+: single non-sporulating hyphae; ++: poorly developed and sporulating mycelium; +++: moderately developed and sporulating mycelium; ++++: good developed, abundantly sporulating mycelium; +++++ very good developed, abundantly sporulating mycelium, evident lysis of leather powder.

Table 4. Ability of 15 *Aspergillus fumigatus* strains to develop on Czapek agar and assimilate fatty acids as the sole source of carbon.

Strain No.	Evaluation of fungal growth and sporulation intensity, visible changes of droplets of olive oil				
	5 days	10 days	15 days	21 days	28 days
1	+++	++++	++++	++++	++++
2	+++	+++	++++	++++	++++
3	+++	++++	++++	++++	++++
4	++	++++	++++	++++	++++
5	+++	++++	++++	++++	++++
6	+++	++++	++++	+++++	+++++
7	+	++++	++++	++++	++++
8	+	+++	++++	++++	++++
9	+++	++++	++++	++++	++++
10	+++	++++	++++	++++	++++
11	+++	++++	++++	++++	++++
12	++	+++	++++	++++	+++++
13	+++	++++	++++	++++	++++
14	++	++++	++++	++++	++++
15	+++	++++	++++	++++	++++

+: single non-sporulating hyphae; ++: poorly developed and sporulating mycelium; +++: moderately developed and sporulating mycelium; ++++: good developed, abundantly sporulating mycelium; +++++: very good developed, abundantly sporulating mycelium, evident lysis of droplets of olive oil.

Quantitative measurement of endo-1,4-β-glucanase activity. All 15 strains of *Aspergillus fumigatus* were able to develop in liquid Czapek medium with 0.7% of cellulose as the sole carbon source under submerged conditions. Analysis of the EGA test revealed that 11 out of 15 cultures produced endo-1,4-β-glucanase (EGA) after 10 days of development. The activities significantly differed among strains ($F(14.30)=366.86$; $p<0.0001$). According to Scheffe test, strains No 3, 4, 5, 7, 9, 13, 14, and 15 fell into a homogenous group by means and showed the highest activity levels; strains No 6 and 11 showed medium activity, while strain No. 8 showed least expressed enzymatic activity. Strains No 1, 2, 10, and 12 did not show the presence of EGA (Fig. 4).

Analysis of the EGA test after 15 days of cultivation revealed that all the values fell into two groups: strains No. 1, 3, 4, 5, 6, 7, 9, 11, 13, 14, and 15 showed high enzymatic activities and fell into a homogenous group by means, while values of enzymatic activities of cultures No. 2, 8, 10, and 12 were lower (Fig. 5). Achieved values significantly differed among the strains ($F(14.30)=182.48$; $p<0.0001$).

The highest values of enzymatic activity were detected after 21 days of cultivation. EGA produced by the strains No. 1, 3, 4, 5, 7, 9, 10, 11, 13, 14, and 15 decreased viscosity of CMC solution more than 50%, and the values fell into one homogenous group by means. The strains No. 6 and 8 showed medium activity levels whereas the strains No. 2 and 12 showed the lowest activity (Fig. 6). There were significant differences among the strains in production of the EGA ($F(14.30)=333.36$; $p<0.0001$).

Production of proteases. Almost all examined strains of *Aspergillus fumigatus* cultivated on Czapek agar with 0.3% of stained leather powder as the sole source of carbon showed restrictive growth and poor sporulation during entire incubation time, up to 28 days (Tab. 3). The only exception were strains No. 2 and 6 which showed a moderate development and sporulating. Nevertheless, cases of obvious lysis of the leather powder in the medium were not recorded (Fig. 2).

Production of lipases. The strains cultivated on Czapek agar with 2.0% of olive oil showed considerable differences in growth and sporulation intensity at the beginning of the test (Tab. 4). After 5 days of incubation, 9 out of 15 strains were ascribed as fast growing and proliferating, and only strains No. 7 and 8 were underdeveloped with pigmentless non-sporulating mycelium. At the age of 15 days, all the cultures became mature with well developed mycelium and abundant sporulation, though lysis of the droplets of olive oil in the medium was not observed. Clear zones without oil droplets in the medium were noticed under developing colonies of the strain No. 6 after 21 days of cultivation, and under colonies of the strain No. 12 after 28 days of cultivation (Fig. 3).

DISCUSSION

Widespread occurrence of *Aspergillus fumigatus* and the ability to colonize various substrates indicate that this fungus possesses well-developed enzymatic systems. High cellulolytic activities of the examined *A. fumigatus* strains found in the present study are in accordance with the data published by other authors [5, 9, 12, 28, 36, 37]. The ability to produce thermotolerant cellulases enables the fungus to develop in the extreme conditions which exist in piles of compost, organic debris, moist hay, and wood pulp.

The examined *A. fumigatus* strains showed no evident proteolytic activity when growing on Czapek agar and assimilating collagen as the sole carbon source. According to literature data, the high collagenic activity of *A. fumigatus* could play a role in the invasion of the tissues by the fungus [24]. Extracellular proteases produced by *A. fumigatus* are able to induce detachments of the epithelial cells and the release of proinflammatory cytokines [13, 31, 38]. Moreover, these proteins act as allergens [31, 33].

It was revealed that some of the tested strains of *Aspergillus fumigatus* are able to produce extracellular lipases and hydrolyze fatty acids of vegetable oil. This correlates with scanty published data [7, 26]. For a more precise evaluation of lipases more sophisticated methods should be applied.

In conclusion, a strong cellulolytic activity of *A. fumigatus* strains isolated from the air at waste landfills, evidenced in the present study by two methods, suggest that the cellulolytic enzymes are abundantly produced by these fungi developing in waste piles. Though from the economic point of view the fast decomposition of waste is beneficial, vast amounts of cellulolytic enzymes should be considered as a potential adverse factor that may be a cause of allergy and increased virulence of *A. fumigatus* strains and hence should be the subject of further studies.

REFERENCES

1. Bilaj TI: Determination of activity of proteinases. **In:** Bilaj V (Ed): *Methods of Experimental Mycology*, 207-211. Naukova Dumka, Kiev 1982 (in Russian).

2. Borovikov VP, Borovikov IP: *Statistics. Statistical Analysis and Data Processing in the Windows Medium*. Filin, Moscow 1997 (in Russian).

3. Clark CS, Rylander R, Larsson L: Levels of gram-negative bacteria, *Aspergillus fumigatus*, dust, and endotoxin at compost plants. *Appl Environ Microbiol* 1983, **45**, 1501-1505.

4. Crook B, Bardos RP, Lacey J: Domestic waste composting plants as sources of airborne micro-organisms. *The Aerosol Society Second Conference "Aerosols, Their Generation, Behaviour and Applications", Bournemouth, 22-24 March 1988*, 63-68. The Aerosol Society Second Conference, Bournemouth, UK 1988.

5. Das MK, Prasad JS, Ahmad SK: Endoglucanase production by paper-degrading mycoflora. *Lett Appl Microbiol* 1997, **25**, 313-315.

6. Denning DW: Invasive aspergillosis. *Clin Infect Dis* 1998, **26**, 781-805.

7. Domsch KH, Gams W: *Compendium of Soil Fungi*. Academic Press, London 1980.

8. Dutkiewicz J, Jabłoński L: *Biologiczne Szkodliwości Zawodowe* (*Occupational Biohazards*). PZWL, Warsaw 1989 (in Polish).

9. Gravesen S, Frisvad JC, Samson RA: *Microfungi*. Munksgaard, Copenhagen 1994.

10. Huuskonen MS, Husman K, Järvisalo J, Korhonen O, Kotimaa M, Kuusela T, Nordman H, Zitting A, Mäntyjärvi R: Extrinsic allergic alveolitis in the tobacco industry. *Br J Ind Med* 1984, **41**, 77-83.

11. Jongman RH, Ter Braak CJF, Van Tongeren OFR: *Data Analysis in Community and Landscape Ecology*. Pudoc, Wageningen, the Netherlands 1987.

12. Joseph JM: Aspergillosis in composting. **In:** DiSalvo AF (Ed): *Occupational Mycoses*, 123-141. Lea & Febiger, Philadelphia 1983.

13. Kauffman HF, Tomee JF, Van De Riet MA, Timmerman AJ, Borger P: Protease-dependent activation of epithelial cells by fungal

allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol* 2000, **105**, 1185-1193.

14. Kiviranta H, Tuomainen A, Reiman M, Laitinen S, Nevelainen A, Liesivuori J: Exposure to airborne microorganisms and volatile organic compounds in different types of waste handling. *Ann Agric Environ Med* 1999, **6**, 39-44.

15. Krysińska-Traczyk E: Pleśnie *Aspergillus fumigatus* jako przyczyna schorzeń płuc o charakterze zawodowym (Moulds *Aspergillus fumigatus* as a cause of respiratory diseases of occupational origin). *Med Wiejska* 1973, **8**, 275-284 (in Polish).

16. Lacey J, Crook B: Review: Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. *Ann Occup Hyg* 1988, **32**, 515-533.

17. Lacey J, Williamson PAM, King P, Bardos RP: *Airborne Microorganisms Associated with Domestic Waste Composting.* Report No. LR 808 (MR). Warren Spring Laboratory, Stevenage, Herts., UK 1990.

18. Lacey J, Williamson PAM, Crook B: Microbial emissions from composts made for mushroom production and from domestic waste. **In:** Jackson DV, Mercllor JM, L'Hernite P (Eds): *Composting and Compost Quality Assurance Criteria*, 117-130. EUR 14254, CEC, Luxembourg 1992.

19. Land CJ, Hult K, Fuchs R, Hagelberg S, Lundström H: Tremorgenic mycotoxins from *Aspergillus fumigatus* as a possible occupational health problem in sawmills. *Appl Environ Microbiol* 1987, **53**, 787-790.

20. Latge JP: The pathobiology of *Aspergillus fumigatus. Trends in Microbiology* 2001, **9**, 382-389.

21. Millner PD, Olenchock SA, Epstein E, Rylander R, Haines J, Walker J, Ooi BL, Horne E, Maritato M: Bioaerosols associated with composting facilities. *Compost Science & Utilization* 1994, **2,** 6-55.

22. Minárik L, Skalsky T, Heyná A, Steklačova E: Plucne aspergilózy profesionálneho povodu (Occupational aspergillosis of the lung). *Rozhl Tuberk* 1969, **29**, 169-173 (in Slovak).

23. Minárik L, Mayer M, Votrubová V, Ürgeová N, Dutkiewicz J: Exogenná alergická alveolitida z precitlivenosti na antigény z plesnivych bukovych stiepok. Popis dvoch pripadov (Allergic alveolitis due to antigens present in mouldy beech chips - description of two cases). *Studia Pneumol Phtiseol Cechoslov* 1983, **43**, 38-45 (in Slovak).

24. Monod M, Jaton-Ogay K, Reichard U: *Aspergillus fumigatus* – secreted proteases as antigenic molecules and virulence factors. *Contrib Microbiol* 1999, **2**, 182-192.

25. Nielsen EM, Nielsen BH, Breum NO: Occupational bioaerosol exposure during collection of household waste. *Ann Agric Environ Med* 1995, **2**, 53-59.

26. Ogundero VW**:** Hydrolysis of vegetable oils and triglycerides by thermotolerant and zoopathogenic species of Aspergillus from Nigerian palm produce. *Mycopathologia* 1982, **77**, 43-46.

27. Prażmo Z, Krysińska-Traczyk E, Dutkiewicz J, Sitkowska J, Skórska C, Tarkowski S, Kończalik J: Mikroflora bioaerozoli powstających na wysypisku odpadów komunalnych (Microflora of bioaerosols present at a municipal waste dumping site). *II Konferencja Naukowa "Rozkład i Korozja Mikrobiologiczna Materiałów Technicznych", Łódź, 30-31 May 2001*. Materiały Konferencyjne, 133-137. Politechnika Łódzka, Łódź 2001.

28. Raper KB, Fennell DI: *The Genus Aspergillus*. Williams & Wilkins, Baltimore 1965.

29. Reese ET, Siu RGU, Levinson US: The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis. *J Bacteriol* 1950, **59,** 485-497.

30. Reese ET: Enzymatic hydrolysis of cellulose. *Appl Microbiol* 1956, **4,** 39-45.

31. Robinson BW, Venaille TJ, Mendis AH, McAleer R: Allergens as proteases: An *Aspergillus fumigatus* proteinase directly induces human epithelial cell detachment. *J Allergy Clin Immunol* 1990, **86**, 726-731.

32. Schelley AW, Deeth HC, MacRae IC: Review of methods of enumeration, detection and isolation of lipolytic microorganisms with special reference to dairy applications. *J Microbiol Methods* 1987, **6**, 123-137.

33. Shen HD, Lin WL, Tam MF, Chou H, Wang CW, Tsai JJ, Wang SR, Han SH: Identification of vacuolar serine proteinase as a major allergen of *Aspergillus fumigatus* by immunoblotting and N-terminal amino acid sequence analysis. *Clin Exp Allergy* 2001, **31**, 295-302.

34. Sivers VS, Musich EG, Lizak YV: Determination of activity of cellulases. **In:** Bilaj V (Ed): *Methods of Experimental Mycology*, 180-186. Naukova Dumka, Kiev 1982 (in Russian).

35. St Leger RJ, Joshi L, Roberts DW: Adaptation of proteases and carbohydrates of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their niches. *Microbiology* 1997, **143**, 1983-1992.

36. Stewart JC, Parry JB: Factors influencing the production of cellulase by *Aspergillus fumigatus. J Gen Microbiol* 1981, **125**, 33-39.

37. Tashpulatov J: Cellulolytic enzymes of thermophilic and mesophilic strains of *Aspergillus fumigatus. Mikrobiologiya* 1966, **35**, 27-34 (in Russian).

38. Tomee JFC, Wierenga AT, Hiemstra PS, Kauffman HK: Proteases from *Aspergillus fumigatus* induce release of proinflammatory cytokines and cell detachment in airway epithelial cell lines. *J Infect Dis* 1997, **176**, 300-303.

39. Vincken W, Roels P: Hypersensitivity pneumonitis due to *Aspergillus fumigatus* in compost. *Thorax* 1984, **39**, 74-75.

40. Watanabe N, Ota Y, Minoda Y, Yamada K: Isolation and identification of alkaline lipase producing microorganisms, cultural conditions and some properties of crude enzymes. *Agric Biol Chem* 1977, **41**, 1353-1358.