Enzyme production by soilborne fungal strains of *Aspergillus niger* isolated from different localities affected by mining

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INTRODUCTION

Enzymes have wide application in industry. Lipase and protease are of great use in several sectors, for example, in the food industry and the leather industry; they are also used in the production of cosmetics, detergents, medicines, etc. [1,2]. Esterase plays an important role in the degradation of natural materials and industrial pollutants, such as wastes from cereals, plastics and other toxic chemicals. It also has uses in the synthesis of compounds, perfumes and antioxidants [3]. The biotechnological function of cellulase is employed especially in agriculture, the food industry, brewing and wine-making, as well as in the refining of biomass, cellulose, paper, textiles and linens [4]. The aim of the study was to compare the production of the enzymes lipase, protease, esterase and cellulase by several strains of *A. niger* isolated from various localities. These localities differ from one another by the value of the soil reaction and by the varying degrees of contamination with heavy metals and potentially toxic elements; most of them are sites of old ecological burdens.

MATERIAL AND METHODS

Strains of the microscopic filamentous fungus A. niger

The localities or substrates from which the A. niger strains were isolated differed mainly in the value of the soil reaction from ultra-acidic (<3.5) to strongly alkaline (8.5 – 9.0) as well as in values of potentially toxic elements (Tab. 1). For monitoring the enzymatic activity, we used sixteen strains of Aspergillus niger (A. niger). We selected A. niger from uncontaminated alluvial forest soil in the Gabčíkovo locality as the control strain.

Enzyme activity

We observed the enzyme activity of the strains on diagnostic nutrient media according to Kraková et al. [5], specified for the determination of individual enzymes: cellulase activity (CA) on CongoRed medium, esterase activity (EA) on Tween 80 medium, lipase activity (LA) on Spirit Blue medium (HiMedia, Mumbai, India) and protease activity (PA) on Gelatine P3 medium. The production of an enzyme is visible in the form of the so-called "halo" effect (CA, LA) or as an opaque (PA) or yellow zone around the growth of the organism (EA) [6]. The growth and colony size of all the *A. niger* strains were monitored for comparison by culture on SDA agar (Sabouraud Dextrose Agar, HiMedia, Mumbai, India).

Table 1. *A. niger* strains sorted based on the range of pH values isolated from soils and substrates and their chemical characteristics.

	Strain	pH	potentially toxic elements in soil/substrate		Strain	pH	potentially toxic elements in soil/substrate
1.	**An-S**	3.0 ultra acidic	Al 727–506 mg/kg	9.	**An-L18**	6.85 neutral	NEL 201 000 mg/kg; PAH C10-C40 121 000 mg/kg; *Cr 182 mg/kg; *Cu 2 102 mg/kg; *Zn 6 946 mg/kg; *Ba 3 652 mg/kg; *Pb 4 066 mg/kg
2.	**An-N**	3.32 ultra acidic	As 400 mg/kg; Mn 302,4 mg/kg; *Zn 21,4 mg/kg	10.	**An-Pop3**	7.45 slightly alkaline	As 25 mg/kg; *Sb 12200 mg/kg; *Zn 150 mg/kg; Cu 60 mg/kg; Pb 70 mg/kg; Hg 0,5 mg/kg
3.	**An-Pop4**	3.85 extreme acidic	As 25 mg/kg; *Sb 5825 mg/kg; *Zn 150 mg/kg; *Cu 60 mg/kg; Pb 70 mg/kg;	11.	**An-ZK**	7.51 slightly alkaline	As 634 µg/g; Zn 47 µg/g; Hg 0,47 µg/g
4.	**An-Pop1**	4.52 very strong acidic	As 25 mg/kg; *Sb 1022 mg/kg; *Zn 150 mg/kg; Cu 60 mg/kg; Pb 70 mg/kg;	12.	**An-G**	7.7 slightly alkaline	-
5.	**An-P**	5.25 strong acidic	As 363 mg/kg; Sb 93 mg/kg; Fe 82.8 mg/kg; Al 5,5 %	13.	**An-KD**	8.25 medium alkaline	-
6.	**An-Sm**	5.4 strong acidic	Mg 344 mg/l; *Fe 463 mg/l; *Mn 36,5 mg/l; *Al 107 mg/l; *Cu 3263 µg/l; *Zn 12600 µg/l; *Cd 15 µg/l	14.	**An-KF**	8.49 medium alkaline	-
7.	**An-Kmi**	5.4 strong acidic	-	15.	**An-SL**	8.6 strong alkaline	As 511 mg/kg; *Cu 8186 mg/kg; *Zn 25108 mg/kg; *Pb 2964 mg/kg; *Mn 2647 mg/kg; *Cd 8,76 mg/kg
8.	**An-Pop5**	6.05 medium acidic	As 200 mg/kg; *Sb 2099 mg/kg; *Zn 200 mg/kg; Cu 70 mg/kg; *Pb 115 mg/kg;	16.	**An-Aral**	8.6 strong alkaline	As, Sb, Cr, Cs

RESULTS AND DISCUSSION

For checking the growth of the *A. niger* strains we used SDA broth. Upon culturing the strains on the control medium, we did not record any large differences in the size and growth rate of the colonies. Pronounced differences were observed after five days of cultivation, particularly in the size of the colonies and the intensity of sporulation of the strains on diagnostic nutrient media. The largest colonies were formed by strains on the nutrient medium for lipase activity, where we also observed the most intense sporulation. Next were the strains on media for esterase activity and protease activity, and the smallest colonies were formed by strains on nutrient medium for cellulase activity (figure 1). The production of the monitored enzymes of the *A. niger* strains is shown in table 2.

Table 2. Growth of *A. niger* (SDA) strains and their lipase (LA), esterase (EA), protease (PA) and cellulase (CA) activity.

Strain	SDA	LA	EA	PA	CA
An-S	++	+	+/-	-	-
An-N	++	+	+/-	-	-
An-Pop 4	++	++	+/-	-	-
An-Pop1	++	+	+/-	-	-
An-P	++	++	+/-	-	-
An-Sm	++	+	+/-	-	-
An-Kmi	++	+	+/-	-	-
An-Pop 5	+	+	+/-	-	-
An-L18	++	++	+	-	-
An-Pop 3	+	+	+/-	-	-
An-ZK 5	++	++	+/-	-	-
An-G	++	++	+/-	-	-
An-KD	+	+	+/-	-	-
An-KF	++	+	+/-	-	-
An-SL	++	++	+/-	-	-
An-Aral	+	++	+/-	-	-

Lipase activity

When culturing the strains on SBA broth, the colonies had an average size of 2 ± 0.1 to 3.1 ± 0.1 cm. The largest colonies were formed by the An – Pop 1, An – Pop 3 and An – KD strains. The smallest colonies were clearly formed by the control strain An – G, which, however, showed the most intense lipase activity, as the medium stained quickly and the so-called "halo" effect was formed. Strains An – Pop 4, An – P, An – L18, An – ZK and An – SL showed only very weak lipase production (figure 3). In other *A. niger* strains, the production of lipase was not confirmed, which means that these *A. niger* strains either do not produce the enzyme at all or that production is so low that discolouration of the SBA diagnostic medium does not occur at all.

Esterase activity

When monitoring esterase activity on the Tween 80 diagnostic medium, rapid growth and intense sporulation of *A. niger* strains were observed, with a mean colony size of 2.5 ± 0.1 to 3 ± 0.1 cm. Yellow staining around the colony growth was observed only in the An – L18 strain, and no similar reaction was observed in the other strains.

Protease activity

When cultured on Gelatine P3 broth for determining protease activity, *A. niger* strains formed colonies with an average size of 1.5 ± 0.1 to 2 ± 0.1 cm. However, no halo effect or an opaque zone, which indicates the formation of this enzyme, appeared around the growth of the colonies. On the basis of some studies, the production of the protease enzyme may be inhibited by the occurrence of above-limit Cd values in the environment [7]. This element was present in the majority of the examined localities.

Cellulase activity

Upon monitoring cellulase activity on the CongoRed medium, very slow growth of *A. niger* strains was observed, with an average colony size of 1.5 ± 0.1 cm. No production of the cellulase enzyme, which would have been found in a similarly opaque arc around the colony, was observed. Similar results were reported with the monitoring of cellulase activity of *Penicillium* species taken from environments with above-limit values of the elements As, Zn, Sb and Pb [8].

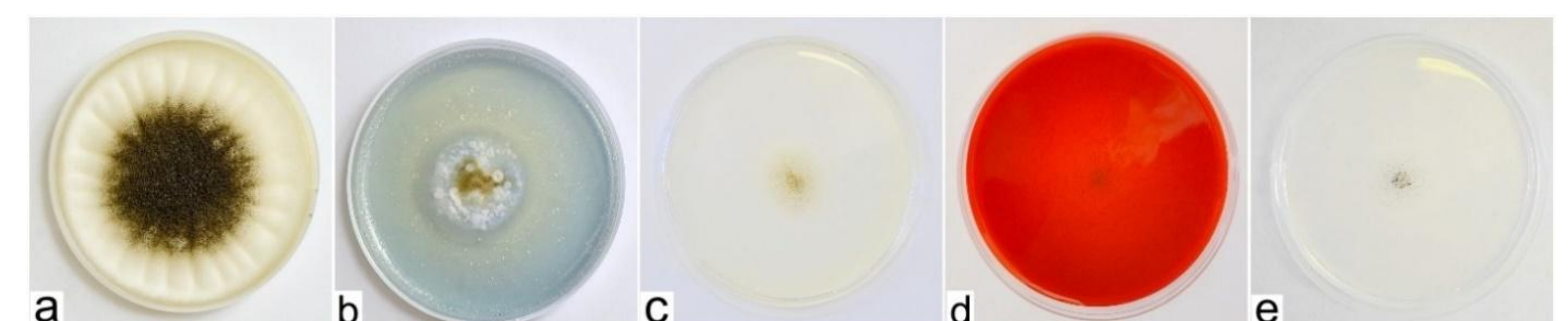

Figure 1. Enzymatic activity of the control strain An – G: SDA (a), LA (b), EA (c), CA (d) and PA (d).

CONCLUSION

Differences in the growth and size of colonies, as well as weak enzymatic activity in strains taken from a contaminated environment, are with the highest probability caused by the negative impact of above-limit values of potentially toxic elements that occurred in the majority of the localities. The achieved results confirmed the direct influence of environmental factors on the physiological properties of the studied strains of *Aspergillus niger*. We recorded enzymatic activity when monitoring lipase production, where we observed the most intense activity in the strain that was isolated from an uncontaminated environment (An – G). As a result of the impact of potentially toxic elements that exceed the limit values in all substrates taken from mining sites, the production of enzymes was probably suppressed in strains taken from contaminatd enivroments.

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