Aspergillus niger Environmental Isolates and Their Specific Diversity Through Metabolite Profiling

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We present a biological profile of 16 Aspergillus niger environmental isolates from different types of soils and solid substrates across a pH range, from an ultra-acidic (<3.5) to a very strongly alkaline (>9.0) environment. The soils and solid substrates also differ in varying degrees of anthropic pollution, which in most cases is caused by several centuries of mining activity at old mining sites, sludge beds, ore deposits, stream sediments, and coal dust. The values of toxic elements (As, Sb, Zn, Cu, Pb) very often exceed the limit values. The isolates possess different macro- and micromorphological features. All the identifications of Aspergillus niger isolates were confirmed by molecular PCR analysis and their similarity was expressed by RAMP analysis. The biochemical profile of isolates based on FF-MicroPlate tests from the Biolog system showed identical biochemical reactions in 50 tests, while in 46 tests the utilisation reactions differed. The highest similarity of strains isolated from substrates with the same pH, as well as the most suitable biochemical tests for analysis of the phenotypic similarity of isolated strains, were confirmed when evaluating the biochemical profile using multicriterial analysis in the Canoco program. The isolates were screened for mycotoxin production by thin-layer chromatography (TLC), as well. Two of them were able to synthesise ochratoxin A, while none produced fumonisins under experimental conditions. Presence of toxic compounds in contaminated sites may affect environmental microscopic fungi and cause the genome alteration, which may result in changes of their physiology, including the production of different (secondary) metabolites, such as mycotoxins.

Keywords: Aspergillus niger environmental isolates, molecular analyses, Biolog FF Microplate™, multi-criteria data analysis, extrolite profile

INTRODUCTION

Aspergillus niger is a cosmopolitan representative of microscopic filamentous fungi. Although the main source of this strain is soil, it frequently occurs in various other sources, such as historical and archaeological objects (Abdel-Kareem, 2010; Pangallo et al., 2012; Aldosari et al., 2019; Geweely et al., 2019) or indoor environments (Egbuta et al., 2017; Omar et al., 2018). The ability of Aspergillus
Aspergillus niger to produce substances of various types, such as low molecular weight organic acids (e.g., gluconic, citric, itaconic, oxalic, malic, acetic, lactic, and others), enzymes (e.g., amylase, aryl-phosphatase, β-glucosidase, cellulase, lipase, and others) as well as other products of metabolism, has great use not only in the food, medicine, pharmaceutical, and chemical industries but also in mineral biotechnology (Steiger et al., 2013; Akhtar et al., 2014; Utobo and Tewari, 2014; Adeleke et al., 2017; Odoni et al., 2017). According to Esteban et al. (2006), up to 6% of A. niger strains synthesise the mycotoxin ochratoxin A, and its degree of production depends on environmental factors, such as the substrate or cultivation medium type, cultivation time, etc. Foodborne A. niger strains are also able to produce the mycotoxins fumonisins, namely fumonisin B1 and B2 (Noonim et al., 2009; Mikušová et al., 2020). The cultivation medium notably affects fumonisin synthesis, as is the case with ochratoxin A production (Frisvad et al., 2007). The ability to synthesise mycotoxins always depends on the expression of the genome of a particular strain of toxic fungal species (Susca et al., 2016).

These metabolic products are mainly used in the biodegradation processes of environmental pollutants, such as crude oil and its products found in industrial water and wastewater (Gulzar et al., 2017). The biosorption and bioleaching of heavy metals and toxic elements from solutions and soils are key processes in bioremediation (Acosta-Rodriguez et al., 2018; Xinhue et al., 2018). This means the treatment of contaminated water, soil and subsurface material by altering environmental conditions to stimulate the growth of microorganisms can degrade the target pollutants. Among the most commonly applied species or strains or certain types of microscopic filamentous fungi in these processes were those that were isolated from the contaminated environment. They are expected to be more effective than species or strains isolated from an uncontaminated environmental source (Hindersah et al., 2018). According to Chávez et al. (2015), the potential for the discovery of novel metabolites in microscopic filamentous fungi is huge. In this context, fungi thriving in harsh environments are of particular interest, since they are outstanding producers of unusual chemical structures.

The Biolog technique, or Biolog FF MicroPlates, has been used in microbiology for more than fifteen years. Klingler et al. (1992) were the first to do so. The Biolog technique was also used in ecological studies to estimate the metabolic potential of microbial communities from rhizospheric soil (Stefanowicz, 2006), substrate utilisation, growth, secondary metabolite, and the antimicrobial profiles of some fungal cultures important for the microbial drug discovery program (Singh, 2009). The Biolog system was used to enable the evaluation of the metabolic profile diversity of microbial populations in environmental samples, which reflects the state of their activity (Gryta et al., 2014; Šimonovičová et al., 2020). Twelve Aspergillus sp. strains were also characterised using Biolog FF MicroPlates to obtain data on C-substrate utilisation and mitochondrial activity (Rola et al., 2015) in testing the functional diversity of root-colonising endophytic fungi (Knapp and Kovács, 2016). Biolog FF Microplate system also identified soil fungal isolates from forest soil for the recovery of flooded soil (Aziz and Zainol, 2018).

The aim of our study was to show the biological profile of 16 Aspergillus niger environmental isolates focusing on the biochemical profiling and physiology of the strains isolated from different types of soils and solid substrates, such as stream sediments, ash layer, coal dust and the surface of artificial adamtite [Zn$_2$(AsO$_4$)$_2$(OH)$_2$], by using the Biolog FF MicroPlate system. Besides metal contamination, the substrates also differed from each other in pH across a range from an ultra-acidic (<3.5) to a very strongly alkaline environment (>9.0).

Contamination of some localities is more than 700 years old and dates back to mining times, so we expected also a changes in the genome associated with the microstructure of study strains, including the production of different (secondary) metabolites, such as mycotoxins.

The biochemical profile of strains based on FF-microplate tests from the Biolog system showed identical biochemical reactions in 50 tests, while in 46 tests the utilisation reactions differed. Data were processed using the PCA method (Principal Component Analysis) in the CANOCO 5 program (Braak and Smilauer, 2012).

In terms of physiological and biochemical properties is Aspergillus niger known as a very good producer of mycotoxins. Therefore all our investigated strains were screened for their ability to produce ochratoxin A (OTA) and fumonisin B1 (FB1) in vitro by rapid thin-layer chromatography (TLC) (Filtenborg et al., 1989; Nelson et al., 1993; Nielsen et al., 2020).

The species Aspergillus niger, along with 16 environmental strains that we isolated, was shown to be a suitable model organism in comparing variable experimental methods with the aim of identifying their common, respectively, different characteristics depending on the environment of origin.

**MATERIALS AND METHODS**

**Aspergillus niger Environmental Isolates**

Sixteen Aspergillus niger (A. niger) strains were isolated from different types of soils (samples 1, 3, 4, 7, 8, 10, 12–14), coal dust (sample 2), stream sediments (samples 5 and 6), an ash layer (sample 11), a tailing pond (samples 9 and 15) and a surface of artificial adamtite (sample 16) (Figure 1). The samples from all solid substrates were taken from three places at each site. Then in laboratory by mixing an average sample was prepared and analysed. From the surface of artificial adamtite was A. niger isolated by using a SWABS swab (1660, Dispolab Brno, Czechia) and subsequent cultivated on Sabouraud Dextrose Agar (M063), HiMedia Labs., Mumbai, India). The chemical characteristics of the localities and substrates from which A. niger strains were isolated are shown in Table 1.

The metallic element composition of the samples from the Banská Štiavnica—Šobov, Pezinok, Nováky, and Smolník localities were analysed by the EL spol. Ltd. accredited test labs in Spišská Nová Ves (Slovakia). Samples from the Poproč, Zemianske Kostoľany, and Slovinky localities were analysed for their metal content at ACME Analytical Laboratories Ltd. (Vancouver, Canada) by ICP-ES or ICP-MS (Šimonovičová et al., 2019). Persistent toxic substances in the Ostrava Lagoons...
locality were analysed in sludge samples (mg/kg of dry weight) using several methods. XRF technology—X-ray fluorescence spectrometry with high sensitivity on the S8 TIGER instrument (Bruker Co., United States) was used for the analysis of metal and metalloid residues. Organic pollution analyses were performed at ALS Czech Republic, Ltd. (a testing laboratory accredited according to ČSN (Czech National Standard) EN ISO/IEC 17025:2005).

All strains were isolated from mixed cultures of fungi by the dilution plate method on SDA (Sabouraud Dextrose Agar, FyHimedia Laboratories, Mumbai, India) at a laboratory temperature of 25 °C for 5–7 days (Šimonovičová et al., 2013, 2017, 2019). All isolates were assigned to A. niger according to molecular analyses and according to the name of the locality, as shown in Table 1. All strains are deposited on SDA in the collection of filamentous fungi in the Department of Soil Science at the Faculty of Natural Sciences, Comenius University in Bratislava (Slovakia). The ITS sequences of the Aspergillus niger isolates were deposited in GenBank under accession numbers MW739953-MW739968.

Macro and Micromorphological Features of the A. niger Environmental Isolates
The macromorphological features, i.e., the diameter of colonies and degree of sporulation, were observed visually on the fifth day of cultivation on SDA in Petri dishes and had a diameter of 6 cm in three repetitions. The micromorphology figures were made with a Canon IXUS 16.1 megapixels camera (Japan). Also, micromorphological features (Figure 2) were observed under an Axio Scope A1 Carl Zeiss Jena light microscope on the fifth day of cultivation in a drop of lactic acid enriched with a cotton blue stain (0.01%).

Genotypic Profile of Aspergillus niger Environmental Isolates
The DNA of A. niger isolates was extracted using a DNeasy Plant mini purification kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The Random Amplified Microsatellite Polymorphism-Polymerase Chain Reaction (RAMP-PCR) was performed according to the protocol of Pangallo et al. (2012). The PCR reaction mixture (25 µL) contained: 2 U (0.4 µL) of SuperHotTag DNA polymerase (Bioron), 1× reaction buffer (2.5 µL), 2.4 mmol−1 MgCl2 (0.6 µL), 200 µmol−1 dNTP (0.4 µL), 60 pmol (0.4 µL) of each primer T14 (AAT GCC GCA G), and K7 (CAA CTC TCT CTC TCT), 17.3 µL of deionised water and approximately 60 ng (3 µL) of DNA. The PCR amplification program included: a denaturation step at 95 °C for 5 min; 30 cycles: 95 °C 45 s; 30 °C for 60 s with ramping 0.1 °C/s to 50 °C, 50 °C for 60 s with ramping 0.1 °C/s to 68 °C, 68 °C for 180 s, and a final polymerisation at 68 °C for 10 min. Five microlitres of RAMP-PCR amplicon were separated on 1.8% agarose gel for 4.5 h at 2.3 V cm−1 in TAE buffer (Trid-acetate-EDTA). The gel was stained with ethidium bromide, visualised under UV light and digitalised.

The RAMP profiles were analysed according to BioNumerics software ver. 6 (Applied Maths, Kortrijk, Belgium) using Pearson correlation and UPGMA clustering.

Physiological Profile of Aspergillus niger Environmental Isolates
The biochemical profile of Aspergillus niger environmental isolates was obtained using the Biolog MicroStation test system (MicroLog3 ver. 4.20.05, Biolog Inc., United States). By using a 96-well Biolog FF MicroPlate, unique phenotypic fingerprinting can be obtained for individual strains; this is a manifestation of
### Table 1: Aspergillus niger environmental isolates obtained from different type of soils and solid sources and its chemical characteristics.

| Order of samples according to pH range | The range of pH according to Čurlík et al., 2003 | An fungal strains | Substrates of studied localities and its chemical characteristics | ITS-product sequence similarity | References |
|----------------------------------------|-----------------------------------------------|-------------------|---------------------------------------------------------------|------------------------------|------------|
| 1.                                     | An–S                                          | Banská Stiavnica—Šobov; Dystric Cambisol (contaminated and eroded) without vegetation; pH 3.12; %C$_{ox}$ 0.49; *Al 727–506 mg/kg | Banska Stiavnica—Shobov; Dystric Cambisol (contaminated and eroded) without vegetation; pH 3.12; %C$_{ox}$ 0.49; *Al 727–506 mg/kg | AM270051 99.6% | Šimonovičová et al., 2013, 2019 |
| 2.                                      | <3.5 Ultra acidic                             | An–N              | Nováky; coal dust; pH 3.32; %C$_{ox}$ 39; *As 400 mg/kg; Mn 302.4 mg/kg; *Zn 21.4 mg/kg | AM270051 99.8% | Šimonovičová et al., 2013, 2019 |
| 3.                                      | 3.5–4.4 Extreme acidic                        | An–Pop 4          | Poproč; Technosol; pH 3.85; %C$_{ox}$ 0.1; *As 25 mg/kg; *Sb 5,825 mg/kg; *Zn 150 mg/kg; *Cu 60 mg/kg; Pb 70 mg/kg; Hg 0.5 mg/kg | KKX01281 100% | Šimonovičová et al., 2019 |
| 4.                                      | 4.5–5.0 Very strong acidic                    | An–Pop 1          | Poproč; Haplic Leptosol; pH 4.52; %C$_{ox}$ 2.62; *As 25 mg/kg; *Sb 1,022 mg/kg; *Zn 150 mg/kg; Cu 60 mg/kg; Pb 70 mg/kg; Hg 0.5 mg/kg | KKX253667 100% | Šimonovičová et al., 2019 |
| 5.                                      | 5.1–5.5 Strong acidic                         | An–P              | Pezinok; stream sediment of Blatina river; pH 5.25; %C$_{ox}$ 7.2; *As 363 mg/kg; Sb 93 mg/kg; Fe 82.8 mg/kg; Al 5.5 % | AM270051 99.8% | Šimonovičová et al., 2013 |
| 6.                                      | 5.6–6.0 Medium acidic                         | An–Pop 5          | Poproč; Technosol; pH 6.05; %C$_{ox}$ 0.14; *As 200 mg/kg; *Sb 2,096 mg/kg; *Zn 200 mg/kg; Cu 70 mg/kg; Pb 115 mg/kg; Hg 0.75 mg/kg | KYX65777 100% | Šimonovičová et al., 2019 |
| 7.                                      | 6.6–7.3 Neutral                               | An–L18            | Lagoon Ostrava, Czechia; sludge from oil refining and other chemical processing; pH 6.85; NEL 201 000 mg/kg; PAH C$_{10}$–C$_{40}$ 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 | KU702453 100% | This publication |
| 10.                                     | 7.4–7.8 Slightly alkali                      | An–Pop 3          | Poproč; Haplic Leptosol; pH 7.45; %C$_{ox}$ 0.91; *As 25 mg/kg; *Sb 12,200 mg/kg; *Zn 150 mg/kg; Cu 60 mg/kg; Pb 70 mg/kg; Hg 0.5 mg/kg | KKK48976 100% | Šimonovičová et al., 2019 |
| 11.                                     | 7.9–8.4 Medium alkali                        | An–KD             | Kuwait; soil sample from desert; pH 8.25 | KX253667 100% | This publication |
| 14.                                     | 8.5–9.0 Strong alkali                        | An–KF             | Kuwait; soil sample from a farm; pH 8.49 | KX253667 100% | This publication |
| 15.                                     | 8.6–9.0 Weak acid                            | An–SL             | Slovíny; Technosol; pH 8.6; %C$_{ox}$ 0.8; *As 511 mg/kg; *Cu 8,186 mg/kg; *Zn 25,108 mg/kg; *Pb 2,964 mg/kg; *Mn 2,647 mg/kg; *Cd 8,76 mg/kg | KFX01303 100% | Šimonovičová et al., 2019 |
| 16.                                     | > 9.0 Very strong alkali                     | An–A              | Surface of artificial adamite [Zn$_2$(AsO$_4$)(OH)$_2$] | MT99437 100% | Kolenčík et al., 2017 |

*Exceeded values of elements; NEL, non-polar extractables; PAH, polyaromatic hydrocarbons, hydrocarbons C$_{10}$ – C$_{40}$ is an indicator for the determination of oil pollution, it is the amount of extractable non-polar hydrocarbons of petroleum and non-petroleum origin with 10–40 carbons per molecule contained in the matrix.

Their catabolic potential. Metabolic reactions, dependent on the production of suitable enzymes for the oxidation of the tested substrates, are quantified by the formation of tetrazolium dye (Bochner, 2009). These results, as fingerprint reaction patterns, provide a lot of information about the individual differences in the metabolic properties of each fungus tested.
All *A. niger* environmental isolates were cultured in tubes on 2% slants of MEA (Malt Extract Agar HiMedia Laboratories, Mumbai, India) at 26°C for 5–7 days. Conidia were then inoculated into FF-IF inoculating fluid (72106 FF-IF inoculating fluid, Biolog, United States) according to the Biolog protocol and the density was adjusted to the recommended value of 65%, which was measured using a turbidimeter. Subsequently, we inoculated 100 µL of conidia suspension onto plates containing a pre-set of test substrates. We incubated the microplates for 72–168 h and then evaluated them using the Biolog MicroStation system.

**Mycotoxins of Aspergillus niger Environmental Isolates**

Strains of *A. niger* were cultivated on MEA (Malt Extract Agar, HiMedia Laboratories, Mumbai, India) agar plates (thickness of the solid medium 0.5 cm) at room temperature for 14 days.

Rings of the fungal cultures (*d* = 1 cm) were placed onto the start line on the TLC silica gel plate (Merck TLC aluminium silica gel, 20 × 20 cm, Merck KGaA, Darmstadt, Germany). The medium side of the rings placed onto the silica gel enables diffusion of the fungal exotoxins (excreted by the fungus into its growing medium, here MEA) into the sorbent (here silica gel), while the biomass side soaked with two drops of extraction mixture methanol/water (80:20, disrupting the fungal cell wall and extracting the toxicants present inside the cell) was used to detect fungal endotoxins (kept in fungal cells). Griseofulvin (10 µL of a solution 100 µg/ml chloroform) (Sigma Aldrich, St. Louis, United States) was applied as the standard. Dried TLC plates were sprayed with 0.5% p-anisaldehyde in a mixture of ethanol/acetic acid/sulphuric acid (17:2:1) and heated at 140°C for 2–3 min. FB1 appears as lilac-deep lilac spots, the standard as a blue spot. All the spots observed were characterised by their retention factors (RF). The ratio of RF of the spot analysed to the RF of the standard griseofulvin (always considered equal to one) was proving the particular mycotoxin production by comparison to the database of these ratio factors according to Filtenborg et al. (1989). The complex method is graphically documented in Figure 3.

**Data Processing**

Multicriteria analysis (Principal Component Analysis—PCA) in the Canoco 5 program (Braak and Smilauer, 2012) was used to analyse the phenotypic similarity of the strains based on biochemical parameters obtained by the Biolog system.
RESULTS AND DISCUSSION
The Characteristics of Localities
All the *Aspergillus niger* isolates from environmental samples from Slovakia, Czechia (Figure 1) and Kuwait used in our study point out to the extreme adaptability of this species across a wide pH range and its high tolerance to metal contamination, despite changes in the macro- and microstructure (Figure 2). Among different type of soils, *Aspergillus niger* strains were isolated from Dystric Cambisol (contaminated and eroded), Technosol, Haplic Leptosol, Cambisol (Calcric), and Eutric Fluvisol, all of which, the last two excepted, were contaminated with heavy metals and toxic elements exceeding the limit values for soils according to Act No. 220 (2004) in Slovakia. This contamination is in most cases caused by several centuries of mining activities, such as quartzite quarrying since 1956 at the Banská Štiavnica—Šobov locality, exploitation of Sb and Au from the seventeenth century at the Poproč locality, or from iron ore mining since the fifteenth century at the Slovinky locality. It can be assumed that both soil samples from Kuwait are also contaminated with petroleum substances due to the discovery of petroleum in 1930 and the rapid development of the oil industry. According to Cho et al. (1997) in the Kuwait desert remains a huge amount of soil contaminated by oil. It was also confirmed by Asem et al. (2016) which studied the impact of crude oil pollution on different types of soils in Kuwait and the contamination was detected up to a soil depth of more than 2 m. Also, all solid substrates are among the contaminated. These include coal dust from the coal mine at the Nováky locality, stream sediments influenced by Sb mining activities from the nineteenth century at the Pezinok locality, the ash slurry present at the locality Zemianske Kostol’any due to a dam failure in 1965, and stream sediment at the Smolník locality, influenced by acid-mining drainage from one of the richest Cu-Fe ore mines in Slovakia, which had its highest activity in fourteenth century. The Ostrava Lagoons locality in the Czechia was created in 1965 and contains sludge and dumped waste from oil refining and separation processes. We did not expect contamination at the Belianske Tatry and Gabčíkovo localities. The Belianske Tatry mountains are part of the Tatra National Park and Gabčíkovo is a cultivated willow-poplar stand on the site of a floodplain forest. The An-G isolate was used as a comparative for further analyses (Table 1).

Genotype Profile of *Aspergillus niger* Environmental Isolates
All environmental isolates of *Aspergillus niger* were compared on the DNA level by the RAMP method. A cluster analysis of RAMP profiles separated the strains into three main groups and several unique branches (Figure 4). The first group contained the An-SL, An-Kmi, An-ZK, and An-Pop 4 strains possessing a similarity...
out on the basis of biochemical test results, in which β-palatinose, sedoheptulosan, D-tagatose, bromosuccinic acid, N-acetyl-β-D-mannosamine, adonitol, D-arabinose, D-arabitol, β-cyclodextrin, L-fructose, D-galactose, D-galacturonic acid, D-glucosamine, glucuronamide, α-D-lactose, lactulose, maltitol, palatinose, sedoheptulosan, D-tagatose, bromosuccinic acid, β-hydroxy-butyric acid, α-keto-glutaric acid, D-lactic acid methyl ester, L-lactic acid, sebacic acid, succinic acid, N-acetyl-L-glutamic acid, L-alaninamide and adenosine-5′-monophosphate. These biochemical tests, in which identical positive or negative results were obtained for all examined A. niger isolates, were not included in the phenotypic comparison of the strains.

The physiological analysis of A. niger isolates was carried out on the basis of biochemical test results, in which different reactions were found between strains (Table 2). All biochemical test results in which A. niger isolates differed in the result (positive reactions, negative reactions, non-identical—ambiguous reactions) were included in this comparison.

In order to analyse the phenotypic similarity of strains based on biochemical parameters obtained by the Biolog system, a multicriteria analysis was used in the Canoco 5 program (Braak and Smilauer, 2012). First, the length of the gradient for the response data was calculated. Based on the calculated gradient of DS = 1.6, where a linear response to the environmental gradient is assumed, Principal Component Analysis (PCA) was selected for component analysis. Input data to the PCA formed two matrices (tables). The first matrix consisted of presence of An strains (columns) in the used biochemical tests of the Biolog system (rows). Presence was coded according to the result of Biolog-biochemical tests by system 2: positive reaction, 1: variable reaction, 0: negative reaction. The second matrix consisted of tested An strains (columns) and the corresponding pH according to used biochemical tests of the Biolog system (rows). The first two ordination axes shown in the ordination diagrams of the PCA analysis (Figures 5, 6) explain about 48% of the data variability (Table 1).

The greatest phenotypic similarity was confirmed between strains isolated from a strong alkaline environment (8.5–9.0), followed by strains isolated from a slightly alkaline, medium alkaline and strong alkaline environment (7.4–7.8, 7.9–8.8, 8.5–9.0) and strains isolated from a strong acidic (5.1–5.5) and a slightly alkaline environment (7.4–7.8). The ordination diagram (Figure 5) did not confirm the phenotypic similarity between A. niger strains isolated from ultra-acidic (<3.5), extreme acidic (3.5–4.4), and very strong acidic (4.5–5.0) environments. Figure 5 visualises the suitability of the FF-biochemical tests from the Biolog system for testing the phenotypic properties of Aspergillus niger isolates in relation to pH. The resulting data show that the most useful were the tests of the utilisation of sugars Results from the biological profile and PCA analysis showed that the sugar utilisation tests (D-Cellobiose—D-Fructose—Maltotriose—β-Methyl-D-Glucoside—Sucrose), are best matched the pH and parameters of the original environment. Tests on sugar derivatives (m-Inositol—α-Methyl-D-Glucoside—Xylitol—D-Psicose), the
### Table 2: Comparison of the results of phenotypic (biochemical) identification profiles of *Aspergillus niger* environmental isolates obtained by the Biolog system.

| Positive reactions                                    | Negative reactions                                      | Different reactions                                      |
|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| (A2) Tween 80                                          | (A3) N-Acetyl-D-Galactosamine                           | (A12) D-Cellobiose                                       |
| (A4) N-Acetyl-D-Glucosamine                            | (A5) N-Acetyl-β-D-Mannosamine                          | (B1) α-Cyclodextrin                                      |
| (A7) Amygdalin                                         | (A6) Adonitol                                          | (B4) i-Erythritol                                       |
| (A9) L-Arabinose                                       | (A8) D-Arabinose                                       | (B5) D-Fructose                                        |
| (A11) Arbutin                                          | (A10) D-Arabitol                                       | (B9) Gentiofuran                                        |
| (B3) Dextrin                                           | (B2) β-Cyclodextrin                                     | (B10) D-Gluconic Acid                                   |
| (B12) α-D-Glucose                                     | (B6) L-Fructose                                       | (C1) α-D-Glucose -1-Phosphate                           |
|                                                        | (B7) D-Galactose                                       | (C3) D-Glucuronic Acid                                  |
|                                                        |                                                        | (C6) m-Inositol                                        |
|                                                        |                                                        | (C7) 2-Keto-D-Gluconic Acid                            |
|                                                        |                                                        | (C11) Maltose                                          |
|                                                        |                                                        | (C12) Maltotriose                                      |
|                                                        |                                                        | (D1) D-Mannitol                                        |
|                                                        |                                                        | (D3) D-Melezitose                                      |
|                                                        |                                                        | (D4) D-Melibiose                                       |
|                                                        |                                                        |                                                       |
| (C4) Glycerol                                          | (C10) Malitol                                         | (F1) γ-Amino-butyratic Acid                             |
| (C5) Glycogen                                          | (C8) α-D-Lactose                                       | (F2) Bromosuccinic Acid                                 |
| (C9) Lactulose                                         | (C9) α-Keto-glutaric Acid                              |                                                       |
| (C11) D-Raffinose                                      | (C10) Maltooligen                                      | (G2) L-Glutamic Acid                                   |
| (E1) D-Ribose                                          | (D9) Palatinose                                        |                                                       |
| (E2) Salicin                                           | (E3) Sedoheptulosan                                   | (G3) Succinic Acid                                     |
| (E4) D-Sorbitol                                        | (E5) L-Sorbose                                        | (G4) Succinic Acid                                      |
|                                                        |                                                        | (G5) Succinic Acid Mono-Methyl Ester                    |
|                                                        |                                                        | (G6) N-Acetyl-L-Glutamic Acid                          |
|                                                        |                                                        | (G7) L-Alaninamide                                     |
|                                                        |                                                        | (G8) L-Alanine                                         |
|                                                        |                                                        | (G9) L-Alanyl-Glycine                                  |
|                                                        |                                                        | (G10) L-Asparagine                                    |
|                                                        |                                                        | (G12) L-Glutamic Acid                                  |
|                                                        |                                                        |                                                       |
|                                                        |                                                        | (H4) L-Proline                                         |
|                                                        |                                                        |                                                       |
|                                                        |                                                        | (H12) Adenosine-5′-Monophosphate                        |

amino acid test of 2-Aminoethanol and tests of the utilisation of organic acids (Fumaric Acid—L-Malic Acid—Glycyl-L-Glutamic Acid—L-Pyroglutamic Acid) were usable too, but require further analysis.

The ordination diagram (Figure 6) is a summary biplot with all strains (included strains with a weak bond to environmental variables. Figure shows the *A. niger* isolates tested and their inclination to centroids representing biochemical similarity tests from the Biolog system and pH (they formed the environment variables). From these results, the An-Pop 3 and An-Pop 1 isolates (different pH of the substrate but the same isolation area of all four *A. niger* strains, namely the Poproč locality) proved to be phenotypically closest. The highest dependence was found between strains An—Pop1, An-A, and An—Pop3, this corresponds to the fact that An-Pop1 and An-Pop3 come from the same site Poproč heavily contaminated with As (and other elements). An—A was isolated from the surface of adamite [Zn₂(AsO₄)(OH)]—also a substrate with a high content of As. Phenotypic similarities were also found among the An-G and An-ZK isolates (both were obtained from a slightly alkaline environment). Strong similarity is further between An-P, An-KF, and An-ZK5 also from different pH of substrate and from different isolation area. The similarity between the An-A (artificial substrate) and An-KD strains is interesting too, it is probably due to the alkalinity of the substrate.

The very high similarity between the An-A and An-N isolates is of interest. The common feature of the origin of both strains is the presence of arsenic. Strain An-A was isolated from the surface of artificial adamite [Zn₂(AsO₄)(OH)] and strain An-N was derived from coal dust with 400 mg/kg of As from
The results presented in Figure 6 replicate the pH limits of the extreme environment of the occurrence of tested *A. niger* isolates in the areas (range) of ultra-acidic (An-S), neutral (An-L18) and (up to) strong alkaline (An-SL) environments.

The An-L18 isolate (Table 1) differs significantly from all other *A. niger* isolates. Only 13 species of microscopic filamentous fungi and yeast have been obtained from the sludge of the Ostrava Lagoons (Vašinková et al., 2017), and it is clear that the high content of organic pollutants in the environment inhibits the vital functions (as growth, reproduction and metabolic activity) of indigenous microorganisms and leads to a significant reduction of their biodiversity compared to the microbiota of uncontaminated sites (Doolotkeldieva et al., 2018).

The An-L18 isolate showed significant phenotypic differences in the tested utilisation reactions, which involve different enzymatic activity, gene expression and thus the formation of secondary metabolites. These likely changes can be due to the high level of tolerance to polyaromatic hydrocarbons (PAH), which has been previously confirmed to be associated with the change in morphology, production of pigments and sporulation activity of microscopic filamentous fungi in contaminated substrates containing large amounts of heavy hydrocarbon fractions (Zafra et al., 2015).

Selvig and Alspaugh (2011) studied the signalling pathways of microscopic filamentous fungi responsive to the pH of the environment and found some degree of signal specificity within the studied species. In general, if organisms grow over a wide pH range, they also adapt their gene expression to their environment. Microscopic filamentous fungi are known to be versatile, adaptable organisms, where their ability to grow over a wide pH range is conditioned by their genetic regulatory system, which adapts gene expression to the pH of the environment (Šimonovičová et al., 2013). The basis for
The role of mycotoxins in soil has not been reliably clarified yet, as there is an enormously rich mixture of all kinds of metabolites present in the natural reservoir of the
microorganisms. Nevertheless, it is assumed that they are important for strengthening the defence reactions of their producers towards other soil (micro)organisms, such as amoebas, nematodes, etc., which are often strong antagonists of fungi (Fox and Howlett, 2008; Karlovsky, 2008; Rohlfis and Churchill, 2011). Mycotoxins perhaps act as soil chemical signals among microorganisms (Rohlfis and Kürschner, 2010).

CONCLUSION

Using the Biolog test system to investigate and understand the metabolic diversity of microorganisms directly related to their genetic profile is an interesting approach to determining their biological profile as a specific identification characteristic. It has been shown that the biological profile of a strain obtained by FF-MicroPlate can be used to assess the level of metabolic properties even between strains of a single microbial species, and their unique fingerprints can be used to detect and evaluate changes in strain adaptability in relation to a particular environmental variable.

This study compares the biological profiles of 16 Aspergillus niger environmental isolates. Strains obtained from different pH sites affected by anthropogenic sources in situ showed significantly higher metabolic similarity. The above-mentioned biological analysis was also found to be useful in distinguishing metabolic diversity between Aspergillus niger environmental isolates, both in relation to environmental factors that most affect phenotypic similarities and in identifying which substrates were most used by the strains. It appears that the Biolog testing system may be a suitable ecological indicator of the interactions of physicochemical changes in an environment in relation to the adaptation of microorganisms to its pH. Detailed knowledge on microscopic fungal metabolism can also provide insight into the complex interactions between microscopic fungi and possible physiological pathways that lead to their mechanisms of adaptation to environmental conditions. Environmental microscopic fungi originated at sites affected by toxic compounds may acquire genetic alterations, which may result in changes of their physiology, including the production of different (secondary) metabolites, such as mycotoxins.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GenBank, MW739953-MW739968.

AUTHOR CONTRIBUTIONS

AŠ and HV isolated all the wild fungal strains. EP and SN did the mycotoxin analyses. LP and DP did the DNA sequencing of the strains. HD did the RAMP analyses. SN, HV, HŠ, and KK did the analyses of the fungal metabolites. HŠ, HV, and BS prepared the multicriterial analysis. SN prepared the microscopic analyses, and photo and graphic documentation. AŠ, HV, EP, and DP prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.658010/full#supplementary-material

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