International Biodeterioration & Biodegradation 125 (2017) 13–23

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Metabolome profiles of moulds on carton-gypsum board and malt extract agar medium obtained using an AuNPET SALDI-ToF-MS method

Justyna Szulc [a, *], Tomasz Ruman [b], Beata Gutarowska [a]

[a] Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Wólczańska 171/173, Łódź 90-924, Poland
[b] Bioorganic Chemistry Laboratory, Rzeszów University of Technology, 6 Powstańców Warszawy Ave., 35-959 Rzeszów, Poland

ARTICLE INFO

Article history:
Received 27 February 2017
Received in revised form
14 July 2017
Accepted 8 August 2017
Available online 29 August 2017

Keywords:
Metabolomics method
Laser desorption/ionization mass spectrometry
Gold nanoparticles
Building materials
Biodeterioration

ABSTRACT

The aim of this study was to compare the metabolite profile of the moulds *Alternaria, Aspergillus, Cladosporium, Penicillium, Stachybotrys,* and *Trichoderma* under model conditions on MEA (malt extract agar) medium and carton-gypsum board (CGB). This was followed by comparison of the metabolite profile of each particular strain and of a mixed culture of all moulds together. Metabolome analysis was performed using a high-resolution surface assisted laser desorption/ionization time-of-flight mass spectrometry based on a gold nanoparticle-enhanced target (AuNPET SALDI-ToF-MS) imaging method. All water extracts tested showed the presence of compounds with molecular weights in the range of 80–2000 *m/z* (mass-to-charge ratio). On CGB, the results obtained showed the existence of 299–453 peaks with higher intensity and 351–487 peaks with lower intensity compared to the peaks obtained from MEA, depending on the mould species. A distinct change in the metabolite profile under the influence of the growth medium was observed for the mixed culture on the MEA medium. The profile showed ~200 peaks with higher intensities of metabolites within *m/z* ranges 80–300 and 450–600; for the culture growing on CGB, the peak intensity was within *m/z* 300–450, and the number of peaks was 600–750. A new metabolomic methodology allowed the identification of 89 metabolites belonging to 48 metabolomic pathways. Compounds belonging to the citrate cycle and penicillin and cephalosporin biosynthesis pathways were identified on MEA. On CGB moulds, the metabolism changed to steroid, ubiquinone and other terpenoid-quinone biosynthesis.

1. Introduction

Worldwide losses of technical materials due to biodeterioration are estimated at a level over 40 billion USD per year (Allsopp, 2011). Biodeterioration is particularly important in the case of building materials, which are susceptible to moisture and growth of fungi. In Australia, Europe, Asia, and North America, the problem of dampness and fungal growth on building material concerns 10–50% of the buildings (Andersen et al., 2011). In the literature, numerous examples of building biodeterioration are described, including historical buildings (Piotrowska et al., 2014; Rajkowska et al., 2016; Sterflinger and Piñar, 2013).

The physiological and morphological properties of moulds and their common occurrence make them the most frequent cause of biodegradation of almost all building materials such as wallpaper, carton-gypsum boards (CGB), emulsion paints, wooden materials, insulation, and finishing materials (Gutarowska and Piotrowska, 2007). Moulds commonly isolated from materials belong to the genera *Acremonium, Alternaria, Aspergillus* (*A. versicolor, A. niger,* and *A. flavus*), *Cladosporium* (*C. cladosporioides* and *C. herbarum*), *Mucor, Penicillium* (*P. chrysogenum, P. expansum, P. aurantiogriseum,* and *P. viridicatum*), *Rhizopus, Stachybotrys, Trichoderma,* and *Ulocladium* amongst others (Andersen et al., 2011; Gutarowska, 2010; Gutarowska and Piotrowska, 2007; Hyvärinen et al., 2002). What is of great importance is that moulds can cause many health effects, including inflammation, respiratory problems, exacerbation of asthma, allergies, dermatomycosis, and sick building syndrome (Górny et al., 2001). Mycotoxins with a broad spectrum of biological effects, including genotoxic and cytotoxic effects, are produced by moulds and are important for the health of the people

* Corresponding author.
E-mail address: justyna.szulc@p.lodz.pl (J. Szulc).

http://dx.doi.org/10.1016/j.ibiod.2017.08.002
0964-8305/

residing in the buildings (Nielsen, 2003). The production of satratoxin, sterigmatocystin, 4-methoxysterigmatocystin and many other secondary metabolites in building materials was confirmed in the published literature (Nielsen et al., 1998; Tuomi et al., 2000).

Building materials are exposed to water, which is the principal solvent for mould metabolites in such an environment. Biodeterioration of building material can start as soon as water activity achieves a minimum limit of approximately 0.7 in the building materials (Pasanen et al., 2000). High water activity may result from technical causes (e.g., faulty structure of the building) or fortuitous events such as floods (Piotrowska et al., 2014). Materials of organic origin (e.g., wood, paper, carton, and wallpaper) can stimulate moulds to produce hydrolytic enzymes. During the biodeterioration of inorganic materials (glass, metal, brick, and gypsum), moulds may produce organic acids (e.g., oxalic, gluconic, citric, lactic, and acetic), which may react with ions of calcium, magnesium, and other minerals to create soluble or insoluble water salts (Gaylarde et al., 2003; Gutarowska, 2014; Rajkowska et al., 2016; Warscheid and Braams, 2000). The result of building material biodeterioration, which, in effect, is the weakening of the functional characteristics such as tensile strength, breaking, cracking and pitting, as well as the loss of colour or staining, is due to dyes produced by moulds (e.g., carotenoids and melanin) (Gaylarde et al., 2011; Gutarowska, 2014; Sterflinger, 2010).

Biodeterioration of building materials involves a multispecies population, where the phenomenon of competition for carbon and other element sources as well as ecological succession occurs. Therefore, in the literature, there is a lack of information on particular metabolites produced by numerous moulds growing on building materials.

In recent years, a metabolomic approach has been developed, especially LDI-MS (laser desorption/ionization mass spectrometry) techniques: MALDI (matrix-assisted laser desorption/ionization) and SALDI (surface-assisted laser desorption/ionization). These techniques have been used successfully to identify bacterial strains using the “metabolic fingerprint” (Carbonnelle et al., 2011; Cherkaoui et al., 2010). Recently, attempts were made to use MALDI techniques based on the medium-high molecular mass of compounds such as proteins and lipids to identify fungal species with clinical significance (Cassagne et al., 2011; Ulrich et al., 2016). However, no attempt has been made to analyse the metabolite profiles of moulds with low molecular weights using LDI techniques. Especially useful in such analysis could be a SALDI-type MS method based on the active surface. Sekuła et al. (2015a, 2015b) used a gold nanoparticle-enhanced target (AuNPET)-based method for mass spectral studies of compounds with different polarities, such as amino acids, saccharides, nucleosides, lipids and glycosides and for the MS imaging of complicated biological mixtures over a wide range of *m/z* (mass-to-charge ratio) values. SALDI AuNPET, in contrast with conventional MALDI, allows the analysis of low molecular weight compounds. The other advantages include a reduction of the background level, a high signal-to-noise (S/N) ratio, and a precise internal spectral calibration (Sekuła et al., 2015a, 2015b). The method described was also used for metabolomic analysis and MS imaging of human renal tissue (Nizioł et al., 2016).

The aim of this study was to compare i) the metabolite profiles of six mould strains under model conditions on mycological MEA medium and CGB and ii) the metabolite profiles of a individual strain and a mixed population of moulds. Particular emphasis was laid on metabolites with destructive potential and secondary metabolites. This work is an example of the first application of a SALDI-type, gold-nanoparticle-based method for the establishment of the metabolomic profile of environmental mould.

2. Materials and methods

2.1. Cultures of moulds

Six mould species were deposited in The Lock Collection of Pure Culture (LOCK CPC, Lodz, Poland): *Aspergillus versicolor* LOCK CPC 1124, *Penicillium chrysogenum* LOCK CPC 1125, *Cladosporium cladosporioides* LOCK CPC 1126, *Stachybotrys atra* LOCK CPC 0560, *Trichoderma viride* LOCK CPC 1127, and *Alternaria linii* LOCK CPC 0616 were selected for studies based on their most frequent isolation from CGB and other building materials (Andersen et al., 2011; Gutarowska, 2010; Gutarowska and Piotrowska, 2007).

CGB was purchased from retail sale outlets (Norgips, Poland); it was unimpregnated with a thickness of 9.5 mm and had an A2 degree of fire resistance. Samples cut into small pieces (20.0x20.0 × 9.5 mm) were conditioned in a climate chamber KBF720 (Binder, Germany) at a temperature of 27 ± 2 °C and a relative humidity (RH) of 80 ± 1% for 31 days.

The inocula of moulds were prepared as follows: colonies from MEA (malt extract agar, Merck, Germany) slants cultured at 27 ± 2 °C for 7 days were washed using 10 mL of distilled water with 0.01% of Tween® 80. The number of mould spores was estimated at 10^7 spores mL^{-1} using a Thom chamber and verified by the plate count method. To obtain a mixed population of moulds, inocula of particular strains were combined in equal volumes (1 mL). The average density of the suspension ranged from 1.9×10^7 to 2.5×10^7 CFU mL^{-1}.

An MEA medium of the same size (20x20 × 9.5 mm) as the CGB pieces was inoculated with 100 μL of the spore suspension. M0 medium (100 μL, 5 g of $MgSO_4$ x $7H_2O$; 3 g of $(NH_4)_2SO_4$; 1 g of KH_2PO_4; 20 g of glucose; 1000 mL of H_2O) was added to the CGB pieces to initiate microbial growth and establish the maximal material moisture (100%) at the beginning of the culture. All samples were incubated for 21 days in a climate chamber KBF720 (Binder, Germany) at 27 ± 2 °C and RH = 80 ± 1%. During the incubation period, the growth of the test strains was observed macroscopically, and metabolome analysis was performed after 21 days. All samples were prepared in triplicate.

2.2. Metabolome analysis

Metabolome analysis was performed using high-resolution laser desorption/ionization time-of-flight mass spectrometry based on an AuNPET plate. For the ToF- and ToF/ToF-MS sample preparation, carton-gypsum and agar with mould growth and control samples (without moulds) were subjected to three freeze (−20 °C)/unfreeze cycles. A volume of 400 μL of distilled water was added to the samples to wash out any water-soluble metabolites. Out of this raw extract, 100 μL was placed in Eppendorf tubes with distilled water (900 μL). Samples were vortexed for 2 min and placed (0.5 μL) on the AuNPET (Sekuła et al., 2015a), and SALDI-ToF-MS experiments were performed using a Bruker Autoflex Speed Reflectron time-of-flight mass spectrometer, which was equipped with a SmartBeam II laser (352 nm) in the *m/z* 80–4000 range. The laser impulse energy was approximately 60–120 μL, the laser repetition rate was 1000 Hz, and the deflection value was set to *m/z* 80 Da. The first accelerating voltage was held at 19 kV, and the second ion-source voltage was held at 16.7 kV. The reflector voltages used were 21 kV (first) and 9.55 kV (second).

Extracts were measured in MS imaging mode with 20,000 laser shots per sample divided into four individual pixels, which were placed on the centre of the sample spot (diameter ca. 2 mm) with 350 μm lateral resolution. Bruker's FlexImaging 4.0 software was used to set the MS imaging experiments. Mass calibration was performed using internal standards (gold ions and clusters from