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The ability of filamentous fungi to produce acids on indoor building materials

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Abstract - Sixty two filamentous fungi isolated from paint coatings, wallpaper, carton-gypsum board, and indoor air in buildings were screened for acid activity. It was found that 64.5% of strains produce acids on medium with bromo-cresol purple, where 18% of the strains were distinguished by very high acid activity (acid activity coefficient Q = 1.32-2.83), including the species: *Aspergillus niger, Aspergillus versicolor, Penicillium expansum, Penicillium brevicompactum, Penicillium chrysogenum, Cladosporium cladosporioides, Stachybotrys chartarum, Mucor globosus, Ulocladium chartarum* and *Alternaria alternata.* Research indicated that filamentous fungi considerably decrease the pH of the medium when that medium containing building material. The greatest acid production and pH decrease of the medium was observed during the growth of filamentous fungi in a medium with mortar, while the production of acids was less in a medium with carton-gypsum board, gypsum, and wallpaper. Filamentous fungi produced succinic, oxalic, malic and fumaric acids in the medium with indoor building materials. It was stated that the type of building material affects the spectrum and quantity of organic acids produced by filamentous fungi.

Key words: filamentous fungi; organic acids; building materials.

INTRODUCTION

Organic acids produced by microorganisms can be the reason for the biodeterioration of construction and technical indoor building materials. Fungi, bacteria, cyanobacteria, and algae take part in this process (Ortega-Calvo *et al.,* 1991; Gaylarde and Morton, 1999; Warscheid and Braams, 2000). The important microorganisms in the biodegradation process are filamentous fungi, considering their frequency of occurrence, high ability for adaptation, and low alimentary requirements. Filamentous fungi destroy wooden constructions, carton-gypsum boards, stone, concrete, bricks, mortars, as well as finishing materials, such as wallpapers and paint coatings (Gu *et al.,* 1998, Gaylarde and Morton, 1999; Wasserbauer, 2003). Those microorganisms are responsible for the physical damage of building materials. The mycelium of filamentous fungi penetrates inside the building materials causing the loss of coherence and crushing (Morton, 2003). Owing to the retention of water, a mycelium growing on the surface of building materials changes the building environment to allow numerous enzymes and metabolites to act. Chemical processes are also the cause of the destruction of indoor building materials (de La Torre and Gomez-Alarcon, 1994; Wasserbauer, 2003). In the corrosion of building materials, organic acids produced by the mycelium are very important (Warscheid and Braams, 2000; Abin *et al.,* 2002). The mycelium can produce acids such as citrate, malate, itaconate tartarate, gluconate, fumarate, oxalate, and succinate (Magnuson and Lasure, 2004). The mechanism of acid activity is based on chemical reactions between compounds of the building material and the acids produced, causing the production of salts that are water soluble or insoluble (Warscheid and Braams 2000). Organic acids can react with cations (Gomez-Alarcon *et al.,* 1994; Warscheid and Braams, 2000), which are rinsed out of the material. Consequently, the material becomes less coherent, it crushes easily and disintegrates; therefore, the level of destruction depends on the type of acid and the duration of its activity. These processes appear on inorganic materials, such as stones (Palmer *et al.,* 1991; May *et al.,* 1993; Warscheid and Braams, 2000) as well on organic materials. The results of scientific research concerning organic acids produced by filamentous fungi on sandstone were presented by de La Torre *et al.* (1991) and Gomez-Alarcon *et al.* (1994). However, there has been no research on organic acid production appearing on other construction and technical building materials applied inside buildings. It is unknown whether the kind of building material (organic or inorganic origin) has an influence on the acidity of filamentous fungi.

The aims of our work were to estimate the abilities of filamentous fungi to produce acid metabolites, to indicate the types of indoor building materials that induce the production of acid

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compounds, and to establish the quantity and types of organic acids produced by filamentous fungi in media containing mortar, gypsum, wallpaper, and carton-gypsum board.

MATERIALS AND METHODS

Filamentous fungi. Filamentous fungi (62 strains) were isolated from 42 buildings in Łódź (Poland) from paint coatings, carton-gypsum board, wallpapers, wooden finishings, and indoor air. Saline smears were taken from building materials on media: DG18 (Dichloran Glycerol agar, Oxoid), MEA (Malt Extract agar, Oxoid) with the addition of chloramphenicol (0.1%). Samples of indoor air of 100 cm^3 volume were taken using a Mass Sampler (Merck) on the media described above. Next, the samples were incubated at 27 °C for 7 days. Then, strains were identified to species on Czapek Dox agar medium (Difco) according to Samson *et al.* (2000) and Flannigan *et al.* (2001).

Estimation of acid activity. The screening method for the estimation of acid activity described by Abin *et al.* (2002) was used. Medium acidity was estimated on a medium with bromocresol purple (glucose 20 g l^{-1}, peptone 1 g l^{-1}, yeast extract 5 g l^{-1}, $CaCO_3$ 5 g l^{-1}, agar 20 g l^{-1}, bromocresol purple 30 mg l^{-1}; pH 6). Filamentous fungi were incubated at 27 °C for 5 days in triple repetitions. After that time, the acidity of medium diameters (acidity = yellow zone around colonies) and the diameters of the colonies were measured. The acid activity coefficient (Q) was estimated:

$$Q = \text{diameter of acidity of medium (mm)} / \text{diameter of colony (mm)}$$

For the purpose of the experimental criteria, the acid activity was established using the following scale: very high activity Q > 1.3; high Q = 1-1.3; medium Q = 0.5-1; low Q = 0.1-0.5; no activity Q = 0.

Cultures of filamentous fungi. Eleven strains of filamentous fungi with the highest coefficient of acid activity (*Aspergillus niger* strain 1 and 2, *Aspergillus versicolor, Penicillium brevicompactum, Penicillium chrysogenum, Penicillium expansum, Cladosporium cladosporioides, Stachybotrys chartarum, Mucor globosus, Ulocladium chartarum, Alternaria alternata*) were incubated in liquid Mo medium as a control ($MgSO_4 \cdot 7H_2O$ 5 g l^{-1}, $(NH_4)SO_4$ 3 g l^{-1}, KH_2PO_4 1 g l^{-1}, glucose 30 g l^{-1}, yeast extract 5 g l^{-1}; pH 5.8) and in four Mo media with the addition of wallpaper, carton-gypsum board, gypsum and mortar. Mo liquid medium was used for initiating filamentous fungi growth. This medium also simulates the inorganic and organic contaminations of building materials.

The building materials were bought in the retail trade. Ground materials were added to Mo medium (7.5 g of building material in 150 ml of medium), in Erlenmeyer's flasks (volume 350 ml) and sterilised at 120 °C for 20 min. Then the media were inoculated with 1 ml spore suspension (density 1-1.5 x 10^6 CFU ml^{-1}). The concentration of spores was measured using a Thoma's chamber. Cultures were incubated at 27 °C for 14 days. In the first day of the stationary phase of filamentous fungi growth, the pH of the medium was measured and the organic acids concentrations were determined.

Estimation of the stationary phase of filamentous fungi growth. The first day of the stationary phase of filamentous fungi growth was estimated based on a mathematical model describing ergosterol synthesis in the mycelium to determine the ergosterol content in the cultures (Gutarowska and Żakowska, 2009). The ergosterol, an indicator of mycelium content in various building materials (Gutarowska and Żakowska, 2002; Hippelein and Rügamer, 2004), was determined on the 3rd, 7th, 10th, 12th and 14th day of culture. Next, the ergosterol synthesis for each strain growing on a medium with a building material was described using the mathematical model. The derivative of model function was evaluated. The period when ergosterol synthesis was slowest (derivative equal zero) indicated the first day of the stationary phase. The computer program Origin 6 (Microcal Software Inc., USA) was used for these calculations. The first days of the stationary phase of growth depending on filamentous fungi strains and kind of building materials are presented in Table 1.

pH measurement. The values of pH in the media with building materials and control medium were measured using a Beckman's Φ310 pH meter. The initial pH values and pH on the first day of the stationary phase of filamentous fungi growth were measured. The pH measurement was done three times for each determination.

Organic acids determination. Organic acids concentrations were determined on the first day of the stationary phase of filamentous fungi growth in a single sample collected from each culture. A volume of 5 ml liquid culture was taken and centrifuged at 910 *x g* for 10 min. The presence of organic acid was determined by HPLC in supernatant liquid. The chromatographic analysis was performed with a SpectraSYSTEM P2000 gradient pump (Thermo Separation Products, Riviera Beach Fl., USA) a Rheodyne 7725i injector valve equipped with a 50 µl loop (Rheodyne, Cotati, USA) and SpectraSYSTEM RI-150 refractive index detector.

The column used was an Aminex HPX 87H, 300 x 7.8 mm id. (HPLC Organic Acid Analysis Column, Bio-Rad, Hercules CA, USA). The mobile phase was water adjusted to a pH between 2.1 to 2.15 with sulphuric acid and filtered through a cellulose mem-

TABLE 1 - The first days of the stationary phase of filamentous fungi growth evaluated with the mathematical model of ergosterol synthesis (Gutarowska, 2009) for control medium (Mo) and Mo added with various building materials

Filamentous fungi	The first day of stationary phase of growth in media (days)				
	Control	Wallpaper	Carton-gypsum board	Gypsum	Mortar
Aspergillus niger 1	4.6	4.9	6.2	7.9	10.5
Aspergillus versicolor	6.5	6.9	7.9	9.6	11.0
Penicillium chrysogenum	6.8	8.5	9.0	10.0	12.7
Penicillium brevicompactum	7.0	7.8	9.5	12.3	14.0
Penicillium expansum	7.8	8.1	9.2	11.2	13.8
Cladosporium cladosporioides	8.0	9.3	9.6	12.2	14.0

TABLE 2 - Frequency of filamentous fungi occurrence characterized by different acid activity and the place of their isolation in buildings

Acid activity coefficient (Q)		Amount of strains*	Amount of strains isolated from different places in buildings*				
			Plaster	Carton-gypsum board	Indoor air	Wallpaper	Wood
Very high	(Q > 1.3]	11	7	1	2	1	-
High	(Q = 1-1.3)	16	9	-	5	2	-
Medium	(Q = 0.5-1)	8	3	-	4	-	1
Low	(Q = 0.1-0.5)	5	2	-	1	2	-
No activity		22	14	-	6	1	1

* Amount of all strains N = 62.

TABLE 3 - Acid activity of the most active acid-producing filamentous fungi, isolated from buildings

Filamentous fungi	Place of isolation in building	Acid activity coefficient (Q; N = 3)
Aspergillus niger 1	Plaster	2.83 ± 0.01
Aspergillus niger 2	Plaster	1.78 ± 0.03
Aspergillus versicolor	Carton-gypsum board	1.57 ± 0.03
Penicillium expansum	Indoor air	2.08 ± 0.02
Penicillium brevicompactum	Plaster	1.65 ± 0.06
Penicillium chrysogenum	Indoor air	1.41 ± 0.01
Cladosporium cladosporioides	Plaster	1.40 ± 0.04
Stachybotrys chartarum	Wallpaper	1.35 ± 0.02
Mucor globosus	Plaster	1.33 ± 0.08
Ulocladium chartarum	Plaster	1.33 ± 0.04
Alternaria alternata	Plaster	1.32 ± 0.05

brane with 0.45 μm micropores (Millipore, Belford, USA). The separation was carried out by isocratic elution with a flow rate of 0.6 ml min^{-1}, and the column temperature was maintained at a constant 60 °C. Quantitation was based on the peak area measurement.

Water from a Millipore Milli-Q system was used for all solutions, dilution, and the mobile phase. Sulphuric acid (95-98%) obtained from J.T. Baker B.V. (Deventer, Holland) was a "Baker instra-analyzed" reagent. Organic acids used as standards were purchased from Supelco (Bellefonte, PA).

A mixture of all acids studied was used to optimise peak resolution. The standard of the individual acid was prepared and chromatographed separately in order to determine the retention time for each acid.

Statistical analysis. The concentrations of organic acids from filamentous fungi culture in media with building materials were subjected to statistical analysis, including determination of the arithmetic mean, standard deviation, minimal and maximal values. In addition, the statistical analysis concerned the frequency of the following observations: acid not detected in medium with building material, concentration of acid in control medium is the same as in medium with building material, concentration of acid is higher or smaller in medium with building material than in control medium. The bivariate distribution test was used to calculate of the frequency of these observations. Calculations were performed with *Statistica* (StatSoft Inc., USA).

RESULTS

The screening analysis of 62 filamentous fungi isolated from 42 buildings indicated that 40 strains were able to acidic metabolite production (Table 2). Very high (Q > 1.3) and high (Q = 1-1.3) acid activity coefficient was estimated for 17.7% (11 strains) and 25.8% (16 strains) respectively. Filamentous fungi demonstrating high acid activity were derived from different places of the buildings; most often they were obtained from plasters and indoor air, whereas two strains with high activity (*Aspergillus versicolor* and *S. chartrum*) were isolated from carton-gypsum board and wallpaper (Table 3). Filamentous fungi belonging to such species as *Aspergillus niger, Aspergillus versicolor, P. expansum, P. brevicompactum, P. chrysogenum, C. cladosporioides, S. chartarum, M. globosus, U. chartarum* and *Alternaria alternata* were distinguished by high acid activity (Table 3). The acid activity coefficient of those filamentous fungi ranged between Q = 1.32 ± 0.05 up to Q = 2.83 ± 0.01. *Aspergillus niger* (strain 1 and 2) and *P. expansum* were distinguished by the highest acid activity.

The ability to produce acids by active strains was confirmed in the culture in Mo medium with added building materials (Table 4). A considerable decrease in medium pH from the initial value was observed on the first day of the stationary phase of filamentous fungi growth in all media with building materials. The initial medium pH was different for the various building materials owing to the varied chemical constitution of the compounds (mainly calcium carbonate in mortar and gypsum). Filamentous fungi were intensively acidifying the control medium (Mo) causing a decrease of pH in the range of 1.98 to 3.46. The most significant changes of medium pH were observed in filamentous fungi cultures in medium with mortar, where pH values decreased to a range between 1.85 to 3.52, depending on the strain. The smallest decrease of pH in comparison with the control medium was noticed in all media with wallpaper, apart from the one inoculated with *Aspergillus versicolor*. The pH decreases in the carton-gypsum board and gypsum media were comparable. Our research confirmed that *Aspergillus niger* considerably decreases the pH value in all experimental variants. Moreover, a significant decrease of the pH value of medium with mortar in culture *C. cladosporioides, Aspergillus versicolor, P. expansum* and *P. chrysogenum* was also observed (Table 4).

Based on the estimation of organic acids produced by filamentous fungi in the Mo medium with building materials and in the control medium, it was stated that mortar induced the production of acid compounds (Table 5). All examined strains in the medium with mortar produced a wider range of organic acids in larger quantities in comparison to the control medium. The appearance of new acids in the medium with mortar, which were not detectable in control medium, including malic, oxalic, and

TABLE 4 - Values of pH in control medium (Mo) and Mo added with various building materials on the first day of the stationary phase of filamentous fungi growth

Filamentous fungi	pH medium in the first day of stationary phase of growth*				
	Control	Wallpaper	Carton-gypsum board	Gypsum	Mortar
	5.78 ± 0.06#	5.79 ± 0.18#	6.43 ± 0.27#	6.92 ± 0.25#	10.24 ± 0.29#
Aspergillus niger 1	2.32 ± 0.05	4.74 ± 0.15	3.62 ± 0.19	4.56 ± 0.18	7.60 ± 0.22
Aspergillus versicolor	3.79 ± 0.06	3.99 ± 0.16	5.33 ± 0.13	5.45 ± 0.33	6.72 ± 0.24
Penicillium chrysogenum	3.82 ± 0.03	4.74 ± 0.28	4.79 ± 0.15	5.82 ± 0.24	7.09 ± 0.15
Penicillium brevicompactum	3.65 ± 0.02	5.28 ± 0.19	5.32 ± 0.24	5.48 ± 0.26	8.39 ± 0.19
Penicillium expansum	3.21 ± 0.02	4.71 ± 0.23	4.81 ± 0.22	5.40 ± 0.21	6.86 ± 0.28
Cladosporium cladosporioides	3.80 ± 0.06	5.36 ± 0.17	5.87 ± 0.25	5.91 ± 0.19	7.19 ± 0.17

* Amount of replicas N = 3; # initial pH of medium.

fumaric acids, was observed in *Aspergillus niger, P. brevicompactum, P. chrysogenum* and *C. cladosporioides* cultures. A significant change in the medium with mortar was also the increased amount of acids: 0.005-0.537 g per 100 ml of the medium, depending on acid and microorganism. The largest amounts of organic acids produced by filamentous fungi that were noted during culture in medium with mortar were as follows: malic acid 0.543 g per 100 ml (*P. chrysogenum*), oxalic acid 0.327 g per 100 ml (*P. brevicompactum*), and succinic acid 0.199 g per 100 ml (*C. cladosporioides*). Fumaric acid was rarely produced and in small quantities, the largest quantity of this acid was produced by the *C. cladosporioides* strain in medium with mortar (0.023 g per 100 ml). The most significant differences between organic acid production in the medium with mortar and the control medium were noticed in the *C. cladosporioides* culture (Fig. 1 and 2).

Moreover, increased amounts of organic acids were also observed on the Mo medium with carton-gypsum board in comparison to the Mo medium (Table 6). A higher quantity of malic acid was produced by 4 of the 6 strains: succinic acid, 3 of the 6, and oxalic and fumaric acid, 2 of the 6 examined strains. It was stated that filamentous fungi produced significantly more malic acid (4/6) in the medium with wallpaper, while *Aspergillus versicolor* produced significantly more oxalic acid in the same medium. In the medium with gypsum, the increased production of malic acid (2/6 strains) was observed as well as succinic and fumaric acids in the *Aspergillus niger* culture.

DISCUSSION

Filamentous fungi most frequently appearing in buildings belong to the following species: *Penicillium, Aspergillus, Cladosporium* and *Alternaria* (Flannigan *et al.,* 1991, 2001). They produce numerous metabolites when growing on paint coatings, building materials of organic origin (wallpapers, carton-gypsum board and wood) and inorganic origin (mortar, gypsum board, and brick). Their ability to produce harmful mycotoxins for human health (Nielsen *et al.,* 1998; Tuomi *et al.,* 2000) and volatile compounds (Van Lancker *et al.,* 2008) is widely known.

In the presented research, it was indicated that filamentous fungi have the abilities to produce organic acids in large quantities (maximum 0.543 g of malic acid per 100 ml medium with mortar). Screening research of 62 filamentous fungi isolated from buildings showed acid activity in 65 per cent of

TABLE 5 - Organic acids produced by filamentous fungi in control medium (Mo) and Mo added with various building materials on the first day of the stationary phase of growth

Filamentous fungi	Organic acid	Quantity of organic acids (g/100 ml)				
		Control	Wallpaper	Carton-gypsum board	Gypsum	Mortar
Aspergillus niger 1	Oxalic	0.536	0.304	nd*	nd	0.086
	Malic	nd	0.038	0.012	0.009	0.055
	Succinic	0.001	nd	0.003	0.012	0.006
	Fumaric	nd	nd	nd	0.001	0.001
Aspergillus versicolor	Oxalic	0.080	0.165	0.117	nd	nd
	Malic	0.014	0.046	0.007	0.015	0.526
	Succinic	0.014	0.008	0.003	0.007	0.033
	Fumaric	nd	nd	0.001	nd	nd
Penicillium chrysogenum	Oxalic	nd	nd	0.241	nd	0.192
	Malic	0.006	0.023	0.022	0.007	0.543
	Succinic	0.003	nd	0.025	0.003	0.051
	Fumaric	0.001	nd	0.003	nd	nd
Penicillium brevicompactum	Oxalic	0.335	0.318	nd	0.156	0.327
	Malic	nd	0.037	0.021	0.022	0.028
	Succinic	0.016	0.015	0.012	0.008	0.052
	Fumaric	nd	nd	nd	nd	0.002
Penicillium expansum	Oxalic	0.906	0.166	0.089	0.103	0.261
	Malic	0.048	0.019	0.054	nd	0.078
	Succinic	0.042	0.004	0.055	0.035	0.068
	Fumaric	0.006	nd	nd	nd	nd
Cladosporium cladosporioides	Oxalic	0.169	0.166	0.032	0.028	0.243
	Malic	0.009	0.003	0.004	nd	0.315
	Succinic	0.007	0.001	0.006	nd	0.199
	Fumaric	nd	nd	nd	nd	0.023

* nd: not detected.

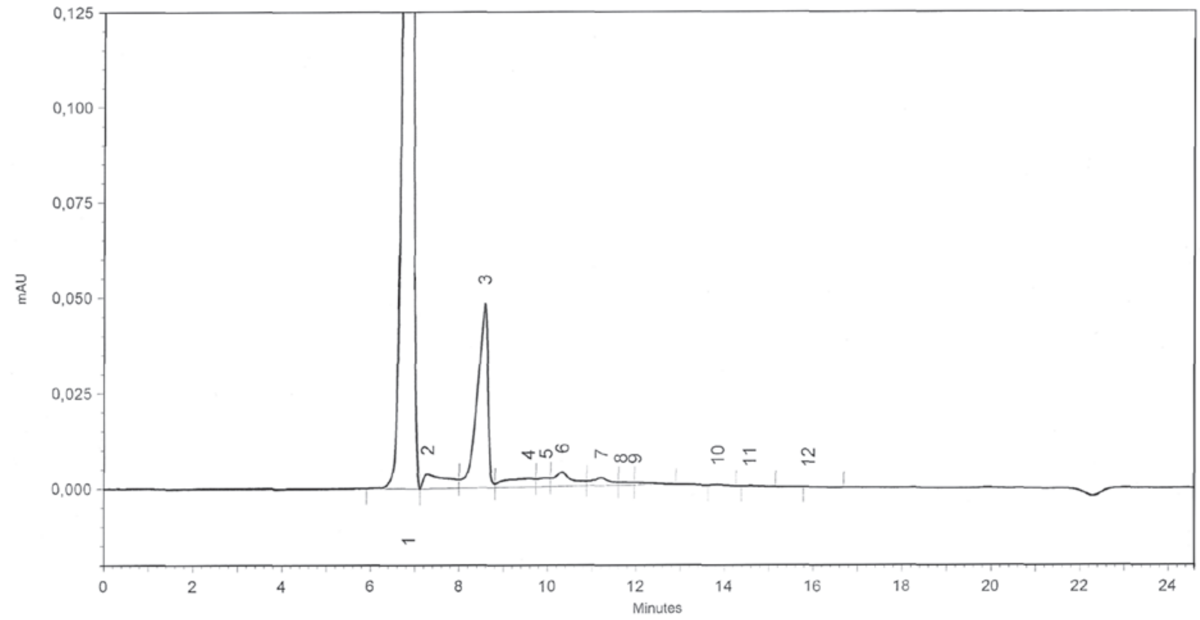

FIG. 1 - Chromatogram of *Cladosporium cladosporioides* metabolites in control medium on the first day of stationary phase of growth (8th day of culture). (2): oxalic acid, (6): malic acid, (7): succinic acid.

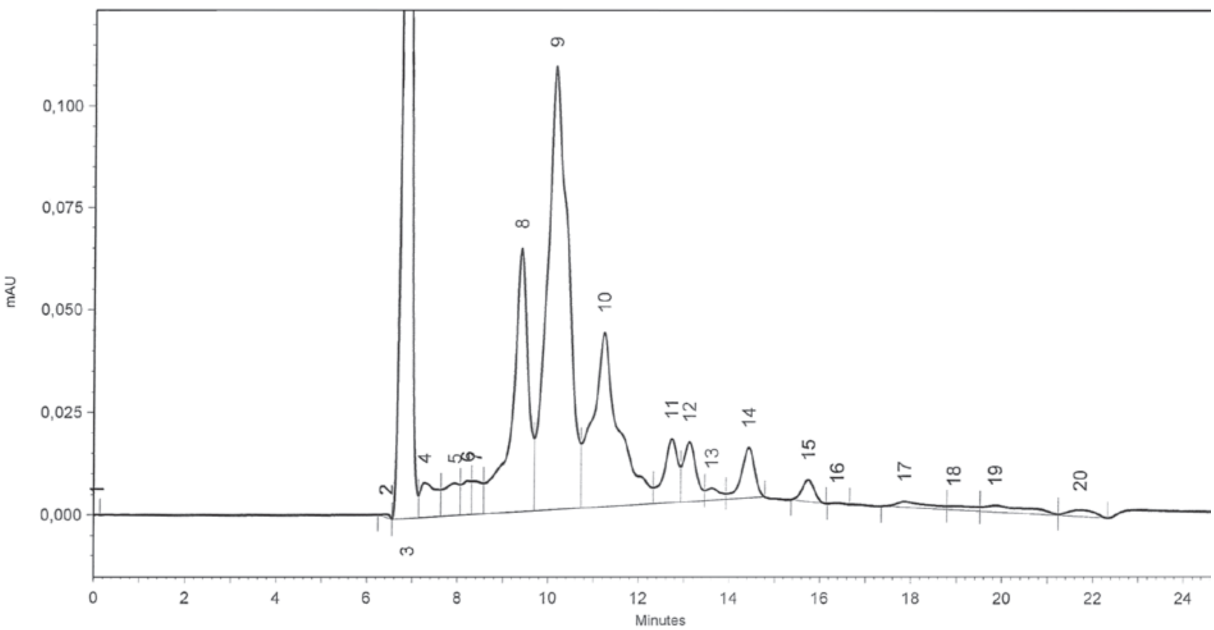

FIG. 2 - Chromatogram of *Cladosporium cladosporioides* metabolites in medium with mortar on the first day of stationary phase of growth (14th day of culture). (4): oxalic acid, (9): malic acid, (10): succinic acid, (14): fumaric acid.

isolates, where 18 per cent of strains indicated activity at a very high level. Acid-producing filamentous fungi isolated from the building belong to the following species: *Aspergillus niger, P. expansum, P. brevicompactum, Aspergillus versicolor, P. chrysogenum, C. cladosporioides, S. chartarum, M. globosus, U. chartarum and Alternaria alternata*. These microorganisms were able to decrease the pH of control medium Mo from the initial level pH = 5.7 to pH = 3.82-2.32. The capacity for acid production by *Aspergillus, Penicillium, Fusarium* and *Trichoderma* genera in laboratory media is known (Abin *et al.,* 2002). Likewise, the capacity to produce organic acids on sandstone with organic matter by such filamentous fungi as *Penicillium, Fusarium* and *Trichoderma* were described by de La Torre *et al.* (1991), Gomez-Alarcon and de La Torre (1994) and Gomez-Alarcon *et al.* (1994). The capability of intensive acidifying by *Penicillium* in medium with mortar was confirmed by a decrease of the pH value from the initial level 10.2 to the level of 6.9 during culture. Additionally, the high ability of *Cladosporium cladosporioides* for production of acid metabolites was also confirmed, particularly in medium with mortar (pH decrease from 10.2 to 7.2). Species of this genus are described in literature as non-acidifying (de La Torre *et al.,* 1991). *Aspergillus versicolor,* which is often isolated in building environment (Flannigan *et al.,* 1991), had strong abilities for acidifying the medium with mortar and wallpaper.

TABLE 6 - Statistical analysis of results of organic acid production in control medium (Mo) and Mo added with various building materials

Organic acid	Statistical determinations of acid production in media[a]	Type of medium				
		Control	Wallpaper	Carton-gypsum board	Gypsum	Mortar
Oxalic	Mean	0.338	0.186	0.079	0.048	0.185
	SD	0.337	0.116	0.092	0.066	0.121
	Min.– Max.	0-0.906	0-0.318	0-0.241	0-0.156	0-0.327
	*Nd		1	2	3	1
	*Building material = Control		2	0	0	1
	*Building material > Control		1	2	0	2
	*Building material < Control		2	2	3	2
Malic	Mean	0.013	0.028	0.020	0.009	0.258
	SD	0.018	0.016	0.018	0.008	0.238
	Min.– Max.	0-0.048	0.003-0.046	0.004-0.054	0-0.022	0.028-0.543
	*Nd		0	0	2	0
	*Building material = Control		0	0	2	0
	*Building material > Control		4	4	2	6
	*Building material < Control		2	2	0	0
Succinic	Mean	0.014	0.005	0.017	0.011	0.068
	SD	0.015	0.005	0.020	0.012	0.067
	Min.– Max.	0.001-0.042	0-0.015	0.003-0.055	0-0.035	0.006-0.199
	*Nd		2	0	1	0
	*Building material = Control		1	2	1	0
	*Building material > Control		0	3	1	6
	*Building material < Control		3	1	3	0
Fumaric	Mean	0.001	Nd	0.001	0.002	0.004
	SD	0.002		0.001	0.0004	0.009
	Min.– Max.	0-0.006		0-0.003	0-0.001	0-0.023
	*Nd		6	4	5	3
	*Building material = Control		0	0	0	0
	*Building material > Control		0	2	1	3
	*Building material < Control		0	0	0	0

[a] SD: standard deviation; Min.–Max.: minimum–maximum values; Nd: not detected.
*Number of positive researches (amount of replicas N = 6).

It was stated that the type of building material can change the spectrum and quantity of organic acids produced by filamentous fungi. Building materials stimulate filamentous fungi to produce organic acids, especially inorganic materials such as mortar (consist of cement and sand). All filamentous fungi produced significantly larger quantities of organic acids in medium with mortar in comparison to the control medium. Perhaps the high initial pH of this medium causes the metabolism of filamentous fungi to be oriented on the production of organic acids. The phenomenon of the influence of increased pH medium on the stimulation of citric acid production is known (Magnuson and Lasure, 2004). Our research has shown that filamentous fungi cultured on building materials produce a number of acids, such as: succinic, oxalic, malic, and small quantities of fumaric acids. Probably other acids were also produced (Fig. 1 and 2); however, due to the interference of other metabolites and building material compounds, it was impossible to identify and estimate the quantities of all organic acids in chromatographic analysis.

The ability of filamentous fungi for production of oxalic, fumaric, succinic, citric, gluconic, malic, and itaconic acids on stones were regarded as the corrosive factor (de La Torre *et al.,* 1991; Gomez-Alarcon and de La Torre*,* 1994; Gomez-Alarcon *et al.*, 1994). Some acids, such as citric, gluconic, and malic with minerals (Ca, Fe, Al, Mg, K, Na) from building materials, can create numerous salts soluble in water. In this process, the minerals are rinsed out of building materials, causing the crushing and destruction of the material (Werscheid and Braams, 2000). In our research, intensive production of malic acid by filamentous fungi was confirmed in all media with building materials. In the reaction between oxalic or succinic acids with calcium (from calcium carbonate in mortar and gypsum) water-insoluble salts can be produced, which result in the destruction of the structure of the building material.

The presence of oxalate crystals on the surface of stones was confirmed in research by Gomez-Alarcon *et al.* (1994). In our research, the increased production of oxalic acid by two examined strains of filamentous fungi in a medium with mortar and carton-gypsum board was noted. Increased production of oxalic acid in the medium with wallpaper in the *Aspergillus versicolor* culture was also confirmed in our research. Production of organic acids on organic materials, such as wallpapers, can lead to hydrolysis of polysaccharides (cellulose, lignin) which causes the material structure to weaken.

It seems that the presence of meaningful amounts of carbonates in mortar and gypsum causes the buffering of acidic filamentous fungi metabolites, which in turn, results in the stabilising of the pH level (Werscheid and Braams, 2000). Perhaps the continuous production of reactive organic acids by filamentous fungi on inorganic building materials could well violate the balance of hydrogen ions, causing a pH decrease and in turn leading to material deterioration.

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