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Biography

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Biocorrosion of materials and sick building syndrome



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The problem of biological damage of mineral building materials and structures based on them is multifaceted and covers all types of industry. The most destructive biocorrosion impacts are on building materials in cities with a large water area. Various types of microorganisms, including

pathogens, and especially the filamentous fungi of the genera *Aspergillus*, *Penicillium*, *Trichoderma*, etc., occupy the surfaces of mineral building materials, cause their destruction, disturb the ecological balance of cities and endanger the human health. The term 'sick building

syndrome' (SBS) is used to describe a situation when the residents of a building experience acute health- or comfort-related effects that seem to be linked directly to the time spent in the building wherein no specific illness or cause can be identified. Biological contaminants, in particular micromycetes, can present one of the possible causes of SBS. Here, we assessed the biodeterioration level of structural materials on the basis of fine-grained concrete widely used in construction practice and architecture. First, we determined the strength characteristics of the material that has been biologically damaged; second, we identified the damaging micromycetes and analysed their metabolic activity related both to the induction of biocorrosion and to the impacts of fungi on human health. Applying a new integrated approach, which combines methods of molecular microbiology and genetic toxicology with standard methods for determining the strength of building structures, we confirmed the relation between biodestructive and pathogenic properties of micromycete isolates.

At present, the world's losses from biodeterioration are estimated at billions of dollars and are more than 7% of the total cost of industrial products. Physical, chemical and biological factors of mortar and concrete corrosion are in close interrelation. Because concrete is a capillary-porous body, microorganisms, settling on the surface, then spread into the interior causing corrosive processes by the secreted metabolites. Biocorrosion of construction materials and structures has now become an important environmental problem, as according to statistics, the urban residents spend about 95% of their time indoors. Since the prospects for the overwhelming majority of the population are in city housing, the relevance of studying the biocorrosion of building materials and its consequences for the environment and human health is constantly increasing. One of the consequences is sick building syndrome (SBS), which is considered to be a multifactorial health problem, being a medical, psychological and social phenomenon¹⁻³ at the same time. The spaces that contain sufficient levels of chemicals, allergens and microorganisms to make those who live or work in the space sick are known as 'sick buildings'. These are mainly incommensurable old buildings contaminated by microorganisms, especially filamentous fungi inducing biocorrosion of construction materials. At least 600 species of fungi are in contact with humans, and less than 50 are frequently identified and described in epidemiologic studies of indoor environments². In this study we found the connection between destructive and health-damaging properties of filamentous micromycetes.

According to the Federal State Statistics Service (2016), the population of the Republic of Tatarstan is 3 868 730 people, of which the urban population is 76.41%. Biological damage to building structures is a common problem, especially in cities. Filamentous

fungi dramatically worsen the characteristics of materials on which they grow causing partial or complete biodegradation. We have isolated micromycetes from the inner walls of some old buildings in Kazan city and found that fungal communities represented the class *Eurocytomyces* and family *Trichocomaceae*. Pan-fungal primers specific for the conserved sequences of 18S and 28S rRNA genes common to all fungi have been used. The predominant genera (97%) were filamentous fungi *Penicillium* and *Aspergillus*: *A. niger* and *A. fumigatus* were the most common species. In some cases, micromycetes of the genera *Alternaria* and *Trichoderma* were found. Simultaneously, our studies of fungal infection in patients who visited the Laboratory of fungal diseases at the Kazan Research Institute of Epidemiology and Microbiology revealed the prevalence of aspergillosis. This is partly due to the contamination of many buildings with micromycetes of the genus *Aspergillus*, which can cause mycogenic allergies, atopic dermatitis and mycotoxicoses⁴, the probability of which is significantly increased in environments with high numbers of these organisms, which contribute to SBS¹.

Three main processes cause biodeterioration: mechanical, assimilative (because building materials are a source of nutrition and energy for microorganisms) and dissimilative (the interaction of building materials with aggressive metabolites of microorganisms)^{5,6}. Since the ability of fungi to produce secondary metabolites like single-, double- and tribasic organic acids and enzymes plays an important role in the process of dissimilative biodeterioration^{7,8} as well as in pathogenesis^{2,9,10}, we focused our experimental work on measurements of secreted proteases and lipases of *Aspergillus* strains isolated from old buildings and from patients diagnosed with onychomycosis and otomycosis.

Pathogenic fungi secrete hydrolytic enzymes that enable them to breach and invade host tissues. The most highly recognised extracellular hydrolytic enzymes include proteases and lipolytic enzymes. Proteases from fungi induce inflammatory responses by altering the permeability of the epithelial barrier and by inducing the proinflammatory cytokines through protease-activated receptors¹⁰. Lipolytic enzymes have been shown to influence growth, morphology, adherence, and dissemination of fungal cells across the host⁹. We found that isolates from buildings showed a higher level of lipolytic activity compared to clinical strains (Figure 1a). The level of proteases was low in both groups, with slight prevalence in the activity of clinical isolates. Growing on keratin-containing substrate, *Aspergillus* strains produced a coat around the hair, but did not destroy the hair itself (Figure 1b). All the isolates studied are able to synthesise oxalic acid. The malic acid was found in culture fluids of 93% micromycetes, acetic acid was synthesised by 67%, citric acid by 60%, lactic acid by 33% and acetic acid by 13% of isolates. Acids promote the wash-out of calcium from concrete thus increasing the biodeterioration. On the other hand,

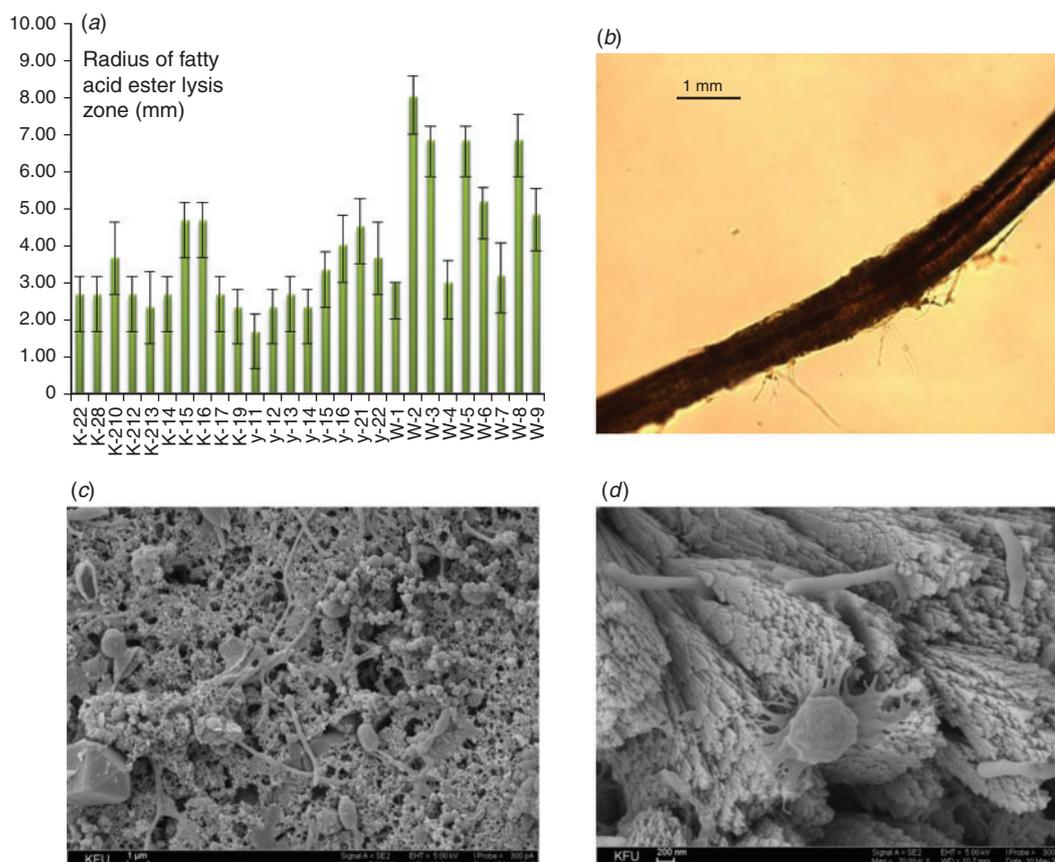


Figure 1. Lypolytic (a) and keratinase (b, light microscopy) activities of *Aspergillus* strains isolated from old buildings (W), from patients with diagnosed onychomycosis (K) and otomycosis (Y) measured by hydrolysis of linoleic acid methyl ester added to Sabouraud agar. Germination of *A. niger* W-2 spores in microcracks of concrete by low (c) and high (d) resolution scanning microscope LSM 780, Carl Zeiss, Germany.

Table 1. Alteration of strength characteristics of concrete during 28 days of exposure in pure Czapek Dox medium and medium inoculated by spores of *Aspergillus* W-2.

Brand of concrete ^A	Characteristics of concrete strengths, MPa ^B					
	Resistance to compression, R _c			Resistance to bending, R _b		
	Initial	Incubated in pure medium	Incubated in inoculated medium	Initial	Incubated in pure medium	Incubated in inoculated medium
M400	6.60	6.55	6.50	32.30	32.25	32.15
M500	7.10	7.05	7.00	42.80	42.75	42.65
M600	9.50	9.45	9.40	60.80	60.70	60.70
M800	10.50	10.49	10.40	74.80	74.80	74.7
M1000	12.20	12.17	12.15	95.20	95.16	95.00

^ABrand of concrete reflects its average characteristics depending on the amount of cement used in a mix.

^BCompression and bending strengths are measured by breaking cylindrical concrete specimens in special testing machine and are calculated as failure load divided by cross-sectional area (reported in megapascals, MPa).

pathogenic fungi acidify the environment as a strategy to damage host tissues, and acidification of the host tissues promotes the expression and activity of fungal proteases¹¹.

To date, there are more than 200 different methods to test biostability of building materials. Along with international standards,

there are national standards for particular countries (STD 141C/6271/2-86 for USA; BS 1133 for UK, DIN 53739-84 for Germany, NFX 41-514-81 for France; JIS Z 2911-87 for Japan, GOST 9.048-89 for Russia). According to our data, the most aggressive strain was *A. niger* W-2 isolated from an old hospital building in Kazan city. This isolate possessed the highest levels of lipolytic activity and acid

production. After 28 days of contact with *A. niger* W-2 in the growth medium, the values of concrete sample stability were determined as described earlier¹². As seen from Table 1, the resistance coefficients to bending and compression (R_b and R_c) after contact with the growing strain decreased by no more than 1%, indicating that there are no significant changes in the concrete specimens. However, these minimal changes determined by the standard test develop over time and gradually contribute to the destruction of concrete. Penetrating into the thickness and germinating in cracks, *A. niger* W-2 destroys the concrete (Figure 1c, d). Moreover, colonisation of the building material by toxigenic fungi raises the question of a subsequent exposure of residents to aerosolised mycotoxins. The greatest part of the aerosolised toxic load is found in particles whose size corresponds to spores or mycelium fragments. However, some toxins were also found on particles smaller than spores that are easily inhaled and can deeply penetrate into the respiratory tract¹³.

A list of major strains in hospital environments (Centre F. Baclesse, Normandy, France) compiled according to their frequency, concentration level, and/or capacity to produce mycotoxins *in vitro*, is as follows: *A. fumigatus*, *A. melleus*, *A. niger*, *A. versicolor*, *Cladosporium herbarum*, *Purpureocillium lilacinum*, and *Penicillium brevicompactum*¹⁴. No mutagenic activity was found in bioaerosols. The results of genotoxicity testing by the reversion of auxotrophic *Salmonella typhimurium* strains to prototrophy under exposure to the culture fluid of *A. niger* W-2 (Ames assay) were also negative. Nevertheless, some *Aspergillus* strains assigned to section Nigri are well known as ochratoxin and/or fumonisin producers and showed toxigenic potential *in vitro*¹⁵. It could be concluded that active biodegraders from the genus *Aspergillus* contribute to the onset of the SBS.

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