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Original Research

Fungi of the Genus *Penicillium* in School Buildings

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Abstract

School buildings typically serve a number of functions simultaneously, owing to which they seem to be especially exposed to the expansion of fungi with wide enzymatic capabilities, including fungi of the genus *Penicillium*. In total, 34 isolates belonging to 20 species were obtained within two schools. The most frequently isolated species included: *Penicillium chrysogenum*, *P. waksmanii*, and *P. citrinum*. Over 65% more fungi were isolated in the heating season than when the heating was off. This period was also characterized by an almost threefold greater taxonomic spectrum. Especially in the heating season, the prevalence and species spectrum of the fungi of the *Penicillium* genus in school buildings should be constantly monitored due to their environmental plasticity as well as toxigenic, allergenic and antibiotic properties.

Keywords: *Penicillium*, school building, walls, indoor air

Introduction

In a temperate climate, maintaining thermal comfort of users of various rooms requires isolating them from the external environment. Temperature and humidity measured outside buildings are subject to greater fluctuations than those measured indoors. The resultant microclimate of room facilities defines the spectrum of organisms, including fungi, likely to colonize a building interior. The term "domestic fungi" refers to species whose ecological niches are consistent with conditions occurring in closed rooms. This conventional group includes mainly fungi of the genus *Aspergillus* and *Penicillium*. Especially the latter is often noted in rooms utilized in various ways.

Fungi of the genus *Penicillium* constitute a permanent element of mycobiota of buildings linked with human economic activity, i.e. breeding farms, mills, factories of floor panels, or compost sites [1]. Their presence has been detected in places visited by a limited number of users: laboratories [2, 3], private apartments [4], social care homes [5], and public utility buildings, including medical care facilities [6], academic buildings [2, 7], libraries [8], or places of religious worhsip [9]. School buildings serve a number of functions simultaneously (learning, playing, sports activities, consuming meals), owing to which they seem to be especially exposed to the expansion of fungi with wide enzymatic capabilities, including the above-mentioned microfungi.

Investigations of organic ontocenoses of schoolchildren carried out since 1997 have demonstrated phenological fluctuations in their colonization by potentially-pathogenic yeast-like fungi [10-12]. These fungi may be acknowledged as bioindicators of the health status of the human body, for their appearance indicates diminished immunity. They have been shown to colonize the oral cavity, throat, and nasal cavity of healthy children much more frequently in the spring than in the autumn season. It is likely that the spring debility of children's organisms is only of physiological origin or is due to the negative impact of a school environment in which the children are spending ca. 1/3 of the day. Hence a study was undertaken to examine habitat preferences and occurrence of fungi of the genus *Penicillium* in school buildings as well as to determine their potential effects on the health of school pupils and school workers.

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Material and Methods

The experimental material were fungi collected from buildings of two schools. Both buildings were built in the early 1970s using prefabricated concrete technology, and were not insulated with polystyrene foam. In the study period, the schools had old wooden windows and door frames. These facilities were free of water damage. The schools

Table 1. Fungi of the genus *Penicillium* in schools – a statement of its own data and literature [8, 17, 19-21, 24, 27, 29].

Species	Number of isolates							Habitat		
	sum	school I May	school I November	school II May	school II November	May	November	occurence in a bulding	human organism (dates of references)	other habitats (dates of referenes)
*Penicillium baarnense** van Beyma	1	0	0	0	1	0	1	a		coniferous litter
*P. chrysogenum** Thom	6	3	1	2	0	5	1	a, r, s	skin, celebrum	atmospheric and indoor air, dust, greenhouses, housing, libraries, soil, lake, pharmaceutical starch, meat, egg, fruits, cheese
*P. citrinum** Thom	3	1	0	1	1	2	1	a, s	skin	atmospheric and indoor air, greenhouses, housing, libraries, soil, pharmaceutical starch, nuts, cereals, fruits
*P. commune** Thom	1	1	0	0	0	1	0	a		atmospheric and indoor air housing, libraries, soil, cheese, nuts, meat, fats
*P. corylophilum** Dierckx	1	0	0	0	1	0	1	r		atmospheric and indoor air, dust, housing, libraries, soil
P. cyaneum (B.& S.) Biourge	1	0	0	0	0	1	0	r		soil
P. egyptiacum van Beyma	1	0	1	0	0	0	1	a		soil
P. frequentans Westling	1	0	1	0	0	0	1	a		atmospheric air, soil, dust, library materials, pharmaceutical starch
Penicillium griseoroseum Dierckx s. *P. cyaneo-fulvum** Biourge	1	0	1	0	0	0	1	a		seeds of trees, libraries
*P. jenseni** Zaleski	1	0	1	0	0	0	1	r		atmospheric air, dust, housing, pharmaceutical starch
P. kapuscinskii Zaleski	1	0	0	0	1	0	1	r		pharmaceutical starch
P. nigricans (Bain.) Thom	1	0	1	0	0	0	1	r	skin	soil
*P. notatum** Westling	2	0	1	0	1	0	2	r		library materials, pharmaceutical starch
*P. solitum** Westling	2	0	0	2	0	2	0	a		atmospheric and indoor air, housing, soil, fruits, nuts, fihs, meat, fats
P. spinulosum Thom	2	0	1	0	1	0	2	p, s		soil, chicken farm, church, fets, oils
*P. steckii** Zaleski	1	0	0	0	1	0	1	r		atmospheric air, library materials
*P. urticae** Bainier	1	0	0	0	1	0	1	a		atmospheric air, pharmaceutical starch
*P. verruculosum** Dierckx	2	0	1	0	1	0	2	r		atmospheric air, dust, soil, lake, cereals, meat, fish
P. viridicatum Westling s.*P. psittacinum* Thom	1	0	0	0	1	0	1	a		atmospheric and indoor air, soil hospital, cereals
P. waksmanii Zaleski	4	1	2	1	0	2	2	a, s		atmospheric and indoor air, housing, church, pharmaceutical starch
sum of isolates	34	6	11	7	10	13	21			
sum of species	20	4	10	4	10	6	17			

s – smooth wall surface , r – rough, a – air
* toxic species [20, 21, 24]
allergic species [27, 29].

selected for our study had a comparable number of users. Simultaneous analyses of the mycological purity of bioaerosol demonstrated the number of fungi to range from 148.09 to 756.23 cfu/m³ [13]. The total count of fungi in most of the school rooms did not exceed standard values recommended by Krzysztofik [14]. Since our previous research [13] demonstrated a limited effect of atmospheric air on the qualitative and quantitative composition of air inside buildings, the analysis of atmospheric aerosol around school buildings was omitted in this study.

The samples of fungi were collected from air and from walls of school buildings. In the case of the first, they were collected with the sedimentation method, with research stations established in two diagonal corners of each room. In the case of the latter, they were collected using surface swabs. At 3 points = stations of each room there were selected surfaces painted with acrylic or emulsion paint, later the text referred to as rough surfaces, as well as surfaces covered with oil paint or glaze (referred to as smooth surfaces). At each station two samples were collected for each of the two assumed temperatures of incubation. No macroscopic changes indicatory of mycelium growth were noted on the walls. Analyses were conducted in May (with heating off) and in November (with heating on). During sampling, temperature and air humidity were measured in the rooms as well. In total, we collected 416 samples from 13 rooms serving various functions.

Cultures from sedimentation and swab samples were run on Chapek-Dox culture medium at 25º and 37ºC, for at least three weeks. The morphology of the fungal colonies was analyzed macroscopically, with attention paid to the colony color and its reverse, the size and structure of mycelium, the shape of conidiophores, and the presence of perithecia. Dimorphic capability was determined by observing the type of mycelium produced at 25º and 37ºC. Attention was also paid to dye production and the presence of mycelium secretions in the form of drops on a colony's surface. In order to identify microscopic characteristics, i.e. length of conidiophores and shapes of their endings, as well as size and structure of walls of conidial spores and potential ascospores, preparations were prepared by staining in lactophenol aniline blue according to Gerlach [15]. Photographic documentation was gathered systematically.

Fungi were matched to species based on the key by Raper et al. [16] and the atlas of fungi noted in clinical material by De Hoog et al. [17].

Results

In total, 34 isolates belonging to 20 species of the genus *Penicillium* were obtained in the study (Table 1). All had grown only at 25ºC. No growth of fungi was observed at 37ºC. The most frequently isolated species included *Penicillium chrysogenum*, *P. waksmanii,* and *P. citrinum*. Over 65% more fungi were isolated in the heating season than when the heating was off. This period was also characterized by an almost threefold greater taxonomic spectrum (Fig. 1A). A similar tendency was noted in both buildings examined. In both experimental seasons, the prevalence of *Penicillium* in respect to fungi of the genus *Aspergillus*, isolated in our previous research [18], reached slightly over 15% (Fig. 1B). Each school had its own species spectrum. Only ca. 18% of the species were common for both schools (Fig. 2).

The fungi were most frequently isolated from bioaerosol of rooms. On surfaces covered with glaze or oil paint, we detected only 4 species, including three predominating in the whole pool of taxa. Out of the isolated fungal

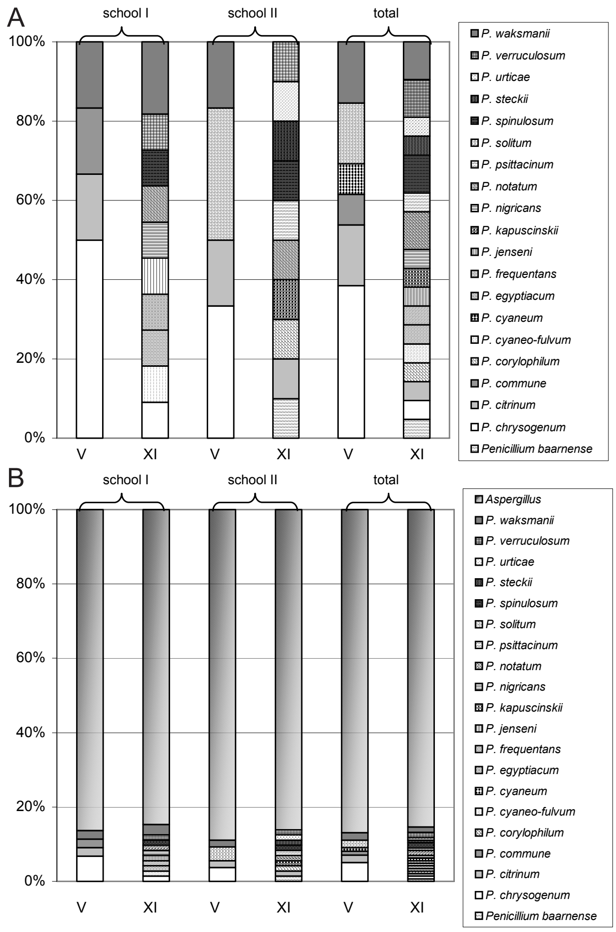

Fig. 1. Frequency of appearance of *Penicillium* types in inspected school buildings in May (V) and November (XI) – A, in comparison to the prevalence of fungi of the type *Aspergillus* – B [18].

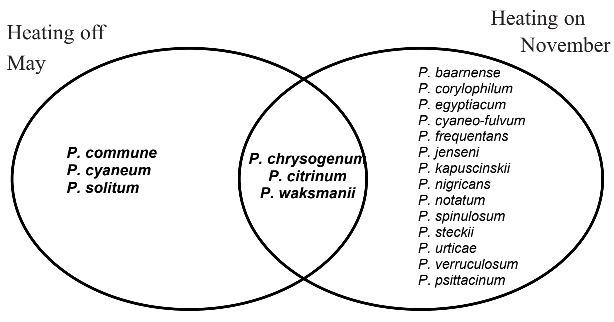

Fig. 2. Fungi of the genus *Penicillium* isolated during the heating season (November) and when the heating was off (May).

species, 12 have been reported in literature as toxigenic and 5 as allergenic (Table 1).

Temperature values in the school buildings examined were alike and ranged from 17 to 23.5ºC, whereas relative air humidity ranged from 39 to 52%.

Discussion of Results

Fungi of the genus *Penicillium* are isolated primarily from natural soils – forest, meadow and those subjected to anthropopression – cultivable and contaminated with organic or inorganic compounds, e.g. cement [19]. They belong to eury-ecological saprotrophs. Wide enzymatic capabilities of these fungi enable the acquisition of coal from a variety of sources. Furthermore, they colonize products of plant origin, including cereal grains, wood, paper and dietary starch [1, 20, 21], or those of animal origin, including milk and dairy products [22], or parchment [8]. Owing to this, representatives of this genus may occur in nature in each environment abundant in organic matter, as long as water activity of a substrate is not lower than 0.79 [23]. Also, construction materials, finishing materials, or those linked with utilization of school buildings may be used by the fungi as a food substrate. These materials are available to the fungi year round, thus their occurrence was noted in both experimental seasons, though with various intensity.

One of the room types discovered to be the richest reservoir of fungi was libraries [8]. This is due to a variety of organic compounds available to fungi in those rooms, both of plant origin (paper, paperboard, plant dyes), and of animal origin (parchment, leather on book covers, egg yolks added to dyes, bone glues used for binding or bodies of dead arthropodas feeding on the library collection). In libraries we isolated as many as 21 species of fungi of the genus *Penicillium*, which corresponds strongly with the total number of species identified in our study. In turn, when analyzing the taxonomic spectrum of particular buildings examined, it was observed that the diversity of the fungi analyzed in schools (12 species) was similar to that noted for chicken farms (11), fattening farms (10) or feed mixing plants (11). In turn, a higher number of fungi species belonging to the genus *Penicillium*, i.e. 17, was observed in a wood panels plant [1]. It seems, therefore, that in rooms predominated by one genus of organisms, the number of *Penicillium* taxa reaches a similar level.

The prevalence being higher by 65% and *Penicillium* species spectrum being three times wider in the heating season could have been linked with less frequent ventilation of the building and, thus, with cutting off of fungi inflow from the outer environment. Most likely, there have appeared new microhabitats, e.g. in corners of rooms or around radiators, with physicochemical parameters being especially convenient for the fungi. In this respect, fungi of the genus *Penicillium* are very much alike to those of the genus *Aspergillus*, which in the heating season increase their prevalence in rooms by 30%, yet with the number of their species being reduced by 54% [18]. Heat enclaves around radiators may, therefore, be suitable for thermophilic species of *Aspergillus*. In turn, moist air at the contact site of ceilings and walls, especially those being simultaneously external partitions of a building, afford ideal conditions for the growth of psychrotrophic fungi of the genus *Penicillium*.

Fungi of the genus *Penicillium* belong to eury-thermal species. They include species growing at 30ºC, as well as isolates observed to vegetate at -4ºC. They reach the optimum of growth at temperatures ranging from 15 to 25ºC. In both experimental seasons, amplitudes of temperatures inside school rooms did not differ significantly and were remaining in the optimal range for the fungi examined. A factor promoting their growth in the heating season, and explaining their higher prevalence as compared to fungi of the genus *Aspergillus* [18], is rather higher relative humidity of air and poorer abstraction of water vapor from buildings, than indoor temperatures.

The fungi examined inhabit construction partitions that simultaneously constitute their feed substrates. They change the medium on which the mycelium is growing by secreting inorganic (CO_2, H_2O_2) or organic compounds (glucuronic acid). This may, on the one hand, lead to suppressed growth of *Penicillium* competitors, while on the other afford favorable conditions for the succession of another groups of organisms. Apart from the direct impact on building quality, owing to their capability to biodegrade construction and finishing materials, species of the genus *Penicillium* may contaminate buildings with secondary metabolites [24]: mycotoxins and compounds exhibiting antibiotic effects. Poor ventilation of buildings in the heating season may lead to the accumulation of especially the latter compounds and may bear an inhibiting effect on the growth of other fungi. The higher prevalence of fungi of the genus *Penicillium* in the autumn results from their amensalism. By producing toxic metabolites, the fungi examined simultaneously protect themselves against their parasites. A more important aspect, however, is protection against being digested by enzymes of other mycelia. This effect is often observed in cultures of fungi of the genus *Penicillium*, in the form of a "buffer" zone between neighboring isolates.

Water and air are claimed to be the best transmission vectors of abundantly-produced conidial spores of *Penicillium* [18, 25]. This may explain more frequent isolation of conidiospores from air than from surfaces of walls of the buildings examined. In addition, light and often rough *Penicillium* spores stop more easily on porous surfaces (plaster covered with acrylic paint) than on glazed surfaces (glaze) or these covered with oil paint. The latter type of finishing does not absorb condensation water and is more difficult for the fungi to colonize. It is all the more important that as many as 12 species detected in the school buildings examined are noted in literature as toxigenic [21, 24]. They may synthesize: citrinin (*P. citrinum*, *P. corylopfilum*, *P. jenseni*, *P. notatum*, *P. steckii*, *P. verruculosum*), penicillic acid (*P. baarnense*, *P. commune*, *P. chrysogenum*, *P. notatum*, *P. viridicatum*), ochratoxin A (*P. commune*, *P. veruculosum*, *P. viridicatum*), patulin (*P. cyaneo-fulvum*, *P. urticae*), verruculone (*P. verrucolosum*), cyclopiasone acid

(*P. chrysogenum*, *P. commune*), viridicattins (*P. viridicatum*, *P. solitum*), roquefortine (*P. chrysogenum*), and xantomegnin (*P. viridicatum*). Uncontrolled growth of *Penicillium* cymelium may bear health implications for users of room facilities: schoolchildren and school workers. At a long-time exposure to spores, the spores drifting in bioaerosol and saturated with mycotoxins easily donate them to human tissues after penetrating lungs or directly through skin [24, 26]. Mycotoxins produced by *Penicillium* may exert a variety of effects on a human body [18]: they may damage particular organs (e.g. kidneys – ochratoxin A and citrinin), affect their neoplasia (ochratoxin A, patulin), paralyze the nervous systems (roquefortine) or, at best, induce gastric disorders, nausea, and vomiting (patulin).

Bioaerosol of buildings may additionally be contaminated by allergens constituting the cell walls of numerous *Penicillium* species [27-29], including those isolated in our study *P. chrysogenum*, *P. citrinum*, *P. commune*, *P. notatum*, and *P. viridicatum*. In children, the hypersensitivity to *Penicillium* allergens constitute ¼ of mycosis-based allergies. Over 75% of cases of allergies are diagnosed in children at the age of 7-12 [30]. Because children at this age stay at school for over 8 hours a day, the quality of indoor bioaerosol and room purity are of the outmost importance.

Conclusion

The schools under scrutiny were characterized by a various taxonomic spectrum of *Penicillium* genus genus, which was determined by ecological diversity of the school environments examined. Although fungi of the genus *Penicillium* occur in the outer environment that constitutes the primary reservoir of these microfungi to building contamination, the possibility of their survival in constructing materials and in bioaerosol of rooms is high as a result of their capability to adapt their vital strategies to conditions of the environment to be colonized. Especially in the heating season, the prevalence and species spectrum of the fungi of the *Penicillium* genus in school buildings should be constantly monitored due to their environmental plasticity as well as toxigenic, allergenic, and antibiotic properties.

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