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Dedicated to Professor Maria Ławrynowicz on the occasion of the 45th anniversary of her scientific activity

Diversity of yeast-like fungi and their selected properties in bioaerosol premises utility

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A total of 69 isolates of yeasts were recorded in the indoor air of the school buildings: 43 in heated rooms and 26 in unheated rooms. Perfect stages prevailed. Fungi isolated in our study belonged to 39 species. These were mostly monospecific isolates although five two-species isolates were noted. Differences in the properties of physiological characters of fungi isolated in both study seasons were observed. As indoor and outdoor air does not mix during the heating season, a specific substrate for prototrophic, non-fermenting yeast-like fungi forms. Acid production allows fungi to dissolve inorganic compounds in building structures and to release needed microcomponents. Abilities to produce carotenoid pigments are clearly promoted in yeast-like fungi living indoor. This may be related to the accumulation of compounds that are indirect stages in the cycle of biosynthesis of carotenoids or a surplus of oxidizing compounds.

Key words: yeast-like fungi, school, indoor air

INTRODUCTION

While fungi of various taxonomic groups constitute a considerable part of the biocoenosis in buildings, they are difficult to detect. Our earlier studies show that yeast-like fungi co-occurring with moulds are notoriously difficult in this respect (Ejdys 2011). Challenges to isolating yeast-like fungi from the indoor bioaerosol can also arise from issues of methodology such as an inappropriately selected incubation temperature and varying nutritive preferences of individual fungi. Colonies of yeast-like fungi, especially in moisture-damaged rooms, are usually overgrown with moulds whose growth rate is higher and nutritive requirements are smaller than those of yeast-like fungi. Pure isolation of the latter may be impossible and consequently a low number of them is reported in studies in various indoor spaces (Awad

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et al. 2010; Krajewska-Kułak et al. 2002; Meklin et al. 2002). Microbiologistis estimate that only 10 to 15% of microorganisms are culturable *in vitro* at best in some environments (Amann et al. 1995). Insufficient knowledge of the ecophysiology of indoor-air isolates is another reason for failure to culture and isolate yeast-like fungi from the indoor bioaerosol. Therefore the aim of this study was to investigate some properties of yeast-like fungi that help them to survive indoors.

MATERIAL AND METHODS

Yeast-like fungi isolated from the bioaerosol in partly modernized school buildings were investigated. Samples were collected during the heating season (November) and after the heating was switched off (May). Twenty six different rooms on all the stories in two school buildings were selected. Two study sites were set up in each room. Samples were collected using Koch sedimentation method. Three substrate types were used for cultures: solid Sabouraud medium with antibiotics, Rose-Bengal medium and Czapek-Dox medium (Ejdys et al. 2011).

The total number of fungi in the air was identified according to Polish Standard PN-89/Z-04111/03 and yeast-like fungi were isolated and identified according Kurtzman et al. (2010). The morphology of fungal isolates was assessed. Special attention was paid to properties of vegetative cells, budding type, size and shape of blastospores. The developmental stage, abilities to form hyphae and chlamydospores, and selected biochemical traits: fermentation abilities, carbon and nitrogen sources, vitamin absorption, acid production and abilities to synthesize pigments, were identified. Methods and techniques recommended by Kurtzmann and Fell (2000) were used.

RESULTS

The total number of fungi in the indoor bioaerosol was between 314 and 3577 cfu/m^3 in spring and between 79 and 1533 cfu/m^3 in autumn. Yeast-like fungi were not recorded in the air in one room during the heating season. A total of 1416 cfu/m^3 was recorded in one room (the library) while it did not exceed 393 colony forming units per 1 m^3 in other rooms. The number of cells of yeast-like fungi in the indoor aerosol ranged from 39.3 to 442 cfu/m^3 after the heating had been turned off.

A total of 69 isolates of yeasts were recorded in the indoor air in the school buildings: 43 in heated rooms and 26 in unheated rooms. Perfect stages prevailed. Fungi isolated in our study belonged to 39 species (Tab. 1). These were mostly monospecific isolates although five two-species isolates were noted:

Magnusiomyces magnusii (syn. *Dipodascus magnusii*) + *Sporobolomyces roseus*

Sakaguchia dacryoidea (syn. *Rhodosporidium dacryoideum*) + *Rhodotorula toruloides*

Wickerhamomyces anomalus (syn. *Pichia anomala*) + *Rhodotorula glutinis*

Kondoa malvinella (syn. *Rhodosporidium malvinellum*) + *Vanderwaltozyma polyspora* (syn. *Kluyveromyces polysporus*)

Cystofilobasidium informominiatum + *Lipomyces lipofer.*

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Table 1
Yeast-like species isolated during the heating season (November) and when it is disabled (May) of indoor air

No	Species	Number of isolates	
		May	November
1	*Arthroascus schoenii* (Nadson& Krassin.) G.I.Naumow, Vustin & Babeva	0	1
2	*Babjeviella inositovora* (Golubev, Blagod., Suetin & R.S. Trots.) Kurtzman & M. Suzuki	1	0
3	*Bueremia inundata* (P.A. Dang) M.S. Reddy & C.L. Kramer	0	1
4	*Candida tropicalis* (Castell.) Berkhout	4	0
5	*Citeromyces matritensis* (Santa María) Santa María	0	1
6	*Cystofilobasidium infirmominiatum* (Fell, I.L. Hunter & Tallman) Hamam., Sugiy. & Komag.	0	1
7	*Debaryomyces hansenii* (Zopf) Lodder&Kreger	0	4
8	*D. robertsiae* (Van der Walt) Kurtzman & Robnett	0	1
9	*Dekkera anomala* M.T. Sm. & Grinsven	3	3
10	*D. bruxellensis* Van der Walt	1	2
11	*Kluyveromyces lactis* (Boidin, Abadie, J.L. Jacob & Pignal) Van der Walt	3	0
12	*K. marxianus* (E.C. Hansen) Van der Walt	1	1
13	*Kondoa malvinella* (Fell & I.L. Hanter) Y.Yamada, Nakagawa & I. Banno	0	1
14	*Leucosporidium scottii* Fell, Statzell, I.L. Hunter & Phaff	0	2
15	*Lipomyces lipofer* Lodder & Kreger ex Slooff	0	1
16	*L. starkeyi* Lodder & Kreger	1	0
17	*Magnusiomyces magnusii* (F. Ludw.) Redhead & Malloch	1	0
18	*Millerozyma farinosa* (Lindner) Kurtzman & M. Suzuki	1	2
19	*Mycotorula rubescens* (Saito) Cif. & Raelli	0	1
20	*Oosporidium margaritiferum* Stautz	0	1
21	*Pichia membranifaciens* (E.C. Hansen) E.C. Hansen	0	2
22	*Pseudozyma fusiformata* (Buhagiar) Boekhout	0	1
23	*Rhodosporidium diobovatum* S.Y. Newell & I.L.Hunter	1	1
24	*Rh. fluviale* Fell, Kurtzman, Tallman & J.P.Buck	1	1
25	*Rh. kratochvilovae* Hamam., Sugiy. & Komag.	1	0
26	*Rh. lusitaniae* A Fonseca & J.P. Samp.	1	2
27	*Rh. paludigenum* Fell & Tallman	1	0
28	*Rh. sphaerocarpum* S.Y. Newell & Fell	1	0
29	*Rhodotorula glutinis* (Frasenius)F.C.Frasenius	0	1
30	*Rh. graminis* Di Menna	0	1
31	*Rh. toruloides* Banno	0	1
32	*Sakaguchia dacryoidea* (Fell S.L. Hanter &Tallman) Y.Yamada, Maeda&Mikata	0	2
33	*Schwanniomyces polymophus* (Klöcker) M. Suzuki & Kurtzman	1	1
34	*Sch. vanrijiae* (Van der Walt & Tscheuschner) M. Suzuki & Kurtzman	1	2
35	*Sporobolomyces roseus* Kluyver & C.B. Niel	1	0
36	*Sporidiobolus salmonicolor* Fell & Tallman	0	2
37	*Vanderwaltozyma polyspora* (Van der Walt) Kurtzman	0	1
38	*Wickerhamomyces anomalus* (E.C. Hansen) Kurtzman, Robnet & Basehoar-Powers	0	2
39	*Yarrowia lipolytica* (Wick., Kurtzman & Herman) Van der Walt & Arx	1	0
	Total	26	43

A vast majority of the isolates did not form pseudohyphae or they were only fragmentary.

Differences in the properties of physiological characters of fungi isolated in both study seasons were observed (Fig. 1). Non-fermenting and pigment producing isolates dominated in heated rooms (Fig. 1B, D) while the majority of fungi recorded after the heating season had the ability fermentation and the production of pigment

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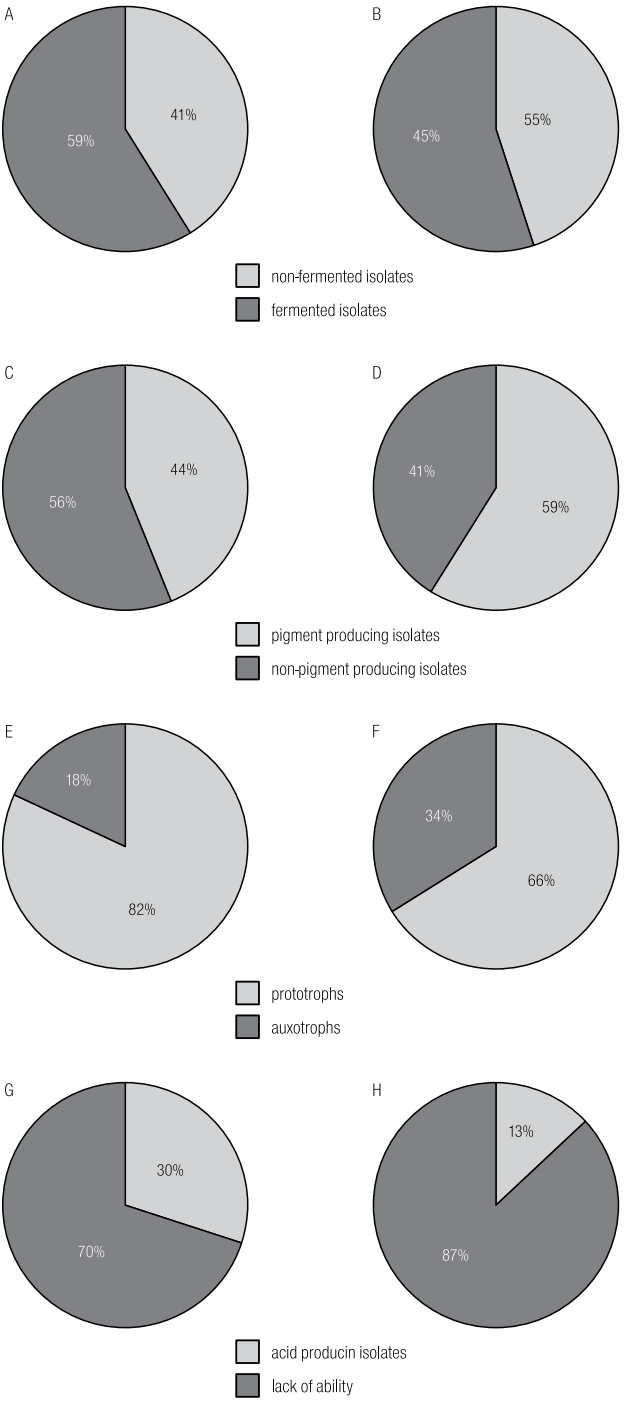

Fig. 1.The frequency of isolates at the time of the heating period and after it is turned off, with the capacity: fermentation (A, B), production of pigments (C, D), vitamins (E, F) and acid (G, H).

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(Fig. 1A, C). 2/3 of isolates did not require any vitamins for growth. Five isolates belonging to four species exhibited acid-forming abilities: *Dekkera anomala, D. bruxellensis, Yarrowia lipolytica, Candida tropicalis (*syn. *Candida citrica)*.

DISCUSSION

Indoor spaces in the temperate climate are a special biotope. Organisms inhabiting it are not influenced by the seasons characterized by atmospheric conditions of this climatic zone. The indoor environment is mostly influenced by the heating or its lack and resulting fluctuations in the humidity and temperature. When the heating is on and the airing frequency decreases, a biotope consisting of a variety of microhabitats with highly varying parameters are formed. It is usually warmer and drier around the heaters than in the remaining part of the room while contact sites between the ceiling and the walls, especially northern and/or external walls, are the most humid and coldest places. A high diversification of indoor biotopes allows fungi with different physiological requirements to survive. While the diversity of taxa of fungi noted in unheated indoor air varies greatly (some species are allogenic), the species composition of the heating season can be considered to be relatively stable (Ejdys et al. 2009) although difficult to determine. Some two- or even three-species isolates can be undetectable if only biochemical properties are examined. They are a sum of properties of individual symbionts and taxa cannot be determined reliably. In our previous studies, multispecies isolates were recorded relatively frequently both in aquatic macrobiocoenoses (Biedunkiewicz, Barańska 2011), land biocoenoses (Ejdys et al. 2009) and in organ ontocoenoses (Dynowska, Ejdys 2000; Biedunkiewicz 2001; Dynowska et al. 2008). While this can reflect a frequent natural phenomenon, the high percentage of synergizing yeast-like fungi isolated from the indoor bioaerosol in our study can rather be attributed to the fact that yeast-like fungi survive on culture media better than fungi adopting a solitary lifestyle.

Metabolism of yeast-like fungi can make them more difficult to culture. Only approximately 20% of yeast-like fungi are vitamin prototrophs (Kurtzman, Fell 2000). They can produce biotin (vit. B_1/H), inositol (vit. B_8), folic acid (vit. $B_{9/11}$), pantothenic acid (B_5), 4-aminobenzoic acid (vit. B_X), niacin (vit. B_1/PP), pyridoxin (vit. B_6), retinol (vit. A), riboflavin (vit. B_2) and thiamin (vit. B_1). A vast majority of yeast-like fungi require regulatory compounds for life and must derive them from the environment. It is therefore interesting that 66% of prototrophic fungi were isolated during the heating season and as much as 82% of prototrophic fungi were detected outside the heating period. Importantly, school rooms (public use indoor spaces) are not deficient in the organic matter. Prototrophy may allow yeast-like fungi to win nutritive competition, especially with moulds.

While simple sugars can penetrate indoor spaces with the atmospheric air in spring, these substrates are not easily available to indoor microorganisms in autumn. When a high and steady supply of oxygen is available, fermentation abilities are not needed for life in indoor environment. This explains a low percentage of non-fermenting species in our studies. On the other hand, substrate acidification encourages the release of ions, e.g. iron ions, needed for life. These abilities were observed

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in 30% of fungi identified although it is a very rare property in yeast-like fungi. Abilities to produce acetic acid or citric acid were confirmed only in eight species of 1414 listed in a study by Kutzman and Fella (2000).

Yeasts produce some characteristic non-carotenoid compounds such as yellow riboflavin produced by *Candida guilliermondii*, pink pulcherrimin, a ferric pigment produced by fungi of the genera *Metschnikowia* (*M. pulcherrimina, M. reukaffi*) and *Kluyveromyces.* Yeast-derived carotenoid pigments include astaxanthin (*Xanthophyllomyces dendrorhous, Phaffia rhodozyma*), carotene, torulen, torularhodin, produced by the genera *Rhodotorula, Rhodosporidium, Sporidiobolus, Phaffia, Protomyces, Pseudozyma, Cystophilobasidium* and *Saitoella* (Pawłowska 2009; Stachowiak, Czarnecki 2006; Latha, Jeevaratnam 2010). Carotenoid pigments are perceived as bioactive substances, strongly anti-oxidizing (Stachowiak, Czarnecki 2006).

Physical and chemical factors and the availability of the nutritive substrate influence carotenogenesis. The highest capacity of carotenogenesis in laboratory tests is obtained at 20-22˚C. Pigment formation is photoregulated although the presence of light is not necessary. This may explain the high percentage of pigmented isolates in autumn, that is during the "short day". The type and concentration of carbon and nitrogen sources and their mutual ratio are mostly given as substrate types. The most recent studies report increased carotenoid production when secondary metabolites of other microorganisms are present in the substrate, even as their extracts (Stachowiak, Czarnecki 2006). If these compounds include enzymes decomposing cell walls, then pigments are a response to free radicals of H_2O_2 produced. This may also be related to the presence of carotenoid precursors. Their presence may promote pigment-forming fungi. This was most probably the case in our study. A high number of pigmented isolates in indoor spaces is indirectly or directly related to the diversity of the biota of the indoor aerosol. Therefore it seems justified that the occurrence of carotenoid-producing yeast-like fungi may be an indicator of mycological, or even microbiological, air purity.

CONCLUSIONS

As indoor and outdoor air does not mix during the heating season, a specific substrate for prototrophic, non-fermenting yeast-like fungi forms.

Acid production allows fungi to dissolve inorganic compounds in building structures and to release needed microcomponents.

Abilities to produce carotenoid pigments are clearly promoted in yeast-like fungi living indoor. This may be related to the accumulation of compounds that are indirect stages in the cycle of biosynthesis of carotenoids or a surplus of oxidizing compounds.

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Wybrane właściwości grzybów drożdżopodobnych na tle ich różnorodności w bioaerozolu pomieszczeń użyteczności publicznej

Streszczenie

Znaczną część biocenozy budynków stanowią trudno wykrywalne grzyby z różnych grup taksonomicznych. U podstawy niepowodzeń w hodowli i izolacji drożdżaków z bioaerozolu wewnętrznego leży zbyt mała wiedza dotycząca ekofizjologii izolatów bytujących w pomieszczeniach. Dlatego celem badań było poznanie niektórych właściwości drożdżaków, ułatwiających im przeżywanie w środowisku pomieszczeń.

Materiałem do badań były grzyby drożdżopodobne uzyskane z bioaerozolu 26 pomieszczeń szkolnych o różnym sposobie użytkowania poddanych częściowej modernizacji. Próby pobierano w okresie grzewczym (listopad) i po wyłączeniu ogrzewania (maj). Określano morfologię uzyskanych izolatów grzybów, stadium rozwojowe, zdolności strzępkowania i wytwarzania chlamydospor oraz wybrane cechy biochemiczne: zdolności fermentacyjne, źródła węgla i azotu, przyswajanie witamin, wytwarzanie kwasów oraz zdolności syntezowania barwników.

Ogólna liczba grzybów w bioaerozolu badanych pomieszczeń wynosiła wiosną od 314 do 3577 jtk/m^3, natomiast jesienią od 79 do 1533 jtk/m^3. Z powietrza wewnętrznego w badanych budynkach uzyskano łącznie 69 izolatów drożdży: 43 z pomieszczeń ogrzewanych i 26 z nieogrzewanych. Dominowały stadia doskonałe. Wyizolowane grzyby należały do 39 gatunków. Na ogół izolaty były jednogatunkowe, natomiast w pięciu przypadkach stwierdzono izolaty dwugatunkowe. Zaobserwowano różnice w cechach fizjologicznych grzybów izolowanych w obu sezonach badawczych.

Nie mieszanie się powietrza zewnętrznego i wewnętrznego pomieszczeń w okresie grzewczym stwarza specyficzne siedlisko dla prototroficznych, nie fermentujących grzybów drożdżopodobnych. Produkcja kwasu umożliwia grzybom rozpuszczanie związków nieorganicznych w konstrukcjach budowlanych i uwalnianie potrzebnych mikroskładników. Zdolności wytwarzania barwników karotenoidowych, są wyraźnie promowane u drożdżaków bytujących w pomieszczeniach, co być może wiąże się z nagromadzeniem związków będących pośrednimi etapami w cyklu biosyntezy karotenoidów oraz nadmiarem związków utleniających.

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