

Original Research

Threats Resulting from the Presence of Potentially Pathogenic Fungi in the Sport Changing Rooms – a Preliminary Study

Tomasz Bałabański *, Anna Biedunkiewicz

Department of Microbiology and Mycology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn, Oczapowskiego 1A St. 10-719 Olsztyn, Poland

Received: 23 November 2022

Accepted: 10 March 2023

Abstract

Mycological cleanliness of rooms directly translates into the health of their users. This is important for athletes who are actively training. The aim of the study was to evaluate the sanitary and epidemiological status of sports changing rooms by determining the species diversity of their mycobiota in the context of prevention of athletes' health. The research was carried out in changing rooms of sports halls used by volleyball players in the city of Olsztyn (NE Poland). Standard mycological isolation and identification methods were used in this study. A total of 87 strains of microfungi were isolated. 52 belonged to the mold fungi, among which 18 species, belonging to 10 were found. The species isolated most frequently were: *Aspergillus fumigatus*, *A. niger*, *Juxtiphoma eupyrena* (previous name: *Phoma eupyrena*) and *Westerdykella minutispora* (previous name: *Ph. minutispora*). 35 yeast strains, belonging to 15 species and 10 genera were isolated. The most common species were: *Candida glabrata*, *C. krusei*, *C. parapsilosis* and *Rhodotorula glutinis*. 5 species were categorized as class BSL-2 fungi. The conducted research justify the need for preventive examinations of athletes and shows the necessity of constant monitoring of the sanitary condition of the sport changing rooms.

Keywords: microfungi, indoor environment, athletes, sport changing rooms, epidemiology

Introduction

Microfungi are characterized by high ecophysiological plasticity enabling them to be adapted to the current conditions in the environment [1]. Due to their wide tolerance to environmental factors, they colonize most of the earth's ecosystems [1, 2]. Microfungi can also occur in buildings, within the

structure of building materials (e.g. wood, plaster or paint) and using them as a source of necessary nutrients [2-5]. Buildings are most often colonized by mold fungi of the genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium* and *Stachybotrys* [6-8]. The most common genera of yeasts in buildings are *Candida*, *Geotrichum*, *Malassezia*, *Rhodotorula*, *Saccharomyces* and *Trichosporon* [5, 9, 10]. The presence of microfungi in rooms leads to damage to building materials and deterioration of the sanitary and epidemiological condition of rooms, which is directly related to the health risk of their users [2, 3, 5, 11].

*e-mail: tomasz.balabanski@uwm.edu.pl

School buildings are particularly at risk of colonization by fungi because they are intended for many different functions. They are places of learning, eating, as well as sports competitions [6, 7]. According to Eurostat data from 2013, over 64 million students and 4.5 million teachers stay in school buildings every day. Statistical research indicates that children spend almost 80% of their time indoors, and school is the second place in the order in which young people stay the longest during the day, suggesting the need for constant monitoring of the sanitary condition of school buildings and take action to prevent the negative effects of indoor air pollution [6, 12].

Currently, school buildings are not used only during classes. Classrooms, changing rooms and gyms are rented or lent to users other than students. In smaller cities, due to the absence of independent sports facilities, competitions and tournaments of various disciplines take place in the gyms of school buildings. The presence of fungi in this type of room causes a special threat to athletes due to their increased sensitivity to microbial factors. This is associated with a weakening of immunity during and immediately after intense physical activity [2, 13]. The risk of infection is also increased by the possibility of damage to the skin surface during training and using the same equipment, showers, changing rooms and their equipment [2]. The literature indicates a relationship between increased sports activity and the frequency of mycosis of nails and

smooth or hairy skin in humans however, this problem has not been sufficiently investigated [14].

Taking into account the adaptive properties of microfungi and the fact that rooms can be considered as separate ecological niches, characterized by an autonomous microclimate, they are given increasing attention in research [7, 15]. Therefore, the study aimed to determine the sanitary and epidemiological status of sports changing rooms, in the context of preventive health of athletes, by analyzing the diversity of the species composition of their mycobiota.

Material and Methods

Period and Area of Research

The study was of a reconnaissance nature, for this purpose, three sports changing rooms within sports halls in the city of Olsztyn (NE Poland) out of approximately 120 were selected (Table 1). The choice of the changing rooms and the dates of sampling for tests were conditioned by the schedule of matches of the Regional Youth Volleyball League (15-18 years). Research material was collected before and after the matches of the mentioned league. The study of sports facilities is particularly difficult due to formal limitations. The managers and owners of the facilities in most cases did not agree to the research.

Table 1. Technical specification of the tested changing rooms.

Parameter	Changing room 1	Changing room 2	Changing room 3
Date of sampling	11.12.2016 and 13.10.2017	17.02.2017	14.10.2017
Construction/ architecture	Changing room in the sports hall of the high school building	Changing room in the sports hall of the high school building	Changing room of the professional sports hall
Usable surface	Approx. 32 m ² (including the shower room)	Approx. 26 m ² (including the shower room)	Approx. 40 m ² (including the shower room)
Equipment	<ul style="list-style-type: none"> • Wooden benches • Metal wall hangers attached to the board • Plastic, open trash can 	<ul style="list-style-type: none"> • Wooden benches • Metal wall hangers attached to the glaze • Plastic, closed trash can 	<ul style="list-style-type: none"> • Wooden benches • Metal wall hangers attached to wooden paneling. • Plastic closed trash can
Walls	Glaze - up to 2/3 of the wall height (above the glaze the wall is covered with paint)	Glaze - entire surface of the wall	Wooden paneling - entire surface of the wall
Floor	Terracotta	Terracotta	Terracotta
Windows	2 narrow windows near the ceiling, opposite the front door	1 long, narrow window near the ceiling, opposite the front door	1 long, narrow window near the ceiling, opposite the front door
Showers room	<ul style="list-style-type: none"> • Terracotta floor • Glaze - entire surface of the walls • 2 narrow windows close to the ceiling 	<ul style="list-style-type: none"> • Terracotta floor • Glaze - entire surface of the walls • 1 long, narrow window close to the ceiling 	<ul style="list-style-type: none"> • Terracotta floor • Glaze - entire surface of the walls • 1 long, narrow window close to the ceiling
Showers room location	Separated from the changing room by a partial wall	Separated from the changing room by a wall with wooden doors	Separated from the changing room by a wall with wooden doors
Showers number	3 showers in a row	3 showers in a row	8 showers in 2 opposite rows (4 in a row)

Collection of Samples

The surface swab method was used, using disposable, sterile, cotton swabs moistened with 0.85% sterile NaCl solution. For each changing room, the research material consisted of 3 swabs from the walls surfaces and elements of its equipment, 3 swabs from the surface of used handshowers and 3 swabs from the surface of the shower trays used by the athletes. In the case of walls and shower trays, the material was taken from an area of 25 cm², using a sterile, aluminum frame. No samples were taken from the air as a space for microfungal transmission. The research was focused only on swabbing samples from walls and usable surfaces in order to identify places of adherence of microfungi spores that come into direct contact with the athletes (skin surface, injuries and abrasions).

Isolation and Identification of Fungi

All microbiological media used in the research were prepared according to [16]. A total of 72 swabs were taken, and the obtained material was inoculated using a swab onto the Sabouraud's dextrose agar with chloramphenicol (glucose 20 g; peptone 5 g; agar 8 g; chloramphenicol 0.25 g; distilled water 500 mL) and incubated at 37°C for 72 hours.

To obtain growth of fungi not observed on Sabouraud's dextrose agar, the used swabs were placed in tubes with Sabouraud's dextrose broth (glucose 20 g; peptone 5 g; sodium chloride 2.5 g; distilled water 500 mL) and incubated at 37°C for 72 h. Then, using an automatic pipette, five 20 µl drops from each Sabouraud's dextrose broth culture were transferred to the Sabouraud's dextrose agar with chloramphenicol (0,05%) and incubated under the same conditions (drop plate method).

Mold Fungi

Macroscopic analysis of mold fungi colonies was made (the color of the bottom and top of the colony, the dye secreted to the substrate and the presence of secondary metabolites in the form of guttation drops were taken into account). Micromorphological features of mold fungi (hypha structure, shape and size of spores and conidiophore or sporangiophore, presence of metulas, phialides, wall structure of conidial spores) were microscopically analyzed using the print preparation stained with aniline blue with lactophenol.

Photographic documentation from microscopic analyses was prepared. Species identification of mold fungi was based on the diagnostic keys [17-19]. The identified species of mold fungi were classified into biosafety levels (BSL) [19].

Yeasts

Life-time preparations were made in a drop of sterile tap water. The exclusion of bacterial colonies from further stages of the diagnostic process was based on the macroscopic analysis of the colonies and microscopic features observed in preparations.

The yeast colonies were analyzed macroscopically, taking into account the following features: size, convexity, color, sheen, surface texture, edge structure, clarity, texture and smell.

A zymograms and carbohydrate auxanograms were performed to determine the ability of isolated yeast strains to ferment and assimilate glucose, galactose, lactose, maltose and sucrose.

To determine the micromorphological structure of yeast colonies (presence, size, position and shape of blastospores, chlamydozoospores, formation of pseudomycelium), microcultures on Nickerson agar (NH₄NO₃ - ammonium nitrate 0.5 g; sodium chloride 0.5 g; biotin 1.25 g; agar 7.5 g; trypan blue 0.05 g; distilled water 500 mL) in moist chambers were performed.

Photographic documentation from microscopic analyses was prepared. Based on microscopic observation of microculture and analysis of the enzymatic properties of the obtained strains, species identification was carried out. Species identification of yeasts was based on the diagnostic keys [19, 20]. Biosafety level (BSL) values were determined for each yeast species [19].

Statistical Methods

Statistical analysis was performed using STATISTICA13.3 software (StatSoft). The significance of the differences in the number of isolates on each of the tested surfaces before and after the match was determined at the level of $p < 0,05$ using Wilcoxon Matched Pairs Test. The significance of the differences in the number of isolates between the 3 tested surfaces was determined at the level of $p < 0,05$ by a non-parametric method Kruskal-Wallis H test (one-way ANOVA on ranks).

Results and Discussion

During the research, a total of 87 strains of microfungi were isolated. Among them, there were 35 yeast strains, belonging to 15 species and 10 genera. In the case of mold fungi, 52 strains were obtained, among which 18 species, belonging to 10 were found (Table 2, 3; Figs 1, 2).

Mold Fungi

The most frequently occurring mold species in the studied rooms were: *Aspergillus fumigatus*, *A. niger*,

Table 2. The number of isolates of individual species of mold fungi isolated from the tested surfaces (along with the percentage share) and the BSL classification.

Species	Walls		Handshowers		Shower tray		Total number of isolates	BSL value
	Before the match	After the match	Before the match	After the match	Before the match	After the match		
<i>Acremonium alabamense</i>	2 (3.85)	0	0	0	0	0	2 (3.85)	1
<i>Caesitomonium hyalinulum</i> (prev. name: <i>Acremonium hyalinulum</i>)	0	0	0	0	1 (1.92)	0	1 (1.92)	1
<i>Sarocladium kiliense</i> (prev. name: <i>Acremonium kiliense</i>)	0	0	1 (1.92)	0	0	0	1 (1.92)	2
<i>Aspergillus flavus</i>	1 (1.92)	0	0	0	0	0	1 (1.92)	2
<i>Aspergillus fumigatus</i>	1 (1.92)	5 (9.62)	0	5 (9.62)	0	0	11 (21.15)	2
<i>Aspergillus niger</i>	2 (3.85)	3 (5.77)	0	0	1 (1.92)	0	6 (11.54)	1
<i>Aspergillus ochraceus</i>	0	0	0	0	1 (1.92)	0	1 (1.92)	1
<i>Cladosporium cladosporioides</i>	1 (1.92)	0	1 (1.92)	1 (1.92)	1 (1.92)	0	4 (7.69)	1
<i>Ochrocladosporium elatum</i> (prev. name: <i>C. elatum</i>)	0	0	0	1 (1.92)	0	0	1 (1.92)	1
<i>Mortierella polycephala</i>	1 (1.92)	0	0	0	0	0	1 (1.92)	1
<i>Corchyceps javanica</i> (prev. name: <i>Paecilomyces javanicus</i>)	1 (1.92)	0	0	0	0	0	1 (1.92)	1
<i>Penicillium griseofuuum</i>	0	1 (1.92)	0	0	0	0	1 (1.92)	1
<i>Juxtiphoma eupyrena</i> (prev. name: <i>Phoma eupyrena</i>)	0	0	3 (5.77)	1 (1.92)	1 (1.92)	4 (7.69)	9 (17.31)	1
<i>Westerdykella minutispora</i> (prev. name: <i>Phoma minutispora</i>)	0	0	1 (1.92)	2 (3.85)	3 (5.77)	0	6 (11.54)	1
<i>Epicoecum sorghinum</i> (prev. name: <i>Phoma sorghina</i>)	0	0	2 (3.85)	0	0	0	2 (3.85)	1
<i>Rhizopus oryzae</i>	0	1 (1.92)	0	0	0	0	1 (1.92)	1
<i>Trichoderma viridae</i>	1 (1.92)	0	1 (1.92)	0	0	0	2 (3.85)	1
<i>Talaromyces sp.</i>	0	1 (1.92)	0	0	0	0	1 (1.92)	1
Sum	10 (19.23)	11 (21.15)	9 (17.31)	10 (19.23)	8 (15.38)	4 (7.69)	52 (100)	-

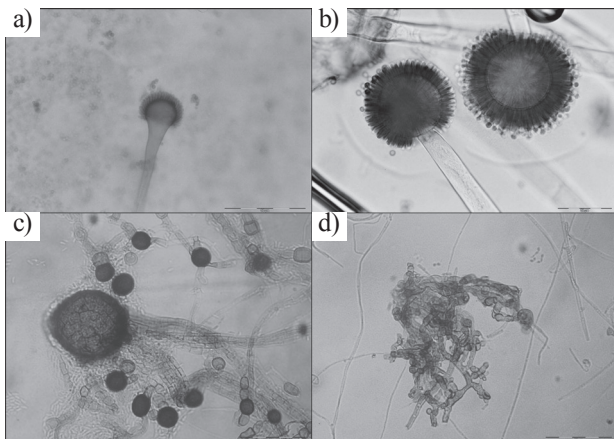


Fig. 1. The most frequently isolated mold fungi – print preparations stained with aniline blue with lactophenol – a) – *Aspergillus fumigatus* (magn. 600x); b) – *Aspergillus niger* (magn. 400x); c) – *Juxtiphoma eupyrena* (prev. name: *Phoma eupyrena*) (magn. 600x); d) – *Westerdykella minutispora* (prev. name: *Phoma minutispora*) (magn. 600x).

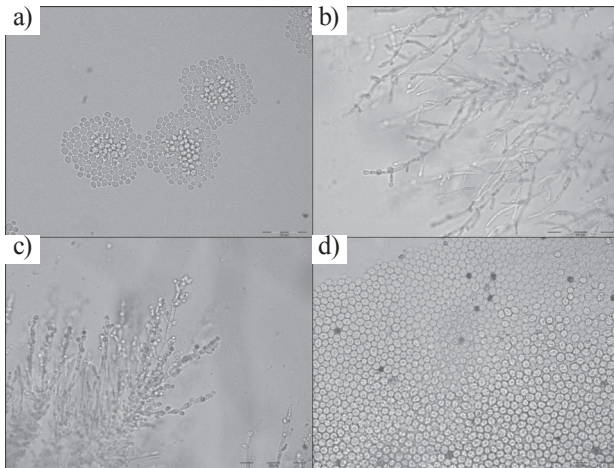


Fig. 2. The most frequently isolated yeasts – microscopic preparations from microculture on Nickerson agar – A – *Candida glabrata* (magn. 1000x); B – *Candida krusei* (magn. 600x); C – *Candida parapsilosis* (magn. 400x); D – *Rhodotorula glutinis* (magn. 600x).

Juxtiphoma eupyrena (prev. name: *Phoma eupyrena*) and *Westerdykella minutispora* (prev. name: *Ph. minutispora*) with the clear dominance of the first of them (Fig. 1, Table 2). In total, slightly more fungi were obtained from the material taken before the start of the match than after it (Table 2). The most isolates of mold fungi were obtained from the walls (21), and the least from the shower tray (12). 19 isolates were obtained from the handshowers (Table 2). The differences in the number of isolates between examined surfaces were not statistically significant ($p = 0.2976$). In the case of walls and handshowers, more fungi were found in the material collected after the matches, while the shower trays were more strongly contaminated before the athletes entered the changing room (Table 2). In all of these cases,

the differences in the number of isolates before and after matches were not statistically significant (walls, $p = 0.858863$; handshowers, $p = 0.735317$; shower trays, $p = 0.294508$). The material taken from the walls was characterized by the largest species diversity while the smallest species diversity was found in the material obtained from the surface of the shower trays (Table 2).

Fungi of the genus *Aspergillus* were present on all of the tested surfaces (Table 2). *A. fumigatus* was most frequently isolated. It occurred on the surface of walls and handshowers. Regardless of the tested surface, *A. fumigatus* was more often isolated from changing rooms after the end of the matches (Fig. 1, Table 2). The second most frequently isolated species belonging to this genus, *A. niger* was detected on the surface of walls and shower trays. As in the previous case, more isolates were obtained from the changing room after the matches were over (Fig. 1, Table 2).

The remaining, most often isolated mold fungi belonging to the genus previously named *Phoma* were present on the surface of handshowers and shower trays, but they did not occur on the walls. Species *Juxtiphoma eupyrena* was most frequently represented (Fig. 1). It was collected more often after the matches. In the case of *Westerdykella minutispora* a reverse trend was observed (Table 2).

BSL classes for isolated species were determined. 15 species were categorized as class BSL-1 fungi, which constituted 83.33% of isolated species. 3 other species (16.67%) were classified into the class BSL-2 (Table 2).

Yeasts

Most commonly recorded species were: *Candida glabrata*, *C. krusei*, *C. parapsilosis* and *Rhodotorula glutinis* (Table 3, Fig. 2). Slightly more yeast strains were isolated from post-match material (Table 3). Most yeast strains were obtained from the shower trays (14) and the least from the walls (8). 13 isolates were obtained from the handshowers (Table 3). The differences in the number of isolates between examined surfaces were not statistically significant ($p = 0.6226$). Handshowers and shower trays were more contaminated after the matches were over, while more yeast was isolated from the walls before the game began (Table 3). No statistically significant differences were found in any of the cases (walls, $p = 0.615811$; handshowers, $p = 0.812943$; shower trays, $p = 0.324012$). The greatest species diversity was noted on the shower tray's surface. In the case of handshowers and walls, the same number of species were isolated, less than from the shower trays (Table 3).

Species *C. glabrata* and *R. glutinis* were present on all tested surfaces (Table 3). *C. glabrata* was most frequently isolated. The same number of isolates of this species were obtained before and after the match (Fig. 2, Table 3). The second most frequently isolated species was *R. glutinis*. More isolates of this species were

Table 3. The number of isolates of individual species of yeasts isolated from the tested surfaces (along with the percentage share) and the BSL classification.

Species	Walls		Handshowers		Shower tray		Total number of isolates	BSL value
	Before the match	After the match	Before the match	After the match	Before the match	After the match		
<i>Candida glabrata</i>	1 (2.86)	1 (2.86)	1 (2.86)	2 (5.71)	1 (2.86)	0	6 (17.14)	2
<i>Candida guilliermondii</i>	1 (2.86)	0	0	0	0	1 (2.86)	2 (5.71)	1
<i>Candida chiroptcherorum</i>	0	0	0	1 (2.86)	0	1 (2.86)	2 (5.71)	1
<i>Candida krusei</i>	0	0	1 (2.86)	1 (2.86)	0	2 (5.71)	4 (11.43)	2
<i>Candida parapsilosis</i>	1 (2.86)	0	1 (2.86)	1 (2.86)	0	0	3 (8.57)	1
<i>Candida utilis</i>	0	0	0	0	0	1 (2.86)	1 (2.86)	1
<i>Issatchenkia orientalis</i>	0	0	0	1 (2.86)	1 (2.86)	0	2 (5.71)	1
<i>Metschnikowia pulcherima</i>	0	1 (2.86)	0	0	0	0	1 (2.86)	1
<i>Rhodotorula glutinis</i>	1 (2.86)	0	1 (2.86)	1 (2.86)	0	2 (5.71)	5 (14.29)	1
<i>Saccharomyces cerevisiae</i>	0	1 (2.86)	0	0	1 (2.86)	0	2 (5.71)	1
<i>Saitoella complicata</i>	0	0	0	0	1 (2.86)	1 (2.86)	2 (5.71)	1
<i>Toluraspora delbrueckii</i>	0	0	0	0	1 (2.86)	0	1 (2.86)	1
<i>Moniliella spathulata</i> (prev. name: <i>Trichosporonoides spathulate</i>)	0	0	0	0	0	1 (2.86)	1 (2.86)	1
<i>Rhodospiridium</i> sp.	0	0	2 (5.71)	0	0	0	2 (5.71)	1
<i>Trichosporon</i> sp.	1 (2.86)	0	0	0	0	0	1 (2.86)	1
Sum	5 (14.29)	3 (8.57)	6 (17.14)	7 (20.00)	5 (14.29)	9 (25.71)	35 (100.00)	-

obtained from the changing room after the matches were over (Fig. 2, Table 3).

Of the remaining most common species, *C. krusei* was not found on walls and *C. parapsilosis* was not isolated from the shower trays (Fig. 2, Table 3). *C. krusei* was isolated more frequently from pre-match material. In the case of *C. parapsilosis*, the opposite trend was noted.

BSL classes for isolated species were determined. 13 species were categorized into the class BSL-1 (86.67%) and 2 others (13.33%) into the class BSL-2 (Table 3).

The presence of microfungi in buildings leads to the biodeterioration of building materials and may pose a threat to the health of room users due to their pathogenic and allergic properties [5, 7, 11]. Bacteria, parasite eggs, antigens, endotoxins, and in the case of microfungi: mycotoxin, spores, hyphae and complex fragments of mycelium can occur in the air as an element of bioaerosol, maintaining their infectious potential [5-8, 21, 22].

Research indicates that people spend about 87% of their time indoors, and according to WHO over 3 billion people suffer from diseases caused by air pollution [21, 23]. In addition, human skin contact with contaminated indoor surfaces is an important way for microorganism transmission [24]. The WHO has distinguished specialized terms that describe a set of non-specific symptoms associated with staying in buildings - SBS and diseases associated with staying in contaminated rooms - BRI [8, 11, 22]. Symptoms of the sick building syndrome include mucosal irritations, wheezing, headaches, coughs, gastrointestinal disorders, fatigue, sensitivity to light, and even depression [8, 11, 22]. The most common diseases of users of contaminated buildings are bronchial asthma, rhinitis and alveolitis [6, 8, 22, 25, 26]. The multi-tasking of school buildings with sports halls and changing rooms makes these rooms particularly susceptible to colonization by microfungi with extensive enzymatic possibilities [7].

Athletes are exposed to various physicochemical and biological factors such as temperature, irradiation, cold air, chlorine, allergens, microorganisms and many more [13]. Given the increased physical effort, which causes periodic weakening of immunity athletes are a group particularly sensitive to microbial infections. The dominant part of diseases diagnosed in athletes concerns skin and mucous membranes. It is related to the direct contact of the body with the surfaces of the rooms and elements of equipment for sports facilities [27]. Studies indicate that exercise can negatively affect lupus erythematosus, psoriasis, porphyria, acne, lichen planus, albinism, other skin diseases and asthma [13]. Skin and nails mycosis occurs more often in athletes than in the rest of the population, and amateur and competitive sports are the factors favoring the occurrence of these diseases [14].

Own research showed a relationship between the type of surface from which material was taken and the number of isolates obtained.

The most mold fungi were obtained from the walls, and the least from the shower trays. The justification for this observation may be the smooth structure and elliptical shape of mold spores isolated from the walls, allowing the adhesion of fungi to this type of surfaces. Similar observations were made by [9]. In addition, spores on the surface of the shower tray may be flushed when the shower is used. For walls and handshowers, more mold fungi isolates were obtained after matches. Such differences can be caused by air circulation and movement of room users who can transfer microbes between rooms on their body [9, 12]. In the case of the shower trays surfaces, more mold fungi were isolated before the match. The probable reason for this observation may be flushing the spores from the shower tray surface during taking a shower. The material obtained from the walls was characterized by the largest species diversity. The probable reason for this observation may be the fact that this environment was not subject to such frequent changes as handshowers and shower tray washed by water. The rooms with showers are often isolated from the rest of the changing room, which practically prevents contact of water with the wall surfaces, and consequently flushing out the spores. However, indoor humidity increases, which can promote spore adhesion to the walls [9, 10].

In turn most yeast strains were isolated from the shower trays, the least from the walls. Athletes rinse off the yeast cells from the body surface along with washing agents. Moreover, the showers are practically not used before the matches start. Another likely cause may be increased humidity around the showers, which promotes the growth of microfungi. The relationship between air humidity and the presence of yeast is confirmed in numerous scientific works [2, 9, 10]. Additionally, attention should be paid to direct and frequent contact body surfaces of athletes with the shower surfaces, while the walls are rarely touched, and contact with them is limited only to hanging clothes.

In own research, *Aspergillus fumigatus*, *A. niger*, *Juxtiphoma eupyrena* and *Westerdykella minutispora*, (for which soil is the natural reservoir) were most often isolated from sports changing rooms. Numerous literature data indicate the dominance of the *Aspergillus* genus also in indoor air [23]. This genus belongs to the "indoor fungi", typical for most rooms used by humans [9]. It is also commonly found in school buildings [9, 10], kindergartens [15], offices and university buildings [9]. Many species of the *Aspergillus* genus can grow at human body temperature and reduced oxidation-reduction potential, characteristic of diseased tissues, causing difficult skin mycosis, lung aspergillosis and even brain infections [9, 28]. In addition, numerous fungi of the *Aspergillus* genus produce strong mycotoxins that can cause mycotoxicosis [9, 29].

The most isolated *Aspergillus fumigatus* is typical for indoor rooms, which is confirmed by numerous studies [9, 10]. This species is thermophilic and can grow at human body temperature [9, 10]. Some strains

produce toxic fumicin, which can enter the structure of the room bioaerosol. Moreover, *Aspergillus fumigatus* has 26 allergens within mycelium and spores, which are a threat to allergy sufferers and asthmatics [6, 9, 21, 29].

Aspergillus niger was isolated at a high frequency too. This species is often isolated from soil and food products. It occurs especially in extremely wet environments. *Aspergillus niger* may be an etiological factor of ear mycosis. It also produces strong secondary metabolites such as aflatoxins and ochratoxin A [29, 30].

The least frequently isolated species of this genus was *Aspergillus flavus*. Research [9] and [10] shows the presence of this species in school rooms. This species may contribute to the development of pulmonary aspergillosis in immunocompromised patients [19, 28, 31]. *Aspergillus flavus* produces aflatoxins with strong carcinogenic properties [10, 29].

In total, 17 isolates of the genus previously named *Phoma* were obtained during the study: *Juxtiphoma eupyrena*, *Westerdykella minutispora* and *Epicoccum sorghinum*. Fungi of these species occur mainly in the soil environment and may be potential pathogens of skin surfaces [32]. Research indicates that they are also isolated from wet rooms [4, 32]. In own research, the strains obtained were isolated only from the shower trays and handshowers, which confirms their high moisture affinity. They were also isolated from the stone, wood and other materials in old (historical) buildings [4]. However, no fungi of these species have been reported in changing rooms and sports halls so far.

In the conducted research 4 isolates of the *Cladosporium cladosporioides* were obtained. This species has strong allergenic and toxin-forming properties [26] and can cause skin and nail infections [9, 23, 25]. There is also a relationship between this species and the occurrence and development of asthma [6, 8, 25, 26]. *Cladosporium* genus belong to the most commonly isolated from indoor rooms (also school buildings) and outside air [8, 12, 23].

Two isolates of *Trichoderma viride* and *Acremonium alabamense* were obtained. *Trichoderma* genus occur in soil, wood, textiles and dump rooms. They are classified as saprotrophs but can also infect the human body. Studies indicate a relationship between this genus and the occurrence of skin and pulmonary infections, peritonitis and periarticular tissue diseases [33]. *Acremonium* genus is often found in soil and plant remains. Most representatives of this genus are saprotrophs. Special attention should be paid to *Sacroladium kiliense* (prev. name: *Acremonium kiliense*) which may be a parasite of humans and animals, causing onychomycosis, keratitis and in extreme cases brain infections [34].

For other species of mold fungi, single isolates were obtained that are not associated with buildings.

Fungi of the genus *Candida* constituted over half of the obtained yeast isolates. Species such as *C. glabrata*, *C. krusei*, and *C. parapsilosis* are human

pathogens [28, 35]. Candidiasis often affects athletes [27]. This result should be considered as expected due to the constantly increasing incidence of candidiasis in recent years. The pathogenicity of the genus *Candida* is conditioned by numerous factors, which include: the ability to create biofilm, the release of hydrolases, and the ability to adhere to various surfaces, including the body surface [28, 35-37]. The mentioned virulence factors may favor the colonization of the studied surfaces by fungi belonging to the genus *Candida*, which is the justification of the received results. Due to the ability to adhere, fungi belonging to this genus can also appear on the surface of costumes and equipment used by athletes during training. Although *Candida* species were mainly isolated from shower trays and handshowers, in comparison with the other isolated species, they constituted the dominant part of the wall surfaces mycobiota. This may be because this genus exhibits greater tolerance to conditions of limited water availability than, for example, *Rhodotorula glutinis* [9]. The species *C. glabrata* was the most common of all isolated yeast. This species may be part of the human mycobiota as it is commensal in nature, and due to its ability to form a biofilm can easily colonize surfaces similar to the tested ones [9, 10, 35-37].

Rhodotorula glutinis have also been isolated frequently. These fungi can be isolated from clinical human material [20]. Their presence in school buildings is therefore associated with human secretions and excretions, which may justify their presence in the tested facilities in changing rooms. This species dominated on the surface of showers which can be considered a correct result, due to the hydrophilic nature of the fungi belonging to the genus *Rhodotorula* [9].

The species *Saccharomyces cerevisiae* was isolated from the surface of the walls and the shower trays area. This result can be confirmed by the fact that this species is one of the extreme xerophylls that can develop with water activity: $wa > 0.75$. The occasional occurrence of these fungi can also be associated with eating food in changing rooms [9, 37].

The genus *Rhodospiridium* and the species *Saitoella complicata* have been found twice. These fungi are mainly isolated from soil [20], so the presence in changing rooms probably had an environmental origin.

The remaining yeast species were isolated individually, therefore it is difficult to demonstrate their relationship with the activity of athletes. Current scientific reports do not classify these species as characteristic of indoor spaces.

A total of 5 of the isolated species *Sacroladium kiliense*, *A. flavus*, *A. fumigatus*, *C. glabrata* and *C. krusei* were classified into the second group of biosafety (BSL-2). This means they can cause severe opportunistic infections in immunocompromised people [36, 37]. These fungi should therefore be considered potentially dangerous for the health of changing users, due to their occurrence in buildings, which is proven by own and other studies [9, 10].

Abbreviations

BRI, Building Related Illnesses; **BSL**, Biosafety level scale; **SBS**, Sick Building Syndrome; **WHO**, The World Health Organization

Conclusions

The current state of knowledge in the field of mycobiota of sports facilities is significantly limited. The results indicate the need for constant monitoring of the sanitary condition of the sport changing rooms, sports halls, as well as the equipment used by the athletes. In addition, own research justifies the need for preventive examinations of athletes in various sports in connection with the cleanliness of sports rooms. Taking into account the preliminary results obtained, it should be indicated that it will be very valuable to conduct further research in the field of mycological cleanliness of sport changing rooms. This is particularly important in the epidemiological context, the constantly growing popularity of indoor sports and an increasing number of young athletes.

Acknowledgments

„Development Program of the University of Warmia and Mazury in Olsztyn”, POWR.03.05.00-00-Z310/17, co-financed by the European Union under the European Social Fund from the Operational Program Knowledge Education Development. First author is a recipient of a scholarship from the Programme Interdisciplinary Doctoral Studies in Biology and Biotechnology (POWR.03.05.00-00-Z310/17), which is funded by the European Social Fund”.

Conflict of Interest

The authors declare no conflict of interest.

Funding

This work was supported by the Department of Microbiology and Mycology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn (12.610.009-300), and European Social Fund (POWR.03.05.00-00-Z310/17).

References

1. TEDERSOO L., BAHRAM M., PÖLME S., KÖLJALG U., YOROU N. S., WIJESUNDERA R., RUIZ L. V., VASCO-PALACIOS A. M., THU P. Q., SUIJA A., SMITH M. E., SHARP C., SALUVEER E., SAIITA A., ROSAS M., RIIT T., RATKOWSKY D., PRITSH K., PÖLDMAA K., PIEPENBRING M., PHOSRI C., PETERSON M., PARTS K., PÄRTEL K., OTSING E., NOUHRA E., NJOUONKOU R., NILSSON H., MORGADO L. N., MAYOR J., MAY T. W., MAJUAKIM L., JEAN LODGE D., LEE S. S., LARSSON K., KOHOUT P., HOSAKA K., HIIESALU I., HENKEL T. W., HAREND H., GUO L., GRESLEBIN A., GRELET G., GEML J., GATES G., DUNSTAN W., DUNK C., DRENKHAN R., DEARNALEY J., DE KESEL A., DANG T., CHEN X., BUEGGER F., BREARLEY F. Q., BONITO G., ANSLAN S., ABELL S., ABARENKOV K. Global diversity and geography of soil fungi. *Science*, **346** (6213), 1078, **2014**.
2. BOONRATTANAKIJ N., YOMCHINDA S., LIN F.J., BELLOTINDOS L.M., LU M.C. Investigation and disinfection of bacteria and fungi in sports fitness center. *Environmental Science and Pollution Research*, **28** (37), 52576, **2021**.
3. MENSAH-ATTIPOE J., REPONEN T., VEIJALAINEN A. M., RINTALA H., TÄUBEL M., RANTAKOKKO P., YING J., HYVÄRINEN A., PASANEN P. Comparison of methods for assessing temporal variation of growth of fungi on building materials. *Microbiology*, **162** (11), 1895, **2016**.
4. MA X., ZHANG B., LIU B. Analysis of fungal diversity of the rotten wooden pillars of a historic building. *Research Square*, **1**, **2020**.
5. NOVAK BABIČ M., ZUPANČIČ J., GUNDE-CIMERMAN N., ZALAR P. Yeast in Anthropogenic and Polluted Environments. In *Yeasts in Natural Ecosystems: Diversity*, Buzzini P., Lachance M.-A., Yurkov A.M. Eds., (pp. 145-169). Springer International Publishing: Switzerland, **2017**.
6. OLUWOLE O., KIRYCHUK S.P., LAWSON J.A., KARUNANAYAKE C., COCKCROFT D.W., WILLSON P.J., SENTHILSELVAN A., RENNIE D.C. Indoor mold levels and current asthma among school-aged children in Saskatchewan, Canada. *Indoor Air*, **27** (2), 311, **2017**.
7. EJDYS E., BIEDUNKIEWICZ A. Fungi of the genus *Penicillium* in school buildings. *Polish Journal of Environmental Studies*, **20** (2), 333, **2011**.
8. MENTESE S., MIRICI N.A., ELBIR T., PALAZ E., MUMCUOĞLU D.T., COTUKER O., BAKAR C., OYMAK S., OTKUN M.T. A long-term multi-parametric monitoring study: Indoor air quality (IAQ) and the sources of the pollutants, prevalence of sick building syndrome (SBS) symptoms, and respiratory health indicators. *Atmospheric Pollution Research*, **11** (12), 2270, **2020**.
9. EJDYS E. Fungi isolated in school buildings. *Acta Mycologica*, **42** (2), 245, **2007**.
10. EJDYS E., DYNOWSKA M., BIEDUNKIEWICZ A., SUCHARZEWSKA E. An overview of the species of fungi occurring in school rooms - A four-year study. *Polish Journal of Environmental Studies*, **22** (6), 1691, **2013**.
11. GHAFFARIANHOSEINI A., ALWAER H., OMRANY H., GHAFFARIANHOSEINI A., ALALOUCHE C., CLEMENTS-CROOME D., TOOKEY J. Sick building syndrome: are we doing enough? *Architectural Science Review*, **61** (3), 99, **2018**.
12. MADUREIRA J., PEREIRA C., PACIÊNCIA I., TEIXEIRA J.P., DE OLIVEIRA FERNANDES E. Identification and levels of airborne fungi in Portuguese primary schools. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, **77**, 816, **2014**.
13. RANKIN A., O'DONAVON C., MADIGAN S.M., O'SULLIVAN O., COTTER P.D. 'Microbes in sport'

- The potential role of the gut microbiota in athlete health and performance. *British Journal of Sports Medicine*, **51** (9), 698, **2017**.
14. BATYCKA-BARAN A., BARAN W., KUCZBORSKA I., SEBASTIAN-RUSIN A., BARAN E. The most common fungal infections of people practicing sports. *Mikologia Lekarska*, **16** (4), 243, **2009** [In Polish].
 15. BRĄGOSZEWSKA E., MAINKA A., PASTUSZKA J.S. Bacterial and fungal aerosols in rural nursery schools in Southern Poland. *Atmosphere*, **7** (11), 142, **2016**.
 16. KULESZA K., BIEDUNKIEWICZ A., NOWACKA K., DYNOWSKA M., URBANIAK M., STĘPIEŃ Ł. Dishwashers as an extreme environment of potentially pathogenic yeast species. *Pathogens*, **10** (4), 1, **2021**.
 17. RAPER K.B., FENNELL D.I. The Genus *Aspergillus*. The Williams & Wilkins Company: Baltimore, **1965**.
 18. RAPER K.B., THOM C., FENNEL D.I. A Manual of the *Penicillia*. The Williams & Wilkins Company: Baltimore, **1949**.
 19. DE HOOG G., GUARRO J., GENÉ J., FIGUERAS M.J. Atlas of Clinical Fungi. 2nd ed.; Centraalbureau voor Schimmelcultures/Universitat Rovira and Virgili: Reus, **2000**.
 20. KURTZMAN C.P., FELL J.W., BOEKHOUT T. The Yeasts: A Taxonomic Study. 5th ed.; Elsevier: Amsterdam, **2011**.
 21. MOSALAEI S., AMIRI H., RAFIEE A., ABBASI A., BAGHANI A.N., HOSEINI M. Assessment of fungal bioaerosols and particulate matter characteristics in indoor and outdoor air of veterinary clinics. *Journal of Environmental Health Science and Engineering*, **19** (2), 1773, **2021**.
 22. BOŽIĆ J., ILIĆ P., ILIĆ S. Indoor air quality in the hospital: The influence of heating, ventilating and conditioning systems. *Brazilian Archives of Biology and Technology*, **62** (e19180295), 1, **2019**.
 23. OGÓREK R., PŁASKOWSKA E. Mycological analysis of air in selected public spaces. Preliminary report. *Mikologia Lekarska*, **18** (1), 24, **2011** [In Polish].
 24. WILKINS D., LEUNG M.H., LEE P.K. Indoor air bacterial communities in Hong Kong households assemble independently of occupant skin microbiomes. *Environmental microbiology*, **18** (6), 1754, **2016**.
 25. NATH R., BARUA S., BARMAN J., SWARGIARY P., BORGHAIN M., SAIKIA L. Subcutaneous Mycosis Due to *Cladosporium cladosporioides* and *Bipolaris cynodontis* from Assam, North-East India and Review of Published Literature. *Mycopathologia*, **180** (5-6), 379, **2015**.
 26. SEGURA-MEDINA P., VARGAS M.H., AGUILAR-ROMERO J.M., ARREOLA-RAMÍREZ J.L., MIGUEL-REYES J.L., SALAS-HERNÁNDEZ J. Mold burden in house dust and its relationship with asthma control. *Respiratory Medicine*, **150**, 74, **2019**.
 27. LIGUORI F., BOCCIA G., LIMONGELLI F. L'igiene nei locali adibiti a spogliatoio. *Medicina dello Sport*, **55** (3), 195, **2002** [In Italian].
 28. LASS-FLÖRL C., SAMARDZIC E., KNOLL M. Serology anno 2021 – fungal infections: from invasive to chronic. *Clinical Microbiology and Infection*, **27** (9), 1230, **2021**.
 29. RÁDULY Z., SZABÓ L., MADAR A., PÓCSI I., CSERNOCH L. Toxicological and Medical Aspects of Aspergillus-Derived Mycotoxins Entering the Feed and Food Chain. *Frontiers in Microbiology*, **10** (2908), 1, **2020**.
 30. MIRHENDI H., ZAREI F., MOTAMEDI M., NOURIPOUR-SISAKHT S. *Aspergillus tubingensis* and *Aspergillus niger* as the dominant black *Aspergillus*, use of simple PCR-RFLP for preliminary differentiation. *Journal de Mycologie Medicale*, **26** (1), 9, **2016**.
 31. LAI C.C., YU W.L. COVID-19 associated with pulmonary aspergillosis: A literature review. *Journal of Microbiology, Immunology and Infection*, **54** (1), 46, **2021**.
 32. BENNETT A., PONDER M. M., GARCIA-DIAZ J. *Phoma* infections: Classification, potential food sources, and their clinical impact. *Microorganisms*, **6** (3), 2, **2018**.
 33. HATVANI L., HOMA M., CHENTHAMARA K., CAI F., KOCSUBÉ S., ATANASOVA L., MLINARIC-MISSONI E., MANIKANDAN P., REVATHI R., DÓCZI I., BOGÁTS G., NARENDHAN V., BÜCHNER R., VAGVÖLGYI C., DRUZHININA I. S., KREDICS L. Agricultural systems as potential sources of emerging human mycoses caused by *Trichoderma*: A successful, common phylotype of *Trichoderma longibrachiatum* in the frontline. *FEMS Microbiology Letters*, **366** (21), 1, **2019**.
 34. PÉREZ-CANTERO A., GUARRO J. *Sarocladium* and *Acremonium* infections: New faces of an old opportunistic fungus. *Mycoses*, **63** (11), 1203, **2020**.
 35. JAMIU A.T., ALBERTYN J., SEBOLAI O.M., POHL C.H. Update on *Candida krusei*, a potential multidrug-resistant pathogen. *Medical Mycology*, **59** (1), 14, **2021**.
 36. BIEDUNKIEWICZ A., SUCHARZEWSKA E., KULESZA K., NOWACKA K., KUBIAK D. Phyllosphere of Submerged Plants in Bathing Lakes as a Reservoir of Fungi – Potential Human Pathogens. *Microbial Ecology*, **79** (3), 552, **2020**.
 37. BIEDUNKIEWICZ A., GÓRALSKA K. Microfungi Potentially Pathogenic for Humans Reported in Surface Waters Utilized for Recreation. *Clean - Soil, Air, Water*, **44** (6), 599, **2016**.