

Original papers

Potentially pathogenic fungi of the *Candida* genus isolated from the Łyna River – a 20-year study

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ABSTRACT. This paper concerns part of a monitoring study and contains the results of 20-year observations on the species diversity and dynamics of the count *Candida* genus fungi which inhabit surface waters. The research material consisted of water samples collected from the Łyna River, the main river of the Warmińsko-Mazurskie Voivodeship, within the administrative boundaries of the city of Olsztyn. The samples were subjected to routine diagnostic testing applied in hydromycological studies. The obtained fungi isolates were determined, documented photographically and deposited in a strain collection of the Department of Microbiology and Mycology at the University of Warmia and Mazury in Olsztyn. A total of 23s of the *Candida* genus were isolated, including six species in the teleomorphic stage. It was noted that over the past decade, the taxonomic diversity of the fungi had increased significantly (from 2 to 23 species). Potential anthropopathogens had also emerged. Over the twenty-year period of observations, the species that were always isolated were *Candida albicans* and *C. guilliermondii*, characterised by high pathogenicity and bioindicative properties, which are important in the epidemiology of fungal infections.

Key words: *Candida*, river, pathogenicity, bio-indicator

Introduction

The author's own study clearly indicated a large diversity of microfungi in waters and their increasing count proportional to the rate and intensity of the general anthropogenic changes occurring in aquatic ecosystems [1–5]. This primarily concerns fungi which prefer heavily polluted waters rich in organic substances of various origin [1,6]. Such fungi include yeasts and yeast-like fungi. Each increase in human pressure entails problems associated with the eutrophication of the aquatic environment. Fungi are usually found in heavily degraded environments. Their ability to adapt to qualitatively changing various types of environment (air, soil, and water) allow them to increasingly occupy new habitats [2]. Most microfungi species isolated from waters to date are potential etiological factors of surface and organ fungal infections [7,8] and their abundant proliferation in the waters under study may pose a serious epidemiological hazard [9–11]. The latter

aspect is of particular importance to the use of the Łyna River for recreational and tourist purposes as a kayaking route. Therefore, the aim of the study was to assess the dynamics of changes in the species composition of fungi of the *Candida* genus over a twenty-year period of observations, with a focus on emerging potential anthropopathogens, particularly within the administrative boundaries of the city of Olsztyn.

Materials and Methods

The Łyna River runs through Olsztyn from south to north, which divides the city into eastern and western parts. Within the city, the river runs through a diverse area. On the outskirts of the city there are suburban developments with numerous woodlots. A little further, it turns into dense development in the Old Town and, beyond the city boundaries, the river flows through a large area of the City Forest. On the river section under study, within the administrative boundaries of the city of Olsztyn, initially four, and



ultimately ten, stations were established. The first of them were located at the point where the river enters the city and the subsequent stations were at even intervals: across the city centre, and up to the City Forest (Fig. 1).

Fig. 1. Map of the city of Olsztyn, including water sampling stations (I: Kalinowskiego Street, II: Tuwima St., III: Obrońców Tobruku St., IV: Niepodległości St., V: John's Bridge St., VI: Knosały-Prosta St., VII: Prosta/ John's Inn St., VIII: Zamkowa St. before stacking, IX: Zamkowa St. after stacking, X: Artyleryjska St.)

Water samples were collected in the years 1989–1993, 1994–1996 and 2009–2010, throughout the year at monthly intervals, except 2010 when, due to low temperatures, the river was covered with ice.

From each station, from a depth of 30 cm, two water samples (each of 1000 ml) were taken: one for quantitative testing and the other for qualitative testing.

The research material was yeast-like fungi obtained by means of membrane filtration following incubation on Sabouraud's medium with chloramphenicol (0.1%) for 48–72 h at a temperature of 37°C. The grown colonies were counted and those which were macroscopically different were transferred to clean Sabouraud's

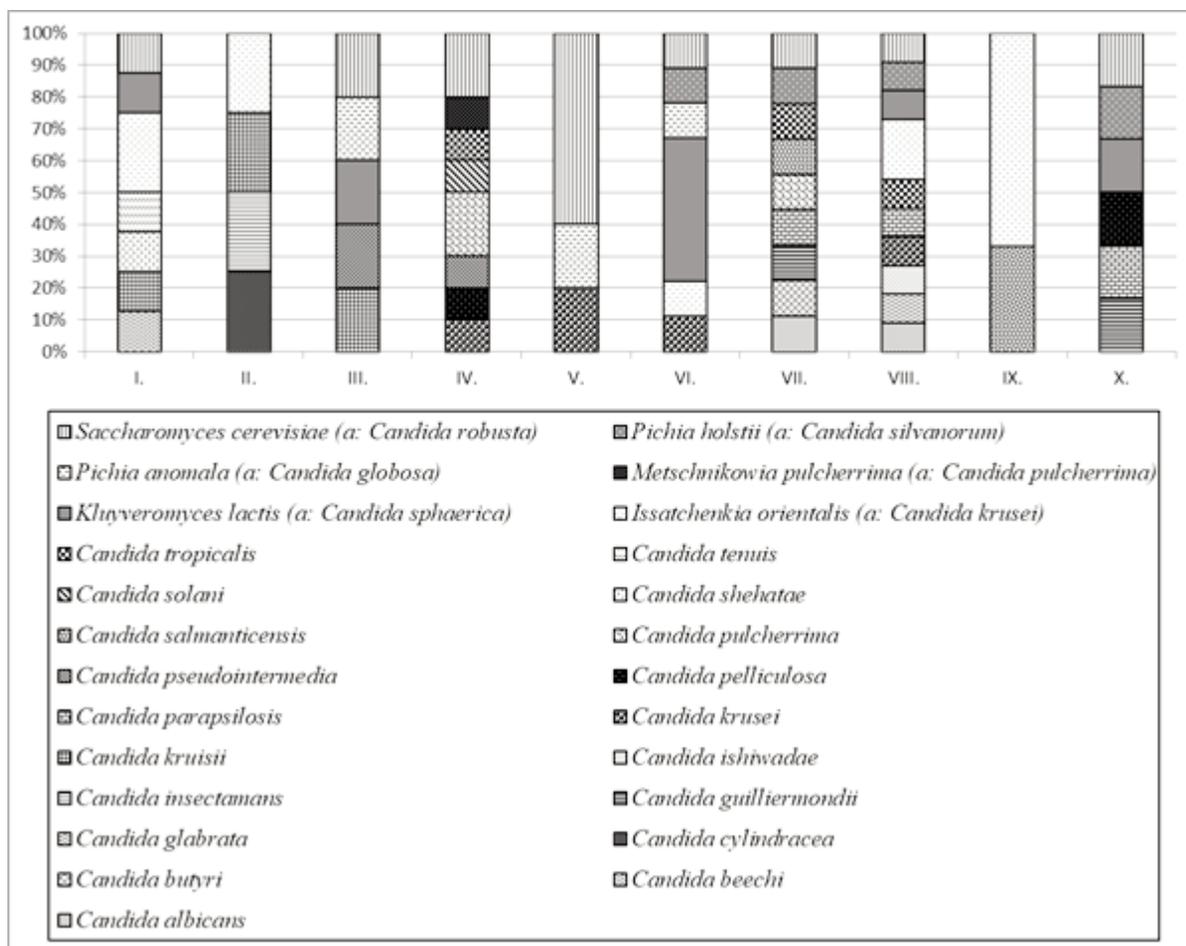


Fig. 2. Species diversity of fungi of the *Candida* genus at particular research stations

Table 1. List of species isolated over the period of 20-year study on the Łyna River, including a summary of literature data

No.	Species	BSL	Selected literature data
1.	<i>Candida albicans</i>	2	Water, lakes [1,9], sewage [7], soil, leaves, cutaneous infection, spittle, interdigital epidermophytosis, laryngitis, nail, vaginitis, urine, catheters [4]
2.	<i>Candida beechi</i>	n	Cider [4]
3.	<i>Candida butyri</i>	n	Lakes [1], abscess at site of penicillin injection, butter [4]
4.	<i>Candida cylindracea</i>	n	Soil [4]
5.	<i>Candida glabrata</i>	2	Urine, fetal fluid, vaginitis, spittle, water, fermenting passion-fruit juice [4]
6.	<i>Candida guilliermondii</i>	1	Water, lakes [9], sewage [7], icicles [8], spittle, alcohol strain, atmosphere, sputum, blood [4]
7.	<i>Candida insectamans</i>	n	Frass of beetle larvae [4]
8.	<i>Candida ishiwadae</i>	n	Soil [4]
9.	<i>Candida kruisii</i>	n	Lakes [1], on <i>Boletus purpureus</i> [4]
10.	<i>Candida parapsilosis</i>	1	Lakes [1], sewage [7], respiratory system, nail, skin, bladder, toe, cucumbers [4]
11.	<i>Candida pelliculosa</i>	1	Lakes [9], sewage [7]
12.	<i>Candida pseudointermedia</i>	n	Fish [4]
13.	<i>Candida salmanticensis</i>	n	Fish [4]
14.	<i>Candida shehatae</i>	n	Soil, wood, insects [4]
15.	<i>Candida solani</i>	n	Lakes [1], sewage [7], icicles [8], potato-starch mill [4]
16.	<i>Candida tenuis</i>	n	Coniferous trees, insects [4]
17.	<i>Candida tropicalis</i>	2	Water, lakes [9], sewage [7], icicles [8], human, interdigitale infection, infected nail, child's feces, blood, fodder yeast, kefir, soil, water, fermentation vats [4]
18.	<i>Issatchenkia orientalis</i> (a: <i>C. krusei</i>)	2	Lakes [9], sewage [7], icicles [8], respiratory system, feces, urine, vaginitis, sea water, fruit flies, beer, tea [4]
19.	<i>Kluyveromyces lactis</i> (a: <i>Candida spherica</i>)	1	Lakes [1], sewage [7], cheese [4]
20.	<i>Metschnikowia pulcherrima</i> (a: <i>Candida pulcherrima</i>)	1	Lakes [1], sewage [7], flowers, fruits, exudates of trees [4]
21.	<i>Pichia anomala</i> (a: <i>Candida globosa</i>)	1	Lakes [9], rivers, sewage, soil, grain, warm-blooded animals, fermenting mushrooms, fruit[4]
22.	<i>Pichia holstii</i> (a: <i>Candida silvanorum</i>)	n	Lichens, soil, lakes, streams, cadaver [4]
23.	<i>Saccharomyces cerevisiae</i> (a: <i>Candida robusta</i>)	1	Lakes [1,9], sewage [7], icicles [8], respiratory system, vineyard, soil, cavern soil, ginger beer, brewery, beer, wine, distillery, sugar cane, sherry, grape must [4]

Explanations: BSL – BioSafety Level; n – BSL not yet determined

medium and re-incubated under the same conditions. The procedure of species identification included an analysis of both macroscopic features on Sabouraud's medium and microscopic features in microcultures on Nickerson's agar, as well as a check on selected biochemical features (carbohydrate zymograms and auxanograms) in accordance with the developed diagnostic process adopted in mycological laboratories [2,12]. In order to confirm the species to which the fungi belonged, commercial biochemical tests API manufactured by

bio-Mérieux (API 20C and API 20C AUX) were used. Fungi were determined using specialised keys and studies [13–16].

Results

Over a period of twenty years, a total of 23 fungi species of the *Candida* genus were noted (Table 1). In the initial period of the study, at the stations located within the city, only *C. albicans* was found and, at the other ones, *C. guilliermondii* was found.

Table 2. Distribution of the seasonal variability of the isolation of species of the *Candida* genus

No.	Species	Spring	Summer	Autumn	Winter
1.	<i>Candida albicans</i>		•		•
2.	<i>Candida beechi</i>	•	•		
3.	<i>Candida butyri</i>	•			
4.	<i>Candida cylindracea</i>			•	
5.	<i>Candida glabrata</i>		•		•
6.	<i>Candida guilliermondii</i>	•	•		•
7.	<i>Candida insectamans</i>			•	
8.	<i>Candida ishiwadae</i>	•			
9.	<i>Candida kruisii</i>		•	•	
10.	<i>Candida parapsilosis</i>		•		•
11.	<i>Candida pelliculosa</i>	•	•	•	
12.	<i>Candida pseudointermedia</i>	•		•	
13.	<i>Candida salmanticensis</i>			•	•
14.	<i>Candida shehatae</i>				•
15.	<i>Candida solani</i>	•	•		
16.	<i>Candida tenuis</i>				•
17.	<i>Candida tropicalis</i>		•	•	•
18.	<i>Issatchenkia orientalis</i> (a.: <i>C. krusei</i>)	•	•	•	•
19.	<i>Kluyveromyces lactis</i> (a.: <i>Candida spherica</i>)	•	•	•	•
20.	<i>Metschnikowia pulcherrima</i> (a.: <i>Candida pulcherrima</i>)		•	•	
21.	<i>Pichia anomala</i> (a.: <i>Candida globosa</i>)			•	•
22.	<i>Pichia holstii</i> (a.: <i>Candida silvanorum</i>)			•	
23.	<i>Saccharomyces cerevisiae</i> (a.: <i>Candida robusta</i>)	•	•	•	•

Explanations: **Bold** – species common to all research seasons

In subsequent years of the study, the count of the species significantly increases (a total of 23). Fungi in the anamorphic stage were dominant. In six species, teleomorph stages were also observed: *Issatchenkia orientalis* (anamorph: *Candida krusei*), *Kluyveromyces lactis* (a.: *C. sphaerica*), *Metschnikowia pulcherrima* (a.: *C. pulcherrima*), *Pichia anomala* (a.: *C. globosa*), *Pichia holstii* (a.: *C. silvicola*) and *Saccharomyces cerevisiae* (a.: *C. robusta*) (Table 1). The highest species diversity was noted in the city centre (stations IV–VI) and in water samples collected from the river approaching the forest area (Fig. 2). The number of species decreased dramatically at an aeration site (damming at station IX) and increased again at the boundary of the City Forest (Fig. 2).

Fungi were isolated during all seasons of the year. In every month of the study, yeast-like fungi belonging to three species, namely *Issatchenkia*

orientalis (a. *Candida krusei*), *Kluyveromyces lactis* (a. *C. spherica*) and *Saccharomyces cerevisiae* (a.: *C. robusta*) were noted (Table 2).

During the entire period under analysis, comparable counts of the fungi per 1 ml water sample were found. Overall, it ranged from 300 CFU/ml in the winter to 30,000 CFU/ml in the spring, except station III located in proximity to allotments situated next to the city centre (Fig. 3).

In summary, approximately 20% species of all isolated ones belong to Biosafety Level two (BSL-2) i.e. are potential pathogens. Of this group, the most often isolated species included: *Candida albicans*, *C. glabrata*, *C. krusei* (teleomorph: *Issatchenkia orientalis*) and *C. tropicalis* (Fig. 4).

Discussion

Rivers are a specific type of water body whose

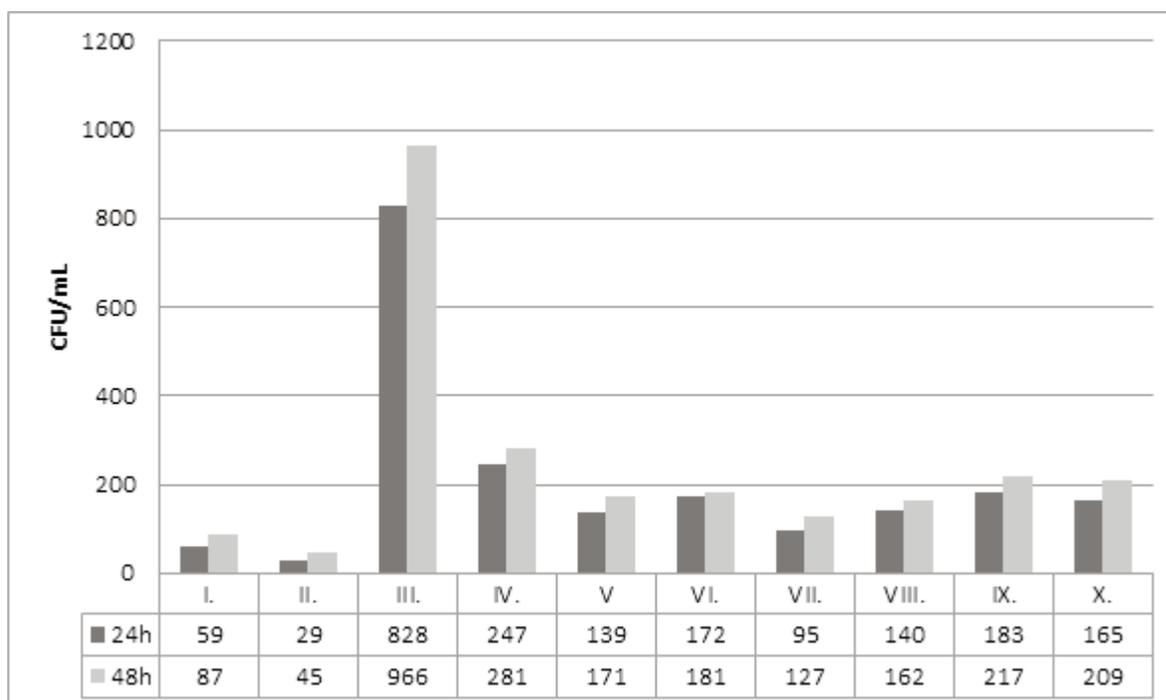


Fig. 3. Count of colonies on two initial days of incubation

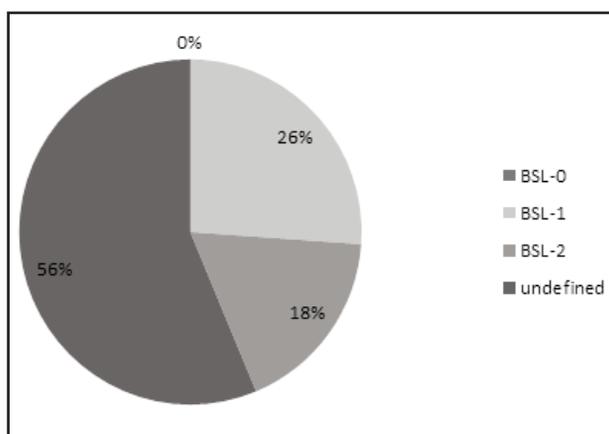


Fig. 4. Percentage of fungi of the *Candida* genus, including BSL classification

quality is determined by incoming pollutants. This is particularly evident in urban areas, where municipal sewage, rainwater, groundwater and processed wastewater are often discharged to rivers. Some authors have noted that the enrichment of water with organic matter results in an increase in the number and changes in the species composition of microfungi [17–19], which indicates their high ability to adapt. This is confirmed by the fact that most fungi were noted in proximity to the allotments from where organic pollutants may penetrate into the river. Nearly half of the species noted in the river were also found in waters from the wastewater treatment plant area and from the

Ślupianka River, into which they had been discharged [1]. Fungi of the *Candida* genus are closely associated with polluted waters [20]. Certain isolates of this genus isolated from the river were found in the teleomorphic stages: *Issatchenkia orientalis*, *Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia anomala*, *Pichia holstii* and *Saccharomyces cerevisiae*. This indicates the flexibility of the reproductive systems of this group of fungi in waters.

A higher species diversity was observed in the autumn, while a lower diversity was noted in the winter and spring; in turn, the situation as regards the count of fungi was opposite, since with the higher count in the winter, significantly fewer species were noted. A higher diversity of microfungi in the autumn period may be determined by the accumulation of organic matter or anthropogenic pollutants following the summer season, and a higher count of the fungi at lower temperatures is associated with better solubility of oxygen in cool water [21].

Most species noted in the Łyna River had been previously isolated from other types of waters, such as marine waters, lakes, wastewater, mains water or icicles [2,5,18,22]. The species isolated from the Łyna River for the first time included *C. beechi*, *C. cylindracea*, *C. insectamans*, *C. ishiwadae*, *C. pseudointermedia*, *C. salmanticensis*, *C. shehatae* and *C. tenuis*. Attention should be paid to *Candida*

kruisii, a species which had not been noted from river waters in hydromycological literature by 2009 but was noted in Olsztyn in Lake Tyrsko [2].

The most often described species *Candida albicans*, often noted in municipal sewage as well, is closely associated with an urban environment [1,6,23,24]. It was found as early as during the first observations in the Łyna River waters [20]. In this study, the species was noted at stations in the city centre, i.e. at sites subjected to constant human pressure. This is one of two species, including *C. guilliermondii*, which have been continuously isolated over the twenty-year period of the study. However, the frequency of noting *C. albicans* in aquatic ecosystems has been decreasing lately and the species is being replaced by more abundant species, e.g. *C. krusei* or *C. tropicalis* [2,5]. Similar changes have been observed in the dynamics of changes in species composition of the fungi of organ ontocenoses [7,8,25].

In this study, higher species diversity, including the presence of potential pathogens, was noted at a station in proximity to a hospital and at a station beyond the conventional boundaries of the “city centre”, where the Central Park – a place for active leisure of city dwellers – is situated. Yeast-like fungi are opportunistic forms [6,20], frequently found in healthy individuals [25]; however, their presence is usually associated with reduced general biological resistance of the body [8]. The multi-annual mycological monitoring surface waters of north-eastern Poland demonstrated the constant presence of microfungi [1,2,5,22]. Their emergence may indicate poor sanitary condition of waters and the isolation of species classified as pathogenic from the environments under study on a massive scale poses a real epidemiological hazard, particularly when they are in contact with the skin or mucous membranes of humans [2,20,26].

Flowing river waters should be regarded as being capable of passively spreading fungi. While flowing across cities, they carry a pollutant load of anthropogenic origin, and become dangerous in case of a direct contact of humans or animals with the contaminated water. Rivers are often a living place for wild birds. On the Łyna River, flocks of mallards (*Anas platyrhynchos*) are often observed, which may be one of the links in the epidemiological chain of fungal infections originating in waters [27]. This is indicated by the isolation of the same fungi species from the Łyna River as well as from the bill and cloaca ontocenoses of the mallard (*C. albicans*,

C. guilliermondii, *C. krusei*, *C. parapsilosis* and *C. tropicalis*).

The fungi species emerging in waters can be regarded, on the one hand, as bioindicators of the ecological conditions of waters, but also, on the other hand, as indicators of anthropogenic pollutants [10]. This is why constant monitoring aquatic environments should be regular and not occasional.

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