

Yeasts and Yeast-Like Fungi as an Element of Purity Assessment of Surface Waters

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Abstract

The aim of our study was to determine species biodiversity of yeasts and yeast-like fungi in the stratum of surface water of Lake Tyrsko, as well as to evaluate the condition of waters of that lake based on the isolated species of fungi.

The study was carried out from June 2007 to June 2008. The isolation and determination of the number of fungal colonies were conducted with the use of the membrane filter method. Fungal diagnostics were conducted based on the morphological and biochemical characteristics of the fungi. These characteristics were additionally used for taxonomic identification.

In the course of the study we isolated 56 species of yeasts and yeast-like fungi belonging to 26 genera, with the predominating genus being *Kluyveromyces* (13 species), followed by less frequent genera: *Candida* (8 species), and *Debaryomyces* (7 species). The highest number of isolates were obtained in the spring (51), fewer in the summer (36), and the lowest in autumn (15). Species indicative of the self-purifying process of the water examined were frequently isolated (*Debaryomyces hansenii*, *Pichia membranifaciens*). Results obtained in the study confirm suggestions of other authors that yeasts and yeast-like fungi may be useful as indicator organisms showing the extent and character of water contamination and as bioindicators of sanitary and hygienic evaluation of water.

Keywords: yeasts and yeast-like fungi, bioindicators, pathogenicity

Introduction

The presence of fungi as well as their capability to adapt to various types of habitats and to relocate make the air, soil, and inland waters a potential source of mycotic infections [1-3]. It refers most of all to fungi linked with the ontosphere of man [3]. Diaspores of fungi penetrate the human body while bathing in waters of lakes, rivers, and swimming pools. They may also reach the body through the digestive tract with, among others, drinking water and water used for the preparation of meals, as well as through inhalation and sexual transmission. A gateway for infection

is often natural orifices: oral cavity, nasal cavity, opening of the urethra, vagina, and rectum [4].

Fungi play a significant role in the functioning of most aquatic ecosystems. As early as in the 1990s, due to increasing contamination of water, a need was observed for the isolation and determination of yeast-like fungi that are capable of decomposing and accumulating toxic compounds of organic and inorganic origin [5], as well as those characterized by high pathogenicity potential [2, 3].

The aim of this study was, therefore, to determine taxonomic diversity of (yeast-like fungi) yeasts and yeast-like fungi occurring in the stratum of surface waters of Lake Tyrsko, with consideration given to fungi potentially pathogenic to man and animals. An attempt was also undertak-

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en to evaluate the condition of waters of that lake based on the species isolated.

Materials and Methods

Area of Studies

The object of our study was Lake Tyrsko (area 19.75 ha, max. depth 38 m), located on the northwestern edge of the city of Olsztyn (longitude 20°25.5'E, latitude 53°48.3'N). The lake is located in the catchment of the Łyna River and is a hydrologically-closed reservoir [6]. It is classified as a lobelia lake and in 1957-87 it was established as a floristic reserve due to the presence of (recently rare in the Masuria Lake District) *Isoetes lacustris* L. – lake quillwort [6], which is contemporarily extinct [7]. Analyses have already been carried out in Lake Tyrsko for macrophytes as bioindicators of environmental changes [7] and for taxonomic diversity of zoobenthos [8, 9].

In the 1930s the lake was classified as oligotrophic. Currently, however, it has been reported to exhibit traits typical of a lake being at the transition stage from β -mesotrophic to eutrophic. The greatest threat to the condition of the lake is posed by excessive recreational exploitation [6].

Water for mycological analyses was sampled from 10 stations:

- Stations I, II – located in the vicinity of a hotel and a farmstead. In the summer, people have been observed sunbathing on the lakeshore in the proximity of the stations.
- Stations III, IV – neighboring with allotment gardens.
- Stations V, VI – the part of the lake with a sandy beach used most frequently for recreational purposes, neighboring with a mixed forest, and exploited as an illegal bathing beach.
- Station VII – located from the side of railways, from which it is separated by a meadow. The site is visited by people, which is indicated by burnt-out bonfires and left-over food products.
- Station VIII – neighboring with a pasture meadow for horses, with access to water provided for the animals.
- Stations IX, X – located near an unsurfaced road, from which it is separated by pine greenwoods. These are common fishing sites.

Yeast Isolation and Identification

The study was carried out from June 2007 to June 2008 in monthly intervals, on the ten stations selected.

The experimental materials were yeasts and yeast-like fungi obtained from the littoral zone of the lake. Water samples (250 ml) were collected into sterilized bottles by means of an extension arm, from a depth of 30 cm, 4.5 m away from the shore.

The isolation of fungi was conducted with the method of membrane filters used in diagnostic mycological laboratories [1]. Species identification was performed based on macroscopic (color, consistency, shape, character of colony

edge, odor), microscopic (shape, size and arrangement of blastospores, presence of pseudomycelium and chlamydospores), and biochemical characteristics [10, 11].

The fungi were determined based on keys by: Lodder and Kreger-van Rij [12] and Kurtzman and Fell [13]. Photographic documentation was made and the strains were catalogued.

Results

In the study, 70 water samples were collected from the surface stratum, and 102 isolates of yeasts and yeast-like fungi were obtained. The highest number of isolates was obtained in the spring (51), fewer in the summer (36), and the lowest in the autumn (15). Out of the isolates, 56 species of fungi were identified that belonged to 26 genera. Furthermore, yeasts and yeast-like fungi belonging to 6 genera (*Candida*, *Debaryomyces*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, and *Rhodospodium*), were isolated in all experimental months. The genus noted both in the spring and summer season was *Saccharomyces*, whereas that detected in the spring and autumn season was *Saccharomycopsis*. The greatest genus diversity of the fungi was recorded in the summer, whereas a lesser one in the spring time and in the autumn (Fig. 1).

The greatest taxonomic diversity of the fungi isolated from the lake was noted at stations III-IV and stations IX-X (21 species each in all analytical periods) as well as in water samples collected from stations V-VI (20 species).

The genus *Kluyveromyces* was found to predominate (13 species), and was followed by the genera: *Candida* (8) and *Debaryomyces* (7). The highest number of species was identified in the spring (36) and summer (32), whereas their lower number was detected in the autumn (14). Among the fungi of the genus *Candida*, the greatest taxonomic diversi-

SPRING		SUMMER	
<i>Kazachstania</i> <i>Kloeckera</i> <i>Lodderomyces</i> <i>Schizosaccharomyces</i>	<i>Saccharomyces</i> <i>Wickerhamomyces</i> <i>Meyerozyma</i>	<i>Brettanomyces</i> <i>Citeromyces</i> <i>Dipodascus</i> <i>Hanseniaspora</i> <i>Issatchenkia</i> <i>Nakazawae</i> <i>Rhodotorula</i> <i>Schefferomyces</i> <i>Zygosaccharomyces</i>	
<i>Saccharomycopsis</i>	<i>Candida</i> <i>Debaryomyces</i> <i>Kluyveromyces</i> <i>Metschnikowia</i> <i>Pichia</i> <i>Rhodospodium</i>		
<i>Lipomyces</i> <i>Oosporidium</i> <i>Yamadazyma</i>		AUTUMN	

Fig. 1. Genus diversity of yeast-like fungi in particular seasons of the year.

Table 1. Continued.

Species	Station/Season																		Remarks*
	I-II			III-IV			V-VI			VII			VIII			IX-X			
	Sp	Su	A	Sp	Su	A	Sp	Su	A	Sp	Su	A	Sp	Su	A	Sp	Su	A	
<i>Pichia jadinii</i> (a)		+																	path
<i>Pichia membranifaciens</i>	+	+																	path
<i>Rhodosporidium lusitaniae</i>				+															sap
<i>Rhodosporidium sphaerocarpum</i>												+							sap
<i>Rhodotorula glutinis</i> var. <i>glutinis</i>					+														path, ind, pur
<i>Saccharomyces cerevisiae</i>		+																+	path, sap
<i>Saccharomyces transvaalensis</i>		+																	sap
<i>Saccharomycopsis capsularis</i>													+				+		path, sap
<i>Saccharomycopsis fibuligera</i>											+								sap
<i>Scheffersomyces segobiensis</i>										+									sap
<i>Schizosaccharomyces octosporus</i>									+										sap
<i>Wickerhamomyces bisporus</i>		+																+	path, sap
<i>Wickerhamomyces bovis</i>	+																		path
<i>Yamadazyma nakazawae</i>						+													sap
<i>Zygosaccharomyces rouxii</i>													+						sap

Sp, Su, A – experimental seasons: spring, summer, autumn

path, ind, sap, pur – pathogenic, indicatory, saprotrophic, purifying

+ – occurrence of a species in a particular experimental season on a particular station

2 – twofold occurrence of a species in a particular experimental season on a particular station

* according to Kurtzmann, Fell 2000

ty was noted at the first group of stations (I-II, 5 species). That genus was not identified at stations VII and VIII. In turn, over the entire experimental period, *Candida albicans* was isolated once at station V (Table 1). In analyzing the results obtained in terms of the most frequently isolated species, the prevailing species turned out to be *Debaryomyces hansenii* – isolated 8 times (6.3% of isolates), followed by the species isolated 6 times: *Candida solani* (4.8%), *Meyerozyma guilliermondii* (4.8%), *Kluyveromyces thermotolerans* (4.8%), and *Metschnikowia pulcherrima* (4.8%).

In the case of two species, teleo- and anamorphous stages were isolated simultaneously (*Pichia jadinii* – anamorphous stage: *Candida utilis*, *Kazachstania exigua* – anamorphous stage: *Candida holmii*).

Worthy of notice is the first-ever isolation of two species of fungi that have not been noted so far in the aquatic environment: *Candida kruisii* and *Dipodascus armillari-ae*.

Six species of fungi isolated in the study have been listed in the classification of biosafety level (BSL) of fungi potentially pathogenic to man and animals: *Candida parapsilosis*, *Candida pulcherrima*, *Candida utilis*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* (belonging to BSL-1 category), and 1 species – *Candida albicans* – belonging to BSL-2 category. The other fungi have been classified as non-hazardous saprotrophs.

The highest counts of the yeasts and yeast-like fungi were recorded in the spring (ca. 6,690 cfu/dm³ in spring 2007 and 2008), and the lowest in the autumn (ca. 410 cfu/dm³ of water). In the first experimental season, in the spring the growth of fungi was often uncountable (Table 2).

Discussion

Fungi constitute a significant link in the circuit of matter and energy in various types of aquifers. They utilize organic substances dissolved in water that are synthesized by phytoplankton. In the trophic chain, they serve as feed to protozoa (*Protozoa*) and turbellarians (*Turbellaria*). They additionally play the role of suppliers of CO₂ to phytoplankton, which is of great importance during blooming of the algae when they cannot utilize the atmospheric carbon dioxide [14]. Fungi are microconsumers, constituting – together with other microorganisms – a group of destruent taking active part in the mineralization of dead organic matter of various origins [3, 15] through the secretion of numerous hydrolytic enzymes degrading polysaccharides, proteins, lipids, and carbohydrates to the environment [4].

In 1971, at the first International Mycological Congress in Exeter, UK, attention was paid to the applicability of fungi as biological indicators of contaminated waters [16]. The yeast-like fungi known to facilitate self-purification process-

Table 2. Mean count of fungi cells per 1 dm³ of water in particular seasons of the year.

Station	Season of the year			
	spring [cfu/dm ³]		summer [cfu/dm ³]	autumn [cfu/dm ³]
	2007	2008		
I	2,200	300	400	200
II	1,200+C	-	300	100
III	2,800+C	600	2,600	100
IV	1,200+C	19,200	900	100+C
V	5,200+C	1,000	1,600	500
VI	200+C	25,700	6,700	300
VII	800+C	200	4,000	-
VIII	1,000+C	700	1,000	1,600
IX	600+C	500	1,800	200
X	3,200+C	300	700	1,000
Mean	1,840+C	4,850	2,000	410+C

cfu – colony forming unit

C – confluent growth

es, owing to their enzymatic activity, are capable of metabolizing a number of compounds toxic in character, including: lactic acid, acetic acid, ethanol, glycerol, nitrates, and phenols. Products of their degradation constitute a source (often the only one) of carbon to fungi [17]. Currently, fungi of the phylum *Ascomycota* have been shown to be more susceptible to heavy metals than fungi of the phylum *Basidiomycota* [18]. They may accumulate them around cell walls and in the granular elements of a cell. Investigations have shown that *Rhodotorula glutinis* is capable of accumulating aluminum [19] and *Debaryomyces hansenii* cesium, while *Candida albicans*, *Saccharomyces cerevisiae*, and *Rhodotorula rubra* accumulate silver [20].

A satisfactory effect achieved in the reported study on Lake Tyrsko waters was the isolation of species referred to as “purifying”, i.e. *Debaryomyces hansenii*, *Rhodotorula glutinis*, and *Pichia membranifaciens* (constituting nearly 11% of all isolates). It should additionally be emphasized that the predominating species was *Debaryomyces hansenii*. Its presence was detected on as many as seven stations, in all analytical seasons. This is likely to indicate the self-purifying processes – facilitated by yeast-like fungi – proceeding in waters of that lake. Literature data report that *Debaryomyces hansenii* has been isolated from dairy produce and cured meat products, but also from wounds and from persons with skin lesions [12]. The presence of that fungus points explicitly to the utilization of the lake by people and to the enrichment of its waters with organic compounds originating from food products.

Investigations carried out in the 1960s and 1970s that addressed the occurrence of fungi in aquatic ecosystems referred mainly to a general concept of mycological monitoring of the environment [16, 21-24]. This was fundamen-

tal researches that, by indicating the count and taxonomic diversity of mycobiota, contributed to establishing global standards of water purity, considering fungi as bioindicators (USA, Canada) [25]. The fundamental studies were continued until the 1990s [2, 3]. Nowadays, the scope of mycological research has changed, with molecular analyses becoming the priority, and interdisciplinary, monitoring, fundamental studies becoming of secondary importance. This is, however, not necessarily the right direction, for without fundamental research molecular analyses would not reflect the full picture of the state of an aquatic ecosystem and resultant risks posed to people.

In our study, *Rhodotorula glutinis* was isolated once from the station adjacent to allotment gardens. Literature data indicate that this fungus was detected at a depth of 130 m in freshwater Lake Ontario [22] and in deposits originating from Lake Michigan [23]. That species has been reported to be capable of utilizing nitrates as a source of nitrogen [24]. Analyses conducted with waters of Lake Ontario demonstrated that the increasing number of yeast-like fungi was accompanied by a decreasing total count of Coli-group bacteria [22]. Grabińska-Loniewska et al. [26] paid attention to the possibility of applying *Rhodotorula glutinis* for wastewater treatment, owing to its capability to decompose methyl phenols detrimental to environmental and human health. Methyl phenols are aromatic hydrocarbons that penetrate water bodies with sewage discharged from the pharmaceutical industry, from the production of paints, as well as distillation of petroleum and gas pitch. They have additionally been demonstrated to exhibit carcinogenic activity [26]. Furthermore, Dynowska [2, 3] emphasized the possibility of applying yeasts and yeast-like fungi, including among others *Rhodotorula glutinis* and *Candida albicans*, as indicators of water purity. They are characterized by preference to fresh, untreated municipal wastes. Many species of the genus *Candida* has been proposed for the detection of fecal contamination of water as well as for the evaluation of purity of drinking water [2], which may turn out to be extremely useful in quality monitoring of waters. Techniques applied in molecular biology (QPCR) enable fast determination of the count of yeast-like fungi in different types of water samples. In recent years, a number of results have been published that confirm suggestions of other authors [2, 3], that the yeast-like fungi present in waters utilized by civilians for recreational purposes and in drinking water may pose a threat to human health [27, 28].

It should be emphasized that the observed increase in mycotic infections is evoked mainly by the yeast-like fungi of the genus *Candida* [29, 30]. In the reported experiment, 4 of 8 species of that genus were isolated from water samples, which constitute 7.35% of all isolates.

It is worth noting that *Candida albicans* – an anthropophilous species considered an etiological factor of most mycoses induced by yeast-like fungi, was isolated only once over the entire experimental period. That species was isolated from water samples collected in the summer (July 2007) from station V (used as a bathing beach), which may point to its anthropogenic origin. Its occurrence should be monitored due to the fact that lake Tyrsko has been classi-

fied as a pure lake [6, 7]. Moreover, sporadic appearances of *Candida albicans* in the samples examined were not sufficient to state that unfavorable changes do proceed in the lake. Those findings correspond closely with results of a study by Rózga et al. [31] conducted on lakes of the Tucholski Landscape Park. Most of the lakes investigated by those authors were not receivers of wastewaters from spot-like sources of contamination. Diametrically opposed results were achieved by Dynowska [3] while assaying a few lakes in the city of Olsztyn fed with municipal sewage, by Czeczuga [32], who investigated lakes located around the city of Elk, and by Wójcik and Tarczyńska [33] while evaluating surface waters of bottom deposits from the Sulejówek Reservoir. In turn, Czeczuga and Muszyńska [34] isolated *Candida albicans* from waters of ponds, whereas Kiziewicz [35] did so from spring waters.

On station X, being a fishing site, the presence of *Saccharomycopsis capsularis* was noted twice. Dynowska [3] was first in Poland to detect its permanent occurrence in waters of lakes Kortowskie, Skanda, and Trackie, and its occurrence in the autumn season in waters of an astatic reservoir located outside the town. In our experiment, *Saccharomycopsis capsularis* was isolated in the autumn (October 2007) and in the spring (May 2008), which confirms – to a great extent – results achieved by Dynowska in other lakes located in the city of Olsztyn [3]. In turn, Biedunkiewicz et al. [36] isolated *Saccharomycopsis capsularis* from waters of lakes Goplana and Głębokie in the city of Szczecin. That species was additionally isolated from human respiratory tracts: from sputum and bronchoscopic specimens, as well as from pharyngeal, nasal, and oral swabs of hospitalized patients [37]. In 2008 it was also detected in human colon [38]. Dynowska and Biedunkiewicz [37] concluded that the occurrence of *Saccharomycopsis capsularis* in human ontocenoses was likely to indicate its increased expansiveness in the extrinsic environment.

Apart from *Saccharomycopsis capsularis*, species of yeast-like fungi observed to extend the spectrum of their occurrence include also *Saccharomyces cerevisiae*. In our study, it was isolated from station II – adjacent to a recreational site, and from station X. It has also been detected in bathing waters of the Sulejówek Reservoirs [33] and in waters of Lake Wysockie located in the northwestern part of Tucholski Landscape Park [31]. *Saccharomyces cerevisiae* is increasingly often isolated from various segments of the ontocenosis of the gastrointestinal tract of man, and from skin of the so-called “risk-group patients,” i.e. oncological patients and those with chronic diseases of the respiratory tract. It has usually been detected in the summer and in the autumn, rarely in the spring and in the winter [39]. The presence of fungi of the genus *Saccharomyces* in waters may be elucidated by the consumption of food products at a lake shore as well as by preparation and casting fish baits based on yeast.

In the reported study, we also managed to isolate species that so far have not been noted in hydromycology: *Candida kruisii* and *Dipodascus armillariae*. Detection of new species in water confirms their euryecological charac-

ter and eases in colonizing new ecological niches, as well as providing immediate reflection of water purity in the species composition of fungi isolated from it.

Rare species detected in water of Lake Tyrsko included also *Citeromyces matritensis* – detected for the first time in the area of Olsztyn by Dynowska in Lake Skanda and Lake Track [3].

Species diversity and the count of fungi in a given aquatic environment are determined by the bulk of accumulated organic matter. The highest number of yeast-like fungi cells per 1 dm³ is usually recorded in the autumn, and the lowest in the spring [1, 40]. The increased count of yeast-like fungi implicates increasing contamination, the progressing process of eutrophization and, consequently, an increase in the level of nitrogen and phosphorus compounds [2, 21, 41-43].

The conducted study does not confirm previous observations made by Niewolak [40] and our earlier findings of 2007 [1] referring to the count of fungi in particular seasons of the year. The mean number of cells of yeast-like fungi determined in the later study reached 1,965 cfu/dm³ in the autumn and 240 cfu/dm³ in the spring. In the reported experiment conducted on Lake Tyrsko, the number of colony-forming units isolated from water was observed to decrease successively from spring to autumn. The highest mean number of fungi cells was achieved in the spring of 2008 (4,850 cfu/dm³) and in the spring of 2007 (1,840 cfu/dm³) (confluent growth was not observed only in one experimental month). In the summer, the respective mean number accounted for 2,000 cfu/dm³. The lowest number of fungi cells was observed in the autumn – 410 cfu/dm³. Presumably, the surprisingly low number of fungal cells in the autumn was due to meteorological conditions occurring in the country. Investigations conducted under the Integrated Monitoring of Natural Environment demonstrate that, in terms of thermal and precipitation ratios, 2007 was warm and humid. A high diversity of atmospheric precipitation was observed in the summer, including persistent rainless periods. Considerable precipitation noted in 2007 (exceeding the mean values of the multi-year 1994-2006), relatively high temperatures of air in the winter period, as well as early melts have led to increased levels of surface and ground waters [44]. Seasonal water runoffs might have additionally contributed to the thinning of organic matter, oxygenation of the ecosystem, and enhanced intensity of the self-purification processes of water. As a consequence, this might have resulted in the numerical decrease of the population of fungi in the autumn [45].

In analyzing the results obtained, attention should be paid to the morphometry of the reservoir. Lake Tyrsko has the greatest maximum depth out of the lakes located in Olsztyn – 38 m [6]. It additionally is characterized by a small water surface area and a great bottom gradient close to depression. Surface runoffs of melting snow from areas around the lake might have also contributed to the carrying of a great bulk of organic matter to the coastal zone of the aquifer the spring samples were collected from. This has been reflected in a high count of yeast-like fungi reported in the spring.

Lake Tyrsko, at least in its part where most of the analytical stations were selected, is surrounded by a pine forest that arrests the spring runoff only to a negligible extent, therefore the lake is becoming their major receiver. Those observations may be confirmed by results of studies by Willoughby [46] on the number of spores of *Saprolegniales*. The highest number of fungal spores (25-5,200 spores/dm³ of water) was reported by that author in the coastal waters of Lake Windermere (the greatest lake of England) after heavy rains and an increased level of water, whereas as little as 11-100 spores/dm³ of water were determined in the central part of the lake.

Because non-decomposed organic matter is successively deposited in the greatest hollows of reservoirs, it may be presumed that in the autumn season in Lake Tyrsko organic matter is deposited at the maximum depth of the lake. For this reason, despite autumn circulation of water, the count of fungi in the coastal waters is low.

Special attention has recently been paid also to the problem of the purity of waters at bathing sites. In Poland, purity standards of those waters are provisioned in the regulation of the Minister of Health of October 16, 2002. It stipulates the time, as well as place and frequency of water sample collection for analyses by state sanitary inspection. Appendix No. 1 to the regulation contains a list of indicators taken into account in basic analyses, as well as a list of indicators and parameters serving for extended assays. Unfortunately, none of those assays considers yeast-like fungi as potentially-pathogenic organisms. Results of investigations by Dynowska [2, 3], Biedunkiewicz [1], and Biedunkiewicz et al. [36] suggest the advisability of exploiting fungi not only as indicators of water purity, but also as excellent bioindicators of the potential sanitary and epidemiological threat.

Biedunkiewicz [1] emphasizes that none of the regulations in force in Poland considers fungi as factors deteriorating the quality of water. Worthy of notice is the standard of *Candida albicans* used in the monitoring analyses, implemented in the USA as early as in 1970 [2, 3]. Therefore, bathing sites as places used by a vast number of people (especially in the summer season) should be subjected to constant mycological monitoring [1].

Conclusions

In summary of the studies conducted on Lake Tyrsko, it should be concluded that its waters – despite year-round exploitation – are in good condition and do not pose a health risk. In the study reported, species indicator of the self-purifying processes of water were identified considerably more often than those claimed to pose a risk to human health.

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