ACTA MYCOLOGICA
Vol. 32 (2): 279–286
1997

Yeast-like fungi possessing bio-indicator properties isolated from the Łyna river

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D y n o w s k a M.: *Yeast-like fungi possessing bio-indicator properties isolated from the Łyna river*. Acta Mycol. 32 (2): 279-286, 1997.

Yeast-like fungi isolated in the Łyna river are constant components of microflora of inland waters. Every increase in their number indicates progress in the process of eutrophication and accumulation of organic and inorganic pollutans. The fungi *Candida albicans*, *Pichia quilliermondii*, *P. anomala*, *Rhodotorula glutinis* i *Trichosporon beigelii*, potentially pathogenic apperred in water with high content of municipal sewage, but *T. aquatile* – in the clean waters only. The tested fungi can be also considered as bio-indicators.

Key words: yeast-like fungi, bio-indicator, municipal sewage, aquatic fungi.

INTRODUCTION

The majority of hydro-mycology studies in Poland refered to lakes while only a few were connected with rivers (P a l u c h 1965; J a n u s z k o et al. 1976; J a n u s z k o and M a ł y s z k o 1977; C z e c z u g a 1990, 1995; W o r o n o w i c z 1991).

The Łyna is the major river in a section of the Masurian Lake District (K o n d r a c k i 1972). While the upper Łyna carries clean waters towards Olsztyn, below Olsztyn municipal and industrial sewage result in a significant level of its contamination. No mycological tests were carried out in the river till 1989. The first mycological assesstment was performed during the years 1989-1993. At the time 12 species of yeasts and yeast-like fungi were isolated from the waters of the Łyna. The number of fungi cells differed very significantly along the banks of the river and the composition of species indicated close correlation with the water purity class (D y n o w s k a 1995).

On these bases clean and polluted sections of the river as well as fungi which potentially could be used for assesstment of the level of human pressure were identified. Own conclusions were closely compatible to those presented by other authors (C o o k e et al. 1960; M e y e r s et al. 1970; S i m a r d and B l a c k w o o d 1971a, 1971b; J a n u s z k o et al. 1976; J a n u s z k o and M a ł y s z k o 1977). The objective of this work was observation of selected yeast-like fungi at different sections of the Łyna with particular focus on places where municipal sewage was released.

MATERIAL AND METHODS

The tested material consisted of fungi possessing bio-indicator properties: *Candida albicans* Berkhout, *Pichia quilliermondii* Wickerham, *P. anomala* Kurtzman, *Rhodotorula glutinis* Harrison, *Trichosporon aquatile* Hendrick et Dupont and *T. beigelii* Vuillemin, isolated from the Łyna during the period from March 1994 till October 1996. The frequency of their appearance was assessed.

Four sections of the river were identified: I – from sources to Olsztyn (5 testing points), II – Olsztyn (3 testing points), III – Olsztyn–Dobre Miasto (3 testing points) and IV – Dobre Miasto (3 testing points). The characteristics of testing points and designation of river sections divided into clean (I) and dirty (II, III and IV) ones were presented in the earlier paper (D y n o w s k a 1995) taking into consideration suggestions by other authors involved in physico-chemical and biological analyses of the river (K o r p a c z 1967; P i o t r o w s k a and W i ę c ł a w s k i 1976).

Physico-chemical analyses of the water were performed at the Institute of Inland Fisheries in Olsztyn according to standard methods by H e r m a n o w i c z (1976). At every testing point 500 ml of water were sampled in sterile conditions from the depth of 10-20 cm. Each sample was tested in two ways: the water was filtered through microbiological filters (FMW – 5/50) which were placed on Sabouraud solid medium with gentamycin and chloromphenicol; *each sample was centrifuged, the effluent was collected from* above the sediments and filtered as above; the sediment was covered with 10 ml liquid Sabouraud medium with the same bacteriostates.

The cultures were incubated for 48 hours at 25°C. Fungi that appeared on liquid and solid medium (sediment or membrane) on the surface were transferred to antibiotic free Sabouraud scarves. After 7 days of incubation at 37°C they were ready for biochemical tests. In the process of identification macroscopic features on Sabouraud agar and microscopic features on Nickerson agar (K u r n a t o w s k a 1995) were considered. Biochemical tests were performed using API – tests bio – Merieux (API 20°C and API 20°C AUX).

Works by B a r n e t t et al. (1990); L o d d e r and K r e g e r–v a n R i j (1967) and K u r n a t o w s k a (1995) were used for identification of the fungi.

RESULTS

It was found that fungi selected for mycological assessment were not present along all the studied length of the river (Tab. 1). Apperance of a specific species of fungi was linked up to the presence of phosphorus and nitrogen containing subtances, electric conductivity and saturation of water with oxygen (Tab. 2). Waters of the Łyna should be considered most severely polluted in section II (Olsztyn) and IV (Dobre Miasto). Phosphorus (phosphates and total phosphorus) and nitrogen (ammonia, nitrates and nitrites) compounds reached there the highest levels. With the length of flow, a clear change in electric conductivicty of water in the Łyna was observed resulting from the level of mineralization of water and an increased level of CO_3^-, HCO_3^-, Hg^{++}, SiO_2 and Fe^{++} ions (corresponding to an increased ion content in the water). Within the most severely polluted section IV it increased twofold as compared to section I values.

T a b l e 1

Frequency of occurrence of fungi investigated in each section of the Łyna river

Species	Sections of the river				Potentially use
	I	II	III	IV	
C. albicans		+ + +	+	+ + +	P, C, T
P. anomala				+ +	D
P. quilliermondii				+ +	P, C, T
Rh. glutinis		+		+ + +	P, C, T, D
T. aquatile	+ +				W
T. beigelii	+	+ +	+	+ + +	P, C, T(NO_2^-, NO_3^-, SO_3^-, SO_4^{--})

\+ – rarely, + + – often, + + + – constantly indicator: P – pathogenic organisms, C – communal sewage, T – trophicity, W – plain water, D – properties detersive

The flow dependent increase of parameters disscused here indicates gradual accumulation of biogenic substances originating mainly from municipal sewage. It contains a high level of detergents, the self-purfication processes are there too weak. The increase in the level of pollutants is coupled with a decrease in oxygen content from as much as 90% within clean section to 61%

and 65% in sections II and IV (Tab. 2). Although from the sanitary point of view the content of CO_2 in water is of no major significance, its level may by be an indication of pollution of water with organic compounds and the intensity of decomposition of such compounds. CO_2 may also originate form geothermal processes or metabolism of water organisms. Abundance of fungi, where high level of chloride (Cl^-), the element which should inhibit development of fungi, was present is worth noticing. Taking into consideration all physical and chemical parameters, pH and temperature, seem to play the last important role. Higher temperature observed within sections II and IV is probably associated with accumulation of municipal pollutants (Tab. 1 and 2).

Table 2
Physico-chemical analyses of the water in each section of the Łyna river

Parameters		Section of the river			
		I	II	III	IV
t	[°C]	7,0	9,5	7,5	10,0
pH		7,9	7,8	7,5	7,4
O_2	[%]	89,0	65,0	89,0	61,0
O_2	[mg/cm^3]	10,5	11,0	10,5	6,9
CO_2	„	5,4	8,2	6,6	30,5
NH_4-N	„	0,40	0,13	0,10	1,895
NO_2-N	„	0,002	0,141	0,050	0,255
NO_3-N	„	0,114	0,595	0,110	5,510
PO_4-P	„	0,042	0,194	0,096	0,457
$P_{tot.}$	„	0,620	1,138	0,285	0,830
CO_3^{--}	„	0,00	0,00	0,00	0,00
HCO_3^-	„	158,80	183,00	165,0	253,0
SiO_2	„	4,05	5,40	5,40	12,84
Cl^-	„	7,95	9,25	7,50	15,44
Ca^{++}	„	45,30	52,10	43,40	66,90
K^+	„	1,54	5,11	4,80	13,35
Mg^{++}	„	7,50	8,50	7,50	12,50
Fe^{++}	„	0,00	0,00	0,00	0,00
SO_4^{--}	„	21,45	32,53	23,00	29,76
oxygen.	„	7,20	8,00	14,20	421,00
el. cond. [µs/cm^{-1}]		267,00	300,00	290,00	17,10

DISCUSSION

Fungi are among the bio-indicators which are least known and rarely used in practice in Poland. Attempts are taken at using the sensitivity of some phytopathogenic fungi to air pollution assessment (B e v a n and G r e e n h a l g h 1976; D y n o w s k a 1996). The issue of using of micro-fungi for assessment of purity (D y n o w s k a 1993) and nature of water ecosystems is discussed increasingly frequently (K o r n i ł ł o w i c z 1995). The observations by C o o k e et al. (1960); H i n z e l i n and B l o c k (1985); S i m a r d and B l a c k w o o d (1971a, b) indicate that yeast-like fungi can function as very good bio-indicators. They may be treated as euryecological orgamisms commonly present in biosphere of all climatic zones within a wide range of temperatures, humidity, pressure, oxygen saturation and acidity. Only a drastic shortage of oxygen or a sudden increase in carbon dioxide content may result in degenerative changes of their cells. However, there are species inhabiting sewage treatment plants and reactors for denitrification of sewage where anaerobic conditions do not influence the process of synthesis of cell enzymes and no changes in the genotype are observed in their case (D y n o w s k a 1995).

According to C o o k (1965) and C o o k e et al. (1960) yeast-like fungi tolerate high levels of pollutants, even organic matter hard to decompose, and some are present in highly degraded systems only. The largest proportions of fungi from *Cryptococcus*, *Rhodotorula* and *Trichosporon* genera were isolated by C o o k e (1965) from untreated sewage, feces, effluents containing remains of decomposing plants and wastes of different origin. Continous presence of *C. albicans*, the species closely linked to human body and bodies of other mammals, in the most severely polluted sections of the river proves a high proportion of municipal sewage in such contamination. The same applies to *T. beigelii*. This fungus possessing keratinophile characteristics used to be considered a permanent component of skin microflora. Currently it is also observed in different parts of the body and may be the cause of many severe mycoses. Presence of *Trichosporon* species may also prove a high content of vegetable wastes, mainly cellulose. M e y e r s et al. (1970), relate presence of *Trichosporon* and *Rh. glutinis* with an increased content of sulfates and sulfides in water, resulting from extensive processes of lignin fermentation. On the other hand, T a b a k and C o o k e (1968) observed accelerated growth of yeast-like fungi (*Geotrichum candidum*, *C. parapsilosis*, *Rh. muscilaginosa*) proportional to the content of nitrogen compounds.

Based on own research conducted since 1986 in different water ecosystems of Olsztyn and its closest vicinity it was established that yeast-like fungi are constant components of microflora of inland waters (D y n o w s k a 1995). Every increase in their number indicates the progress in the process of

eutrophication and accumulation of organic and inorganic pollutants, mainly nitrogen and phosphorus compounds. The majority of isolated fungi are potentially pathogenic. A close correlation between apperance of the same fungi in water with high content of municipal, tourist, agricultural and industrial sewage and their presence in the clinical material collected from residents in the province of Olsztyn can be seen (D y n o w s k a 1995).

As early as in seventies yeast-like fungi are used in the USA and Canada as generally understood bio-indicators and monitoring organisms. M i t c h e l (1978); H a a s et al. (1985) and H i n z e l i n and B l o c k (1985) confirmed and stressed the need for appyling fungi, such as *Candida*, as bio-indicators for detection of contamination of water with feces, contamination with sewage and presence of pathogenic organisms in aquatic environment. *C. albicans* proved to be a particulary good indicator, even for assessment of chlorinated water (H a a s et al. 1985). Results and observations from the authors own studies lead to identical conclusions and sugest that besides *C. albicans*, *T. beigelii* could also be considered for sanitary — higienic assessment of water (D y n o w s k a 1993, 1995). S i m a r d and B l a c k w o o d (1971a, b) conducting their ecological studies on yeast-like fungi in the St. Lawrence river which collects sewage from Montreal and Quebec, noticed a clear increase in metabolism of fungi at the places of discharge of sewage. The development of *Rh. glutinis*, *Rh. muscilaginosa* and *C. guilliermondii* was particularly abundant. Simultaneously to degradation in number of coli type bacteria was observed. Similar observations had been made earlier by C o o k e et al. (1960) and H e d r i c k and S o y g e n c (1967) in lake Ontario and in Poland by M a ł y s z k o et al. (1978) while studying water from wells contaminated with feces.

Results of this study correspond closely to the results obtained by the quoted authors and confirm earlier observations from the Łyna river (D y n o w s k a 1995).

Apperance of *T. aquatile*, a species characteristic for clean waters (B a r n e t t et al. 1990) only within the section of the Łyna from its sources to Olsztyn deserves particular attention. In view of what has been said earlier about *T. beigelii*, its presence in the cleanest section of the river also requires explanation. This may result from long term operation of a saw mill at the sources of the Łyna and wood wastes. Finally a significant role of yeast-like fungi in the processes of self-purification of waters and use of these organisms in sewage treatment plants (G r a b i ń s k a–Ł o n i e w s k a 1990) should be stressed. Many of them metabolize lactic acid, acetic acid, ethanol, glycerol, nitrates, phenoles and other poisonous substances using them as a sourse of carbon (G r a b i ń s k a–Ł o n i e w s k a 1990; G r a b i ń s k a–Ł o n i e w s k a and S l á v i k o w a 1990; S l á v i k o w a and G r a b i ń s k a–Ł o n i e w s k a 1987; 1988-1989). In view of the above *P. anomala*

and *Rh. glutinis* are of major importance among the examined species. It is possible that other species may have similar properties, in particular as they appear immediately when pollution level of water increases.

Assessing the consequences of pollution of the natural environment generation of reliable quick information is important. The biological indicators are of more use the higher their sensitivity and specificity of reaction. From that point of view the studied fungi deserve attention.

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Grzyby drożdżopodobne o właściwościach bioindykacyjnych izolowane z rzeki Łyny

Streszczenie

Kilkuletnie obserwacje grzybów drożdżopodobnych w rzece Łynie pozwoliły wyodrębnić gatunki mogące mieć potencjalne zastosowanie jako wskaźniki troficzności i zanieczyszczeń komunalnych oraz mogące być przydatne w ocenie sanitarno-higienicznej wody. Do grzybów o właściwościach bioindykacyjnych należą: *Candida albicans*, *Pichia anomala*, *P. quilliermondii*, *Rhodotorula glutinis*, *Trichosporon aquatile* i *T. beigelii*.