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SOME BIOCHEMICAL PROPERTIES OF FUNGI FROM GENERA *RHODOTORULA* AND *TRICHOSPORON* ISOLATED FROM SULEJÓW RESERVOIR BATH WATER[1]

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ABSTRACT. The hydrolytic activity of *Rhodotorula* and *Trichosporon* strains isolated from Sulejów Reservoir water and sediments were studied. Also tolerance of high concentrations of natrium chloride and biogenic compounds was evaluated. Fungi from genera *Rhodotorula* and *Trichosporon* showed the activity of 12 and 10 from 19 hydrolases of API Zym test, respectively. Tolerance tests showed high resistance of *Rhodotorula glutinis* to hypertonic saline solutions and very high resistance of both *Rhodotorula* and *Trichosporon* strains to nitrate, ammonium and phosphate compounds. These properties of fungi may influence the distribution and the quantity of fungi in aquatic reservoirs.

Key words: biogenic compounds, pathogenic fungi, Sulejów Reservoir, yeast-like fungi.

INTRODUCTION

In years 2000 and 2001 we were studying surface samples of water and sediments collected from litoral zones of Sulejów Reservoir used as a recreation site. The quantitative evaluation and identification of fungi species revealed 28 species from the genera: *Candida*, *Cryptococcus*, *Geotrichum*, *Kloeckera*, *Rhodotorula*, *Saccharomyces* and *Trichosporon* (Wójcik et al. 2003). *Rhodotorula glutinis* was the dominant species in 2000 (found in 39.8% of samples) and in 2001 (in 47.7%). *Trichosporon cutaneum* was the third in frequency of occurrence in 2000 (found in 14.6%), in 2001 more rarely found (in 7.74% of samples). Also *Rhodotorula rubra* and *Trichosporon pullulans* were identified in samples in both years.

Rhodotorula glutinis and *Trichosporon cutaneum* were found in many freshwater basins, which undergo pollution and degradation (Dynowska 1995, Dąbrowski et al. 1998, Rózga et al. 1999). These two species were proposed, with *Candida albicans*, as mycological indicators of water contamination (Dynowska 1997).

The aim of this study was to evaluate some biochemical features of fungi – hydrolytic activity and tolerance of high concentrations of natrium chloride and bio-

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genic compounds – which may have influence on distribution and the quantity of fungi in aquatic reservoirs.

MATERIAL AND METHODS

The fungi strains were isolated from samples of pelagic water and sediments of Sulejów Reservoir as described previously (Wójcik et al. 2003). The hydrolytic activity was examined using API Zym tests (bioMérieux), which contain substrates for identification of 19 hydrolases.

Also the ability of fungi to grow in the presence of high concentrations of natrium chloride (concentrations in the medium: 8%, 10%, 12%, 14%), natrium nitrate (the nitrate nitrogen concentrations: 0.2%, 0.5%, 0.7%), ammonium sulfate (the ammonium nitrogen concentrations: 0.2%, 0.5%, 0.7%), and natrium phosphate (the phosphate phosphorus concentrations: 0.1%, 0.2%, 0.3%) was evaluated on the medium consisting of 1% of peptone, 4% of glucose and 1.8% of agar.

RESULTS

All isolated fungi from genus *Rhodotorula* showed the activity of 12 from 19 hydrolases of API Zym test. Low activity (5-10 nmoles of hydrolysed substrate) revealed: esterase lipase C8 (e4), naphtol-AS-BI phosphohydrolase (e12) and β-glucosidase (e17). The activity of esterase C4 (e3) was found in all isolated strains like the activity of acid phosphatase (e11). Only few *R. glutinis* and *R. rubra* strains showed the activity of arylamidases valine (e7) and cystine (e8), trypsin (e9), α-glucosidase (e16). The highest activity (over 40 nmoles) showed leucine arylamidase

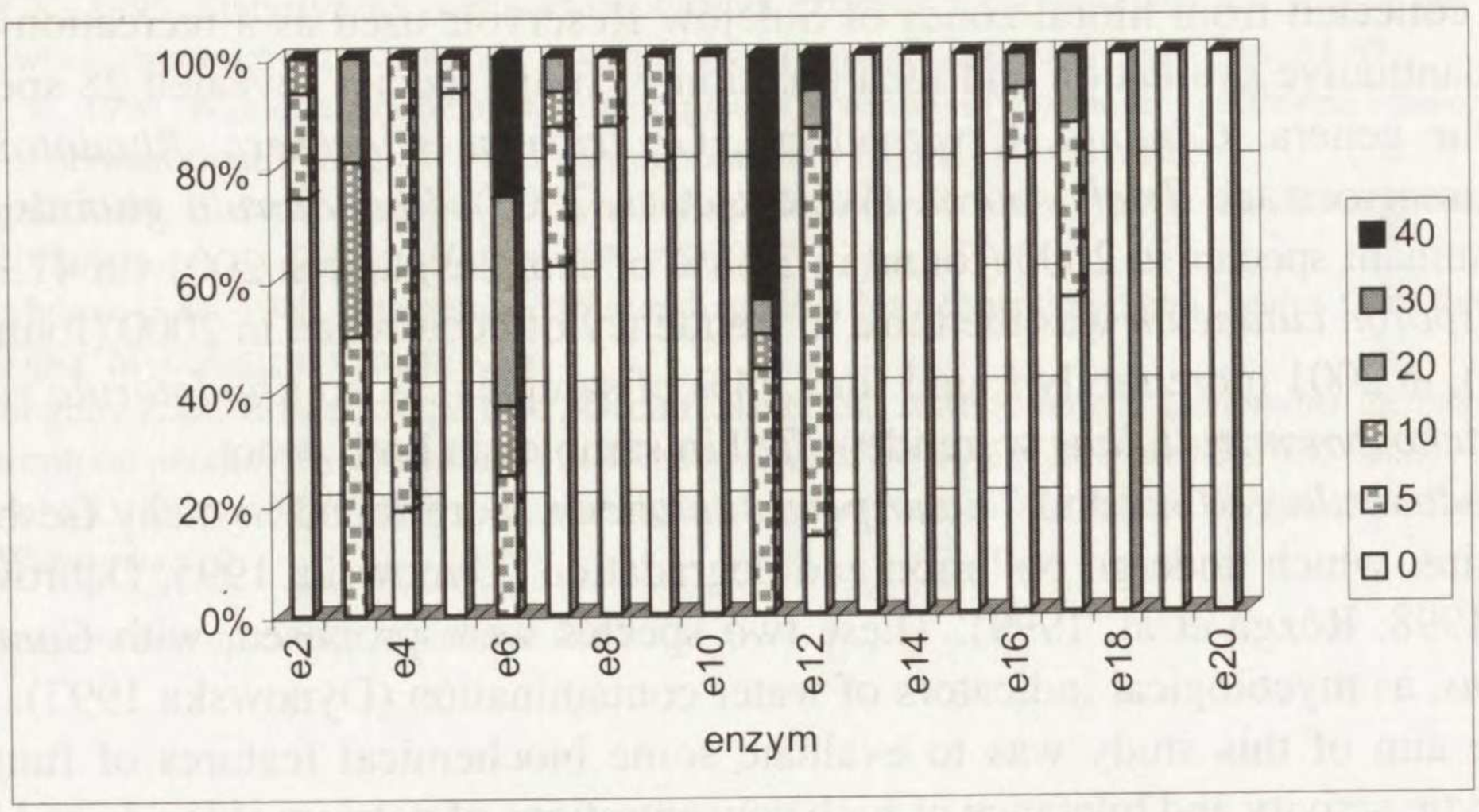

Fig. 1. Hydrolytic activity of *R. glutinis* strains isolated from Sulejów Reservoir water

(e6) and acid phosphatase (e11), but alcaline phosphatase (e2) was detected in *R. rubra* and *R. glutinis* rarely (Table 1, Fig.1).

Table 1. Hydrolytic activity of *Rhodotorula* and *Trichosporon* strains isolated from Sulejów Reservoir water

No	Enzyme	Mean activity in nmoles of hydrolysed substrate		
		R. glutinis n=14	*R. rubra* n=10	*T. cutaneum* n=6
e2	Phosphatase alcaline	1.56 ± 0.724	10.5 ± 3.68	4.16 ± 1.54
e3	Esterase (C 4)	9.38 ± 1.43	9.5 ± 1.89	9.17 ± 2.91
e4	Esterase lipase (C 8)	4.06 ± 0.507	4.80 ± 0.718	5.83 ± 1.54
e5	Lipase (C 14)	0.312 ± 0.312	0	0
e6	Leucine arylamidase	20.0 ± 3.35	25.0 ± 5.00	9.17 ± 4.55
e7	Valine arylamidase	3.13 ± 1.36	3.5 ± 1.30	0.833 ± 0.833
e8	Cystine arylamidase	0.625 ± 0.427	1.00 ± 0.667	0
e9	Trypsin	0.937 ± 0.503	2 ± 1.33	0
e10	Chymotrypsin	0	0	0
e11	Phosphatase acid	35.0 ± 5.53	28 ± 4.16	21.7 ± 5.16
e12	Naphthol-AS-BI phosphohydrolase	9.06 ± 2.45	19.0 ± 4.18	6.76 ± 6.99
e13	α galactosidase	0	0	0.833 ± 0.833
e14	ß galactosidase	0	0	0
e15	ß glucuronidase	0	0	0
e16	α glucosidase	1.88 ± 1.28	7.00 ± 4.16	3.33 ± 1.67
e17	ß glucosidase	4.06 ± 1.66	9.5 ± 3.67	0.833 ± 0.833
e18	N-acetyl-ß glucosaminidase	0	0.5 ± 0.5	0
e19	α mannosidase	0	0	0
e20	α fucosidase	0	0	0

Most *Trichosporon cutaneum* strains demonstrated low activity (0-20 nmoles) of alkaline phosphatase (e2), and very differentiated activity of acid phosphatase (e11): from 5 up to over 40 nmoles of hydrolysed substrate. Other enzymes in the species examined by API Zym test: esterase C4 (e3), esterase lipase C8 (e4), leucine arylamidase (e6) and naphtol-AS-BI phosphohydrolase (e12) showed, in the majority, low activity. Only single strains revealed, also low, the activity of valine arylamidase (e7), α-galactosidase (e13), α-glucosidase (e16) i ß-glucosidase (e17) (Table 1, Fig. 2).

For testing of fungi tolerance to high saline concentration, the growth on Sabouraud medium with 8, 10, 12 i 14% NaCl was evaluated (Table 2).

The majority of *Rodotorula rubra* strains did not grew on medium with 8% of saline, but 81.3% of R. *glutinis* strains showed the ability to growth on medium with 8% of NaCl and 31.3% of strains – with 14% of NaCl. 17.6% of *Trichosporon cutaneum* strains were not susceptible to 14% of NaCl, but 58.8% – revealed no growth on medium with 8% of NaCl.

To determine the fungi growth on media with high concentrations of biogenic compounds, the concentrations exceeded up to 4 to 14 times the nitrate nitrogen

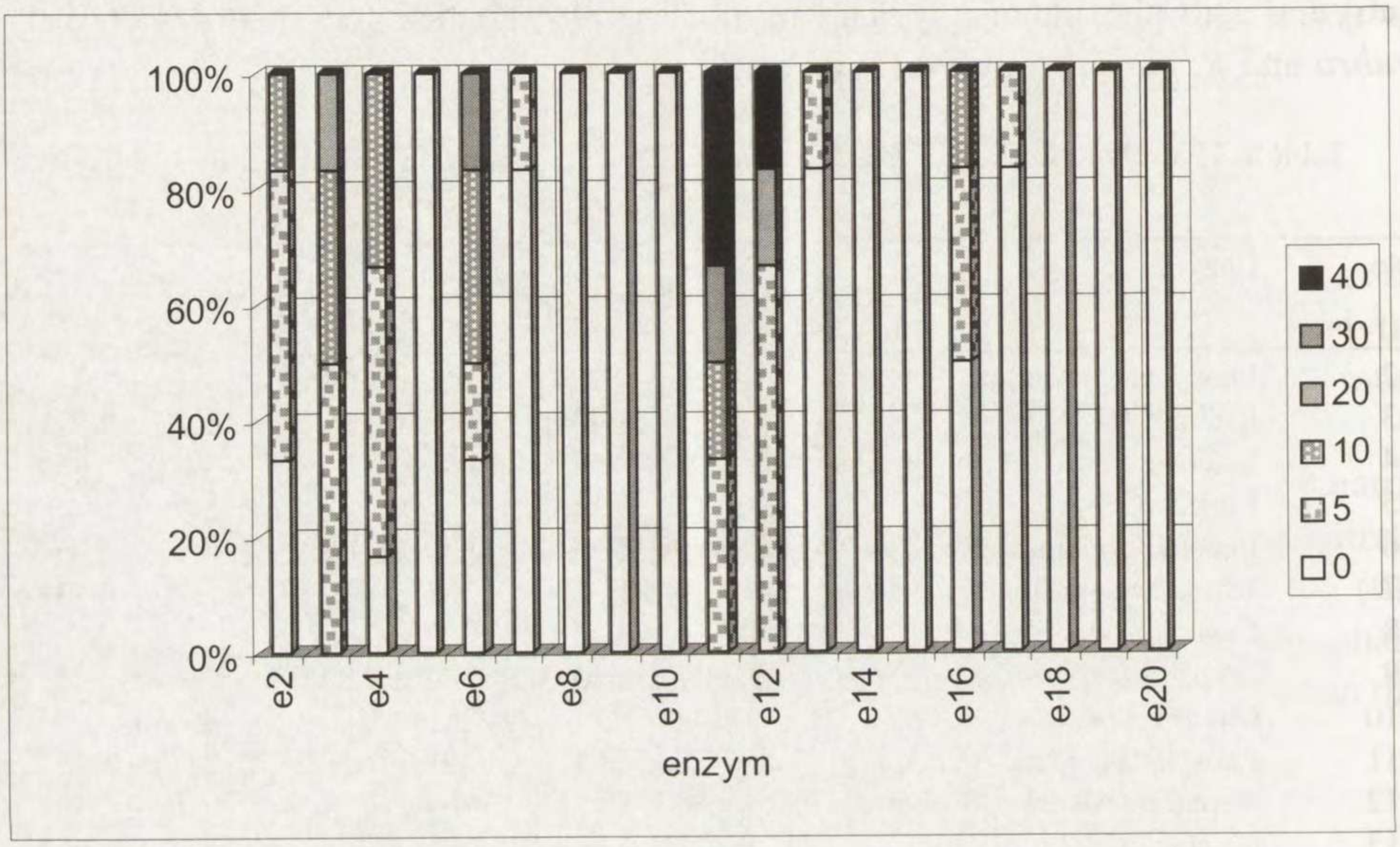

Fig. 2. Hydrolytic activity of *T. cutaneum* strains isolated from Sulejów Reservoir water

concentrations and up to 5 to 16 times the phosphate phosphorus concentrations in the Čapek medium were chosen. On the other hand, the concentrations of nitrate nitrogen exceeded up to 400 to 700 times the standard values for water purity classification, ammonium nitrate over 1000 to 2000 times and the phosphate phosphorus concentrations up to 10000 to over 16000 times (the standard values for classification of inland superficial water purity Dz. U. no 16, 1991, p.503).

Table 2. Percentage of *Rhodotorula* and *Trichosporon* strains showing tolerance of high NaCl concentrations.

Species	NaCl			
	8%	10%	12%	14%
R. glutinis	81.3	62.5	37.5	31.3
R. rubra	33.3	11.1	11.1	0
T. cutaneum	41.2	29.4	23.5	17.6

Table 3. Percentage of *Rhodotorula* and *Trichosporon* strains showing tolerance of high biogen compounds concentrations.

Species	$N - NH_4$			$N - NO_3$			$P - PO_4$		
	0.2%	0.5%	0.7%	0.2%	0.5%	0.7%	0.1%	0.2%	0.3%
R. glutinis	100	100	85.7	100	100	71.4	85.9	85.9	85.7
T. cutaneum	100	100	100	100	100	100	100	100	0

It was found that the majority of evaluated strains well tolerate the chosen concentrations (Table 3). All *R. glutinis* and *T. cutaneum* strains showed growth on the medium with different nitrate nitrogen concentrations. Similar results were received for both species for ammonium nitrate concentrations. More differences were observed for phosphate phosphorus concentrations. Some *R. glutinis* strains did not show growth on medium with high concentrations of the compound.

DISCUSSION

The obtained results of hydrolytic activity in *Rhodotorula rubra* strains from Sulejów Reservoir revealed some differences in comparison with the activity of fungi isolated from skin lesions (Plomer-Niezgoda et al. 1998). The activity of α-glucosidase (e16) and higher activity of acid phosphatase (e11) was showed. In cells of *R. rubra* from the Szczecin Lagoon high activity of lipase C14 (e5) and trypsin (e9), low chymotrypsin (e10), and lack of naphtol-AS-BI phosphohydrolase (e12) was found (Bogusławska-Wąs et al. 2000). But the strains from herring salads showed other enzymograms. The activity of leucine arylamidase (e6), naphtol-AS-BI phpsphohydrolase (e12) and ß-glucosidase (e17) was similar to the activity in strains from the Sulejów Reservoir. There were lack of trypsin (e9), chymotrypsin (e10) and α-glucosidase (e16) activity. Yet, in our investigations, any of isolated *R. rubra* strains did not split the substrate for lipase C14 (e5) and chymotrypsin (e10).

Trichosporon cutaneum (syn. *T. beigelii*) strains from Sulejow Reservoir showed the activity of naphtol-AS-BI phosphohydrolase (e12) and some of α-glucosidase (e16) – enzymes revealed in strains from herring salads. But the activity of lipase C14 (e5), trypsin (e9) and chymotrypsin (e10) present in fungi cells from the Szczecin Lagoon was not seen in our investigations, like in strains from herring salads (Bogusławska-Wąs et al. 2000).

The presented data on enzymograms of strains from the same genera may suggest the ability of the fungi for adaptation to different environments.

In our experiments we have discovered high tolerance of the majority of investigated fungi to biogenic compounds. The concentrations of nitrate, ammonium and phosphate compounds used in the tests, many times exceeded the concentrations in fungal cultivate media or the standard values for III class of water purity. The natural presence of some fungi is related to the extensive process of lignin fermentation. *R. glutinis* and *Trichosporon* species were showed in water with high content of plant remains, mainly cellulose (Meyers et al. 1970). But Kiziewicz and Czeczuga (2001) found *T. cutaneum* in diverse ecological environments: lakes and ponds with strong poluted water and in good oxygenated waters of springs. There are also data that number of fungi arises with the nitrate and phosphate compounds increase e.g. in Łyna River (Dynowska 1995), in Supraśl River (Januszko et al. 1996) or in Mazury Lakes (Czeczuga and Woronowicz 1991-1992). Thus it may be

supposed that not only the biogenic compounds concentration conditioned the presence of fungi in aquatic reservoirs, but also that the presence of the organisms is correlated with the increasing pollution of the reservoirs especially with municipal sewage.

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