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SPECIES DIFFERENTIATION OF YEASTS AND YEAST-LIKE ORGANISMS IN THE WATER AND BOTTOM SEDIMENTS OF THE SZCZECIN LAGOON

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Abstract

The aim of the work was qualitative evaluation of occurrence of yeasts and yeast-like organisms in the water and bottom sediments of the Szczecin Lagoon. One ascertained occurrence of 24 species classified as yeasts and yeast-like organisms on the basis of analyses of the water and bottom sediment mycoflora of the Szczecin Lagoon. 21 species were common for the water and sediment samples, 1 found only in the water, 4 were present only in the sediment. The most often isolated yeasts at all stations in a period of investigations were *Candida* and *Rhodotorula.*

Key words: yeasts, yeast-like organisms, *Candida*, *Rhodotorula*, water, bottom sediments

INTRODUCTION

There are huge concentrations of different species of fauna and flora in the aquatic environment. The most frequently its functioning is disturbed by advanced processes of reservoir eutrophication. Fungi also settle such an anthropologically influenced environment. Ecological flexibility of yeasts, their genotypical variability, settled correlations between growth rate, as well as physiological activity and the level and kind of pollution discarded into water, have led to conditions that yeast-like organisms are regarded as bioindicators of water purity. These fungi are a rare group of mycocenosis structures, easily developing in superficial layers of bottom sediments, as well as in depths of water which provide them not only with the suspension environment but also with a source of food. The Szczecin Lagoon is a typical lagoon estuary and it plays an important role in preventing pollution from getting to the sea because it accumulates useful nutrients as well as various chemical

compounds and biologically toxic substances contained in the flowing water (Klarer and Mille 1989). The principal contamination supplier for the Szczecin Lagoon is the Odra river. The water flowing from the industrialised river basin carries considerable amounts of communal, industrial and agricultural sewage which run into the river hardly purified and flow directly in to the lagoon.

The aim of the following work was to present a qualitative assessment of the occurrence of yeasts and yeast-like organisms in the water and sediments of the Szczecin Lagoon.

MATERIALS AND METHODS

The samples of the water and sediments were taken in four fixed places, marked as: BT-3, WW-E, W-4, and MO-S (Fig. 1) in monthly intervals in 1997 (from April till December). The designated sites were different from each other in respect to the bottom structure, water mixing processes, hydrochemical and biological conditions. The isolation of the yeasts and yeast-like organisms was carried out on the YM medium, at 20-22^{o}C, for five days (MacGillivary and Shaiaris 1993). The identification of the isolated strains was conducted by standard methods according to the Lodder's key (1971). The macroscopic features were estimated on the basis of colony growth on Sabouraud medium. Corn Meal Tween-80 Agar medium (Elmer at al. 1986) was used to carry out the microculture. The species identity was determined and confirmed by identification methods using ID 32C (bioMerieux) tests.

RESULTS

24 species of fungi classified as yeasts and yeast-like organisms were identified on the basis of the analyses of mycoflora conducted in the water and bottom sediments in the Szczecin Lagoon. 21 species were found in samples of the water and bottom sediment. One species was present only in the water and four were present solely in the sediment (Table 1). During the tests in all the research units the most frequently isolated genera were *Candida* and *Rhodotorula* (Fig. 2). The species differentiation of the strains isolated from the sediment samples demonstrated that the most often isolatedspecies were *Candida famata* and *Rhodotorula glutinis*. However, in the water samples there was a preponderance of *Rhodotorula glutinis* (Table 1). Regarding the examined mycocenoses following species were identified as constantly present – *Candida famata*, *Cryptococcus albidus*, *Rhodotorula rubra*, *Trichosporon cutaneum* and *Saccharomyces cerevisiae*. The presence of coexisting forms was occasional (Table 2).

DISCUSSION

The intensive growth and physiological activity of the isolated micro-organisms were significant. Undoubtedly it is a result of the quality and quantity of the pollu-

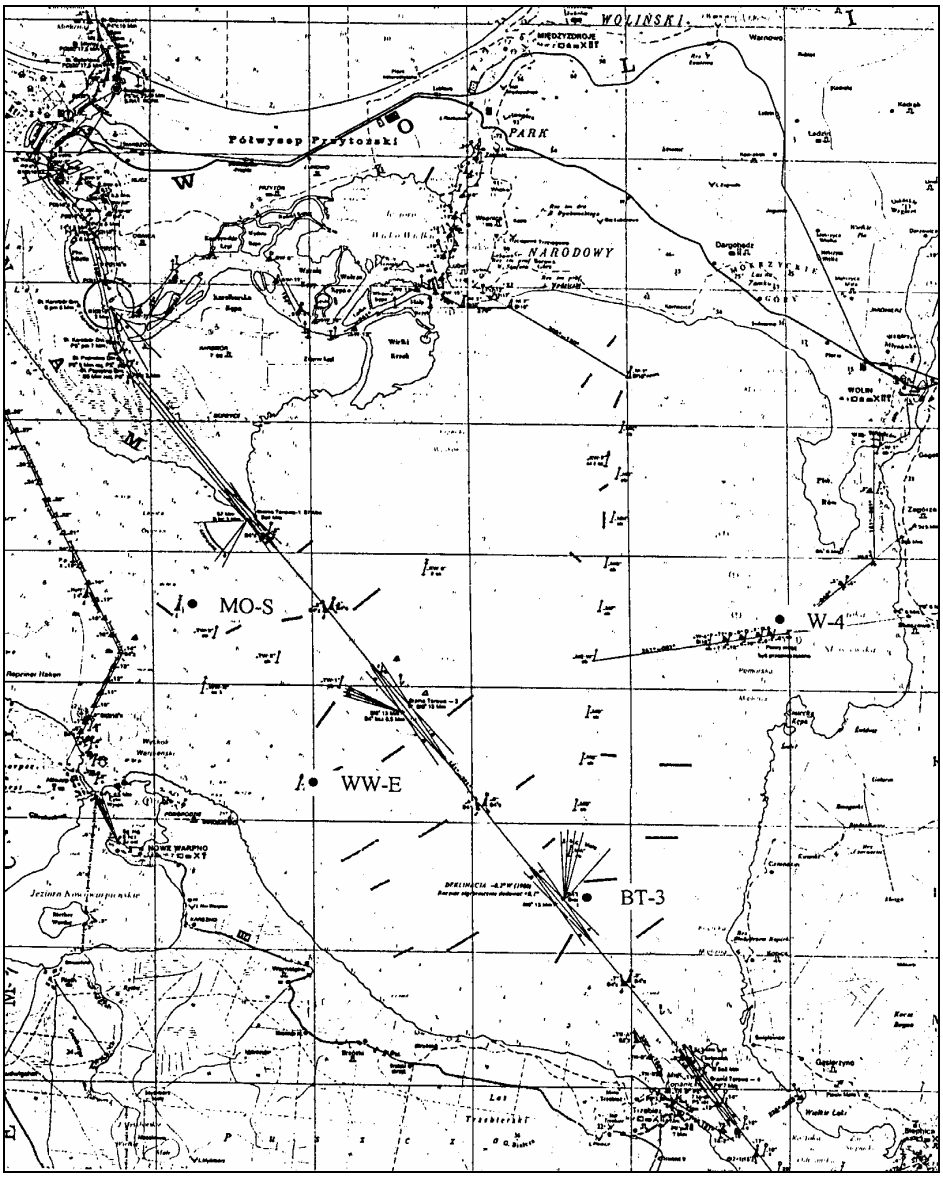

Fig. 1. The sampling stations on the Szczecin Lagoon

Table 1

Percentage of the yeasts species in the water and bottom sediments samples of the Szczecin Lagoon

Strain	BT-3		WW -E		W-4		MO -S	
	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water
	%	%	%	%	%	%	%	%
Aureobasidium pullulans	0,0	0,5	0,0	0,0	1,6	3,5	0,0	2,3
Candida colliculosa	4,4	4,4	2,3	0,6	0,0	0,2	2,1	1,2
Candida famata	13,1	17,1	13,0	15,4	12,1	10,5	18,1	7,5
Candida glabrata	2,7	4,5	0,0	2,5	0,0	1,0	4,3	1,3
Candida holmii	0,1	0,0	0,0	0,0	0,0	0,1	0,0	0,0
Candida inconspicua	0,0	0,0	2,9	0,0	1,0	0,0	1,2	0,0
Candida lipolytica	5,6	0,0	6,5	0,5	0,0	1,8	7,0	0,0
Candida lusitaniae	0,9	0,0	0,0	0,0	1,9	0,0	0,0	0,0
Candida parapsilosis	2,1	0,0	1,0	0,0	1,8	0,0	0,3	2,8
Candida sake	5,5	4,1	4,1	4,4	4,7	3,3	3,7	4,2
Candida tropicalis	5,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Candida utilis	2,1	0,0	5,3	0,0	2,8	0,0	5,9	0,0
Candida zeynoides	0,0	2,6	0,0	0,0	0,0	0,0	0,0	0,2
Cryptococcus albidus	4,9	3,1	8,2	6,8	7,6	3,1	4,9	5,2
Cryptococcus laurenti	4,2	2,1	4,3	2,8	3,5	1,6	5,5	2,8
Cryptococcus neoformans	0,0	0,0	0,0	0,5	1,5	0,0	0,1	0,0
Hansenula saturnus	9,2	14,6	11,2	11,0	10,5	4,8	9,8	8,4
*NOZN**	0,6	0,4	0,5	0,8	0,3	0,4	1,2	0,3
Pichia carsonii	4,1	0,0	8,4	0,0	6,0	0,0	4,3	0,0
Rhodotorula glutinis	13,9	23,9	15,8	34,9	25,7	55,3	16,1	39,7
Rhodotorula rubra	3,3	4,1	2,9	6,6	7,2	7,0	3,2	5,9
Saccharomyces cerevisiae	3,9	6,5	0,6	2,8	1,6	2,9	4,9	7,9
Saccharomyces kluyverii	0,0	0,0	0,3	0,0	0,7	1,2	0,0	0,0
Sporobolomyces sp	3,6	3,1	4,6	2,7	2,9	0,3	3,8	3,6
Trichosporon cutaneum	9,9	9,7	8,0	7,7	4,7	3,3	3,2	6,5
Zygosaccharomyces sp	0,4	0,1	0,0	0,2	0,0	0,0	0,5	0,0

* not identified

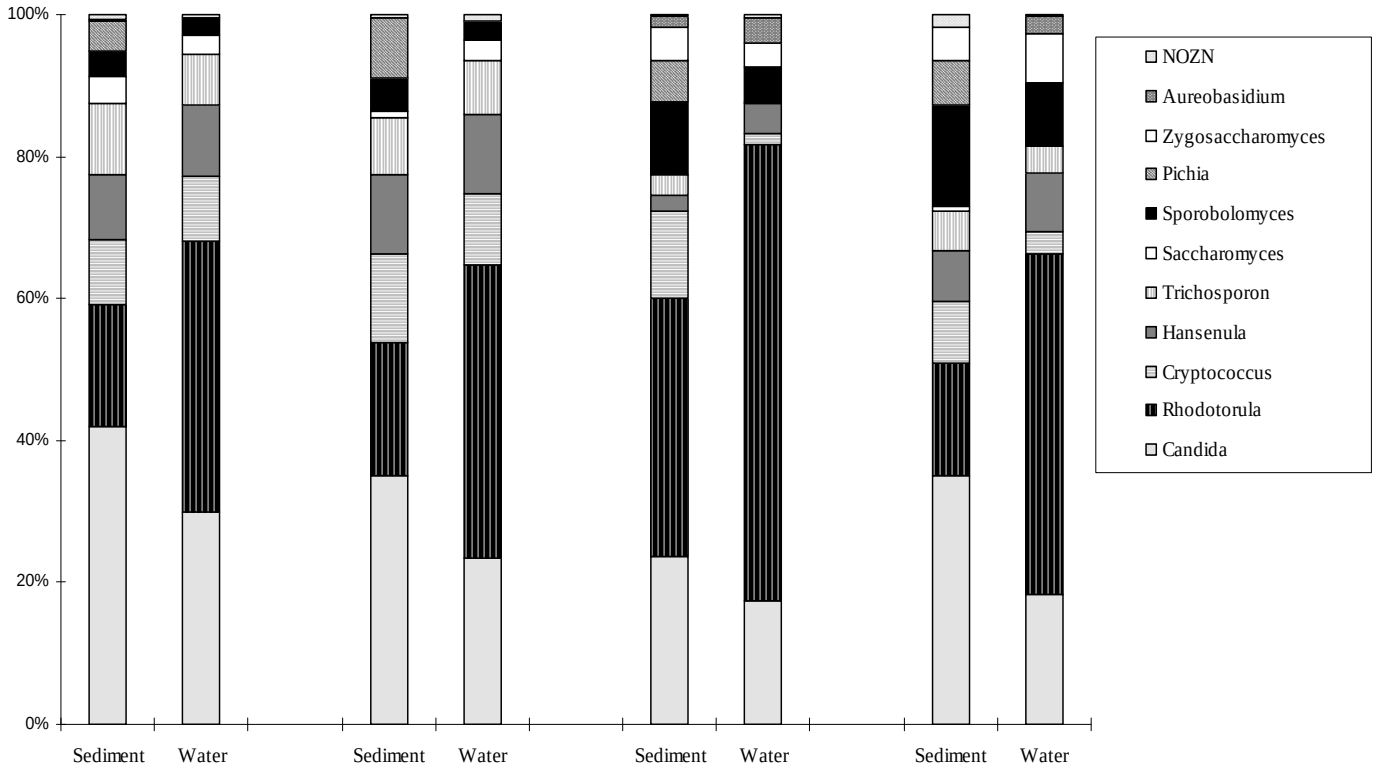

Fig. 2. Species differentiation of yeasts organisms in the water and bottom sediments of the Szczecin Lagoon
NOZN - not identified

Table 2

Frequency of the yeasts species in the water and bottom sediment samples of the Szczecin Lagoon

Strain	Sampling site							
	BT-3		WW-E		W-4		MO-S	
	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water
Aureobasidium pullulans	sporadic	sporadic	absent	absent	sporadic	sporadic	absent	sporadic
Candida colliculosa	sporadic	stable	sporadic	sporadic	absent	sporadic	sporadic	absent
Candida famata	stable	stable	stable	stable	stable	stable	stable	stable
Candida glabrata	sporadic	stable	absent	sporadic	sporadic	sporadic	sporadic	stable
Candida holmii	sporadic	absent	absent	absent	absent	sporadic	sporadic	sporadic
Candida inconspicua	absent	absent	sporadic	absent	sporadic	absent	stable	absent
Candida lambica	absent	absent	absent	absent	absent	sporadic	absent	sporadic
Candida lipolytica	sporadic	absent	sporadic	sporadic	sporadic	sporadic	sporadic	absent
Candida lusitaniae	sporadic	absent	absent	absent	sporadic	absent	absent	absent
Candida parapsilosis	sporadic	absent	sporadic	absent	sporadic	absent	sporadic	sporadic
Candida sake	stable	sporadic	sporadic	sporadic	sporadic	stable	stable	stable
Candida tropicalis	sporadic	absent	absent	absent	sporadic	absent	absent	absent
Candida utilis	sporadic	absent	stable	absent	sporadic	absent	sporadic	absent
Candida zeynoides	sporadic	sporadic	absent	sporadic	sporadic	sporadic	absent	sporadic
Cryptococcus albidus	stable	stable	stable	stable	stable	stable	stable	stable
Cryptococcus laurenti	stable	stable	stable	stable	sporadic	stable	sporadic	stable
Cryptococcus neoformans	sporadic	absent	sporadic	sporadic	sporadic	sporadic	sporadic	sporadic
Hansenula saturnus	stable	stable	stable	stable	stable	stable	stable	stable
Pichia carsonii	stable	absent	stable	absent	stable	absent	stable	absent
Rhodotorula glutinis	stable	stable	stable	stable	stable	stable	stable	stable
Rhodotorula rubra	stable	stable	sporadic	stable	stable	stable	stable	stable
Saccharomyces cerevisiae	stable	stable	sporadic	sporadic	sporadic	sporadic	sporadic	sporadic
Saccharomyces kluyverii	sporadic	absent	sporadic	absent	sporadic	sporadic	absent	absent
Sporobolomyces sp	stable	stable	sporadic	sporadic	sporadic	sporadic	stable	stable
Trichosporon cutaneum	stable	stable	stable	stable	sporadic	sporadic	sporadic	stable
Zygosaccharomyces sp	sporadic	sporadic	sporadic	sporadic	sporadic	sporadic	sporadic	sporadic

tion accumulated in the Szczecin Lagoon. According to Cook et al. (1960) and Dynowska (1997) micro-organisms of the genera *Candida, Rhodotorula, Trichosporon* and *Cryptococcus* are micro-organisms typical of strongly eutrophised water. The origin of most of them may be communal sewage. It is very difficult to explain the lack of expected significant specific differentiation in places selected for the sample collection (Fig. 1). One might draw a conclusion that the Odra river introduces to the lagoon considerable amounts of versatile species which are characteristic of the terrestrial environment (fields, forests) and typical of communal contamination. Thus, the samples coming from the BT-3 site should be characterised by the greatest species diversity. In the other research units far more distant from the mouth of the Odra river the number of species, the terrestrial ones in particular, should gradually diminish. Such a phenomenon was not observed. The causes of the occurrence of certain species only in the sediment have not been mentioned in journals, yet (Table 1). The phenomenon may result from the environmental preferences. Food accessibility is more extensive and therefore yeasts and yeast-like organisms find the sediment more abounding with nutrients than the water. Another reason is that the sediment constitutes the main source of phosphorus (approximately 90%). For the complete development of yeast-like organisms the presence of this element is essential. Phosphorus is a fundamental component for the forming pseudohyphae and thalli of certain fungi from the genus *Candida* (Dynowska and Giełwanowska 1991-1992).

The environment of species from the genus *Candida* may be water, soil, fruit, tree bark, animals and humans (Barnett et al. 1990). According to Dynowska (1997) some species of this genus may point to the level of the environmental contamination and may be a health hazard. It is assumed that 80% of mycotic infections are caused by *Candida albicans* (Zaremba and Borowski 1997*).* It is interesting that the presence of this species in the samples taken from the lagoon was not confirmed. Among budding yeasts this microorganism is the most frequently isolated fungus from reservoirs characterised by a considerable level of fertilisation (Dynowska 1997). Hinzelin and Block (1985) determined that the number of fungi from the genus *Candida* was an indicator of the faecal and sewage contamination of the water and the presence of pathogenic micro-organisms in the environment. In chlorinated water the presence of these fungi, primarily *Candida albicans*, as an indicator gave a more reliable assessment of the water contamination than the coli test. In the water and sediment samples from the Szczecin Lagoon this genus constituted the largest percentage of the isolated genera (Fig. 2). The dominant was a cosmopolitan species - *Candida famata.* It is a fungus that develops easily both in the fresh and salt water. Its presence is sometimes associated with human infections (McGinnis 1980, Barnett et al. 1990), though the causality is questioned (McGinnis 1980). The contamination introduced into the Odra river as well as the sewage whose suppliers are the other localities located along the lagoon coast e.g. Świnoujście, considerably affect the growth intensity of this species. Analyses indicated a substantial percentage participation of *Candida famata* in the sediment

at the sites of the biggest influence of municipal and industrial contamination (BT-3 and MO-S) (Fig. 1).

This substantial contamination of the Szczecin Lagoon with communal sewage is also indicated by the percentage of so called „pink yeast", fungi from the genus *Rhodotorula- Rhodotorula rubra* and *Rhodotorula glutinis* (Table 1). It is a group of organisms, equally mentioned as *Candida*, used as an indicator of the environmental contamination. Dynowska (1997) perceives communal and industrial sewage to be the source of these micro-organisms. Additionally, she states that *Rhodotorula glutinis* and *Rhodotorula muscilaginosa* which were not found in the samples should be regarded as faecal and sewage contamination indicators. Simard and Blackwood (1971) proved that the volume of unprocessed sewage affected the growth intensity of the species from the genus *Rhodotorula*, especially *Rhodotorula glutinis*. The presence and development intensity of the *Rhodotorula* genus in the reservoir should be correlated with the amount of organic compounds introduced into the lagoon. Taking into consideration the volume and places of the discarded pollution such a correlation were not established. The Szczecin Lagoon is generally a reservoir rich in organic compounds and the differentiation of their concentration dependent on the polluted area is likely to have had no influence on the quantity of *Rhodotorula glutinis* in the water and sediment. According to Barnett et al.(1990) this species is a form as often isolated from the water as it is from the soil. The spatial quantity differentiation of the fungi will also be correlated with passively transmitted allochtonic structures. The observed considerable participation of *Rhodotorula glutinis*, mainly in the water samples from the W-4 and MOS sites (Table 1) may result from stronger influence of the salt water from the Pomeranian Bay. Fungi from the genus *Rhodotorula* are the sea saprophytes isolated from different depths and relatively far away from the coast (Batko 1975).

Cook et al. (1960) mentioned that the genus – *Trichosporon* is usually found in reservoirs with an increased concentration of sulphates and sulphites. The presence of these compounds is correlated with intensive processes of lignin fermentation. The fungi are then a very useful indicator of vegetable contamination as they particularly point to considerable levels of cellulose sewage (Meyers et al. 1969). The genus *Trichosporon* is frequently described as a keratinphylic ground fungus and also as an allochtonic form in the water reservoirs, especially rich in organic compounds mixed together with municipal sewage (Dynowska 1996). Slavikova and Vadkertiova (1995) draw attention to an intensive development of *Trichosporon cutaneum* in fish breeding ponds. The authors of the work explain that the rapid development of the micro-organisms may be caused by bird faeces and decomposition of dead fish. A considerable number of these micro-organisms (Table 1) was found in the BT-3 site which was situated closest to the mouth of the Odra river (Fig. 1). Its water and sediment samples are the least diluted by the lagoon water. The inflowing water carries municipal and industrial sewage including sewage from the paper plant in Szczecin Skolwin. This plant discharges sewage whose main compounds are cellulose fibers and kaolin. The attention is also drawn to the fact that the sample collection site is in the direct contact with Trzebież which

is a port for fishing boats. Also the MOS site located near Świnoujście (Fig. 1) seems to present optimal conditions for the development of *Trichosporon cutaneum*. These conditions are due to the introduction of unprocessed sewage from a fish processing plant, and sewage pre-purified by fat clarifiers and collectors from a fish meal plant. The sewage is dumped into the Świna river which introduces it to the lagoon (Landsberg-Uczciwek 1995).

Another of analysed genus was *Cryptococcus*. Only two representatives of this genus– *Cryptococcus laurenti* and *Cryptococcus albidus* were discovered to be present in the samples taken from the water and sediment of the Szczecin Lagoon (Table 2). Views concerning the correlations of the occurrence of species from this genus depending on the water purity are contrasting. Slavikova and Vadkertiova (1995) suggest that *Cryptococcus laurenti* is closely associated with the clean, offshore sea water. However, some authors of works on mycological subjects (Rosa et al. 1995) claim that the occurrence of this micro-organism is associated with contaminated water. In the aquatic areas heavily burdened with sewage, including Szczecin Lagoon an intensive development of the species *Cryptococcus albidius* (Table 1) is observed. This is noted by Rosa et al. (1995), with special emphasis on the substantial quality domination of this species in eutrophised water constantly provided with communal and industrial contamination (Kwaśniewska 1998).

Presented data show that the specific differentiation of yeasts and yeast-like organisms is characteristic to the eutrophised fresh water reservoirs. One may assume that they can play a significant role in the elimination of the contamination discharged into the Szczecin Lagoon. Also a considerable volume of biogenes is introduced into the water that flows into the reservoir. According to Jayasree and Saramma (1996), *Candida parapsilosis* and *Debaryomyces hansenii*, which has been found in the samples taken from the Szczecin Lagoon, take part in the biodegradation of the organophosphoric pesticides. These compounds are used as the only source of phosphorus. The ability of assimilation of this element is dependent on the specific features of fungi (Naik et al. 1982). As far as sewage elimination is concerned, the role of yeasts and yeast-like organisms is more often realized. They use difficult-to-decompose compounds occurring in industrial contamination as growth substrates. Grabińska-Łoniewska et al. (1996) pointed out that: *Candida famata*, *Candida sake*, *Candida tropicalis*, *Candida utilis*, *Rhodotorula rubra*, *Trichosporon cutaneum* and *Yarrowia lipolytica* (*Candida lipolytica*) have an ability to use aromatic hydrocarbons as the only source of carbon. Moreover, these micro-organisms are characterised by a relatively large resistance to high concentrations of heavy metals and an ability to incorporate them into their own metabolism (Rosa et al. 1995). It is a common fact that the Odra river introduces to the reservoir the following: 198,0 tons of Pb, 182,0 tons of Cu, 20,4 tons of Cd, 14,6 tons of Hg, and 1039 tons of Zn (Heybowicz and Rybiński 1998). The resistance to these metals depends mostly on their type and concentration, as well as the species which are influenced by them (Jayasree and Saramma 1996). According to the research done by Rosa et al. (1995), it is suggested that species isolated from the Szczecin Lagoon: *Aureobasidium pullulans, Candida famata*, *Cryptococcus albidius*, *Crypto-*

coccus laurenti, *Rhodotorula rubra* and *Trichosporon cutaneum* are also factors in the elimination process of heavy metals in this environment.

The specific and quantity differentiation of yeasts and yeast-like organisms may point out concentration and a type of discharged sewage. First and foremost, it is essential to underline the substantial role of these forms in the elimination of sewage by an active accumulation and mineralisation processes, especially the sewage introduced into the Szczecin Lagoon, the Pomeranian Bay and the Baltic Sea.

SUMMARY

On the basis of analyses of the water and bottom sediment mycoflora of the Szczecin Lagoon one ascertained:

1. Occurrence of 24 species classified as yeasts and yeasts-like organisms.
2. 21 species were common for the water and sediment samples, 1 was found only in the water, 4 were present only in sediments.
3. The most often isolated yeasts at all stations in period of investigations were *Candida* and *Rhodotorula*. Species differentiation of isolated strains from samples of bottom sediments show that the most frequently identified species were *Candida famata* and *Rhodotorula glutinis*. The majority of isolations in the water samples was *Rhodotorula glutinis*.
4. Species constantly present in examined micocenoses were *Candida famata*, *Cryptococcus albidus*, *Rhodotorula glutinis*, *Rhodotorula rubra*, *Trichosporon cutaneum* and *Saccharomyces cerevisiae*. The other forms of yeasts were sporadic in the studied environment.

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ZRÓŻNICOWANIE GATUNKOWE DROŻDŻY I GRZYBÓW DROŻDŻOPODOBNYCH W WODZIE I OSADACH DENNYCH ZALEWU SZCZECIŃSKIEGO

Streszczenie

Celem pracy była jakościowa ocena występowania drożdży i grzybów drożdżopodobnych w wodzie i osadach dennych Zalewu Szczecińskiego.

Na podstawie przeprowadzonych analiz mykoflory wód i osadów dennych Zalewu Szczecińskiego stwierdzono:

1. Występowanie 24 gatunków grzybów należących do drożdży właściwych i grzybów drożdżopodobnych.
2. W próbach wody i osadów dennych wspólnych dla ocenianych środowisk było 21 gatunków, 1 występował jedynie w wodzie, 4 były obecne wyłącznie w osadach.
3. Na wszystkich stacjach badawczych w okresie przeprowadzania badań najczęściej izolowanymi rodzajami były *Candida* i *Rhodotorula*. Zróżnicowanie gatunkowe szczepów wyizolowanych z prób osadów dennych wskazuje, że najczęściej identyfikowanym gatunkiem była *Candida famata* i *Rhodotorula glutinis*, w próbach wody natomiast ilościowo przeważała *Rhodotorula glutinis*.
4. W badanych mykocenozach wyróżniono na podstawie częstotliwości występowania gatunki stale obecne - *Candida famata*, *Cryptococcus albidus*, *Rhodotorula glutinis*, *Rhodotorula rubra*, *Trichosporon cutaneum* i *Saccharomyces cerevisiae* oraz formy towarzyszące których obecność była sporadyczna.