THE EFFECT OF CONCENTRATION OF SODIUM CHLORIDE ON ENZYMATIC ACTIVITY OF *Candida* sp.

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Abstract: The effect of various concentrations of salt (0%, 0.7%, 1.5% and 3.6% of sodium chloride) on enzymatic activity of *C. famata, C. lipolytica, C. colliculosa, C. holmi* and *C. albicans* isolated from the Szczecin Lagoon was tested. The activity revealed low enzymatic activity of *Candida* isolated deceasing with the rise in concentration of NaCl in the medium. *C. colliculosa* expressed the lowest hydrolytic activity regardless of the salt concentration in the medium. The highest enzymatic activity at 0% of NaCl was demonstrated by *C. lipolytica* (15.16 nmol) and *C. albicans* (14.79 nmol).

Key words: enzymatic activity, yeasts

INTRODUCTION

Biogeochemical cycles and the flow of energy in the aquatic environment depend mostly on micro-organisms present due to a key role they play in production of organic matter and its mineralization. Reduction of components essential for living organisms is based on physicochemical processes e.g. photolysis [Aquatic... 1993] or enzymatic decomposition. At any given moment several enzymes are produced by a cell regardless of its growth stage or the environmental conditions. The others known as inducible enzymes are produced in a strictly controlled way in response to the presence of their inducers. Regulation of the metabolic pathways is controlled at the level of enzyme synthesis or modifications of their activity. The variety and the total amount of DOM (dissolved organic matter) is a result and indicator of enzymatic activity in aquatic eco-systems. It is an important energy source for the micro-organisms and a starting point for a microbiological loop [Lampert and Sommer 1996]. Enzymatic activity is also determined by physicochemical factors, e.g. temperature, pH, salt concentration. Recurrent fluctuations of chloride concentrations in aquatic ecosystems occur most frequently in estuaries. Consequently, our study was undertaken in order to analyse the effects of changes of NaCl concentrations on hydrolytic activity of yeasts and yeast-like fungi isolated from the secondary estuary. These micro-organisms form a significant part of

biodiversity as they play a leading role in the decomposition of macromolecule substrates and they are generally the first link of DOM breakdown which creates medium for subsequent conversions carried out by bacteria [Rheinhaimer 1987].

MATERIALS AND METHODS

Dominant species of yeasts: *C. famata*, *C. lipolytica*, *C. colliculosa*, *C. holmii*, *C. albicans*, isolated from the Szczecin Lagoon, were examined. The Szczecin Lagoon and its strains comprise the secondary estuary. Yeasts were isolated on a synthetic medium at 22°C. The strains were identified according to Barnett et al. [1990] key. Tests ID32C (bioMerieux) were used to confirm the species and genus identification.

The effects of various concentration (0%, 0.7%, 1.5%, 3.6%) of sodium chloride were tested on a salt-shock liquid medium recommended by Lodder [1971]. Cultures were shaken continuously and incubated for 72 hours at 22°C then enzymatic activity of analysed strains was estimated.

The hydrolytic activity was conducted with the API ZYM set (bioMerieux). The results were measured after addition of ZYM A and ZYM B reagents. Interpretations of enzymatic activity were based on a settled range of nanomoles of a decomposed substrate: 0 nmol – activity not observed, 0-20 nmol – low activity, > 20 nmol – high activity.

RESULTS

On the basis of our studies it was stated that the strains of *Candida* sp. isolated from the Szczecin Lagoon demonstrated low average enzymatic activity. The increase of salt concentration corresponded with reduction of average enzymatic activity. The lowest hydrolytic activity was found in *C. colliculosa* regardless of the salt concentration in the medium (Fig. 4). *C. lipolytica* and *C. albicans* showed the highest activity for 0% of sodium chloride – 15.16 nmol and 14.79 nmol, respectively (Fig. 2, 5). *C. colliculosa* turned out to be the most susceptible to sodium chloride. Significant decrease of its enzymatic activity was observed at 0.7% of NaCl (Fig. 4) whereas for the other strains it occurred at 1.5% (Fig. 1, 2, 3) and in case of *C. albicans* at 3.6% (Fig. 5).

The analysis of activity of particular enzymes showed that the largest amount of substrate was broken up by chemotrypsin without regard to the salt concentration. The same as in case of acid phosphatase its enzymatic activity did not change significantly when the salt concentration increased (Tab. 1). Glycoside hydrolases proved to be the most sensitive group of enzymes (Tab. 2).
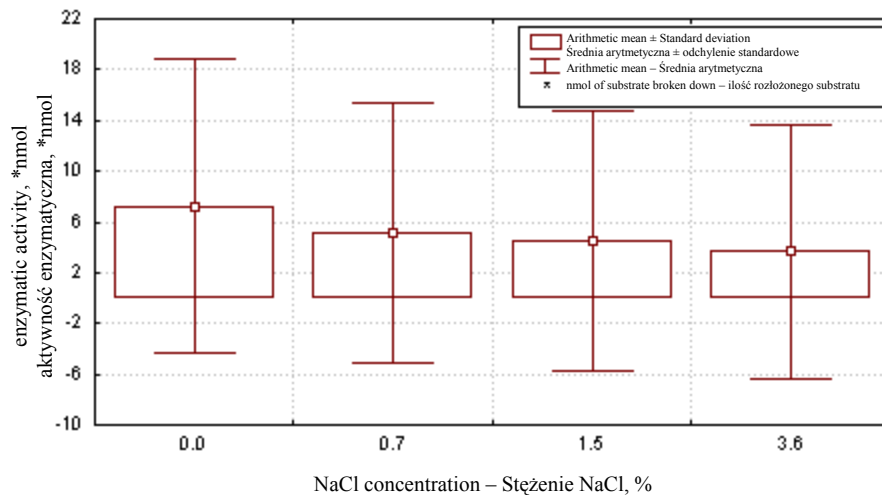
Fig. 1. Enzymatic plasticity of *Candida famata* under different NaCl concentrations
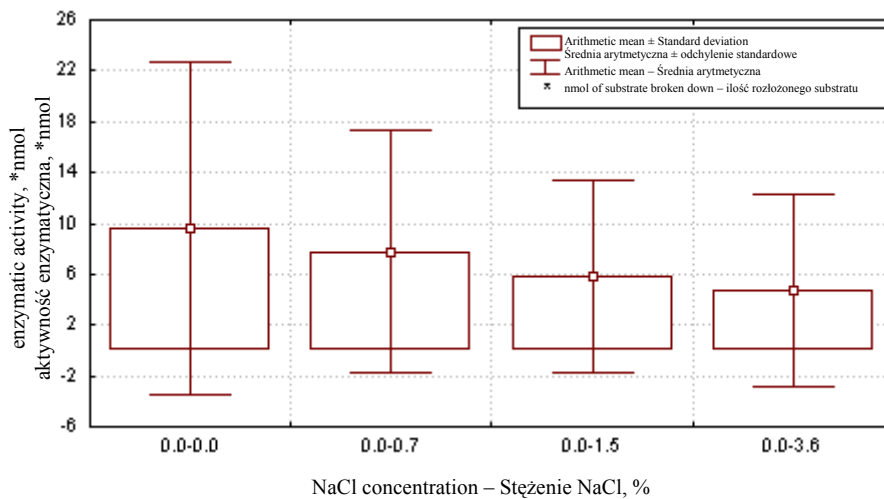Rys. 1. Wpływ stężenia NaCl na plastyczność enzymatyczną *C. famata*

Fig. 2. Enzymatic plasticity of *Candida lipolytica* under different NaCl concentrations
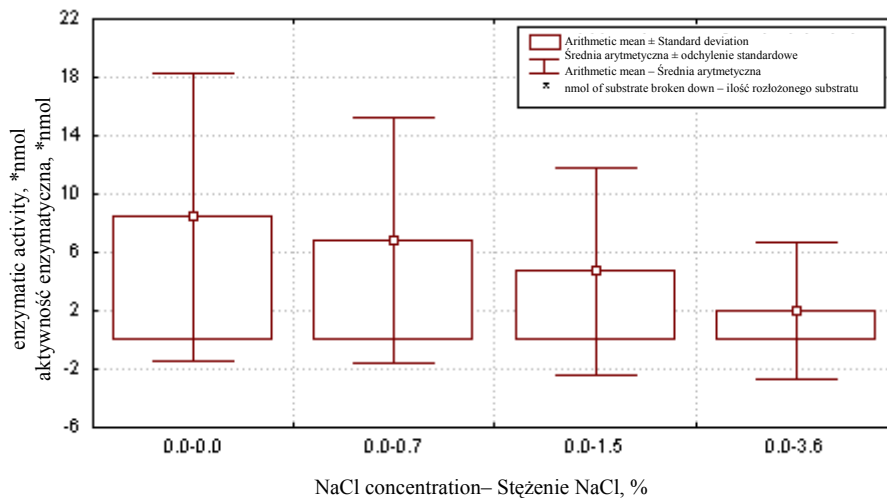Rys. 2. Wpływ stężenia NaCl na plastyczność enzymatyczną *C. lipolytica*

Fig. 3. Enzymatic plasticity of *Candida holmi* under different NaCl concentrations
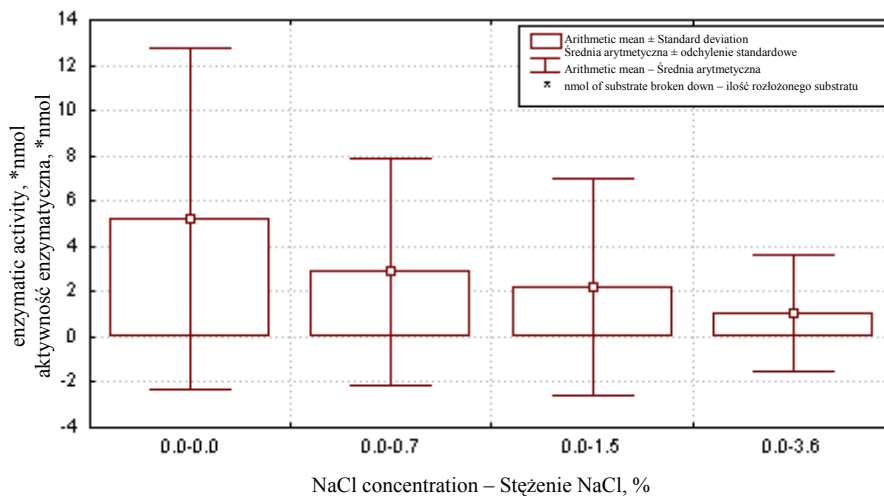Rys. 3. Wpływ stężenia NaCl na plastyczność enzymatyczną *C. holmi*

Fig. 4. Enzymatic plasticity of *Candida colliculosa* under different NaCl concentrations
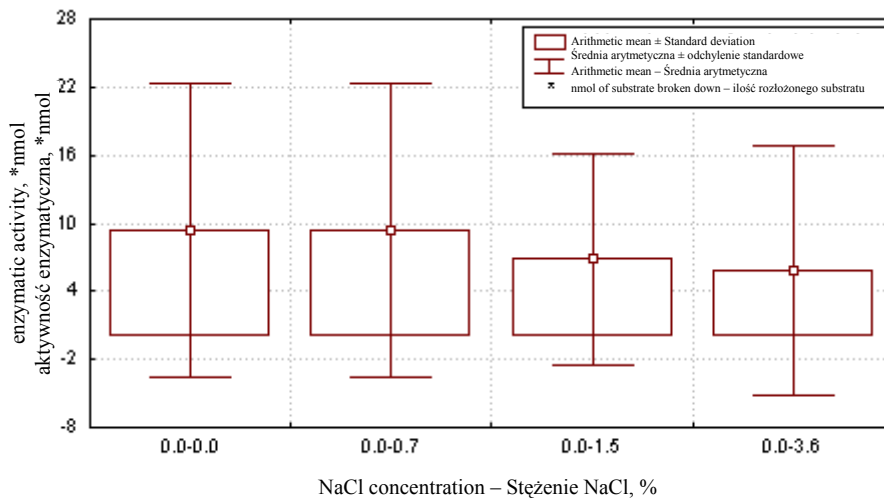Rys. 4. Wpływ stężenia NaCl na plastyczność enzymatyczną *C. colliculosa*

Fig. 5. Enzymatic plasticity of *Candida albicans* under different NaCl concentrations
Rys. 5. Wpływ stężenia NaCl na plastyczność enzymatyczną *C. albicans*

DISCUSSION

Three straits of Piana, Świna and Dziwna merge the Baltic Sea with the Szczecin Lagoon. The distinctive ecosystem of an estuary is mainly the result of a mixing of the Świna and the Piastowski Canal waters [Zalew... 1994]. The impressive quantitative flow of tidal currents from the sea to the Szczecin Lagoon occurs generally in autumn and winter season supplying 60% of its water. It is caused by strong north and north-west winds which induce superficial tidal currents. This phenomenon known as a windward flood limits the flow of the Odra river which supplies 97% of freshwater [Monografia... 1991]. Since fresh inland waters and salt sea tide combine, the ecosystems is characterised by a great variety of physicochemical conditions. The major limiting factor of the ecosystem is salinity which varies greatly even at the same spot between high and low tides. Mycoflora quickly adapt to such conditions due to their ecological flexibility. As stated by Bogusławska-Wąs and Dąbrowski [2000 b] yeasts and yeast-like fungi present in the Szczecin Lagoon are components of mycoflora typical of freshwater ecosystems at a very high trophic level. Therefore, the range of adaptation of yeast and yeast-like fungi to cyclic changes caused by salinity and desalinity should be particularly considered.

A cell wall of yeasts and yeast-like fungi plays an important role in regulation of intracellular pressure. Chemical and enzymatic analyses show that it consists of two chemically-separate layers. Their common feature are mannans deposited in a rigid net of glucans [Wolska-Mitaszko 1988]. The average percentage of particular components in the cell wall of yeasts and yeast-like fungi differs widely considering the fungus species, its growth stage and the kind of fungus cells. Polysaccharide structures of the cell wall is a medium for many enzymes, mostly hydrolases [Kockova-Kratochvilova 1990]. Enzymes are deposited in an outer layer which is composed of a mannan-protein complex.

Table 1. The effect of concentration of NaCl on average activity of *Candida* sp.
Tabela 1. Wpływ stężenia NaCl na średnią aktywność *Candida* sp.

Strains Szczep	NaCl concentr. Stężenie NaCl %	AlP	Ph	Est	El	Lip	Leu	Val	Cys	Try	Chy	AcP	LGa	BGa	BGk	LGl	BGl	Nac	LMa	LFu
Candida famata	0.0-0.0	30.0	0.0	16.2	0.0	15.6	5.0	5.0	0.0	0.0	40.0	15.6	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0
	0.0-0.7	30.0	0.0	12.5	0.0	5.0	0.0	0.0	0.0	0.0	30.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0-1.5	5.0	0.0	15.0	0.0	5.0	0.0	0.0	0.0	0.0	40.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0-3.6	0.0	0.0	5.0	0.0	5.0	0.0	0.0	0.0	0.0	40.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Candida lipolytica	0.0-0.0	40.0	20.0	15.0	5.0	15.0	15.0	2.5	0.0	0.0	40.0	20.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
	0.0-0.7	30.0	20.0	15.0	5.0	10.0	15.0	2.5	0.0	0.0	20.0	20.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
	0.0-1.5	20.0	5.0	12.5	5.0	12.5	2.5	2.5	0.0	0.0	20.0	20.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
	0.0-3.6	20.0	5.0	10.0	0.0	5.0	0.0	0.0	0.0	0.0	20.0	20.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Candida colliculosa	0.0-0.0	5.0	5.0	15.0	0.0	5.0	20.0	20.0	0.0	0.0	5.0	5.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0
	0.0-0.7	2.5	2.5	5.0	0.0	5.0	10.0	5.0	0.0	0.0	20.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0-1.5	0.0	0.0	5.0	0.0	2.5	5.0	5.0	0.0	0.0	20.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0-3.6	0.0	0.0	5.0	0.0	2.5	0.0	0.0	0.0	0.0	10.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Candida holmii	0.0-0.0	5.0	20.0	20.0	0.0	20.0	5.0	5.0	0.0	0.0	30.0	5.0	0.0	0.0	0.0	10.0	20.0	20.0	0.0	0.0
	0.0-0.7	5.0	20.0	20.0	0.0	5.0	5.0	5.0	0.0	0.0	20.0	5.0	0.0	0.0	0.0	5.0	20.0	20.0	0.0	0.0
	0.0-1.5	5.0	5.0	5.0	0.0	2.5	0.0	2.5	0.0	0.0	20.0	5.0	0.0	0.0	0.0	5.0	20.0	20.0	0.0	0.0
	0.0-3.6	5.0	2.5	2.5	0.0	0.0	0.0	2.5	0.0	0.0	20.0	5.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0
Candida albicans	0.0-0.0	20.0	5.0	20.0	0.0	30.0	2.5	5.0	0.0	0.0	30.0	5.0	0.0	0.0	0.0	20.0	0.0	40.0	0.0	0.0
	0.0-0.7	20.0	2.5	20.0	0.0	20.0	0.0	5.0	0.0	0.0	40.0	5.0	0.0	0.0	0.0	5.0	0.0	40.0	0.0	0.0
	0.0-1.5	20.0	2.5	20.0	0.0	20.0	0.0	2.5	0.0	0.0	20.0	20.0	0.0	0.0	0.0	5.0	0.0	20.0	0.0	0.0
	0.0-3.6	40.0	2.5	20.0	0.0	0.0	0.0	2.5	0.0	0.0	20.0	20.0	0.0	0.0	0.0	5.0	0.0	2.5	0.0	0.0

Enzymes – Enzymy

Table 2. Average enzymatic activity of hydrolases
Tabela 2. Średnia aktywność enzymatyczna hydrolaz

Strains Szczep	NaCl concentr. Stężenie NaCl %	Acting on phosphor. ester Wpływ na estry fosforowe E.C. 3.1.3,4. nmol	Acting on carboxyl. ester Wpływ na estry karboks. E.C. 3.1.1. nmol	Acting on peptide bonds Wpływ na estry peptydowe E.C. 3.4. nmol	Acting on glycosidase bonds Wpływ na wiązania glikozydazowe E.C. 3.2.1. nmol
Candida famata	0.0-0.0	15.2	10.6	16.6	3.3
	0.0-0.7	16.6	5.8	10.0	0.0
	0.0-1.5	8.3	6.6	13.3	0.0
	0.0-3.6	6.6	3.3	13.3	0.0
Candida lipolytica	0.0-0.0	26.6	11.7	19.2	3.3
	0.0-0.7	23.3	10.0	12.5	3.3
	0.0-1.5	15.0	10.0	8.3	3.3
	0.0-3.6	15.0	5.0	6.6	3.3
Candida colliculosa	0.0-0.0	5.0	6.7	15.0	6.7
	0.0-0.7	3.3	3.3	11.7	0.0
	0.0-1.5	1.7	2.5	10.0	0.0
	0.0-3.6	0.8	2.5	3.3	0.0
Candida holmii	0.0-0.0	10.0	13.3	13.3	16.7
	0.0-0.7	10.0	8.3	10.0	15.0
	0.0-1.5	5.0	2.5	7.5	15.0
	0.0-3.6	4.2	0.8	7.5	0.0
Candida albicans	0.0-0.0	10.0	16.7	12.5	20.0
	0.0-0.7	9.2	13.3	15.0	15.0
	0.0-1.5	14.2	13.3	7.5	8.3
	0.0-3.6	20.8	6.7	7.5	2.5

Reduction of average enzymatic activity corresponding with the increase of salt concentration was observed in our studies (Fig. 1, 2, 3, 4, 5). It suggests that in the outermost layer, which is the most susceptible to chemical and enzymatic interactions, the partial inactivation of its enzymes may occur [Wolska-Mitaszko 1988]. Glucan molecules are more stable components of the cell wall. They protect against lysis in a hypotonic fluid and determine the shape of cells. The effects of different salt concentrations on polysaccharides in the cell wall were analysed by Katoda et al. [1991]. The authors cultured *Zygosaccharomyces rouxii* in media with 20% and 0% of NaCl. Comparative analysis of the cell wall proved the changes within chemical linkage of glucans and mannans. As stated by Wolska-Mitaszko [1988] structural changes of mannoproteins may lead to reduction of survival rate of some micro-organisms. In our studies salt concentrations applied to the yeasts were within the range of their tolerance to salt-stress. It is proved by a level of acid phosphatase (Tab. 1). According to Kockova-Kratochvilova [1990] it is typical of the active outgrowth of *S. mellis* or halotorelant yeasts and yeast-like fungi. It follows from our results that the changes of salt concentration comparable to the environmental fluctuations either stimulate the growth of *C. albicans* or are neutral (Tab. 1). This mannoprotein enzyme is present in the cell wall, periplasm or may be secreted to the environment. Activity of basic phosphatase found in the tonoplast or spherosomes was also estimated. Theory of the common regulation of both enzymes was described for *S. cerevisiae* by Tohe and Oshima in 1971 [Kockova-Kratochvilova 1990]. Considering enzymatic activity of investigated strains

such correlation may be observed in *C. lipolytica*, *C. holmii* and *C. albicans* (Fig. 2, 3, 5). The non-specific enzymes mentioned above can break up hydrolytically phosphomono-esters, e.g. glycerophosphorates and phenylphosphorates [Enzymes... 1993]. Particular attention should be paid to low enzymatic activity of naftol-AS-BI-phosphohydrolase declining with the rise of NaCl concentration. Our results covering the differentiation of activity of phosphohydrolases (Tab. 2) correspond to the results of analysis of sea-water microflora carried out by Wcisło and Chróst [1997]. The stability of phosphohydrolases is critical in analysis of *Candida* sp. as accessibility to phosphorus in the environment is a major factor which determines the growth ability and enables forming of mycelium [Dynowska and Giełwanowska 1991-1992].

Proteins are an elementary component of organic matter in aquatic ecosystems. They constitute about 50% of POM and 1-10% of DOM as stated by Wcisło and Chróst [1997]. Microbiological proteolytic enzymes are responsible for splitting protein mac-romolecules to amino acids used up to manufacture cell proteins. The efficiency of a particular enzymatic group should be an indicator of protein utilisation. Our experi-ments revealed that proteolytical activity of tested strains is characterised by its narrow spectrum and low level of activity of aminopeptidases (Tab. 1). Along with the increas-ing NaCl concentration, inactivation of this group of enzymes was observed. Mahmoud et al. [1982] established the correlation between NaCl concentration, which is a regula-tor of water activity (a_w), and the amount of proteins in *S. cerevisiae*. The amount of proteins in a yeast cell lowered together with the increasing salt concentration [Mah-moud et al. 1982] what might be an indicator of proteolytic activity of tested micro-organisms. In our work the increase of NaCl concentration caused reduction of activity of valine and leucine arylamidases. In this case in natural conditions the biosynthesis pathway in which both enzymes are responsible for separating an aryl group from an amid group of a particular amino acid may be regulated by repression. Our research showed that all strains presented high activity of chymotrypsin not modified even by various salt concentrations applied. According to Haard and Simpson [2000] this en-zyme is a typical catalytic protein found in sea organisms living at 36‰ of salt concen-tration. Chymotrypsin was characterized by high catalytic activity and its hydrolytic interactions with different protein bonds were not interfered. Chymotrypsin is an intra-cellular enzyme so the changes in salt concentration which occurs in the environment are modified by physiological osmoprotective cell mechanisms. The researchs carried out by Adler [1994] suggests that the cell adaptation to high salt concentration depends on the efficient method of elimination of Na^+ from cytosol. This process is determined by changes in the increase of production and accumulation of glycerol, which is an internal osmotic regulator [Hernandez-Saavedra et al. 1995]. The cell may increase the amount of stored glycerol only if there is a particular substrate, e.g. glucose, in the envi-ronment [Andre et al. 1991]. Analysis of amylolytic activity shows that *C. famata*, *C. colliculosa*, *C. holmii*, *C. albicans* can use up starch if the salt is absent in a medium. Efficiency of this hydrolytic process declines or terminates along with the increase of NaCl (Tab. 1). Decline or lack of the possibilities to utilise starch may be compensated by the activity of β-glucosidase which concentration is not influenced by salt concentra-tion. It is one of the cellulases which are capable of splitting cellulose into glucose. This metabolic pathway may play a key role in building up of glucose in the estuary ecosys-tem. Cellulose is the most abundant organic substance found in nature. It comprises about 50% of biomass produced in photosynthesis [Schlegel 1996].

The second most common polysaccharide is chitin. Results of our research showed the stability of N-acetyl-β-glucoamidase in the environments of varied NaCl concentrations. The presence of enzymes which split cellulose and chitin linkage by hydrolysis and are not sensitive to seasonal changes of salt concentrations will significantly influence the reduction of stored organic matter in the Szczecin Lagoon. It is of crucial importance as according to Bogusławska-Wąs and Dąbrowski [2000 b] yeasts and yeast-like fungi isolated from the Szczecin Lagoon are characterised by a narrow spectrum and low amylolytic activity.

Glycerol plays an essential role in osmoregulation. Its production in combination with various fatty acids may be induced by the presence of fat in the environment. Their amount is regulated by plant and animal productivity and the level of sewage pollution. Lipases start the breakdown of fat into fatty acids and glycerol. Their activity changes depending on species and the salt concentration in a medium (Tab. 1). Undoubtedly, despite the species classification, the source of isolation of tested strains influenced their lipolytic activity significantly [Kockova-Kratochvilova 1990, Malik and Freitas 1991]. In case of gradual reduction of lipolytic activity caused by an increasing NaCl concentration, acid and basic phosphatases can be included in the lipolytic pathway. The rate of substrate split up by both enzymes does not change when the salt concentration increases.

Comparison of average enzymatic activity of *Candida* sp. shows the gradual reduction of hydrolytic efficiency along with the increase of sodium chloride. The lowest hydrolytic activity was found for *C. colliculosa*. As stated by Barnett et al. [1990] it is not often isolated from the aquatic environment, especially from the freshwater ecosystems. It is usually found on vegetable products. The essential enzymatic changes are noticeable at 0.7% of NaCl concentration (Tab. 1) and correlated with the pace of micro-organism multiplication [Bogusławska-Wąs and Dąbrowski 2000 a]. For the rest of *Candida* strains the level of enzymatic activity at 0% of NaCl was comparable. Hydrolytic activity lowered gradually and only for *C. holmii* it decreased dramatically at 3.6%. The highest enzymatic flexibility and the stable pace of biomass productivity was found for *C. albicans* and *C. famata* (Tab. 2). They are considered to be ubiquitous for fresh and sea waters and proposed to be classified as bioindicators characteristic of clean waters.

CONCLUSIONS

1. The increase in concentration of sodium chloride causes reduction of enzymatic activity of *Candida* strains isolated from the Szczecin Lagoon.

2. *C. colliculosa* expresses the lowest hydrolytic activity regardless of NaCl concentration in the medium.

3. The highest enzymatic activity at 0% NaCl was demonstrated by both *C. lipolytica* and *C. albicans*.

4. *C. colliculosa* is most sensitive to NaCl influence.

5. Chemotrypsin is the enzyme less sensitive to NaCl.

6. Glucosidases are the enzymatic group most susceptible to NaCl concentration.

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WPŁYW STĘŻENIA CHLORKU SODU NA AKTYWNOŚĆ ENZYMATYCZNĄ GRZYBÓW Z RODZAJU *CANDIDA*

Streszczenie: Badano wpływ różnych stężeń soli (0%, 0,7%, 1,5% i 3,6% NaCl) na aktywność enzymatyczną – *C. famata, C. lipolytica, C. colliculosa, C. holmi* i *C. albicans*, wyizolowanych z Zalewu Szczecińskiego. Ocenę aktywności hydrolaz

powyższych szczepów przeprowadzono za pomocą zestawu API ZYM (bioMerieux). Na podstawie badań stwierdzono, że wyizolowane szczepy *Candida* charakteryzuje niska aktywność enzymatyczna, która spada wraz ze wzrostem stężenia chlorku sodu w podłożu. Ustalono, że najniższą aktywność hydrolityczną wykazuje *C. colliculosa* bez względu na zawartość soli w podłożu. Najwyższą aktywność enzymatyczną przy stężeniu 0% NaCl stwierdzono w przypadku *C. lipolytica* – 15,16 nmol i *C. albicans* – 14,79 nmol.

Słowa kluczowe: aktywność enzymatyczna, drożdże

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