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# Comparison of enzymatic activity of selected yeast-like fungi isolated from lakes and astatic reservoirs

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The strains of *Candida albicans* and *Trichosporon beigelii* isolated from astatic reservoir show a higher enzymatic activity than the strains isolated from lake. The results obtained confirm earlier suspicions that the morphological differentiation of micro-fungi living in different water reservoirs is generally accompanied by physiological differences, which are, to the largest extent, reflections of the chemical composition of the environment.

**Key words:** yeast-like fungi, enzymatic activity.

## INTRODUCTION

Yeast-like fungi form a permanent element of microflora of inland waters and every increase in their numbers directly related to enzymatic activity indicates a progress in the process of eutrophication and human pressure.

Some years of research on presence of yeast-like fungi in different types of water allow a statement that the largest differentiation in species of such fungi is encountered in lakes and astatic reservoirs rich with organic and inorganic matter. Analysing their ecological properties in astatic reservoirs, it was noticed that higher degree of morphological and physiological differences were observed among isolated fungi of the same species than in case of the same fungi isolated from lakes or rivers (Dynowska 1995).

It was decided to compare enzymatic activities of a number of strains of *Candida albicans* Berkhout and *Trichosporon beigelii* Vuillemin isolated from a selected lake and an astatic reservoir where earlier detailed mycological assessment had been completed. The objective of that work was to confirm the results of reconnaissance studies suggesting a higher enzymatic activity of fungi from astatic reservoirs than from lakes (Dynowska 1995).

## MATERIAL AND METHODS

The research material consisted of strains of *Candida albicans* and *Trichosporon beigelii* isolated from a severely contaminated astatic reservoir and eutrophicated Lake Kortowskie located within the area of Olsztyn, characterised by a high concentration and abundance of yeast-like fungi (D y n o w s k a 1995). Two strains from each reservoir representing extreme physiological characteristics were considered. Using biochemical identification tests by bio-Mérieux (API 20 C, API 20 C AUX) sugar fermenting capacities of the studies strains – the zymogram – and their ability to assimilate carbon from sugars as well as selected nitrogen compounds – the auxanogram – were tested (K u r n a t o w s k a 1995). The initial cultures of fungi were incubated on Sabouraud agar medium with gentamicin and chloramphenicol at 25°C for 48–72 hours.

## RESULTS

As a result of the studies, high level of sugars (GLU, GAL, MAL, SAC, TRE, MEL) fermenting abilities of both strains of *Candida albicans* from the astatic reservoir was confirmed as well as a slightly lower sugars (GLU, GAL, MAL) fermenting ability of the same strains isolated from Lake Kortowskie (Tab. 1). Strains of *Trichosporon beigelii* showed selective abilities of fermenting sugars (GLU, LAC, MAL, SAC, TRE, MEL) without the ability of forming $CO_2$. In case of strain 1 from Lake Kortowskie no fermenting reactions were observed (Tab. 1).

Positive reactions were accompanied by the growth of the fungus [+] accompanied by mild acidification [(+Ag)] or strong acidification and simultaneous formation of $CO_2$ [+Ag].

Based on the results of the hydrocarbons auxanograms it can be said that the ability to assimilate carbon from sugars was manifested by strains 1 and 2 of *Candida albicans* from the astatic reservoir (GAL, GLU, LAC, MAL, SAC) and strain 1 from Lake Kortowskie (GAL, GLU, MAL, SAC) while strain 2 gave no positive reaction in presence of all sugars (with the exception of SAC) (Tab. 2). Strain 1 of *Trichosporon beigelii* from the astatic reservoir assimilated carbon from all tested sugars (GAL, GLU, LAC, MAL, SAC) while the same strain isolated from Lake Kortowskie gave ambivalent results – in presence of GLU and LAC full assimilation occurred while in case of GAL, MAL and SAC the assimilation was only partial (Tab. 2). Strain 1 of *Candida albicans* isolated from the astatic reservoir and from Lake Kortowskie proved to be fully capable of assimilating nitrogen from media containing all studied nitrogen compounds ($KNO_3$, $(NH_4)_2SO_4$, urea, asparagine, peptone).

Table 1

Fundamentals of the zymograms strains *Candida albicans* and *Trichosporon beigelii* isolated from astatic reservoir and lake

| Zymogram | *Candida albicans* | | | | *Trichosporon beigelii* | | | |
|---|---|---|---|---|---|---|---|---|
| | astatic reservoir | | lake | | astatic reservoir | | lake | |
| | strain 1 | strain 2 | strain 1 | strain 2 | strain 1 | strain 2 | strain 1 | strain 2 |
| Glucose (GLU) | + Ag | + Ag | + Ag | + | − | + or − | − | − + or (A) |
| Galactose (GAL) | + (A) | + Ag | + (A) | + | − | − | − | − |
| Maltose (MAL) | + Ag | + Ag | + (A) | + | − + or (A) | − | − | |
| Lactose (LAC) | + | − | − | − | − + or (A) | − + or (A) | − | + (A) |
| Saccharose (SAC) | + (A) | + Ag | − | + (A) | − | − + or (A) | − | − |
| Raffinose (RAF) | − | − | − | − | − | − | − | − |
| Trehalose (TRE) | + | + Ag | − | − | − + or (A) | − | − | − |
| Melibiose (MEL) | + Ag | + Ag | − | − | − + or (A) | − | − | − |

Symbols: + the growth of the fungus, − absent the growth, A only acidification, (A) mild acidification, Ag strong acidification and formation of $CO_2$

Table 2

Fundamentals of the hydrocarbons auxanograms (A) et nitrogenous (B) strains of fungi isolated from astatic reservoir and lake

| Auxanogram | | *Candida albicans* | | | | *Trichosporon beigelii* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | astatic reservoir | | lake | | astatic reservoir | | lake | |
| | | strain 1 | strain 2 | strain 1 | strain 2 | strain 1 | strain 2 | strain 1 | strain 2 |
| A | Galactose | + | + | + | − | + | + | + or − | + |
| | Glucose | + | + | + | − | + | + | + | + |
| | Lactose | + or − | + | − | − | + | + | + | − |
| | Maltose | + | + | + | − | + | + | + or − | + |
| | Saccharose | + | + | + | + or − | + | | + or − | + |
| B | $KNO_3$ | + | + or − | − | − | + | + | − | − or + |
| | $(NH_4)_2SO_4$ | + | + | + | − | + | − | − | − |
| | Urea | + | + | + | − | + | + | − | − or + |
| | Aspargine | + | + or − | + | + | − | − | − | |
| | Peptone | + | + | + | + | − | − | − | − |

Symbols: + turbidity of the base, − absent of the turbidity

In majority of tests, strains of *Trichosporon beigelii* did not manifest the ability to assimilate nitrogen (strain 1 from Lake Kortowskie) of that ability was poor – strain 2 from Lake Kortowskie on the medium with $KNO_3$ and urea (Tab. 2). Concluding, it can be said that the studied strains of *Candida albicans* and *Trichosporon beigelii* isolated from a astatic reservoir show a higher level of ability to ferment and assimilate than the same strains isolated from Lake Kortowskie.

## DISCUSSION

Yeast-like fungi have a significant place in literature devoted to enzymatic processes (Dennis and Buhagiar 1973; Foda 1980; Sanfacon, Rouillard, Goupil and Heick 1972; Sobieszczański, Rutkowski and Wojtanowicz 1981; Wątorek and Kwiatkowska 1983; Wojtanowicz, Sobieszczański and Rutkowski 1981). They may support processes of heterotrophic denitrification (Grabińska-Łoniewska 1990; Grabińska-Łoniewska and Sláviková 1990; Sláviková and Grabińska-Łoniewska 1989) and have a significant influence upon circulation of matter and energy within different aquatic ecosystems, effectively competing with bacteria (Dynowska 1995).

Besides numerous criteria taken into consideration while determining the systematic position of yeast-like fungi their enzymatic activity played the major role. Already Bouthilet, Neilson, Mrak and Phaff (1949) drew attention to the diagnostic significance of fermenting abilities of yeast-like fungi. Lee (1990) following the biochemical characteristics of different strains of *Candida* and *Kluyveromyces* and comparing their karyotype identified numerous variations differing in chromosomal DNA. Gunasekaran and Hughes (1980) used the fatty acids for diagnostic purposes also. In the latest work devoted to yeast-like fungi, Barnett, Payne and Yarrow (1990) collected suggestions by many researchers, updated and expanded the physiological code based of fermentation of sugars and assimilation of sugars and nitrogen compounds. These latest properties were used in this paper.

The results obtained confirm earlier suspicions that the morphological differentiation of micro-fungi living in different water reservoirs is generally accompanied by physiological differences which are, to the largest extent, reflections of the chemical composition of the environment (Dynowska 1995). The environment influences directly the primary metabolism when intake and use of nutrients are balanced. With accumulation of products of metabolism and further changes in the external conditions the balance is

disturbed and the secondary metabolism is initiated. The way of using the nutrients from the environment by the fungi is based on excretion of enzymes by the fungi into their medium. The enzymes digest the nutrients which, partially or fully decomposed, are then assimilated by the cells of the fungus. The type of adaptive enzyme produced depends on the medium on which the fungus starts to support itself. The enzyme production has the ability of very fast adaptation, generally in the young cells (D y n o w s k a and G i e ł w a n o w s k a 1992). Studies on the process of ageing of yeast-like fungi indicated a clear drop in enzymatic activity in 30 days old cultures as compared to 7—14 days old cultures. Changes in the enzymatic activity were accompanied by clearly visible changes in the morphology of the fungi.

Analysing the enzymatic activity of many fungi, in particular yeast-like fungi, may assess the viability, rate of reproduction and potential for adaptation to changing conditions of the environment. The studied fungi also originated from different environments. Fungi residing in a small reservoir manifested faster metabolism and larger flexibility than the some species coming from lakes. This was most probably related to their greater potential for production of adaptive enzymes required for adaptation to rapid, sometimes drastic changes in astatic reservoirs (D y n o w s k a 1995).

*Candida albicans* and *Trichosporon beigelii* play the most important role in etiology of mycotic infections and result in numerous serious diseases. As a consequence of producing a variety of enzymes, they are able to develop within human tissues. Hydrolytic enzymes damage the cellular membrane leading to the dysfunction and physical damages, which facilitate penetration by pseudohyphae and hyphae. It was shown that *Candida albicans* has very strong proteolytic properties while *Trichosporon beigelii* also keratinolytic properties and there are statistically variable differences in enzymatic activity of different strains correlated to the degree of their invasiveness (K u r n a t o w s k a 1995). And further, the level of their invasiveness depends on the health status of the organism with which the fungus is linked, i.e. its environment (D y n o w s k a 1995). This statement corresponds closely with the results obtained in studies on strains originated from water. Also in this case the environment is the engine for enzymatic activity limiting the scope of its increase or decrease.

Fermenting and assimilating properties of strains of *Candida albicans* and *Trichosporon beigelii* originating from a lake are almost identical as those of strains obtained from clinical material (K u r n a t o w s k a 1995). Differences exist in case of strains isolated from the astatic reservoir. This may result from a significant flow of municipal sewage from Olsztyn into that lake and it is also possible that the strains studied originate from that sewage. This would be in agreement with the principle of continuous circulation of pathogenic fungi between human body and the external environment (D y n o w s k a 1995).

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## Porównanie aktywności enzymatycznej wybranych grzybów drożdżopodobnych izolowanych z jezior i zbiorników astatycznych

Streszczenie

Porównano zdolności fermentacyjne i asymilacyjne kilku szczepów *C. albicans* i *T. beigelii* izolowanych z wybranego jeziora i zbiornika astatycznego. Uzyskane wyniki potwierdzają badania rekonesansowe i wskazują na wyższą aktywność grzybów pochodzących ze zbiorników astatycznych niż z jezior. Świadczy to o szybszej przemianie materii i większej plastyczności adaptacyjnej grzybów zasiedlających drobne zbiorniki w porównaniu z jeziorami.

Zauważono także, że właściwości fermentacyjne i asymilacyjne szczepów pochodzących z badanego jeziora są prawie identyczne jak grzybów uzyskanych z materiałów klinicznych. Być może jest to związane z dużym dopływem do jeziora ścieków komunalnych i nie wykluczone, że badane szczepy pochodzą właśnie z nich. Byłoby to zgodne z założeniem ciągłego krążenia grzybów chorobotwórczych między organizmem ludzkim a środowiskiem zewnętrznym.