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Enzymatic activity of fungi of the genus *Candida* isolated from the skin and the digestive tract in people and from municipal sewage

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Enzymatic activities of *C. albicans*, *C. guilliermondii* and *C. tropicalis* from the skin and the digestive tract and from municipal sewage were compared using the API ZYM system (bio Mérieux). Activities were examined in relation to potential pathogenicity of these fungi, strictly connected with their enzymatic properties, especially the production of proteolytic and lipolytic enzymes, as well as acidic and basic phosphatase. The proteolytic activity was high in strains of *C. albicans* from the digestive tract and from municipal sewage, as well as in *C. guilliermondii* isolated from sewage. The highest lipolytic activity was recorded in the case of *C. guilliermondii* from sewage, and *C. albicans and C. tropicalis* from the digestive tract. Acidic and basic phosphatases and phosphohydrolase were secreted by strains of all species isolated from municipal sewage in the greatest degree (the only exception is a high activity of acidic phosphatase of *C. albicans* and *C. tropicalis* in the digestive tract). A higher enzymatic activity was observed in the same species in municipal sewage than in the human body.

Key words: enzymes, *Candida* sp., digestive tract, municipal sewage.

INTRODUCTION

It was observed upon analysis of fermentative and adaptive characteristics of strains of *Candida* and *Trichosporon* isolated from various aquatic communities that biochemical properties of fungi found in a lake into which municipal sewage was disposed were similar to those of fungi obtained from various clinical material (Dynowska and Biedunkiewicz 1998). A hypothesis was formulated that the majority of fungi occurring in the lake most probably came from municipal sewage and were associated with a continuous circulation of potentially pathogenic fungi between people and the external environment (Dynowska 1995). The skin and the digestive tract, as well as

fungi of the genus *Candida*, which most frequently colonise both ontocenoses in an asymptomatic way, deserve the greatest attention in this context. Therefore, some species considered to be common saprobes, belonging to opportunistic fungi and important aetiological factors in a number of serious fungal infections, were examined.

MATERIAL AND METHODS

Study material comprised three species of the genus *Candida*: *C. albicans*, *C. guilliermondii* and *C. tropicalis*. The fungi were isolated from the skin (mouth area, groin, feet) and from the digestive tract (oral cavity, oesophagus, anus) in patients being treated at the Independent Public Centre for Pulmonology and Oncology in Olsztyn, or obtained from various waters in Olsztyn into which municipal sewage from the city is disposed (D y n o w s k a 1995, 1997).

Species were identified using the standard IDC 32 test (bio Mérieux), in keeping with the instructions of the CHROMagar Candida manufacturer, and on the basis of the analysis of the properties obtained in microcultures on Nickerson agar according to K r e g e r - v a n R i j (1984) and B a r n e t t P a y n e and Y a r r o w (1990).

Enzymatic activities were assayed using API ZYM tests (bio Mérieux 1997). The test contains substrates for the detection of 19 hydrolases (Table 1).

Cultures were grown on Sabouraud agar for a period of 48 hours after which a suspension at a density of 5° on the McFarland scale was prepared. Sixty-five µl of the suspension were plated on API ZYM test strips and incubated at 37°C for 4 hours. After incubation a drop of ZYM A and ZYM B reagents, which produce coloration on culture, was added. Enzymatic activities were determined in nanomols (nmol) of the substrate hydrolysed according to the intensity of the coloration reaction. A three-degree scale was used: 0 nmol = no activity, 0–10 nmol = low activity, 11–20 nmol = average activity, > 20 nmol = high activity. Ten isolates of each species were analysed from each ontocenosis and from sewage, 90 strains altogether.

RESULTS AND DISCUSSION

The observations carried out show that the fungi examined, both from the biological material from people and from municipal sewage, have a very wide enzymatic spectrum that depends not only on the source of origin but also on the type of conditions within the same biocenosis. This not only proves the environmental versatility of fungi, reflected in their biodiversity, but first of all shows their adaptive properties and the flexibility of the adaptive enzyme production.

The majority of the strains analysed secreted as many as 16 of the 19 hydrolases examined, and some of them even 18 enzymes (4 strains of

Table 1
The list of hydrolitic enzymes and their substrates

Enzyme no.	Enzyme name	Hydrolitic substrate
1	Phosphatase alcaline ALP	2-naphtyl phosphate
2	Esterase (C4) Est	2-naphthyl butyrate
3	Esterase lipase (C8) El	2-naphthyl caprylate
4	Lipase (C14) Lip	2-naphthyl myristate
5	Leucine arylamidase Leu	L-leucyl-2-naphthylamide
6	Valine arylamidase Val	L-valyl-2-naphthylamide
7	Cystine arylamidase Cys	L-cystyl-2-naphthylamide
8	Trypsin Try	N-benzyl-DL-arginine-2-naphthyloamide
9	α-chymotripsin α Chy	N-glutaryl-phenylalanine-2-naphthylamide
10	Phosphatase acid AcP	2-naphthylphosphate
11	Naphthol-AS-BI-phosphohydrolase Ph	Naphthol-AS-BI-phosphate
12	α-galactosidase α Ga	6-Br-2-naphthyl-α-D-galactopyranoside
13	β-galactosidase β Ga	2-naphthyl-β-D-glukopyranoside
14	β-glukuronisade β Gk	Naphthol-AS-BI-β-D-glucuronidase
15	α-glukosidase α Gl	2-naphthyl-α-D-glukopyranoside
16	β-glucosidase β Gl	6-Br-2naphthyl-β-D-glukopyranoside
17	N-acetyl-β-glukosamidase Nac	1-naphthyl-N-acetyl-β-D-glucosamide
18	α-mannosidaze α Ma	6-Br-2-naphthyl-α-D-mannopyranoside
19	α-fucosidase α Fu	2-naphthyl-α-L-fucopyranoside

C. albicans and *C. guilliermondii* from the skin and 5 strains *C. guilliermondii* from municipal sewage). Two strains of *C. guilliermondii* obtained from sewage produced the entire set of the enzymes studied (Table 2).

Table 2

Enzymatic activity (nmol) of *Candida albicans*, *C. guilliermondii* and *C. tropicalis* isolated from the skin (I), the digestive tract (II) and municipal sewage (III)

Enzyme	*C. albicans*			*C. guilliermondii*			*C. tropicalis*		
	I	II	III	I	II	III	I	II	III
ALP	**20-[3]-22[5]**	15[4]-18[2]	**25[4]-45[5]**	**10[5]-20[4]**	**10[5]-25[3]**	**35[4]-45[4]**	5[6]-10[4]	**15[4]-20[6]**	**20[5]-30[5]**
AcP	**5[3]-20[3]**	**20[6]-25[4]**	**35[5]-40[5]**	**5[5]-20[5]**	**15[4]-20[4]**	**32[5]-45[3]**	10[4]-15[2]	**20[4]-25[6]**	**20[5]-30[5]**
Ph	**4[6]-7[2]**	**5[3]-10[3]**	**20[4]-32[4]**	5[6]-10[2]	5[2]-10[2]	**35[4]-45[3]**	0[5]-5[5]	5[4]-10[4]	**20[5]-30[5]**
Est	8[5]-10[5]	**25[4]-30[4]**	5[2]-15[5]	**10[2]-20[5]**	**15[4]-20[4]**	**20[6]-35[2]**	5[6]-10[4]	**20[3]-25[3]**	10[5]-15[5]
El	12[6]-15[4]	**22[3]-29(5]**	8[4]-15[2]	10[4]-15[4]	**20[3]-22[5]**	**10[2]-30[5]**	10[6]-15[4]	**20[3]-29[3]**	**10[5]-20[5]**
Lip	**20[4]-28[2]**	**15[5]-23(2]**	**10[4]-35[4]**	**20[5]-25[2]**	8[2]-10[2]	**20[5]-35[3]**	**20[2]-25[6]**	**20[3]-25[3]**	**10[5]-18[2]**
Leu	0[2]-10[5]	**20[4]-35[4]**	**18[6]-20[2]**	0[3]-5[7]	5[4]-10[6]	**15[4]-40[4]**	0[1]-5[5]	**5[5]-25[1]**	10[5]-15[5]
Val	0[2]-8[4]	10[2]-15[5]	10[6]-15[2]	0[2]-5[8]	5[4]-10[6]	**20[4]-35[4]**	0[2]-5[5]	5[5]-15[4]	10[5]-15[5]
Cys	0[2]-8[7]	8[8]-10[2]	10[2]-12[5]	0[1]-5[9]	5[4]-10[6]	**10[4]-25[2]**	0[2]-5[5]	5[5]-10[4]	10[5]-15[5]
Try	0[2]-4[6]	0[4]-6[4]	0[5]-5[5]	0[10]	0[4]-5[6]	0[5]-5[5]	0[10]	0[1]-5[4]	2[10]
αChy	0[2]-4[6]	0[2]-6[2]	0[4]-6[5]	0[10]	0[2]-5[5]	0[8]-5[2]	0[10])	0[1]-5[4]	2[10]
αGa	0[4]-3[6]	0[2]-3[6]	4[5]-5[5]	0[5]-2[4]	0[2]-5[8]	0[5]-4[5]	0[5]-4[5]	0[2]-5[5]	0[6]-2[4]
βGa	0[4]-4[2]	0[4]-3[4]	0[4]-2[6]	0[5]-2[5]	0[2]-5[8]	0[5]-4[5]	0[5]-4[5]	0[1]-5[5]	0[2]-4[4]
βGk	0[1]-4[3]	0[3]-4[5]	0[6]-5[4]	0[5]-2[5]	0[2]-5[8]	0[2]-2[5]	0[5]-4[5]	0[2]-4[6]	0[2]-4[4]
αGl	**0[1]-4[6]**	**0[4]-4[5]**	**0[6]-5[4]**	**0[5]-2[5]**	**0[1]-5[5]**	**0[2]-2[5]**	**0[2]-4[5]**	**0[2]-4[2]**	**0[4]-2[4]**
βGl	0[1]-4[4]	0[1]-8[6]	0[5]-5[5]	0[5]-4[5]	0[1]-5[5]	0[1]-5[5]	0[2]-4[5]	0(2]-4[2]	0[4]-4[4]
Nac	0[10]	0[4]-5[5]	0[7]-4[3]	0[5]-6[2]	0[3]-1[7]	0[1]-5[5]	0[2]-2[5]	0[10]	0[2]-4[5]
αMa	0[10]	0[6]-3[4]	0[10]	0[10]	0[2]-5[4]	0[2]-5[5]	0[10]	0[10]	0[10]
αFu	0[10]	0[10]	0[10]	0[10]	0[10]	0[8]-4[2]	0[10]	0[10]	0[10]

Explanations: strain number; bold – high activity

The analysis of the other species, in the same habitat, clearly shows that the activities of proteolytic enzymes (leucine, valine, cystine), lipolyptic enzymes (esterase, esterase lipase, lipase), acidic and basic phosphatase and phosphohydrolase are comparable in all cases (Figs 1, 2, 3).

An overall higher enzymatic activity of fungi from polluted waters, especially in the case of enzymes that influence the course of infection with these micro-organisms, corroborates the claim that municipal sewage constitutes a highly dangerous reservoir and is a source of a number of serious mycoinfections. Therefore, particular attention should be paid to aquatic ecosystems that are subject to anthropopressure and in which the enzymatic activity of fungi potentially pathogenic for people may be significantly higher than in the human body. This may be related to the nutritive competition between fungi and bacteria the physiological spectrum of which is often narrower than that of fungi (D y n o w s k a 1995).

Already H e d r i c k and S o y g e n c (1967) observed that an inverse proportion existed between the overall number of bacteria and the number of yeast-like fungi in some ecological systems of polluted waters. Simard and B l a c k w o o d (1971 a, b), in an ecological analysis of yeast-like fungi in the St. Lawrence River, to which municipal sewage from the biggest cities in Canada was carried off, also recorded a significant increase in the number and the activity of fungi at the place of sewage disposal. The growth of *C. guilliermondii* and of fungi of the genus *Rhodotorula* was particularly abundant. A high enzymatic activity of the association of *Rhodotorula* spp. and other yeast-like fungi was also observed by B o g u s ł a w s k a - W ą s (1998) in her analysis of the mycoflora of the waters and bottom sediment of the Szczeciński Basin. The results obtained by her correspond to those obtained in this study.

Ample research proves that the enzymatic activity of fungi is an indicator of their pathogenicity and expansiveness in the human body (B i a ł a s i e w i c z, G ł o w a c k a and K u r n a t o w s k a 1996; B a t u r a - G a b r y e l and M ł y n a r c z y k 2000). Basic phosphatase, for instance, impairs the migration of neutrophils to the focus of infection. Lipase is considered to be particularly important at the early stages of infection, as lipids may serve as a source of carbon necessary for the growth and development of infection for fungi of the genus *Candida*. A high hydrolytic activity, especially in the case of proteolytic enzymes (R a y, P a y n e and M o r r o w 1991), may bring about an imbalance in the interaction between the fungus and its host as the body responds to infection, during its development and a possible spread of fungi (B a t u r a - G a b r y e l and M ł y n a r c z y k 2000).

The analysis of the production of hydrolases shows that pathogenicity of the three fungi, highly significant in candidiasis, is similar. This is most clearly demonstrated in the case of *C. albicans* and *C. tropicalis*, whose general and particular enzymatic activities in the digestive tract are almost identical (Table 2, Fig. 3). Both species may live as saprobes on mucous membranes and wait

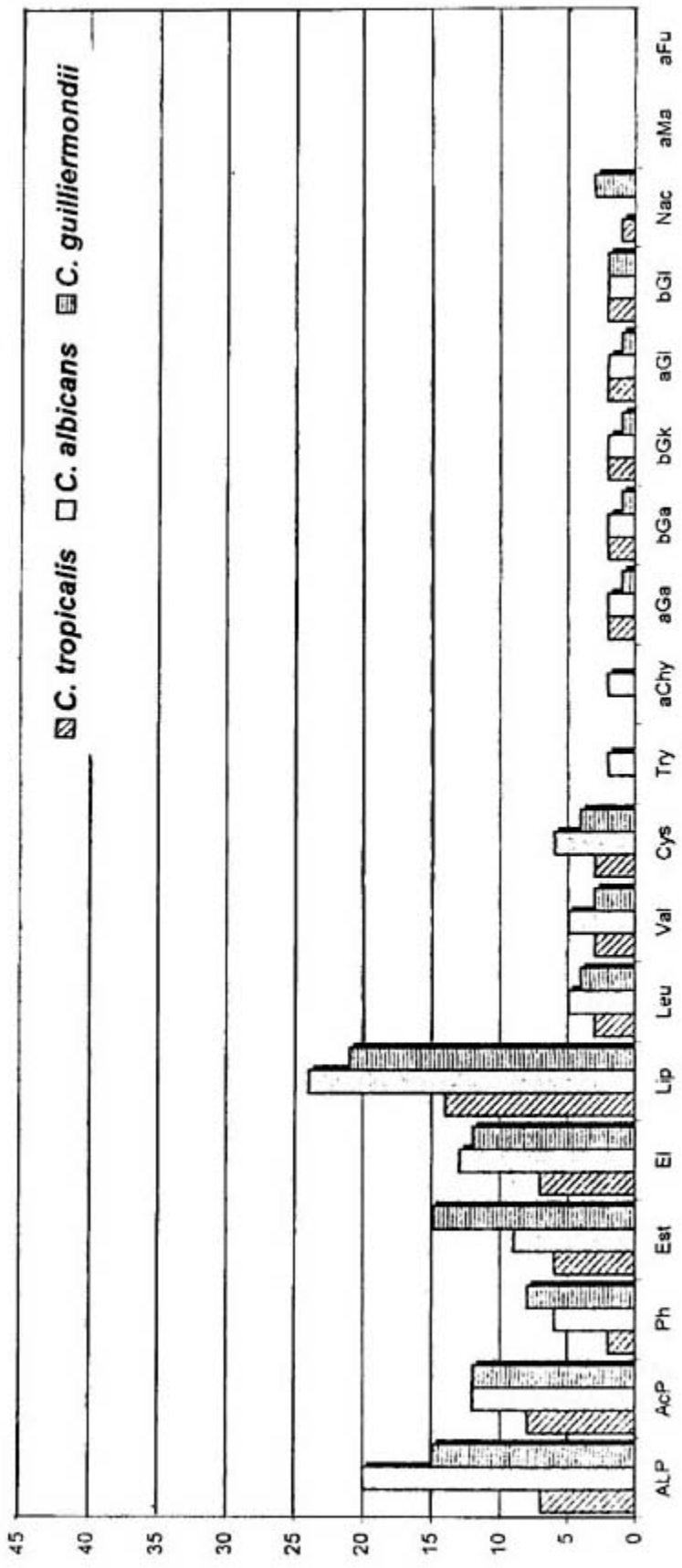

Fig. 1. Average enzymatic activity of *Candida albicans*, *C. guilliermondii* and *C. tropicalis* isolated from the skin

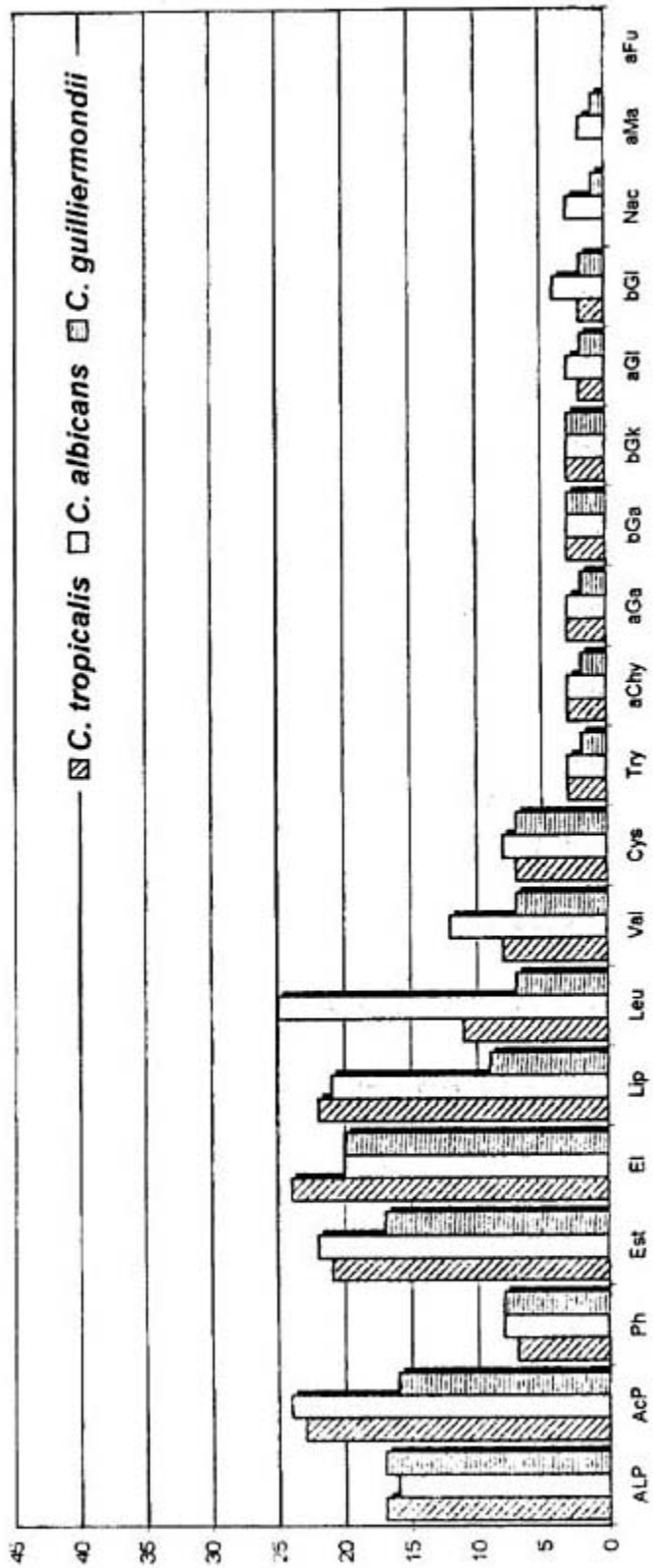

Fig. 2. Average enzymatic activity of *Candida albicans*, *C. guilliermondii* and *C. tropicalis* isolated from the digestive tract

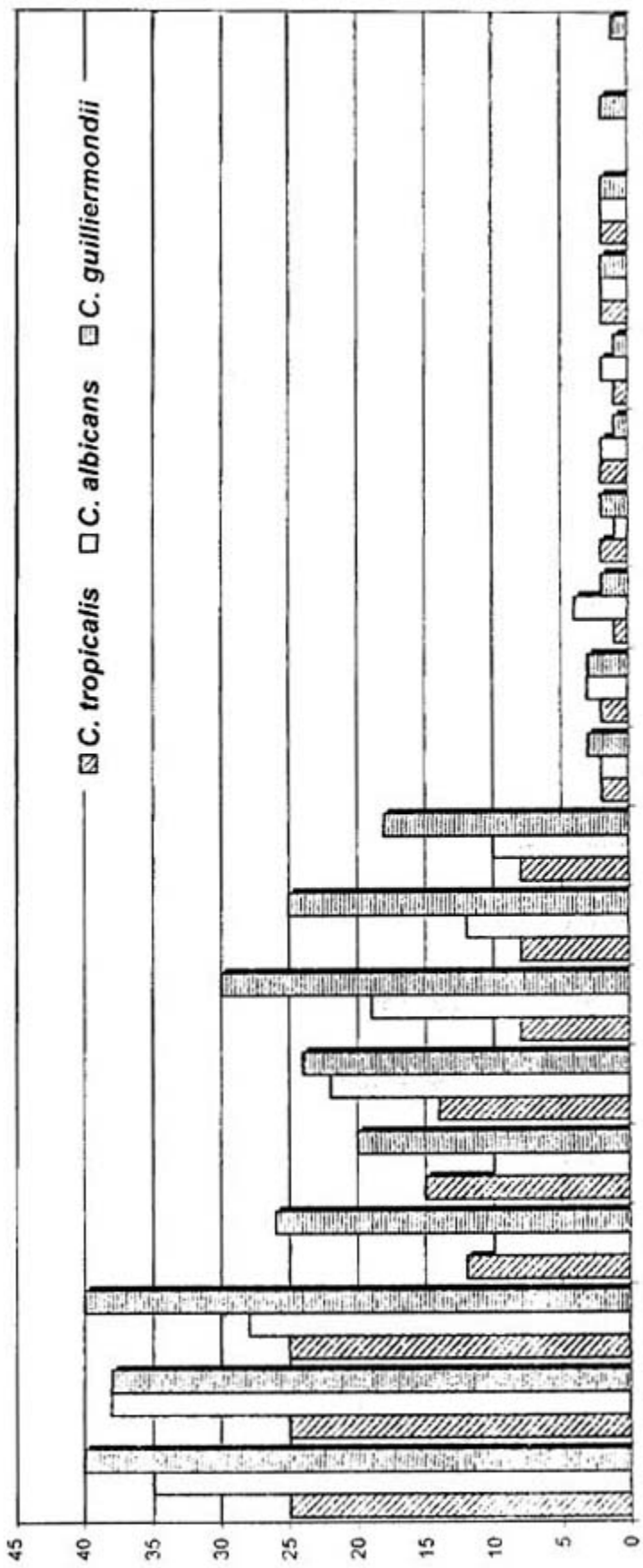

Fig. 3. Average enzymatic activity of *Candida albicans*, *C. guilliermondii* and *C. tropicalis* isolated from municipal sewage

for the moment favourable for the development of infection. A considerably lower enzymatic activity of the strains isolated from the skin (Table 2) shows that the natural protective barrier of this ontocenosis functions well, even in immunocompromised people, such as the patients from whom the fungi were isolated. The skin which is directly exposed to all environmental contaminants is usually characterised by a richness of micro-organisms, which facilitates the preservation of its biological balance. Such a balance is significantly more difficult in the digestive tract in which the enzymatic flexibility of mycoflora often depends on idiosyncratic features of an individual than on the skin.

In the context of the criteria accepted and the scale of the enzymatic activity, strains of *C. albicans* isolated from the digestive tract (leucine up to 35 nmol) and from sewage, as well as strains of *C. guilliermondii* from municipal sewage (leucine up to 40 nmol, valine up to 35 nmol), show a high proteolytic activity. Strains of *C. guilliermondii*, also in sewage (esterase and lipase up to 35 nmol) show the highest activity for lipolyptic enzymes, similarly to strains of *C. albicans* and *C. tropicalis* in the digestive tract (esterase, esterase lipase and lipase over 20 nmol). Acidic and basic phosphatases and phosphohydrolase, however, are secreted to the greatest extent by strains of all species isolated from municipal sewage. A high activity of acidic phosphatase of *C. albicans* and *C. tropicalis* in the digestive tract is the only exception.

It should be noticed that two strains of *C. albicans* obtained from the skin produce carbopeptidases (tripsin, chymotripsin) the activity of which is low, while two strains of *C. guilliermondii*, isolated from sewage, secrete small amounts of α-glucosidases.

Studies on the enzymatic activity of potentially pathogenic fungi not only attempt to determine its value or to ascertain differences in the intensity of the extracellular secretion of hydrolytic enzymes among various fungi, but also endeavour to examine the relationship between the presence of fungi in the body, enzyme secretion and infection occurrence (Batura-Gabryel and Młynarczyk 2000).

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Aktywność enzymatyczna grzybów z rodzaju *Candida* izolowanych ze skóry i układu pokarmowego człowieka oraz ze ścieków komunalnych

Streszczenie

Porównano aktywność enzymatyczną *C. albicans*, *C. guilliermondii* i *C. tropicalis* pochodzących ze skóry i układu pokarmowego oraz ze ścieków komunalnych stosując test API ZYM firmy bio Mirieux. Aktywność tę rozpatrywano w aspekcie potencjalnej patogeniczności tych grzybów, która ściśle skorelowana jest z ich możliwościami enzymatycznymi zwłaszcza w zakresie produkcji enzymów proteolitycznych, lipolitycznych oraz kwaśnej i zasadowej fosfatazy. Wysoką aktywność proteolityczną wykazały szczepy *C. albicans* pochodzące z przewodu pokarmowego i ze ścieków komunalnych oraz szczepy *C. guilliermondii* wyizolowane ze ścieków. Najwyższą aktywność lipolityczną uzyskano także w przypadku *C. guilliermondii* pochodzącej ze ścieków oraz *C. albicans* i *C. tropicalis* z przewodu pokarmowego. Natomiast kwaśne i zasadowe fosfatazy oraz fosfohydrolaza w najwyższym stopniu były wydzielane przez szczepy wszystkich gatunków izolowane ze ścieków komunalnych (wyjątek stanowi wysoka aktywność kwaśnej fosfatazy *C. albicans* i *C. tropicalis* w przewodzie pokarmowym). Generalnie, w ściekach komunalnych te same gatunki charakteryzuje wyższa aktywność enzymatyczna niż w organizmie człowieka.