ACTA MYCOLOGICA
Vol. 32 (1): 99 – 106
1997

Comparative analysis of yeast-like species potentially useful to environmental biotechnology

ANNA GRABIŃSKA-ŁONIEWSKA, ELŻBIETA PAJOR* AND ELENA SLÁVIKOVÁ**

* Institute of Environmental Engineering Systems
Warsaw University of Technology, Nowowiejska 20, 00-653 Warsaw, Poland
** Institute of Chemistry, Slovak Academy of Sciences
Dubravska cesta 9, 842-38 Bratislava, Slovakia

Grabińska-Łoniewska A., Pajor E., Slávikovà E.: *Comparative analysis of yeast-like species potentially useful to environmental biotechnology*. Acta Mycol. 32 (1): 99-106, 1997.

Five yeast-like species recommended for enhancing the bioremediation of wastewaters, soils and exhaust gases contaminated with recalcitrant compounds were analysed with repect to their morphologal characteristics and physiology.

Key words: yeast-like microorganisms, biodegradation.

INTRODUCTION

Recently, much attention has been paid to the application of microscopic fungi in bioremediation of wastewaters, soils and exhaust gases contaminated with recalcitrant compounds. This is due to the fact that this group of microbes is able to extensively metabolize many hardly biodegradable organic substances. Some of these compounds are present in the environment in parts per million range or even less. This makes bacterial biodegradation impossible because the levels of compounds are too low to induce the biosynthesis of enzymes required in these processes. Moreover, the bacterial enzymes rarely posses very high affinity (low K_s value) necessary for significant degradation of organic compounds such low substrate concentration.

Our prior investigations demonstrated that yeast-like microorganisms from the genera: *Geotrichum*, *Candida*, *Trichosporon*, *Rhodotorula* and *Sporobolo-*

myces occurring in wastewater treatment system biocenoses were able to utilize benzene and its derivatives, i.e. aniline, p-nitroaniline and acetanilide as a sole C-source. On the basis of the results obtained the following strains were recommended for enhancing the removal of these compounds from point sources of pollutants: *Geotrichum candidum* strain A2D1, *G. sericeum* strain P5D5, *Candida famata* strain DCZ-1, *C. boidinii* strain M18D3 and *Rhodotorula rubra* strain M4D4 (Grabińska-Łoniewska et al. 1996, Słomczyński, Grabińska-Łoniewska 1996).

In the present study a comparative analysis of morphological, culture and biochemical features of these species is given.

MATERIALS AND METHODS

The strains described in the study were isolated from activated sludges of aeration tanks applied for the purification of municipal sewage in a mixture with different industrial wastewaters (Grabińska-Łoniewska et al. 1993) and from the denitrifying biocenosis of anaerobic UASB-type reactors (Grabińska-Łoniewska, Sláviková 1990).

Morphological and physiological characteristics of the strains were examined by the methods described by van der Walt and Yarrow (1984) and were identified according to Kreger-van Rij (1984) and de Hoog et al. (1986).

The ability to utilize of pollutants occurring in wastewaters: i.e. formaldehyde (FA), phenol (P), benzene (B), toluene (T), o-xylene (o-X), m-xylene (m-X), p-xylene (p-X), p-cresol (p-C), 4-nitrotoluene (4-NT), naphthalene (N), aniline (A), p-nitroaniline (p-NA) and acetanilide (AC) as a sole C-source for growth of microorganisms was examined according to the method given in a previous paper (Grabińska-Łoniewska et al. 1995). Concentration of FA in the growth medium was 100 mgl^{-1}, and that at other organic substances $-250\ mgl^{-1}$.

The strains were deposited in the Culture Collection of Yeasts, Institute of Chemistry Slovak Academy of Sciences in Bratislava.

RESULTS AND DISCUSSION

The cell and culture morphology as well as some physiological characteristics decisive for identification of the selected strains are described below. Fermentation and assimilation of different sugars and the assimilation of some nitrogen compounds are summarized in Table 1.

Table 1
Physiological characteristics of the species

Character of strains	*G. candidum* strain A2D1	*G. sericeum* strain P5D5	*C. famata* strain DCZ-1	*C. boidinii* strain M18D3	*Rh. rubra* strain M4D4
Fermentation:					
Glc	–	–	+ very weak	+	–
Mal	–	–	–	–	–
Sac	–	–	+ very weak	–	–
Lac	–	–	–	–	–
Gal	·	·	–	·	·
Raf	·	·	–	·	·
Tre	·	·	–	·	·
Assimilation of carbon compounds:					
Glc	+	+	·	+	+
Mal	–	–	+	–	+
Sac	–	–	+	–	+
Lac	–	–	–	–	–
Raf	–	–	+	–	+
Mlz	–	–	+	–	+
D-Xyl	+	–	+	+	+
L-Ara	–	–	+	–	+
Tre	–	–	+	–	+
Cel	–	–	+	–	–
Sol st	–	–	–	–	–
Inl	–	–	–	–	–
Ins	–	–	–	–	–
Ert	·	–	·	·	·
Assimilation of nitrogen compounds:					
KNO_3	–	–	–*	+	–*
L-Lys	+	+	·	·	·
Try	+	–	·	·	·

Abbreviations: Glc – glucose; Mal – maltose; Gal – galactose; Sac – sucrose; Lac – lactose; Raf – raffinose; Mlz – melezitose; D-xyl – D-xylose; L-Ara – L-arabinose; Cel – cellobiose; Tre – trehalose; Inl – inulin; Ins – inositol; Ert – erythritol; Sol st – soluble starch; L-Lys – L-lysine; Try – tryptophane; – test negative; + test positive; * test positive during incubation on nitrate broth

Geotrichum candidum Link – anamorph; teleomorph – *Galactomyces geotrichum* (Butler et Petersen) Redhead Malloch – strain A2D1 (CCY 16-1-22).

The shape of budding cells was ovoid to cylindrical. Usually they are occurring singly or in pairs (Fig. 1a). After 3 days of growth at 25°C, the hyphae and arthric condia were formed on malt agar extract medium; condia measured

(4,9-11,5) × (5,0-11,6) μm. A hairy pellicle was present. After one month, the streak culture was on malt agar white, flat, fimbriate, expanding with dry aerial mycelium. Separate hyphae with arthroconidia were formed. The radial growth

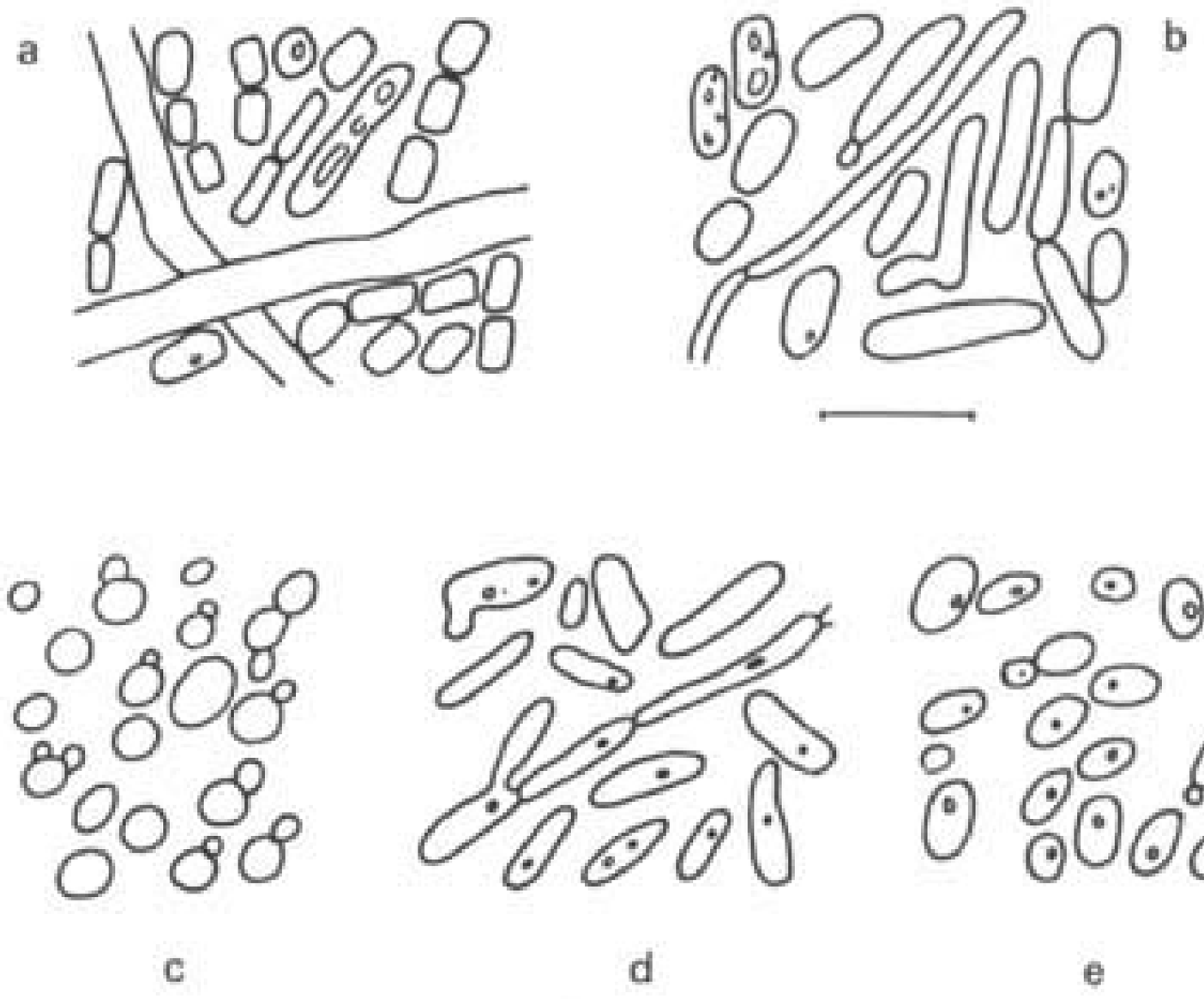

Fig. 1. Cell morphology: *a* — *Geotrichum candidum* strain A2D1; *b* — *Geotrichum sericeum* strain P5D5; *c* — *Candida famata* strain DCZ-1; *d* — *Candida boidinii* strain M18D3; *e* — *Rhodotorula rubra* strain M4D4; (bars — 10 μm)

rate of the gaint colony was: $r_t = 42$ mm in 100 hr. (Fig. 2b). No amylose-like polysaccharide was present in the cell wall. They did not hydrolize urea but grew in the presence of 50% glucose and in vitamin free medium. They show the grow at of 5°C, 28°C and 37°C but not at 42°C. The main natural habitats of these species in nature are various kinds of water bodies like artificial lakes, fish-ponds and rivers (S l á v i k o v á et al. 1992, 1995, 1997) as well as milk, cheese, plants, fruits, soils, insects, humans and other mammals (B a r n e t t et al. 1990). We isolated these organisms from aerobic activated sludge tank biocenosis treating wastewaters with aniline.

Geotrichum sericeum (Stautz) de Hoog, Smith, Guého — anamorph; teleomorph — *Dipodascus ovetensis* (Pelãez et Ramirez) v. Arx — strain P5D5 (CCY 30-2-8). After 3 days growth at 25°C the cells on the malt extract medium, were long-oval to elongate and measured: (2,5-5,0) × (4,1-9,9) μm (Fig. 1b). A wrinkled pellicle was present. After one month the streak culture on malt agar was butyrous, slighty fimbriate, expanding. Septate hyphae with arthroconidia were formed. The radial growth rate of the giant colony was: $r_t = 13,3$ mm in 100 hr (Fig. 2a). No amylose-like polysaccharides were present in the cell wall. This organism neither hydrolized the urea nor grew in the presence of 50% glucose and in vitamine-free medium. It grew at 28°C but not

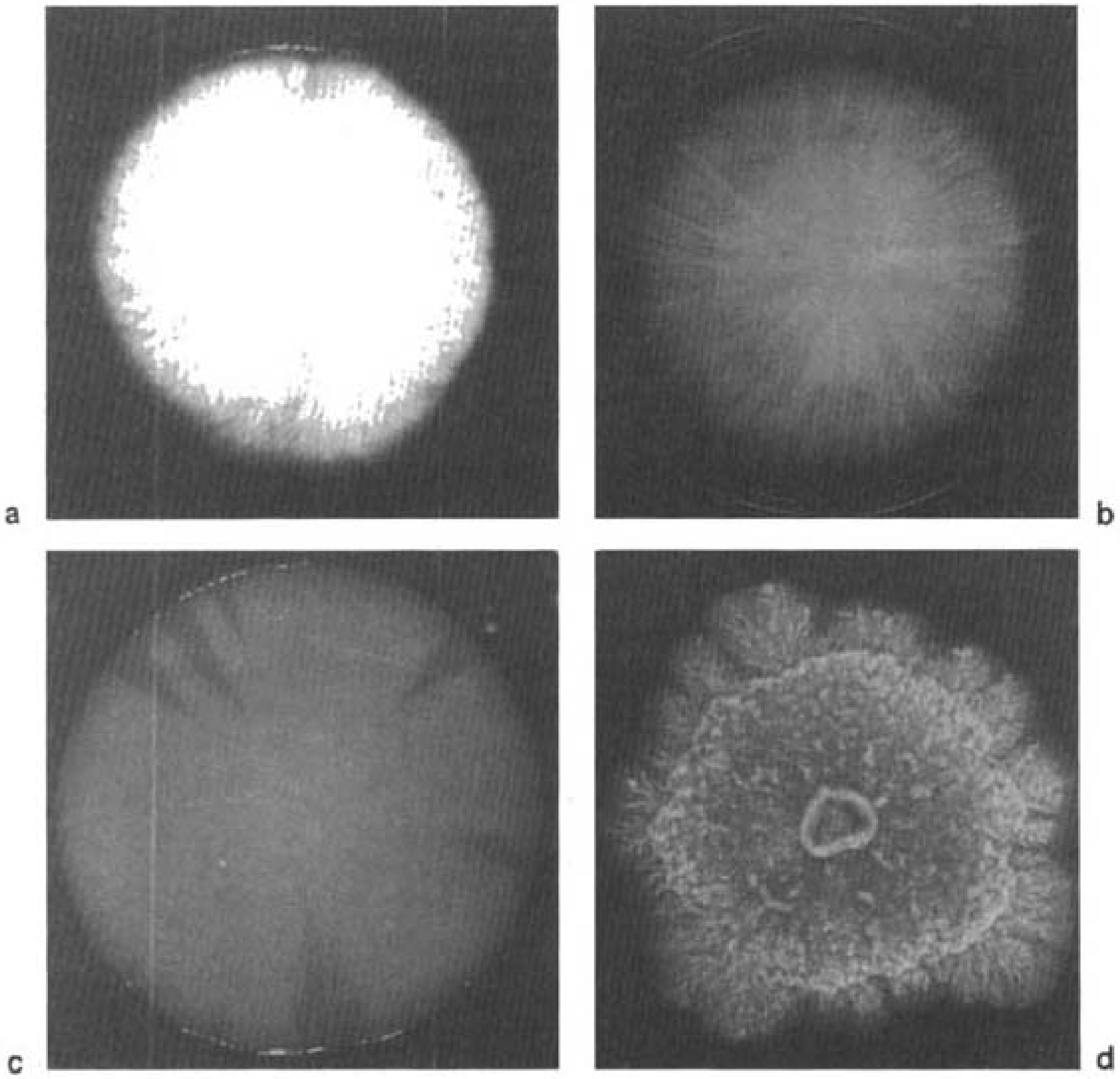

Fig. 2. Giant colonies: *a* — *Geotrichum sericeum* strain P5D5; *b* — *Geotrichum candidum* strain A2D1; *c* — *Rhodotorula rubra* strain M4D4; *d* — *Candida boidinii* strain M18D3

at 5°C, 37°C. According to Barnett et al. (1990) this species occurs frequently in tannin concentrate and exudate of oak tress. We found this organism to be a stable component of fungal consortium of aerobic activated sludge tank purifying petrochemical wastewaters (Treatment Plant in Płock near Warsaw).

Candida famata (Harrison) Meyer et Yarrow — strain M4D4 (CCY 26-9-20)

The cells were spherical to short-ovoidal in malt extract after 3 days at 25°C. They measured: (2.5-5.8) × (2.5-6.6) μm (Fig. 1c). Sediment was formed in this medium. After one month the streak culture on malt agar was white, smooth and soft. The radial growth rate of the giant colony was $r_t = 4.86$ mm

in 100 hr. Pseudomycelium was very primitive. No ascospores were observed. No amylose-like polysaccharides were present in the cell wall. Hydrolysis of urea was not observed. The growth in the presence of 50% glucose was slow, there was no growth in vitamin free medium. This species grew at 28°C, slow growth at 5°C was observed but there was no growth at 37°C and 42°C. The natural habitats of this fungus are: water bodies humans and other mammals, milk, cheese, wine, cucumber brine, sausages, meat, and mushrooms (B a r-n e t t et al. 1990). Our strain was isolated from the aerobic activated sludge tank purifying mixture of municipal and industrial wastewaters (Treatment Plant „Czajka" in Warsaw).

Candida boidinii Ramirez — strain M18D4 (CCY 29-37-7).

The cells in malt extract medium were long-ovoid after 3 days of cultivation at 25°C and measured: (1.7-3.2) × (4.2-9.8) μm (Fig. 1d). A pellicle was present. After one month, the streak culture on malt agar was cream coloured wrinkled and soft. Pseudomycelium was formed. The radial growth rate of the giant colony was: r_t = 6.15 mm in 100 hr (Fig. 2d). No amylose-like polysaccharide was present in the cell wall. Urease was not produced: the cells exhibited weak growth in the presence of 50% glucose and in vitamin free medium. The strain grew at 28°C, weak growth was observed at 37°C but not 5°C and 42°C. This species was found earlier in tanning fluid, soil, sea, soft drinks and wine (K r e g e r-van R i j 1984). We found it in an anaerobic denitrification unit (UASB — type) biocenosis treating wastewaters with nitrates and methanol as a C-source.

Rhodotorula rubra (Demme) Lodder — strain M4D4 (CCY 20-7-15).

After 3 days of growth at 25°C on malt extract medium the cells were short-ovoidal and measured: (2.2-5.4) × (2.7-6.6) μm (Fig. 1e). Sediment was formed in this medium. After one month the streak culture on malt agar was pink, glistening and soft. Pseudomycelium was not formed. The radial growth rate of the giant colony was r_t = 4,60 mm in 100 hr (Fig. 2c). No amylose-like polysaccharide was present in the cell wall. This organism produced urease. The strain showed no growth in the presence of 50% glucose and in vitamin free medium. It grew at 28°C and 37°C, poor growth was observed at 5°C but not at 42°C. This species was previously isolated from various sources, which included: fresh water, sea-water, flowers, air (G o t o et al. 1974; K r e g e r-van R i j 1984; K w a ś n i e w s k a 1988). We stated that this species was a stable component of anaerobic biocenosis treating wastewaters with nitrates and methanol as C-source in the UASB-type reactor.

Comparison of the growth response of the selected strains to different hazardous organic compounds (Fig. 3) showed that consortium consisting of *G. candidum* strain A2D1, *G. sericeum* strain P5D5, *C. famata* strain DCZ-1 and *Rh. rubra* strain M4D4 may be recommended to enhancing of bioremediation of wastewaters from chemical industry containing phenol and formaldehyde.

The use of the culture of *G. candidum* strain A2D1, *G. sericeum* strain P5D5 and *C. boidinii* strain M18D3 should allow more efficient on-site removal of aniline and to derivatives from the industrial wastewaters from production of organic dyes, photographic reagents, aromatic substances and drugs. It may be mixed culture of the *C. famata* strain DCZ-1 and *Rh. rubra* strain M4D4 with contribute to the removal of aromatic hydrocarbons from the tar working plants, explosive materials and petroleum productions. The use of selective fungal populations enables more efficient treatment of these intractable wastes on-site or at special biotreatment centres and the determination of the basic growth parameters of such populations, and can therefore, yield information relevat to developing the most effective management strategies.

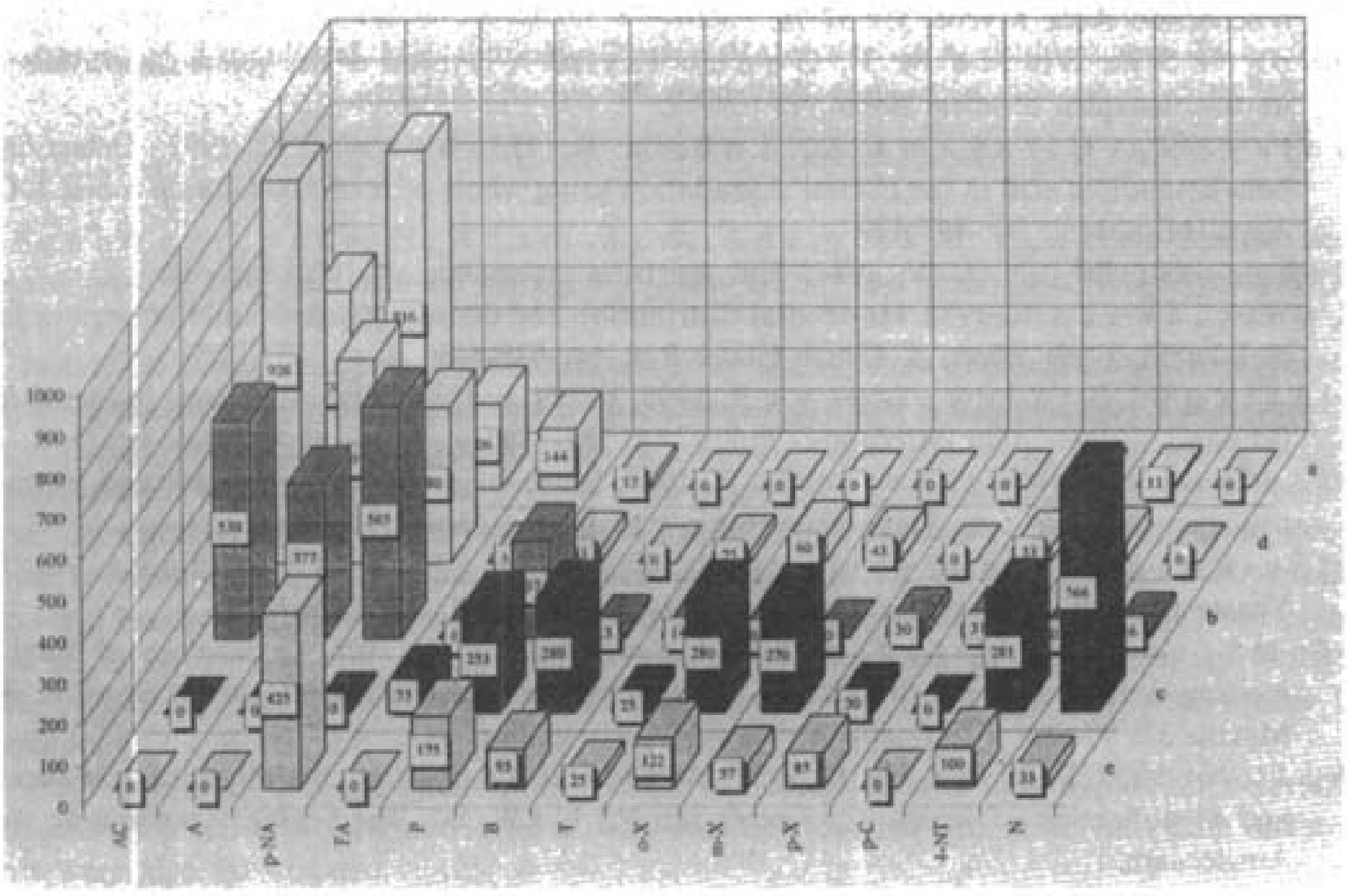

Fig. 3. Intensity of the utilization of pollutants occurring in wastewaters by yeast-like strains recomended for enhancing the treatment process (expressed as CFU yield in the growth medium comparing with control cultures)
FA – formaldehyde; P – phenol; B – benzene; T – toluene; o-X – oxylene; m-X – m-xylene; p-X – p-xylene; p-C – p-cresol; 4-NT – 4-nitrotoluene; N – napthalene; A – aniline; p-NA – p-nitroaniline; AC – acetanilide; *a* – *Geotrichum candium* strain A2D1; b – *Geotrichum sericeum* strain P5D5; *c* – *Candida famata* strain DCZ-1; *d* – *Candida boidinii* strain M18D3; *e* – *Rhodotorula rubra* strain M4D4

This research supported in part, by Grant No 504/830 from the Polish Committee for Scientific Research (KBN) and Grant No 2/1099/96 of the G.A.S. for Biological and Ecological Sciences (Slovakia).

REFERENCES

Barnett J. A., Payne R. W., Yarrow D. 1990. Yeasts: Characteristics and identification. Cambridge University Press. Cambridge.

Goto S., Ohwada K., Yamasato K. 1974. Identification of yeasts isolated from seawater and sediment in Aburatsubo Inlet. J. Gen. Microbiol. 20: 317-322.

Grabińska-Łoniewska A., Slávikovά E. 1990. Fungi in denitrification unit biocenosis. Wat. Research, 24: 565-572.

Grabińska-Łoniewska A., Slávikovά E., Furmańska M., Słomczyński T. 1993. Fungi in activated sludge biocenosis. Acta Microbiol. Polon. 42: 303-313.

Grabińska-Łoniewska A., Slávikovά E., Pajor E., 1995. *Geotrichum capitatum*, a new isolate degrading phenol. Acta Mycol. 30 (2): 207-211.

Grabińska-Łoniewska A., Słomczyński T., Pajor E., Kołosowska K. 1996. Occurrence of fungi degrading aromatic hydrocarbons in activated sludge biocenoses. Acta Mycol. 31: 67-75.

Hoog G. S. de, Smith M. T., Guého E. 1986. A revision of the genus *Geotrichum* and its teleomorphs. Studies Mycol. 29: 51-57.

Katayama-Hirayama K., Tobita S., Hirayama K. 1992. Detection of beta-ketoadipic acid in the phenol degradation using whole cells of *Rhodotorula rubra*. J. Gen. Appl. Microbiol. 38: 497-499.

Kreger-van Rij N. J. W. 1984. The yeasts, a taxonomic study. Amsterdam.

Kwaśniewska K. 1988. Horizontal distribution and density of yeasts and filamentous fungi in lake St. Clair water. J. Great Lakes Res. 14: 438-443.

Slávikovά E., Vadkertiovά R. 1995. Yeasts and yeast-like organisms isolaeted from fishpond waters. Acta Microbiol. Polon. 44: 181-189.

Slávikovά E., Vadkertiovά R. 1997. Seasonal occurrence of yeasts and yeast-like organisms in the river Danube. Antonie van Leeuwenhoek, in press.

Slávikovά E., Vadkertiovά R., Kočkovά-Kratochvilovά A. 1992. Yeasts isolated from artificial waters. Can. J. Microbiol. 38: 1206-1209.

Słomczyński T., Grabińska-Łoniewska A. 1996. Occurrence of fungi degrading aniline and it's derivatives in biocenoses of wastewater treatment systems. Acta Mycol. 31: 77-86.

Walt van der J. P., Yarrow D. 1984. Methods for the isolation, maintenance, classification and identification of yeasts. In: Kreger-van Rij N.J.W. Ed. The yeasts, a taxonomic study. Amsterdam.

Porównawcza charakterystyka szczepów grzybów drożdżopodobnych potencjalnie przydatnych w biotechnologii środowiskowej

Streszczenie

Scharakteryzowano właściwości morfologiczne i fizjologiczne 5 szczepów grzybów drożdżopodobnych zalecanych do wspomagania procesu unieszkodliwiania ścieków, gleby i gazów odlotowych zanieczyszczonych związkami trudnorozkładalnymi.