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Occurrence of fungi degrading aromatic hydrocarbons in activated sludge biocenoses

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A set of 21 strains of yeast-like microorganisms isolated from biocenoses of aerobic and anaerobic wastewater treatment systems were assayed for their ability to utilize aromatic hydrocarbons as a sole C-source. Basing on the achieved results, the highly biochemically active strains for application in enhancing of wastewaters and exhaust gases purification as well as soil bioremediation were selected.

Key words: yeast-like microorganisms, biodegradation, aromatic hydrocarbons.

INTRODUCTION

The pollution of surface and groundwaters, marine environments, soil and air by aromatic hydrocarbons (AHs) is of concern to public health because many of them or their metabolites are mutagenic, carcinogenic, or both. These compounds are discharged to the water reservoirs with wastewaters from tar working plants, fertilizers, dyes and explosive materials productions, petroleum and pharmaceutical industries, mine waters etc. The soil contamination is mainly a result of petroleum pipelines damages and outflows of petrochemicals form storage tanks. AHs are emited into the atmosphere with exhaust gases from petrochemical plants.

A very promising methods for AHs removal from these point sources of pollutants are technologies applying reactors with microbes attached on the porous media (Páca, Koutský, 1994). It makes possible the maintenance a high concentration of active biomass in reactors. The stability and dynamics of populations is essential for controling the reactor systems and selective interference in the case of a process failure. In this aspect, the gathering a collection of highly biochemically active strains has the great significance. It is especially important in the case of AHs degrading

microorganisms because the natural ability to utilization of these compounds is not widely distributed among both bacteria and fungi.

The aim of our investigations was to select the highly active yeast-like microorganisms degrading AHs from a set of 21 strains previously isolated from biocenoses of different wastewater treatment systems (Grabińska-Łoniewska, Sláviková, 1990; Grabińska-Łoniewska et al., 1993). This study is an introduction to futher investigations on the application of the selected strains in enhancing of wastewaters or exhaust gas purification as well as soil bioremediation.

MATERIALS AND METHODS

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

The ability to utilize of AHs and their derivatives (phenol, benzene, toluene, o-, m-, p-xylenes, o-, m-, p-cresoles, 4-nitrotoluene and naphthalene) as a sole C-source for growth was examined according to method given in previous our paper (Grabińska-Łoniewska et al., 1995). Concentration of the C-source in the growth medium was 250 mg l^{-1}.

RESULTS AND DISCUSSION

Literature data imply that phenol belongs to one of readily metabolized aromatics by fungi. Among the yeast-like species able to degrade this compound are: *Candida albicans, C. catenulata, C. humicola, C. maltosa, C. mesenterica, C. rugosa, C. sake, C. tropicalis, C. utilis, Cryptococcus terreus, Lodderomyces elongisporus, Rhodotorula aurantiaca, Rh. glutinis, Rh. gracilis, Rhodosporidium toruloides, Sporidiobolus ruinenii, Sporobolomyces salmonicolor, Trichosporon cutaneum, T. beigelii, Torulopsis dattila, T. utilis, Pichia guilliermondii, Yarrowia lipolytica* (Leibnitz et al., 1960; Mills et al., 1971; Kočková-Kratochvilová, 1982; Krug et al., 1985, 1986; Kocwa-Haluch, 1986; Hofmann, Schauer, 1988; Hofmann, Vogt, 1988). According to Klug et al. (1985, 1986) especially active in phenol degradation (up to concentration of 2.5 g l^{-1}) is *Candida tropicalis*. Among the molds, the growth on this substrate was noted for *Chaetomium cupreum, Drechslera oryzae, Fusarium oxysporum* and wood rotting fungus – *Phanerochaete chrysosporium* (Aitken et al., 1989; Boominathan et al., 1989).

Our investigations have confirmed these data suggesting that the natural ability to utilize phenol is rather common among the fungi. The majority of 14 isolates from the genera *Geotrichum, Trichosporon* and *Candida* have intensively grown on

a mineral medium with this compound as a sole C-source. Particularly active in this respect were *Geotrichum capitatum* strain G1D2, *G. klebahnii* strain P6D6, *G. sericeum* strains P2D2 and P5D5, *Candida famata* strain DCZ-1 and *C. tropicalis* strain O11D8 (Table 1, Fig. 1).

Less numerous were strains able to utilize other tested aromatics. Benzene was metabolized by *Geotrichum candidum* strain A2D3, toluene – *Candida boidinii* strain M18D3, whereas both these compounds – by *C. famata* strain DCZ-1, *Rhodotorula rubra* strain M4D4 and *Trichosporon cutaneum* strain M5D5, Included among o- and m-xylenes metabolized species were *Candida famata* strain DCZ-1, *C. boidinii* strain M18D3, *C. lambica* strain O14D7 and *Rhodotorula rubra* strain M4D4, whereas p-xylene – besides the latest of above strains also, *Candida boidinii* strain M4D3 and *Geotrichum klebahnii* strain P6D6. The growth of the 4-nitrotoluene as a sole C-source was noted for *Candida famata* strain DCZ-1 and *Rhodotorula rubra* strain M4D4 – which intensively metabolized benzene and other it's derivatives, as well as for *Candida boidinii* strain M18D3, *C. lambica* strain O14D7 and *Geotrichum candidum* strain A2D3 (Table 2, Fig. 1). Up to the present, the biodegradation of benzene and it's derivatives has not been demostrated among fungi. So, our findings have revealed unfamiliar physiological properties of these microorganisms.

Table 1

Growth of different yeast-like microorganisms on phenol (evaluated on the basis of CFU yield in the mineral medium with this compound as a sole C-source)

Original name	Number of CFU x 10^6 ml^{-1} after 7 days of incubation at 26°C	
	control cultures (growth medium without C-source)	growth medium with phenol as a C-source
Geotrichum candidum Link strain A2D1	0.62	0.73
strain AC2D3	1.52	1.66
Geotrichum klebahnii (Stautz) Morenz strain P6D6	3.62	14.30
Geotrichum sericeum (Pelaez et Ramirez) von Arx		
strain P2D2	2.60	9.41
strain P5D4	5.68	16.70
strain P5D5	1.70	5.73
Geotrichum capitatum (Diddens et Lodder) von Arx, de Hoog strain G1D2	1.40	7.16
Trichosporon cutaneum (de Beurmann, Gougerot et Voucher) Ota strain M5D5	2.64	5.09
Candida boidinii Ramirez strainM18D3	0.60	0.80
Candida farmata (Harrison) Meyer et Yarrow strain DCZ-1	4.60	16.24
Candida lambica (Lindner et Genoud) van Uden et Buckley strain O14D7	6.20	7.20
strain O7D7	4.90	6.00
strain O5D5	2.50	2.73
Candida tropicalis (Castellani) Berkhout strain O11D8	4.40	13.95

Table 2

Growth of different yeast-like microorganisms on benzene and its some derivatives
(evaluated on the basis of CFU yield in the mineral medium with this compound as a sole C-source)

Original name	Number of CFU x 10^6 ml^{-1} after 7 days of incubation at 26°C						
	control cultures	growth medium containing as a C-source					
		benzene	toluene	o-xylene	m-xylene	p-xylene	4-nitrotoluene
Geotrichum candidum Link strain A2D3	1.37	2.16	1.51	1.47	1.44	1.44	1.96
strain AC2D3	1.51	1.51	1.51	1.68	1.51	1.51	1.37
Geotrichum klebahnii (Stautz) Morenz strain P6D6	4.80	5.60	5.68	4.80	4.80	6.68	1.52
Geotrichum sericeum (Pelaez et Ramirez) von Arx strain P5D5	2.16	2.44	2.46	2.16	2.16	2.80	1.37
Geotrichum capitatum (Diddens et Lodder) von Arx, de Hoog strain G1D2	1.37	1.46	1.46	1.54	1.54	1.46	1.52
Trichosporon cutaneum (de Beurmann, Gougerot et Voucher) Ota strain M5D5	0.45	0.58	0.59	0.50	0.50	0.48	0.52
Candida boidinii Ramirez strainM4D3	0.91	0.91	1.06	1.02	1.12	1.23	0.91
strain M18D3	1.37	1.37	1.71	2.20	1.96	1.37	1.96
Candida famata (Harrison) Meyer et Yarrow strain DCZ-1	1.37	5.21	1.71	5.21	5.08	1.78	5.22
Candida lambica (Lindner et Genoud) van Uden et Buckley strain O14D7	0.54	0.61	0.61	0.75	0.86	0.70	2.28
Candida tropicalis (Castellani) Berkhout strain O11D8	3.82	4.28	4.28	4.08	4.32	3.93	1.37
Candida inconspicua (Lodder et Kreger – van Rij) Meyer et Yarrow strain O9D7	1.37	1.48	1.52	1.54	1.42	1.49	1.52
Rhodotorula rubra (Demme) Lodder strain M4D4	1.37	2.68	1.71	3.04	2.15	2.54	2.74
strain M18D3	1.37	1.56	1.66	1.37	1.56	1.37	1.37

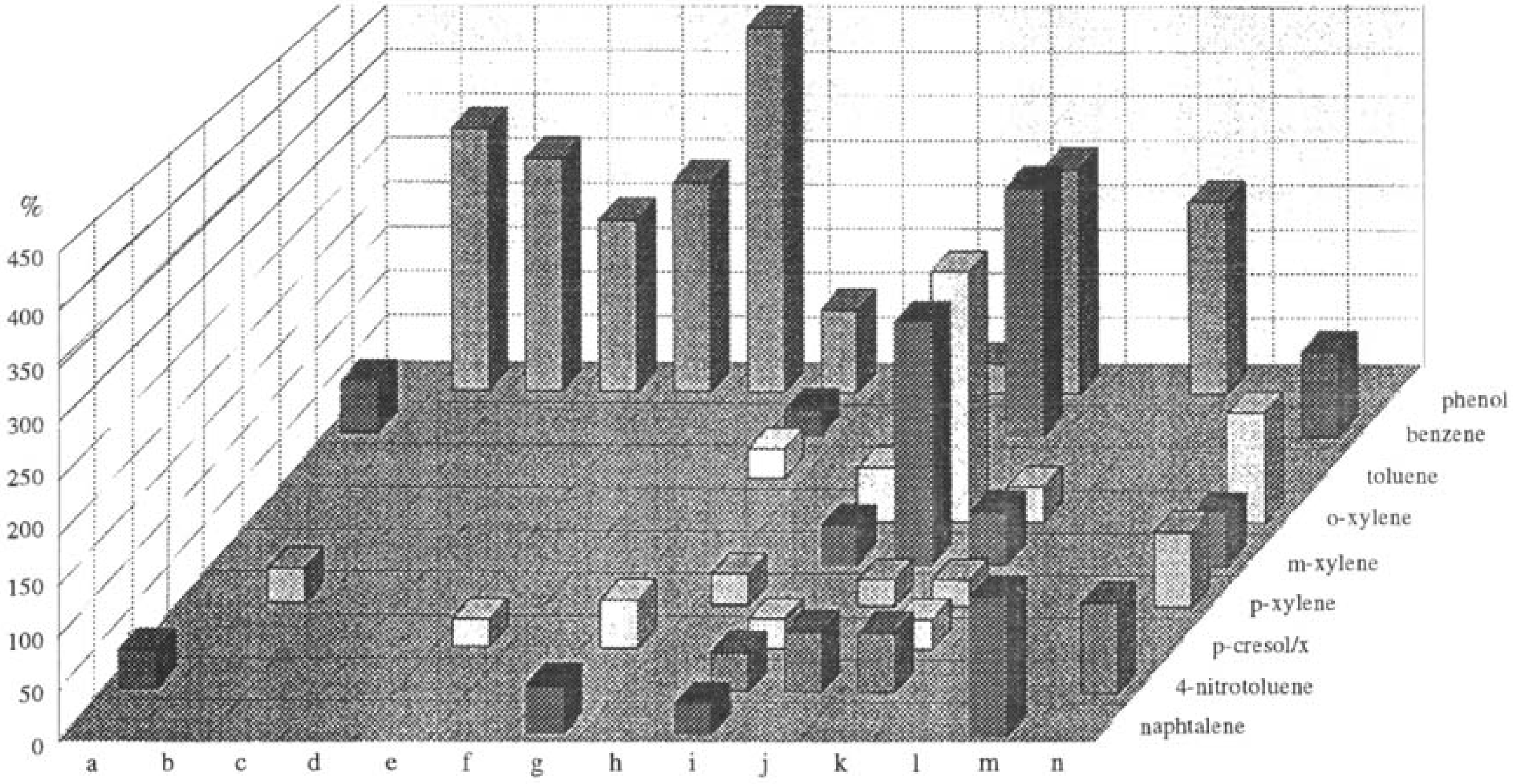

Fig. 1. Intensity of some aromatic hydrocarbons utilization by yeast-like strains isolated from wastewater treatment systems biocenoses measured by CFU yield in mineral medium containing these compound as a sole C-source
(CFU yield in the growth medium comparing with control cultures)

Geotrichum candidum strain A2D3 (a); *G. klebahnii* strain P6D6 (b); *G. sericeum* strain P2D2 (c), strain P5D4 (d), strain P5D5 (e); *G. capitatum* strain G1D2 (f); *Trichosporon cutaneum* strain M5D5 (g); *Candida boidinii* strain M4D3 (h), strain M18D3 (i); *C. famata* strain DCZ-1 (j); *C. lambica* strain O7D7[/k] or strain O14D7 (k); *C. tropicalis* strain O11D8 (l); *Sporobolomyces lactosus* strain P3D2 (m); *Rhodotorula rubra* strain M4D4 (n)

Table 3

Growth of different yeast-like microorganisms on cresoles
(evaluated on the basis of CFU yield in the mineral medium with this compound as a sole C-source)

Original name	Number of CFU x 10^6 ml^{-1} after 7 days of incubation at 26°C					
	o-cresol		m-cresol		p-cresol	
	control cultures (growth medium without C-source)	control cultures (medium with o-cresol as a C-source)	control cultures (growth medium without C-source)	control cultures (medium with m-cresol as a C-source)	control cultures (growth medium without C-source)	control cultures (medium with p-cresol as a C-source)
Geotrichum candidum Link strain A2D1	2.30	2.30	1.37	1.37	0.62	0.66
strainA2D3	1.20	1.32	1.67	1.67	1.52	1.78
strain AC2D3	1.93	1.95	1.47	1.47	1.52	1.66
Geotrichum klebahnii (Stautz) Morenz strain P6D6	4.80	5.50	1.57	1.57	3.62	3.62
Geotrichum sericeum (Pelaez et Ramirez) von Arx						
strain P5D4	1.57	1.57	1.77	1.77	5.68	6.36
strain P5D5	1.87	1.87	1.87	1.87	1.70	2.23
Trichosporon cutaneum (de Beurmann, Gougerot et Voucher) Ota strain M5D5	1.57	1.57	0.45	0.50	2.64	4.03
Candida boidinii Ramirez strain M18D3	0.60	0.82	0.60	0.70	0.60	0.80
Candida lambica (Lindner et Genoud) van Uden et Buckley strain O5D5	1.37	1.37	1.67	1.67	2.50	2.90
strain O7D7	1.42	1.62	1.57	1.57	4.90	6.52
strain O14D7	0.54	0.69	0.54	0.76	6.20	6.57
Candida tropicalis (Castellani) Berkhout strain O11D8	3.82	4.08	3.82	4.08	4.40	5.02
Candida maltosa Komagata, Nakase et Katsuya strain M6D7	1.47	1.47	1.78	1.78	5.88	6.53

The problem of biodegradation of cresoles by fungi is better known. It was evidenced for *Candida tropicalis, Rhodotorula glutinis* and *Phanerochaete chrysosporium* (L e i b n i t z et al., 1960; K l u g et al., 1985; A i t k e n et al., 1989). Our studies have been shown that among these compounds, p-cresol was the most readily utilized. As a p-cresol-utilizers were included *Geotrichum sericeum* strain P5D5, *Trichosporon cutaneum* strain M5D5, *Candida boidinii* strain M18D3 and *C. lambica* strain O7D7 and O14D7 (Table 3, Fig 1). The growth on o-cresol was noted only for two strains: *C. boidinii* strain M18D3 and *Candida lambica* strain O14D7 (cell yield 36 and 28 %, respectively) whereas on m-cresol for the latest of above strains (cell yield 40 %) (Table 3).

Three of 14 isolates namely, *Sporobolomyces lactosus* strain P3D2, *Trichosporon cutaneum* strain M5D5 and *Candida boidinii* strain M18D3 showed intensive growth on naphthalene (Table 4, Fig. 1).

T a b l e 4

Growth of different yeast-like microorganisms on naphthalene (evaluated on the basis of CFU yield in the mineral medium with this compound as a sole C-source)

Original name	Number of CFU x 10^6 ml^{-1} after 7 days of incubation at 26°C	
	control cultures (growth medium without C-source)	growth medium with naphthalene as a C-source
Geotrichum candidum Link strain AC2D3	1.52	1.81
Geotrichum sericeum (Pelaez et Ramirez) von Arx strain P5D5	1.70	1.97
Trichosporon cutaneum (de Beurmann, Gougerot et Voucher) Ota strain M5D5	2.64	4.03
Candida boidinii Ramirez strainM18D3	0.60	0.80
Candida famata (Harrison) Meyer et Yarrow strain DCZ-1	4.60	5.11
Candida lambica (Lindner et Genoud) van Uden et Buckley strain O5D5	2.50	2.68
strain O7D7	4.90	5.98
strain O14D7	6.20	6.82
Candida tropicalis (Castellani) Berkhout strain O11D8	4.40	4.80
Candida inconspicua (Lodder et Kreger – van Rij) Meyer et Yarrow strain O9D7	4.10	4.67
Candida maltosa Komagata, Nakase et Katsuja strain M6D7	5.88	6.53
strain M18D7	3.41	4.19
Sporobolomyces lactosus Slávikova et Grabińska-Łoniewska strain P3D2	1.26	3.20
Rhodotorula rubra (Demme) Lodder strain M4D4	3.92	4.31
strain M18D3	2.26	2.42

These species weren't previously reported as naphthalene-utilizers. The list of naphthalene degrading fungi, reported earlier included 19 species, among them the following: *Saccharomyces cerevisiae, Endomyces magnusii, Candida lipolytica, C. tropicalis, Cunninghamella elegans, Aspergillus niger, Penicillium notatum* and *Mucor hiemalis* (Cerniglia et al., 1977, 1978, 1981; Kočková-Kratochvilová, 1982; Maliszewska-Kordybach, 1987).

Comparison of the growth response of isolates originated from wastewater treatment systems biocenoses on different AHs led to assumption that among them *Candida famata* strain DCZ-1 and *Rhodotorula rubra* strain M4D4 may be usefull in enhancing wastewaters and exhaust gases purification. They were characterized by the ability to intensive growth on a wide variety of AHs. The development and study of systems designed to treat AHs contaminated wastes with these strains are currently in progress.

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Występowanie grzybów rozkładających węglowodory aromatyczne w biocenozach osadów czynnych

S t r e s z c z e n i e

Przebadano zdolność wykorzystywania węglowodorów aromatycznych przez 21 szczepów grzybów drożdżopodobnych wyizolowanych z biocenoz urządzeń do oczyszczania ścieków w warunkach tlenowych i beztlenowych. Na podstawie uzyskanych wyników badań wytypowano wysokowydajne biochemicznie szczepy mogące znaleźć zastosowanie do intensyfikacji procesu oczyszczania ścieków i gazów odlotowych.