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Geotrichum capitatum, a new isolate degrading phenol

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Geotrichum capitatum strain G1D2, highly active in degradation of phenol was isolated from the biocenosis of rotating discs reactor treating petrochemical wastes. It was also able to utilize ethanol, glycerol, acetate, citrate and p-, m-cresoles as sole C-sources.

Key words: *Geotrichum capitatum*, phenol.

INTRODUCTION

The role of microscopic fungi inhibiting wastewater treatment systems in the biodegradation of pollutants has been so far poorly studied. In previous papers of Grabińska-Łoniewska et al. (1986/1988, 1987, 1990, 1988/1989, 1993) the quantitative and taxonomic characterization of mycoflora of different anaerobic wastewater treatment systems was given. These studies showed that in an anaerobic UASB-type reactor, yeasts and yeasts-like microorganisms enhanced the biochemical activity of denitryfying bacteria and utilized for growth both, organic carbon compounds serving as electron donors for denitrification nitrates and N_2 arising during nitrate dissimilation.

The aim of this study was to evaluate the role of *Geotrichum capitatum* strain G1D2 (Teleom.: *Dipodascus capitatus* de Hoog et al. 1986) in the removal of pollutants occurring in industrial wastewaters. This fungus was a stable constituent of rotating discs mycoflora fed with petrochemical wastes containing high quantities of various impurities, mainly aromatic and alyphatic hydrocarbons, fats, phenols, suspended matter and odorous compounds. These studies can be considered as a preliminary step for the selection of highly biochemically active species that might be used for the intensification of the removal of specific impurities occurring in wastewaters.

MATERIALS AND METHODS

The strain G1D2 investigated in the study was isolated from the biofilm of the rotating discs serving both as biomass supporting material and aeration system in technical unit treating highly concentrated wastes (COD in the range of 12-15 g O_2 l^{-1}) grom the production of ethylene oxide and polypropylene glycol in the petrochemical factory (Krzysztofik et al., 1994).

The methods employed for isolation and identification were those described by Grabińska-Łoniewska and Slávikovά (1986/1988). Classification was carried out according to de Hoog et al. (1986). The strain *G. capitatum* G1D2 has been deposited in the Culture Collection of Yeasts, Institute of Chemistry Slovak Academy of Sciences, Bratislava as CCY 30-6-6.

The ability of utilization of specific organic compounds occurring wastewaters as sole C-sources was examined in the medium containing (g l^{-1}): KH_2PO_4 – 2.0; $MgSO_4$ – 0.17; $(NH_4)_2SO_2$ – 2.0 and $CaCl_2$ – 0.3 in distilled water. The medium was supplemented with solutions of microelements (ml l^{-1}) – 0.25 and vitamins – 1.0 (according to Kočková-Kratochvilová, 1977). Concentration of C-source (methanol, ethanol, glycerol, ethylene glycol, formate, acetate, citrate, phenol, benzene, toluene, p-, m-, o-xylenes, p-, m-, o-cresoles and p-nitrotoluene) was 250 mg l^{-1}. Standard inoculum for all tests was 10^3 wash-out cells (72-h culture at 26°C on beer wort) per ml of culture. The cultures were incubated for 7 days at 26°C on stirring bath. Control cultures were conducted on the same media but without C-source. In these tests the cell yield above 20 % as against the control cultures was regarded as a positive result. Cell density of culture medium was determined by measuring the absorbance at 546 nm in "Specol" spectrophotometer with a light path of 5 cm.

RESULTS AND DISCUSSION

Geotrichum capitatum strain G1D2 was a component of rotating discs reactor biocenosis consisting of true and filamentous bacteria (2.8 x 10^8 CFU – Colony Forming Units – per cm^2 of rotating disc surphace), microscopic fungi (2.0 x 10^8 CFU cm^{-2}) and protozoa (Krzysztofik et al., 1994). The synonyms of this species are: *Endoblastomyces thermophilus* Odinzowa et Kudriavzev, *Geotrichum linkii* Vöros-Felkai and *Trichosporon capitatum* Diddens et Lodder. The teleomorph of this organism is *Dipodascus capitatus* (de Hoog et al., 1986). Besides this isolate being the dominating species among yeast-like microorganisms, mycoflora consisted of different molds species. High total fungal count in biofilm, similar to bacterial one, could indicate that fungi play an essential role in wastewater purification in rotating discs reactor.

The cell and culture morphology and physiological characteristics of *Geotrichum capitatum* strain G1D2 isolated from this biocenosis are given below.

Cells after 72 hr at 25°C in malt extract medium were long-oval and measured 2.5-4.1 x 8.2-18.1 µm (Fig. 1). During the growth in this medium a wrinkled pellicle was formed. After one month the streak culture on malt agar was cream coloured and wrinkled, finely funiculose aerial mycelium was formed (Fig. 2). The radial growth rate of the giant colony was: r_t = 10.97 nm in 100 hr. Septate, branched hyphae and arthrospores were observed. No amylose-like polysaccharide was present in the cell wall.

Physiological features were the same as given in standard description of this species (de H o o g et al., 1986). This fungus did not ferment saccharides. Among the sugars, it was able to assimilate glucose, galactose and L-sorbose (weak), but D-xylose, L-arabinose, raffinose, melezitose, maltose, sucrose, lactose, cellobiose, trehalose, inositol, inulin and soluble starch were not utilized. This strain was not able to assimilate potassium nitrate and to grow in vitamin-free medium and in the presence of 50 % glucose. Urease was not produced. It grew well at 28-42°C, but at 5°C its growth was inhibited.

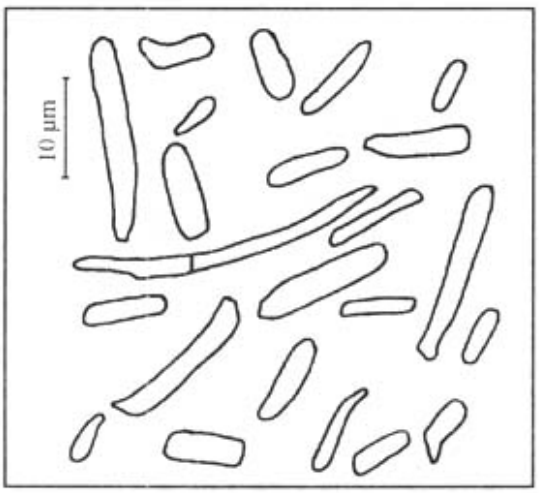

Fig. 1. Cell morphology *Geotrichum capitatum* strain G1D2

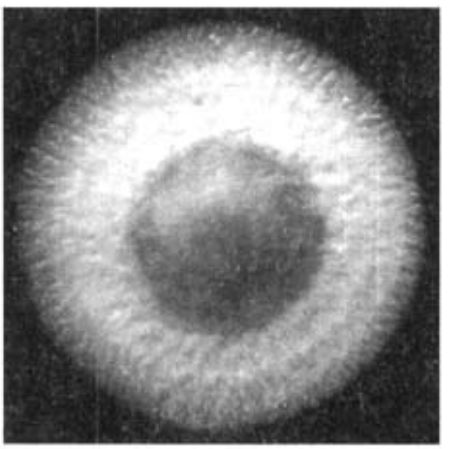

Fig. 2. Giant colonies of *Geotrichum capitatum* strain G1D2

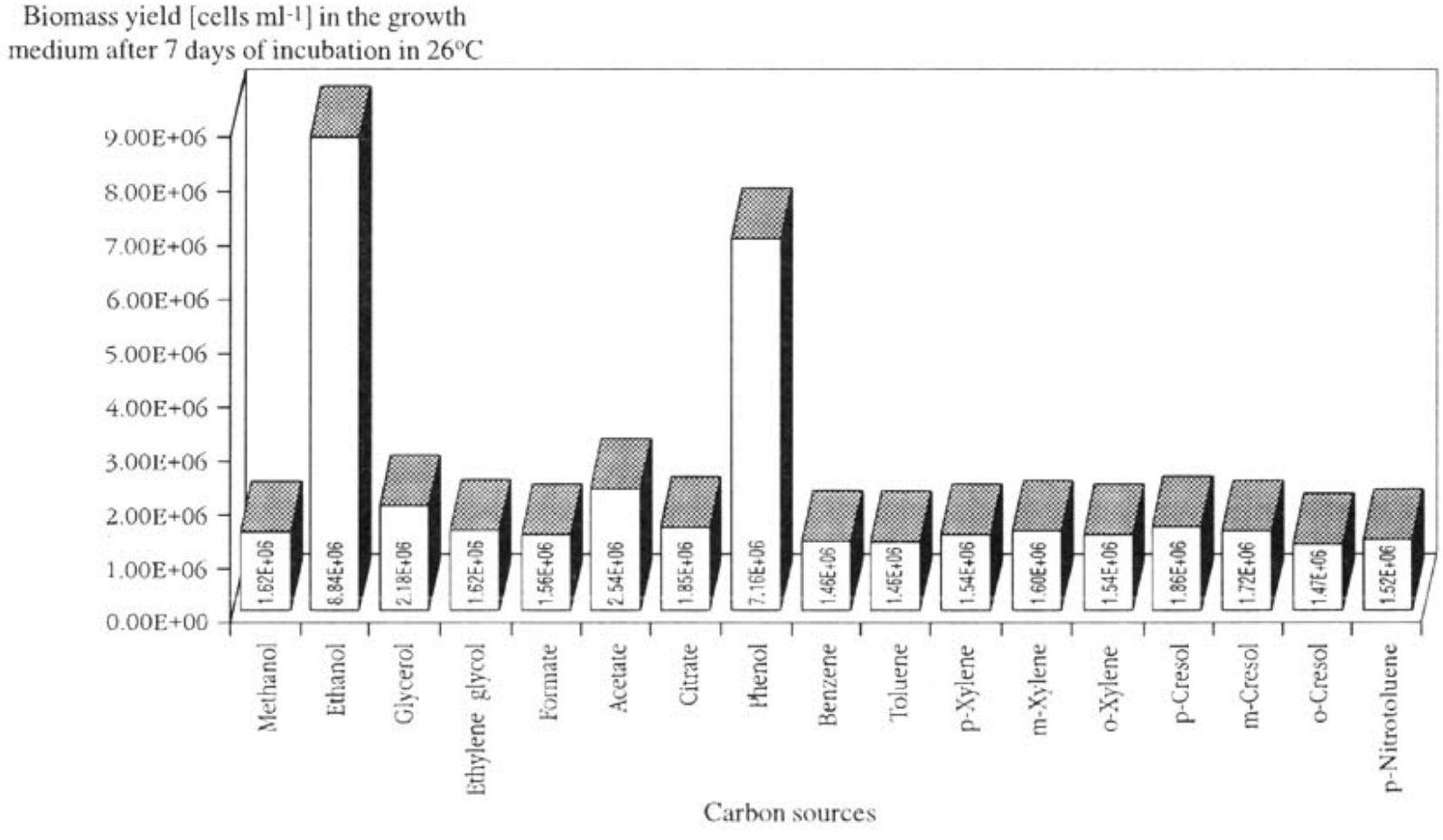

Fig. 3. Utilization of some specific organic compounds occurring in wastewaters by *Geotrichum capitatum* strain G1D2 (evaluated on the basis of cell yield in the mineral medium with specific compounds as sole C-sources)

The studies of special physiological features of the reported isolate showed its high phenol degrading activity. The cell yield of this fungus in the growth medium with this compound as sole C-source was 422 % higher than in control culture (Fig. 3). Phenol is a stable constituent of petrochemical wastes (concentration in the range of 30-260 mg l^{-1}) (Grabińska-Łoniewska et al., 1993). This could suggest that *C. capitatum* plays an important role in removal of this compound during wastewater treatment process. It is also noteworthly that this organism intensively metabolized ethanol, and to a lesser degree acetate, glycerol, citrate and p- and m-cresoles. The ability of assimilation of ethanol and glycerol by *C. capitatum* confirmed the data of de Hoog et al. (1986). According to Lodder (1970) this fungus is frequently associated with humans (e.g. patients with broncho-pulmonary diseases) and warm-blooded animals. It also occurrs in the air of wood pulp factory. Previously its presence in biofilm of tricling filters and in the wastewaters was recorded by Cooke et al. (1960) and Cooke and Matsuara (1963) – cyted after to Lodder (1970). Considering the high biochemical activity of *G. capitatum* strain G1D2, it is apparent that this fungus holds a great deal of promise as a biological treatment tool, particularly in phenolic waste application.

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Geotrichum capitatum, nowy izolat rozkładający fenol

Streszczenie

Z biocenozy złoża tarczowego oczyszczającego ścieki petrochemiczne wyizolowano *Geotrichum capitatum* G1D2 (Teleom.: *Dipodaseus capitatus* de Hoog et al.) intensywnie rozkładający fenol. Organizm ten wykorzystywał także jako jedyne źródło C: etanol, glicerol, cytrynian oraz p- i m-krezole.