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Phenolic biotransformation by native yeast species and correlation with the ubiquinone types.

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Abstract.

Yeast is an important group of microorganisms whose significance in xenobiotic metabolism has been recognized. Contaminated sediment samples collected from industrial sewage were screened for yeast strains able to grow on hydrocarbon and phenol. Although these abilities had been described, the correlation between both degradation abilities with the coenzyme-Q structure of the respective yeast groups, is not well defined. The yeast species were isolated on agar-mineral basal medium with pyrene, and them they were assayed for their ability to grow on phenol and *n*-hexadecane as carbon source, both of them given via vapour phase. Several *Candida* and *Rhodotorula* species were phenol and *n*-hexadecane positive. While the *Rhodotorula* species were able to oxidate both substrates, the *Candida* strains were more effective on phenol culture than in the *n*-hexadecane ones. These abilities were in concordance with the yeast ubiquinone structure, as *Candida* spp. possess Co-Q_9 and *Rhodotorula* spp. were Co-Q_{10}.

Introduction.

Chemical industries, petroleum refineries and agrochemistry plants discharge a variety of organic wastes, including aromatics substances that are structurally related compounds to phenol, toluene and catechol; and these xenobiotics have been listed as priority pollutants by the US Environmental Protection Agency (Ghisalba, 1983; US. EPA, 1990). Although phenol itself is not recalcitrant, many synthetic aromatics have substituted phenol rings (Otte, 1994; Schingler, 1996).

Phenol can be removed by biological treatment, since many bacteria and fungi species are capable of using it as sole source of carbon and energy (Limbert & Betts, 1995). Although some yeast species degrade phenol, and the kinetics and the assimilation sequence had been studied (Middelhoven, 1993; Kemp *et al.*, 1994), if this ability is widely distributed among autochthonous microorganisms is still uncertain. So, the purpose of this report was to screen a diversity of hydrocarbon assimilating yeasts isolated from contaminated sediments able to grow on phenol, and to compare with the *n*-alkanes oxidation ability and ubiquinone type.

Materials and methods.

Diluted sediment samples were assayed for the presence of hydrocarbon utilizing yeasts according to Romero *et al.* (1998). The isolated strains were inoculated on agar plate with mineral basal salts (MB) amended with pyrene as substrate and on malt extract-agar, adjusted to pH 7.0 with HCl, and with streptomycin (0.5 mg.ml^{1}); they were incubated at 28 ± 1° C, during 10 days. Sterile controls of both media were simultaneously incubated.

Yeast species were identified by colony appearance, cell morphologies, pellicle in broth, pseudo-true hyphae, assimilation and physiological differences, and additional tests and growth characteristics were also done according to Kurtzman & Fell (1998). Then, the species were assayed for their ability to grow on phenol and *n*-hexadecane as sole carbon source, both of them given via vapour phase. The inoculation of the plates was performed with yeast cells precultures for 24 h on malt extract-agar and basal medium plus pyrene.

To minimize intracellular reserve compounds c.a. 10^7 cells/ ml were suspended in mineral salt medium without any carbon source and incubated for 20h at 28°C. Then the inoculated agar plates, without any carbon source, were put into cylindrical glass vessels with covers, and a small 25 ml vessel containing 50 mg phenol.

The substrate *n*-hexadecane (0.4 ml) was dropped onto pieces of sterilized filter paper placed in the lid of the petri dishes. The assays were performed during 20 days, at 28° C in darkness, with weekly readings; at the end the results were obtained by comparing the growth with the negative control plates.

Results and discussion.

The isolated yeasts on hydrocarbon agar-plate were identified as *Rhodotorula aurantiaca* *Rhodotorula glutinis*, *Rhodotorula graminis*, *Rhodotorula mucilaginosa* (three strains), *Rhodotorula* spp., *Candida albicans*, *Candida krusei* (two strains), *Candida parapsilosis* (two strains), *Candida rugosa* (three strains), *Geotrichum candidum*, *Debaryomyces hansenii*, *Williopsis californica*, *Pichia anomala*, and *Pichia bovis* and *Pichia cactophila*.

Although they were isolated on this medium, not all of them develop significantly biomass with phenol and *n*-hexadecane as carbon source (Table I).

The *Candida* and *Rhodotorula* species were phenol and *n*-hexadecane positive; however, a significantly increase to assimilate both substrates were observed when the strains were precultured in the aromatic medium. Comparing the *Candida* species with the *Rhodotorula* strains, the first ones were more effective than the second no matter the preculture was.

Despite *R. mucilaginosa* that one strain showed no growth on phenol and the other two strains a weak response, the *Rhodotorula* species were strong degraders; furthermore, *R. aurantiaca* was also active when the cells were precultured in malt-agar. By the other hand, *D. hansenii* was only *n*-hexadecane positive and the preculture in aromatic media enhance the activity of the three *Pichia* species. *G. candidum* and *W. californica* did not show degrading activity on both substrates.

Coenzyme Q structure is considered an important mark which reflects relationship among yeasts, and useful for classifying yeasts at the generic level (Yamada & Kondo, 1971). No hydrocarbon utilizing yeasts have been found in yeast species with CoQ_6 and CoQ_7 (Hofmann & Schauer, 1988); as *Debaryomyces* spp., *Williopsis* spp,. *Geotrichum* spp. and *Pichia* spp. However, nearly all hydrocarbon utilizers possess coenzyme Q_9 or Q_{10}, the *Candida* and *Rhodotorula* species isolated in this study showed possitive results in both treatments and these metabolic ability is in concordance with the Co-Q type.

Trichosporon cutaneum, *Dipodascus tetrasperma*, *D. armillariae*, *C. tropicalis*, *C. maltosa* *R. aurantiaca* and *R. glutinis* had been reported as phenol utilizing organisms (Guého *et al.*, 1992, Polnisch *et al.*, 1992; Middelhoven, 1993, Chang *et al.*, 1995). In this study, the phenol degrading ability of twenty-one autochthonous yeasts were tested; and althuogh some *Rhodotorula* species had been mentionated by other authors, the other strains were not already reported as phenol degrading yeasts.

In conclusion, the phenolic compounds removal from polluted sediments would be speed up by the activation of the indigenous population of these yeast species; furthermore, they are already adapted to phenol and they represented a significant micoflora in this habitats.

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Table I: Experimetal results with the isolated yeasts and Co-Q structure.

[negative; positive; strong positive result; ph phenol, ***n***-hex hexadecane].

	agar-malt		MB+ pyrene		
yeast species	**ph**	***n*-hex**	**ph**	***n*-hex**	**Co-Q**
R. graminis		positive	positive	positive	10
R.mucilaginosa (strain 1)				positive	10
R.mucilaginosa (strain 2)		positive		positive	10
R.mucilaginosa (strain 3)					10
R. glutinis		positive	positive	strong positive	10
R. aurantiaca	strong positive	strong positive	strong positive	strong positive	10
Rhodotorula spp.		positive	positive	positive	10
G. candidum					?
C. parapsilosis (strain 1)		positive		strong positive	9
C. parapsilosis (strain 2)	positive	strong positive	positive	strong positive	9
C. krusei (strain 1)			positive	positive	9
C. krusei (strain 2)		strong positive	strong positive	strong positive	9
C. rugosa (strain 1)	positive	positive	positive	strong positive	9
C. rugosa (strain 2)	positive	positive	positive	strong positive	9
C. rugosa (strain 3)	positive	positive	strong positive	positive	9
C. albicans	positive	positive	strong positive	positive	9
D. hansenii		strong positive		strong positive	9
W. californica					7
P. anomala				positive	?
P. cactophila			positive		7
P. bovis				positive	7