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Filamentous soil fungi with enhanced phenol utilization precultured in hydrocarbon medium.

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Abstract.

Phenolic compounds, like mono-, di-, poly-chlorophenols and nitrophenols, have been classified as priority pollutants by the EPA; so the degradation ability of phenol by filamentous soil fungi is relevant in the environmental biotechnology area. Phenol-degrading fungi were isolated from polluted sediments, and they were cultured on malt extract-agar and on aromatic hydrocarbon-agar. *Aspergillus terreus, Cladosporium herbarum, Cylindrocarpon didymum, Fusarium oxysporum, Pestalohopisis* spp., *Penicillium* spp., *Phoma* spp., *Talaromyces helicus* and *Trichoderma harzianum* were able to grow on phenol given in vapour phase. In most of the cases the mycelia developed on the agar in presence of phenol, and spores were also observed in A. *terreus, C. herbarum, T. harzianum* and *Penicillium* spp. strains. In these assays an increase in the phenol biodegradation were observed when the strains were precultured in aromatic hydrocarbon-agar instead of using medium with conventional carbon sources. So, the presence in contaminated habitats of other compounds, may improved the degradation rate of phenolic pollutants.

Introduction.

The extensive use of phenolic related compounds, like mono-, di-, poly-chlorophenols and nitrophenols, as a result of rapid industrial developments over the past few decades, had produced the deliberately or accidentally released of the xenobiotics into the environment. Many of them are recalcitrant because of their chemical structure and these sites remain polluted for long periods (US. EPA, 1991; Swannell *et al.*, 1996).

Bioremediation of these soils is a conside-

rable challenge due to the complex mixture of the pollutants and it must be remarked that the standing stock of filamentous fungi in these habitats is about tenfold higher than bacteria (Yanagita, 1990). Nevertheless, numerous studies had provided evidence for fungi cross-acclimation, in which exposure to one compound increase the metabolic rates of another of similar structure; being this phenomenon attributed to the broad specificity of the selected organisms and the existence of common pathways catabolism (Leahy & Colwell, 1990; Brunsbach & Reineke, 1995). Therefore, we focused our study on indigenous filamentous soil fungi of contaminated sites; and the aims were to isolate mycelial fungi and to analyze the capacity to grow on phenol as carbon source.

Materials and methods.

Diluted sediment samples were assayed for the presence of hydrocarbon-utilizing species according to Romero *et al.* (1998). The isolated fungi were cultivate 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 amended with streptomycin (0.5 $mg.ml^{-1}$), they were incubated at 28 ± 1° C, during 30 days. Sterile controls of both medium were simultaneously incubated.

The filamentous fungi were identified by morphology and colony appearance through microscopic observation. All the isolated fungi were tested for their ability to grow on phenol and *n*-hexadecane as sole carbon source, given via vapour phase.

The plates were inoculated with a loop with mycelial biomass precultured for 48 h on malt extract–agar, and on basal medium plus pyrene (MB+pyrene).

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, 28 ± 1° 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.

Twenty mycelial soil fungi were isolated from polluted sediments that received the discharge of the Oil Refinery YPF- La Plata, in aromatic hydrocarbon medium. They were identified as *Aspergillus terreus*, *Cladosporium herbarum*, *Cylindrocarpon didymum*, *Fusarium oxysporum* (three strains), *Geotrichium candidum*, *Penicillium* spp. (two strains), *Pestalotiopisis* spp., *Phoma* spp. (two strains), *Talaromyces helicus* (three strains), *Trichoderma harzianum* (four strains); and a sterilia pigmented mycelium was also cultivated.

Among the isolated fungi, some strains were able to grow on phenol given in vapour phase, and an increase in the phenol utilization was observed when the strains were precultured in hydrocarbon-agar instead of using medium with conventional carbon sources, in the *Talaromyces helicus* strains and *Trichoderma harzianum* strains.

In the positive reactions, the mycelia developed onto the agar in presence of phenol, and in two *Talaromyces helicus* strains-plates, a red pigmented substance was observed in the surface agar. According to Hofrichter *et al.* (1993) a characteristic red colour was accumulated in the medium when the phenol metabolite, 4-nitrocatechol, was detected in the fungal experiments.

On the other hand, spores germinated in most of the fungal cultures, as in *A. terreus*, *C. herbarum*, *T. harzianum* and *Penicillium* spp. strains; sporulated cultures were also observed with this substrate, but with *Penicillium* spp. (Hofrichter *et al.*, 1993).

The growth behaviour of the *T. harzianum* strains must be stand out, as they developed a strong mycelia and numerous spores on phenol, no matter the preculture medium was.

Anselmo *et al.* (1989) described the growth of *Fusarium flocciferum* and Madhosnight (1958), *F. oxysporum* with phenol and dinitrophenol; Scow *et al.* (1990) researched the mineralizarion of phenol by *Penicillium* strains. *A. niger* and *A. japonicus* were studied by Shailubhai *et al.* (1984) and Sahasrabudhe *et al.* (1987), respectively.

In summary, the results presented in this study, mentionated filamentous fungi species able to grow on phenol as carbon source, not already cited by other researchers. Moreover, an increase in the adaptation to pollutants when some species

were precultivated in the presence of similar chemical structure was observed; and this can be due to the lack of specificity of the enzymes involved in the phenol degradation (Hofrichter & Fritsche, 1996; Saparrat *et al.* 2000).

Although additional work is needed to determine the significance of these filamentous fungi for soil self-purification or remediation technologies; it is nothworthy the presence in contaminated habitats of numerous fungi species able to degrade phenol, as well as other xenobiotics. This particular event may improved the degradation rates of phenolic pollutants.

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Table I: Experimetal results.[▭ negative; ▨ positive; ▩ strong positive result; ph: phenol, *n*-hex: hexadecane].

Fungi species	**malt-agar**		**MB+pyr**		**phenol cultures**
	ph	**n-hex**	**ph**	**n-hex**	
Talaromyces helicus (strain 1)	▨	▨	▩	▩	red colour medium
Talaromyces helicus (strain 2)	▨	▩	▩	▩	
Talaromyces helicus (strain 3)	▨	▩	▩	▩	red colour medium
***Phoma* spp.** (strain 1)	▨	▨	▩	▩	
***Phoma* spp.** (strain 2)	▨	▨	▨	▩	
Geotrichium candidum	▭	▭	▭	▨	
Aspergillus terreus	▨	▨	▩	▩	spores
Cladosporium herbarum	▨	▩	▭	▩	spores
***Penicillium* spp.** (strain 1)	▨	▩	▩	▩	spores
***Penicillium* spp.** (strain 2)	▨	▩	▭	▩	spores
Trichoderma harzianum (strain 1)	▩	▩	▩	▩	spores
Trichoderma harzianum (strain 2)	▩	▩	▩	▩	spores
Trichoderma harzianum (strain 3)	▨	▩	▭	▩	spores
Trichoderma harzianum (strain 4)	▭	▩	▭	▩	spores
Fusarium oxysporum (strain 1)	▭	▭	▨	▩	
Fusarium oxysporum (strain 2)	▭	▨	▨	▩	
Fusarium oxysporum (strain 3)	▭	▭	▩	▩	
Cylindrocarpon didymum	▭	▨	▩	▩	
***Pestalotiopisis* spp.**	▭	▨	▨	▨	
mycelia sterilia pigmented	▨	▨	▩	▩	

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