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Isolation of Two Fungal Strains Capable of Phenol Biodegradation

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Abstract: In this study, we aimed at isolating fungal strains from the indoor environment that tolerate or/and degrade phenol as sole carbon and energy source. Subsequently, we isolated two fungi (IF2 and IF3, identified as *Alternaria* sp. and *Penicillium* sp., respectively) showed the ability to degrade phenol as sole carbon and energy source in mineral medium. Growth with phenol was monitored by increase in biomass measured as dry weight and depletion of phenol measured by gas chromatography. Both strains were also grown with lactose as reference. The IF2 showed higher phenol degradation capacity ($18.6 \mu\text{g L}^{-1}$) than IF3 ($5.4 \mu\text{g L}^{-1}$).

Key words: Phenol, biodegradation, fungi, mycoremediation, pollutants, aromatics

INTRODUCTION

Phenol is a monosubstituted aromatic compound and is recognized as major environmental pollutant. It is produced through natural and anthropogenic processes. It is found in nature as a constituent of coal tar and is also formed during natural decomposition of organic material (WHO, 1994; EPA, 2002). However, the major part of phenol present in the environment is due to industrial use. Currently, the largest use of phenol is as an intermediate in the production of phenolic resins, which are used in the plywood, adhesive, construction, automotive and appliance industries (EPA, 2002).

Phenol poses several toxic cellular effects (EPA, 2002). Membrane toxicity is the most pronounced effect (Sikkema *et al.*, 1995). Phenol toxic effect on membranes is mainly due to its lipophilicity. Thus, phenol tends to accumulate into the lipophilic layer of the cell membrane disturbing its integrity. Hence, it is not surprising to use phenol in disinfectants (EPA, 2002).

Despite phenol toxicity, a number of microorganisms tolerates and degrades it as source of carbon and energy. Subsequently, several enrichment cultures were obtained and many pure cultures were isolated. Among these isolates are prokaryotic and eukaryotic microorganisms. In the world of prokaryotes, phenol degradation was demonstrated under both conditions, aerobic (Baraniecki *et al.*, 2002; Bej *et al.*, 2000; Kotturi *et al.*, 1991) and anaerobic conditions (Shinoda *et al.*, 2000; Khoury *et al.*, 1992; Tschek and Fuchs, 1987; Healy and Young, 1978).

In respect to biodegradation of phenol by eukaryotes, phenol has been shown to be degraded by several fungal strains including mold strains (Leitao *et al.*,

2007; Bergauer *et al.*, 2005; Hofrichter and Scheibner, 1993) and yeasts (Fialova *et al.*, 2004; Santos and Linardi, 2001). The commonly isolated fungal genera include *Fusarium*, *Alternaria* and *Penicillium* (Leitao *et al.*, 2007; Hofrichter and Scheibner, 1993) and most of these isolates originated from soil.

We aimed in this study initially at exploring the capacity of indoor fungi to degrade phenol to demonstrate the use of sick walls as source of aerobic eukaryotic aromatic-compounds degraders. Since, the paint-coated walls are expected to harbor various aromatic compounds, any growing microorganism is expected to be either tolerant to the toxic properties of these aromatics or posses the potential for degrading such compounds. Such degraders are of particular interest in the field of mycobioremediation.

MATERIALS AND METHODS

Fungi sampling and cultivation: Isolated fungi originated from painted sick-building walls located in Irbid, Jordan during March, 2009. The samples were retrieved by applying the adhesive side of a clear cellotape onto surfaces showing different molds. Molds were transferred to plates containing the growth medium, Potato-Dextrose Agar (PDA). Molds were then incubated at room temperature for at least one week in dark. Growth was inspected daily. Based on colony morphology, three different mold isolates could be obtained. Microscopic observation revealed that they belong to two genera. The identification was guided by comparing the key characteristics to those explained by Watanabe (2002). The two isolates were selected for further experiments, i.e., preparation of spore suspension and quantitative analysis of phenol.

Preparation of spore suspension: Fungal isolates were subcultured on PDA slants (50 mL screw capped bottles containing 20 mL PDA) and incubated for one week. Spores were then suspended in 10 mL 0.8% Tween 80.

Cultivation with phenol and lactose as sole carbon and energy source: Mineral medium lacking carbon and energy sources was prepared to test phenol degradation capacity. The medium contained per liter distilled water: CaCO_3 (4 g), MgSO_4 (2 g), KH_2PO_4 (7 g), NaCl (0.5 g), NH_3SO_4 (6 g), CuSO_4 (0.5 g), ZnSO_4 (0.5 g), Na_2SO_4 (0.5 g), MnSO_4 (0.2 g) and FeSO_4 (0.1 g). The medium was dispensed into 250 screw-capped flasks. Each flask contains 100 ml mineral medium. The medium was then supplemented with 10 and 20 $\mu\text{g L}^{-1}$ liquid phenol as sole carbon and energy source. Parallel flasks containing mineral medium supplemented with lactose (20 g L^{-1}) were also included in the experiment. Additionally, an abiotic control containing only phenol was also included. Complete mineral media containing either phenol or lactose were inoculated with 1 mL of spore suspension of the two selected mold isolates. Duplicate cultures were incubated in shaking incubator (50 rpm) at room temperature over 14 days. Samples (0.5 mL) were retrieved from phenol-supplemented cultures that showed mycelia growth and increase in biomass for GC analysis by the means of sterile syringes.

Biomass measurements: Growth of the isolated fungi was monitored by increase in mycelial biomass measured as dry weight. Semi-permeable filter papers were dried in oven for at least 2 h at 50°C and weighed. Cultures were passed through the pre-weighed dry filter papers under vacuum. The loaded wet filter papers were allowed to dry overnight in oven at 50°C. Dry weight was then estimated by balancing.

Quantitative analysis of phenol by gas chromatography: Concentrations of phenol were determined by Gas Chromatography (GC). The used GC system was GCMS QP5050A (Shimadzu), ionization mode EI. The used column was TRB-1MS (30 m×0.25 mm×0.25 μm). GC program was 60-350 with rate of 35°C min^{-1} and hold for 15 min. Total flow was 20.6 and the column pressure was 100 kPa with split mode (1: 10). Injection temperature was 220°C and detector temperature was 250°C.

RESULTS

Isolation and characterization of fungal strains: Based on colony morphology and microscopic analysis, two fungal strains, IF2 and IF3, were isolated. IF2 and IF3 were

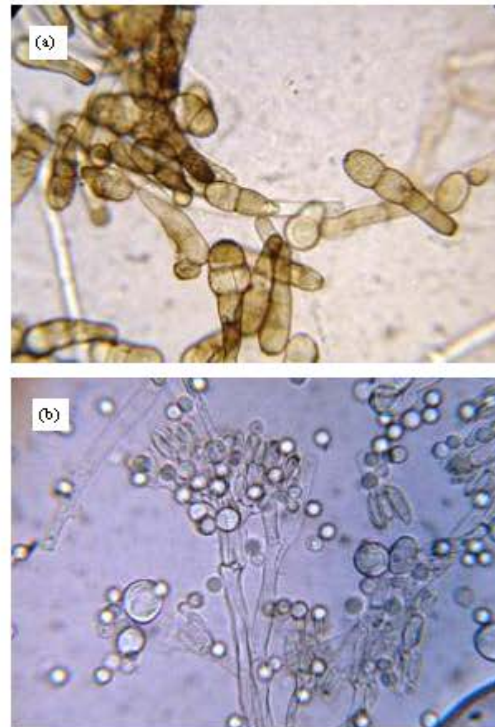


Fig. 1: Light micrograph of the fungal strains (a) IF2 and (b) IF3. Image is magnified 1000x

identified as *Alternaria* sp. and *Penicillium* sp., respectively. *Alternaria* sp. is characterized by pale brown conidiophores. Conidia are cylindrical and catenulate with walls, while *penicillium* sp. showed hyaline, erect conidiophores with hyaline round conidia (Fig. 1a, b).

Mycelial growth utilizing phenol and lactose: The ability of the fungal isolates to grow with phenol as sole energy and carbon source was first monitored by following mycelia growth. Cultures of IF2 containing 10 and 20 $\mu\text{g L}^{-1}$ phenol showed an increase in mycelial biomass after 14 days of incubation (Table 1). However, IF3 showed increase in biomass only in cultures containing 10 $\mu\text{g L}^{-1}$. Increase in biomass indicates the ability of strains to degrade phenol even though IF2 was able to tolerate higher concentration. The two strains were also grown with lactose as sole carbon and energy source as reference. A significant growth was obtained in case of lactose. The biomass obtained in lactose-containing cultures was always higher than that obtained in phenol-containing cultures (Table 1). It is about the 2 folds in case of IF2 and about 3 folds in case of IF3.

Phenol consumption: Cultures of IF2 and IF3 that showed mycelial growth were further analyzed by gas

Table 1: Dry weights of mycelia of the two fungal strains IF2 and IF3 grown with lactose and phenol

Fungal strain	Carbon source (concentration)	Dry weight (g)
IF2	Lactose (20 g L ⁻¹)	1.18
	Phenol (10 µg L ⁻¹)	0.46
	Phenol (20 µg L ⁻¹)	0.45
IF3	Lactose (20 µg L ⁻¹)	1.59
	Phenol (10 µg L ⁻¹)	0.48

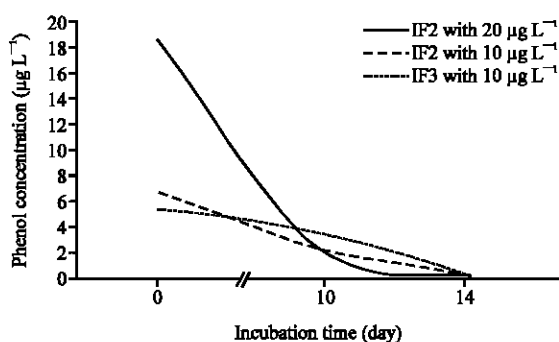


Fig. 2: Phenol consumption by cultures of IF2 growing with 20 µg L⁻¹, 10 µg L⁻¹ and IF3 growing with 10 µg L⁻¹

chromatography to demonstrate phenol consumption over the course of growth. A significant decrease in phenol concentration was detected in these cultures over the time of incubation (Fig. 2). The parallel decrease in phenol concentration and the increase in mycelial growth indicate the phenol degradation capacity of IF2 and IF3.

DISCUSSION

We isolated two fungi: IF2 and IF3 identified as *Alternaria* sp. and *Penicillium* sp., respectively. The two strains were obtained from indoor environment and showed the capacity to grow with phenol as sole energy and carbon source. The capacity of *Alternaria* and *Penicillium* to degrade aromatic compounds was reported by Nambudiri *et al.* (1970) and Leitao *et al.* (2007). However, phenol-degrading *Penicillium* was more frequently reported in the literature. Most of *Penicillium* isolates originated from soil samples. For example, the halotolerant *Penicillium Chrysogenum* was isolated from soil samples of salt mine (Leitao *et al.*, 2007) and *Penicillium* strain Bi 7/2 was isolated from contaminated soil (Hofrichter and Scheibner, 1993). The latter strain was also able to degrade other aromatic compounds. However, among the early work that demonstrated the mycobiodegradability of phenolic compounds was that of Lyr in 1963. Hammel (1995) showed that the fungus *Trametes versicolor* was able to degrade polychlorinated phenols.

Present findings support the findings of others about the biodegradability of phenol by fungi. Most of phenol-degrading fungi reported in the literature, however, originated from soil, whereas our strains originated from indoor environment.

Growth of IF2 with phenol concentration up to 18.6 µg L⁻¹ (measured in the medium) and IF3 with less concentration (5.4 µg L⁻¹, measured in the medium) indicates that IF2 has higher tolerance or/and degradation capacity than IF3.

The toxic effect of phenol on both strains was apparent when the growth yields of cultures of both strains growing with lactose as sole carbon and energy source with those growing with phenol over the same time of incubation were compared assuming no substrate limitation. However, we primarily prepared lactose-containing cultures to check the viability of spore suspension and the suitability of mineral growth medium.

At the cellular level, the most notable effect of phenol is directed to cell membranes. Membrane-directed toxicity of aromatic compounds correlates with the logarithm of its partition coefficient with n-octanol and water (log Pow), which is used as a measure of the relative lipophilicity of a compound. Phenol has a log Pow of 1.46. Organic compounds with a logPow between 1 and 4 generally have a high affinity for cellular membranes and interfere with the functions of the cytoplasmic membrane (Sikkema *et al.*, 1995). This group of compounds also includes the toxic BTEX (benzene, toluene, ethylbenzene, xylene) compounds.

Exploring the diversity of aerobic aromatic-compound biodegraders broaden the organism basis for studies interested in the diversity of aromatic-compounds biodegradation pathways and bioremediation applications.

Phenol degradation pathways were studied in certain prokaryotic and eukaryotic microorganisms. In fungi, phenol was found to be initiated by either ortho or meta cleavage (Leitao *et al.*, 2007; Leonard and Lindley, 1998; Muller and Babel, 1994) where phenol is degraded to catechol. Both pathways have a phenol hydroxylase in the first step of degradation, but in the second degradation step, eukaryotes generally utilize catechol 1, 2-(ortho) dioxygenase and prokaryotes use 2, 3-(meta) dioxygenase (Leitao *et al.*, 2007; Muller and Babel, 1994; Neujahr and Gaal, 1973). On the basis of enzymatic studies, two pathways were proposed for the metabolism of phenol in *Aspergillus fumigatus*. In one route, phenol undergoes ortho-hydroxylation to give catechol; in the other, phenol is hydroxylated in the *para*-position to produce hydroquinone, which is converted to 1, 2, 4-trihydroxybenzene and then, by the action of 1, 2 4-trihydroxybenzene dioxygenase to maleylacetate (Leitao *et al.*, 2007).

CONCLUSION

We isolated two fungal strains from indoor environment with phenol biodegradation capacity. This demonstrates that some indoor fungal strains tolerate phenol and can degrade it as source of carbon and energy.

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