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Biodegradation of phenol in static cultures by *Penicillium chrysogenum* ERK1: catalytic abilities and residual phytotoxicity

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ABSTRACT

A phenol-degrading fungus was isolated from crop soils. Molecular characterization (using internal transcribed spacer, translation elongation factor and beta-tubulin gene sequences) and biochemical characterization allowed to identify the fungal strain as *Penicillium chrysogenum* Thom ERK1. Phenol degradation was tested at 25 °C under resting mycelium conditions at 6, 30, 60, 200, 350 and 400 mg/l of phenol as the only source of carbon and energy. The time required for complete phenol degradation increased at different initial phenol concentrations. Maximum specific degradation rate (0.89978 mg of phenol/day/mg of dry weight) was obtained at 200 mg/l. Biomass yield decreased at initial phenol concentrations above 60 mg/l. Catechol was identified as an intermediate metabolite by HPLC analysis and catechol dioxygenase activity was detected in plate assays, suggesting that phenol metabolism could occur via ortho fission of catechol. Wheat seeds were used as phytotoxicity indicators of phenol degradation products. It was found that these products were not phytotoxic for wheat but highly phytotoxic for phenol. The high specific degradation rates obtained under resting mycelium conditions are considered relevant for practical applications of this fungus in soil decontamination processes.

Key words: *Penicillium chrysogenum*, soil fungus, phenol, biodegradation, phytotoxicity

INTRODUCTION

Soil and water contamination is now considered a serious problem in many industrialized countries. Phenol contamination may arise from a variety of sources of industrial wastewater, such as those from coal refineries, phenol manufacturing pharmaceuticals, industries of resins, paints, dyes, petrochemicals, textiles, pulp and paper mill (9). These phenolic effluents are being discharged into water bodies and this water is used for agriculture and other purposes. Reports of incidents on phenol contamination in the area are scarce. In 2004, some information sources reported that the Iguazú River (Argentina) was affected by the discharges of petrochemical effluents which contain 0.14 mg/l of phenol and other compounds like manganese and aluminium. For these reasons, wastewaters...
containing phenol and phenolic compounds need an appropriate treatment before discharging them into the receiving water bodies (20). Many physico-chemical techniques are available to degrade these pollutants before discharging them (10). Some of these techniques are effective, but most of them are expensive and may lead to the formation of secondary toxic materials or lower mineralization, or need severe operating conditions (10). For these reasons, biological degradation is a viable and economic alternative, which leads to the complete mineralization of the xenobiotic.

On the other hand, bioremediation is a well known treatment for soil contamination, which employs the use of microorganisms that are either naturally occurring or introduced into the soil in order to degrade pollutants (13). Several fungal strains have been reported to degrade phenol as the only source of carbon and energy (5, 12, 18, 19, 24), but many of them are phytopathogens or have high nutritional requirements or fail to colonize the soil and are difficult to apply for soil bioremediation. For these reasons, there is interest in the study of new, non-pathogenic soil fungal isolates.

*Penicillium* species are commonly found in food, indoor air and soils. Particularly, the *Penicillium chrysogenum* has been found on dried cereals, salted meat and many other low water activity foods, but is also common in indoor air environments and salty soils (22). Several members of the genus *Penicillium* are good hydrocarbon-assimilating organisms and there are many reports showing their ability to transform xenobiotic compounds into less mutagenic products (12, 15). *P. chrysogenum* is also used as a complement to identify the fungal isolate (1, 8).

In order to confirm that the fungus was a *Penicillium chrysogenum* isolate, its ability to produce β-lactam antibiotics was analyzed by a diffusion bioassay using *Micrococcus luteus* (ATCC 9341) as described by Castellari et al. (1), since the diameter of the growth inhibition zone is characteristic for each species and is also used as a complement to identify the fungal isolate (1, 8).

**MATERIALS AND METHODS**

**Chemicals**

All the reagents used during this study were of analytical grade, except for phenol and catechol that were of chromatographic grade (purity 99 %) from Sigma-Aldrich (St. Louis, USA). HPLC-grade acetonitrile was from Sintorgan (Argentina).

**Microorganism isolation and growth conditions**

Soil samples were collected from commercial crop soils from Balcarce, Buenos Aires province, Argentina. The soil samples (3 g weight) were mixed with 10 ml sterile water. Serial dilutions of the culture were prepared and spread on mineral medium agar plates supplemented only with 25 mg/l of phenol as a carbon source. The plates were incubated at room temperature for 3 days. The suspected type of colony was purified and was maintained in potato dextrose agar (PDA, Gibco) at room temperature for 14 days (without phenol).

For the degradation assays, the fungus was inoculated directly from the PDA plate into 150 ml of liquid minimal salt medium (LMS) supplemented with different concentrations of phenol as the only source of carbon and energy. The LMS contained: deionized water 1000 ml, MgSO4·7H2O 0.1 g, K2HPO4 0.1 g, NH4NO3 1 g, KCl 0.1g, and 25 µl of trace element solution (in mg/l: MnSO4 15.4, FeCl3 40, ZnSO4 7H2O 6.3, CuSO4·5H2O 2.5, NH4·MO7.O2·4H2O 0.5); pH was adjusted to 6.0.

The selected isolates were identified by physiological, biochemical and molecular tests.

**Morphological characteristics**

The fungus was inoculated onto different culture media: Malt Extract Agar (MEA, Britania, Argentina), Czapek Yeast extract Agar (CYA, Britania, Argentina) and 25 % Glycerol Nitrate (G25N, Britania, Argentina) to observe the different morphological characteristics (colony diameter, color, pigments, exudates, etc.) for its identification according to Pitt (17), Pitt and Hocking (16) and Samson et al. (21).

**Antibiotic production**

In order to observe the number of nuclei per cell, small portions of mycelia previously grown on PDA in darkness for 24 h at 25 ºC were submerged in 0.01 % acridine orange aqueous solution during 10 seconds. The method applied is a modification from the Yamamoto and Uchida’s staining method (28). The stained mycelium was observed under epifluorescent light with an OLYMPUS BX 51 microscope. Digital photographs were taken using the Cool Snap-Pro System. The number of nuclei was counted in 20 cells.
Ehrlich test

The fungal isolate was examined for production of cyclopiazonic acid and other alkaloids reacting with Ehrlich’s reagent using a filter paper method as described by Frisvad and Samson (2).

DNA extraction

The ERK1 strain was grown on potato dextrose broth (PDB) for 3 days. The mycelium obtained was dried, freeze-dried, and disrupted with a hand-operated pellet pestle and DNA was obtained with DNeasy Plant Mini Kit QIAGEN according to the manufacturers’ protocol. The resuspended DNA was stored at -20°C. The DNA was quantified by electrophoresis gel with 0.8 % agarose and Nanodrop™ 2000 (Thermo Scientific).

Polymerase Chain Reaction

Internal transcribed spacer (ITS). Genomic DNA was amplified with primers ITS1 (5′-TCCGTAGGTGAACTTGGG-3′) and ITS4 (5′-TCCTCCTGCTTATTGATATGC-3′) to amplify the region ITS1-5.8s-ITS2 to construct the phylogenetic analyses (Fig. 3); the sequences were chosen to represent sections of Penicillium related with the ERK1 strain, following the classification of Samson et al. (22). Eupenicillium breveldianum was applied as outgroup taxa.

Phenol degradation

Degradation of phenol was analyzed in submerged cultures in 250 ml flasks with 150 ml of LMS medium supplemented at different phenol concentrations as the only source of carbon. Each flask was inoculated with 4 PDA agar discs containing the fungal mycelium. The cultures were incubated at room temperature in the dark in order to avoid phenol destruction under resting mycelium conditions.

Phenol degradation was determined by filtering the mycelium through a Whatman GF/A filter, rinsing twice with distilled water and drying at 100 °C until constant weight. Biomass was calculated as mg of dry weight per litre of reactor volume.

Phylogenetic Analysis

Alignments of the partial beta-tubulin gene sequences were constructed automatically using a built-in CLUSTALW implementation in MEGA software version 4 (27).

Phylogenetic and molecular evolutionary analyses were conducted using MEGA 4.1. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). The evolutionary history was inferred using the Maximum Parsimony method; bootstrap analysis was performed in 1000 replicates with random sequence addition (10 replicates) to estimate branch support. The MP tree was constructed using MEGA 4.1. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion). The evolutionary history was inferred using the Maximum Likelihood method (proteins) implemented in MEGA software version 4 (27).

Plate assays to assess enzymatic activity

Qualitative assays were performed on agar plates to determine the enzymatic activity involved in phenol degradation. Laccase, peroxidase (manganese peroxidase and lignin peroxidase) activities were determined by the colorimetric test with a chromogen such as 4-AMBP and 4-AMPB. The activity was determined at 550 nm using a microplate reader.

Analytical methods

The concentration of phenol was measured using a Waters HPLC system (Millipore, Waters Division, Milford, Massachusetts, USA) consisting of a Model 590 pump, equipped with a UV detector Model 484 variable-wavelength detector set at 270 nm. A computer equipped with HPLC System Manager Software for windows CSW 32 v.1.4 (2002 DataApex Ltd. Czech Republic) was used to acquire and process chromatographic data. The separation was achieved with a Water Spherisorb ODS2 C18 (5 µm) 4.6 x 250 mm analytical column Millipore Corporation, Milford, Massachusetts, USA. Deionized water: acetonitrile (70:30, vol/vol) isocratic system was used as solvent and the flow rate was maintained at one ml/min. These compounds were identified by comparing their retention time with those of similarly treated external standards and by co-chromatography. Under the above conditions, the retention time of phenol and catechol were 10.09 and 5.57, respectively.

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The fungal isolate was examined for production of cyclopiazonic acid and other alkaloids reacting with Ehrlich’s reagent using a filter paper method as described by Frisvad and Samson (2).
peroxidase) and catechol dioxygenase activities were determined as described by Rubilar-Araneda (19), Levin et al. (11) and Shiffman and Cohen (26), respectively.

Phytotoxicity studies
The toxicity of the original and the degraded phenol was assessed by measuring the phytotoxicity effect of LMS, LMS supplemented with phenol 400 mg/l and the residue of phenol degradation on seed germination of wheat (Triticum aestivum), according to Osma et al. (15). Five replicates of 10 seeds were used for each treatment. Germination index (GI) was calculated as follows: \( GI = GP \times \frac{La}{Lc} \), where \( GP \) is the number of germinated seeds expressed as a percentage of control values (LMS). \( La \) is the average value of root length in the phenol solutions and \( Lc \) is the average value of root length in the control.

RESULTS
Identification of the fungal isolate
The fungus was grown on MEA, CYA and G25N. Mycelia showed white obverse color in all the media tested (Figure 1). The reverse colors varied from white to yellow. In CYA at 25 °C, the fungus showed yellow exudates and colonies were radially sulcated (Figure 1).

The fungus grown in MEA medium did not show green color or penicillia at 25 °C. However, when these plates were stored at 4 °C for one month, green color and penicillia were observed (Figure 2 A). Microscopically, penicillia are terverticillate, smooth walled, the conidia width was 3.75 µm and phialide length 12.3 µm (Figure 2 B). The nuclear number per cell was 1 nucleus in hyphal tips and 2-8 nuclei in mature hyphae.

The fungus also showed inhibitory activity against Micrococcus luteus (ATCC 9341), producing a growth inhibition zone with a diameter of 45 mm in diffusion bioassays, suggesting the production of \( \beta \)-lactam antibiotics (Figure 2 C).

The isolate was observed for production of cyclopiazonic acid and other alkaloids by the Ehrlich’s test, but no reaction was observed.

Taxonomic identification of the fungus based on Pitt and Hocking (16) shows that the fungus isolated in this work is a Penicillium strain.

Genomic DNA obtained with the extraction procedure yielded 35 ng/µl. A fragment of 600 bp in size was obtained with ITS1/4 primer pair. A sequence of 441 bp was obtained from the contig assembly of the sequences in both directions. The comparison with Megablast showed 100 % homology (E value 0.0) with P. chrysogenum and Penicillium comune. A fragment of 455 bp in size was obtained from the amplification with pair primers Bt2a/Bt2b. The TEF amplification product was over 500 bp in size, although the corresponding sequence obtained was 265 bp in size due to technical difficulties with the pair primers tested; therefore, it was rejected for further analyses.

![Figure 1](image1.png)  
**Figure 1.** Growth of the fungus on CYA and MEA. A: CYA, 7 days, 25 °C. B: reverse CYA, 25 °C. C: MEA, 7 days, 25 °C. D: reverse MEA, 25 °C.

![Figure 2](image2.png)  
**Figure 2.** Biochemical characteristics of the isolate. A: colony growth (one month old) in MEA. B: Penicillin 1000X. C: Antibiotic-susceptibility test with Micrococcus luteus (ATCC 9341) (susceptible to \( \beta \)-lactam antibiotics). Clear zones indicate growth inhibition around the disk containing the fungal isolate.
Phenol degradation in static cultures by *P. chrysogenum*

From the Parsimony Analysis of the beta-tubulin gene sequences, 4 most parsimonious trees with L=242 steps were obtained, CI=0.643836, RI=0.603053 and Composite Index=0.473472. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches. The most parsimonious assignments of ancestral states for site #1 are shown next to each node. There were a total of 359 positions in the final dataset, out of which 64 were parsimony informative (Figure 3). The clades corresponding to the sections showed high bootstrap values, with 70 % for section *Chrysogena*, 99 % for *Roquefortii* and 74 % for *Viridicata* with the exception of *Penicillium* with 27 %.

**Phenol degradation**

For kinetic studies, the fungus was grown in LMS medium supplemented with phenol as the only source of carbon and energy. Phenol degradation was tested at nominal values of 6, 30, 60, 200, 350, 400 mg/l under resting mycelium conditions (Figure 4). The actual values of phenol concentrations measured by HPLC.

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**Figure 3.** Phylogenetic tree. Maximum parsimony consensus tree with beta-tubulin gene sequences of type strains of *Penicillium* taxa. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. *Eupenicillium brefeldianum* NRRL 710 was applied as an out group. The bars at the right of the cladogram indicate the sections of subgenus *Penicillium* classified by Samson *et al.* (2004).

**Figure 4.** Growth and biodegradation of phenol by *P. chrysogenum* in submerged culture. Degradation of phenol was analyzed by HPLC. LMS medium was supplemented at different phenol concentration as the only source of carbon and energy and inoculated with *P. chrysogenum*. The cultures were incubated in the dark at 25 °C in order to avoid photodestruction of phenol and under resting mycelium conditions. Noninoculated flasks with LMS supplemented with phenol were used as controls. A, B, C, D, E and F corresponding to 6, 30, 60, 200, 350 and 400 mg/l of initial phenol concentrations, respectively.
were 6.28, 31, 60.9, 200, 347 and 400 mg/l. The incubation of *P. chrysogenum* ERK1 in the medium containing phenol as the sole carbon source resulted in an increase in the dry weight of the mycelia over time, concomitant with the decrease in the concentration of phenol in the culture medium (Figure 4). In the case of 60, 200, 350 and 400 mg/l of phenol, the biomass increases until reaching a plateau value which means that the fungal growth has reached the stationary phase (Figure 4). Biomass yield was calculated for each initial phenol concentration and it decreases with the increase of the initial concentration of phenol until 200 mg/l, after which it remained essentially constant (Table 1).

No further dry weight increase was observed following phenol depletion, which demonstrated the ability of the organism to grow on phenol as the only source of carbon.

The time required for complete degradation of the phenol varied at different initial phenol concentrations (Figure 4). Degradation took longer at higher phenol concentrations. For example, on day 6 the degradation percentages were 100 %, 75 %, 51 %, 25.7 %, 26 % and 8 % for 6, 30, 60, 200, 350 and 400 mg/l, respectively. For the initial phenol concentration of 200 and 350 mg/l, the conversion percentages were similar. These results agree with the degradation rates observed for both initial phenol concentration (Table 1). Figure 4 also shows that while the biomass remains constant, the degradation of phenol continues.

Specific degradation rates increase until 200 mg/l, where a maximum is observed, and then decrease showing an inhibitory effect (Table 1). These results agree with that observed for biomass yield.

The inhibitory activity against *M. luteus* (ATCC 9341) by *P. chrysogenum* was analyzed when phenol was used as a carbon source. In these experimental conditions, the fungus did not inhibit *M. luteus* (ATCC 9341) growth, suggesting that *P. chrysogenum* did not produce β-lactam antibiotic when phenol is used as carbon source.

HPLC analysis showed that phenol was completely degraded when used at an initial concentration of 200 mg/l (Figure 5 B). Chromatographic profiles revealed the presence of phenol metabolites formed by the fungus at very low concentrations (less than one mg/l) during the kinetic assay (on day 18) (Figure 5 C). One of the major detected compounds had the same retention time (rt) as the external standard catechol. Other products of phenol degradation were found to be minor, but could not be identified structurally. These minor compounds showed peaks around rt: 5.9, 3.29, 3.77 and 3.9, respectively.

Neither laccase nor peroxidase activities (Mn peroxidases and Li peroxidases) were detected. However, catechol dioxygenase activity was observed in qualitative assays (data not shown). Fungal mycelium developed a yellow color on the plate sprayed with catechol.

**Phytotoxicity assay**

The results of phytotoxicity showed that seed germination was 100 % for wheat in the LMS medium used as a control, 95 % for seeds treated with the products of phenol degradation and 6.25 % for 400 mg/l of phenol. No root elongation was observed in the latter case. Seeds treated with phenol degradation products and control seeds showed similar root elongation, about 14 ± 7.1 mm. Therefore, GI values were: 100 % for both, while seeds treated with 400 mg/l of phenol showed a GI value of 0.4 %.

**DISCUSSION**

Taxonomic identification of the fungus based on Pitt and Hocking (16) shows that the fungus isolated in this work is a *Penicillium* strain, which produces a β-lactam antibiotic showing an inhibition zone in

<table>
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<tr>
<th>Initial phenol concentration (mg/l)</th>
<th>Biomass yield (mg of dry weight/ mg of phenol)</th>
<th>Specific degradation rate (mg of phenol/day/mg of dry weight)</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>2.29±0.016</td>
<td>0.19±0.012</td>
</tr>
<tr>
<td>30</td>
<td>2.31±0.030</td>
<td>0.33±0.021</td>
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<td>60</td>
<td>1.24±0.082</td>
<td>0.48±0.043</td>
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<td>200</td>
<td>0.30±0.013</td>
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<td>350</td>
<td>0.29±0.056</td>
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<tr>
<td>400</td>
<td>0.38±0.040</td>
<td>0.62±0.043</td>
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</table>
Phenol degradation in static cultures by *P. chrysogenum*

Diffusion bioassays lacking phenol, typical of *P. chrysogenum*, as described by Castellari et al. (1).

The phylogenetic analysis based on beta-tubulin gene sequences grouped the species belonging to the same sections together as expected by the comparison with the parsimony analysis performed by Samson et al. (22), although the topology of the tree obtained showed differences with Section *Chrysogena* as poliphyletic. ERK 1 strain grouped with 93% bootstrap with *P. chrysogenum* CBS 306.48. These results are consistent with the high homology observed when analyzed in Megablast with ITS sequences. Although the ITS sequences also yielded high homology with *P. commune*, the sequence analyses of the beta-tubulin gene allowed to differentiate it in a separate cluster from *P. chrysogenum*. The relevance of Samson et al.'s phylogenetic analysis (22) (ibid.) lies in that the authors supported the phylogenetic clades based not only on DNA characters but also on phenotypic characters (morphology and cultural characters).

Therefore, from the agreement in molecular and biochemical characterization, it has been demonstrated that the fungal isolate is very closely related to *P. chrysogenum*.

There are many studies describing the biodegradation of phenol using *Penicillium* isolates (5, 9, 12, 24, 25). Scow *et al.* (25) have described the mineralization of phenol at low initial concentrations by a non-identified *Penicillium* species. On the other hand, *Penicillium frequentans* Bi 7/2 and *P. simplicissimum* SK9117 also use phenol as the only source of carbon and energy (5, 12). *P. chrysogenum* CLONA 2 isolate also completely degrades 300 mg/l of phenol in the presence of sodium chloride (58.5 g/l) (9). Moreover, in this work characterization and degradation potential of a new *P. chrysogenum* ERK1 strain was studied. This fungus shows high specific degradation rates under resting mycelium conditions; which makes it attractive for practical applications in soil decontamination processes. In addition, preliminary results showed that the fungus was able to grow and degrade phenol in artificially contaminated soils (Data not shown).

The specific degradation rates values obtained showed that an inhibition effect occurs. The comparison of the specific degradation rate between *P. chrysogenum* ERK1 and *Penicillium frequentans* Bi 7/2 (5) indicates that the rates are in the same order of magnitude, between 500-1000 mg phenol/g dry weight/day. The

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**Figure 5.** HPLC-chromatogram of phenol biodegradation by *P. chrysogenum*. Kinetics at 200 mg/l of initial phenol concentration was used for this analysis. A: time zero showing the retention time of phenol (Phe: 10.01 min). B: biodegradation of phenol after 20 days. C: metabolite formed during the kinetic assay on day 18 after inoculation time.
degradation rates obtained for *P. chrysogenum* ERK 1 are high considering that cultures were carried out under resting mycelium conditions. In addition, the *P. chrysogenum* strain used in this work was not previously acclimated; for this reason future kinetics studies using the fungus with a previous acclimation period, different inocula sizes and in the presence of different co-substrates could improve degradation rates.

Leitao *et al.* (9) described that the degradation ability of *P. chrysogenum* CLONA2 did not correspond to the visible growth of the mycelia. In this work, the results showed that there is a good correspondence between growth and phenol degradation until biomass remains constant. The same was observed for *Aspergillus fumigatus* degrading 200 mg/l of phenol (7).

Biomass yield ranges between 2.2932 and 0.2965 (Table 1) depending on the initial phenol concentration. These values are in range with that reported for *P. frequens* Bi 7/2 by Hofritcher *et al.* (9). The results showed that above 200 mg/l, biomass growth remained essentially constant; however *P. chrysogenum* continues degrading phenol, suggesting that at this stage phenol is used only for energy requirements. Guedes *et al.* (4) also described that phenol concentration higher than 300 mg/l completely inhibited fungal growth of *P. chrysogenum* CLONA 2.

A number of toxic compounds are formed during industrial processes, giving multicomponent composition of wastewaters. Therefore, the strains used for decontamination processes should not only be highly active to one contaminant but they should also be tolerant of other pollutants or possess different biodegradation abilities (24) and be adaptable to be used in mixed cultures. *P. chrysogenum* ERK1 showed to produce β-lactam antibiotic. However, when it was grown with phenol as a carbon source it did not show antibiotic production. In addition, the fungus was also able to degrade 2, 4, 6-trichlorophenol (data not shown). For this reason, this fungus could be used in mixed cultures to improve phenol degradation or to contribute to the degradation of multi-substrates.

HPLC analysis detected an intermediate product with identical retention time to catechol; this result suggests that phenol metabolism could occur via ortho fission of catechol. On the contrary, Leitao *et al.* (9) could not detect catechol as intermediary metabolite. However, they showed the presence of hydroquinone but only when the fungus was grown in the presence of phenol and glucose as co-substrate. When the culture had only phenol as carbon source no intermediary metabolites were detected. Marr *et al.* (12) identified catechol, hydroquinone and cis, cis-muconic acid as intermediary metabolites during the mineralization of phenol by *P. simplicissimum* SK9117, showing that phenol degradation occurs via the beta-ketoacipdate pathway. In the present work, other metabolites were also observed in HPLC chromatograms. However, future studies have to be done to determine their chemical structures.

Catechol dioxygenase activity observed in plate assays agree with HPLC results, suggesting that phenol metabolism could occur via ortho fission of catechol.

Phenolic effluents are being discharged into water bodies and this water can be used for agriculture. These effluents may cause serious environmental problems and health hazards if not treated appropriately. Thus, it is relevant to assess the phytotoxicity of the phenol before and after degradation. To this purpose, the phytotoxicity of plant growing media based on the germination index (GI) of seeds was evaluated as described by Osma *et al.* (15). This is one of the most common phytotoxic assays used in the literature.

The GI combines measurements of relative seed germination and relative root elongation that are both sensitive to the presence of phytotoxic compounds.

Several species have been traditionally used for evaluating phytotoxicity. However there are no standardized seed species in use worldwide (15). For this reason, wheat (*Triticum aestivum*) was used for this assay because it is a common crop in Argentinean fields. According to Osma *et al.* (15), GI values lower than 50 % mean high phytotoxicity, while values between 50 % and 80 % mean moderate phytotoxicity and values over 80 % indicate that the material is not phytotoxic. Therefore, phenol degradation products were not phytotoxic for wheat.

Finally, the *P. chrysogenum* strain ERK1 described in the present work degrades phenol as the only source of carbon and energy, with high degradation rates and satisfactory biomass yield under resting mycelium conditions. In addition, phenol degradation products did not show any phytotoxic effects. These characteristics make *P. chrysogenum* ERK1 attractive to be used in phenol decontamination of soils.

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REFERENCES


