UTILIZATION OF MYCELIAL GROWTH TO STUDY THE TOLERANCE OF SOME WHITE ROT FUNGI TO PHENOLIC COMPOUNDS

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ABSTRACT: *Phanerochaete chrysosporium*, *Aureobasidium pullulans*, *Coriolus versicolor*, *Pleurotus ostreatus* and *Dichomitus squalens* were some white-rot fungi selected to study the effect of increasing concentrations of phenol, catechol and resorcinol on the mycelial growth on solid media. The increasing concentrations of the phenolic compounds added to a mineral Czapec-Dox agar medium were progressively inhibitory up to a certain maximum value beyond which growth became impossible. *P. ostreatus* was the most affected fungus by the high concentrations used, following by *C. versicolor*, while *P. chrysosporium*, *A. pullulans*, and *D. squalens* tolerated better the presence of the phenolic compounds. It was observed that the toxicity of these compounds towards the microorganisms progressed in accordance to the following order: resorcinol< phenol<cathecol. Furthermore, the capability of growing at the expense of agar, agarose and gelatine as sole carbon and energy source was demonstrated by all the fungi.

KEY WORDS: White-rot fungi; Phenolic compounds; Mycelial growth; Growth inhibition

INTRODUCTION: The white rot fungi have been shown to degrade a wide variety of industrial pollutants, such as phenolic compounds from oil refineries and plastic manufactories. Taking advantage of the ability of these fungi to degrade phenolic compounds, some white rot fungi were screened in order to study the effect of different concentrations of phenol, catechol and resorcinol on their mycelial growth (Santos, 1995).

MATERIALS AND METHODS: Microorganisms - *Phanerochaete chrysosporium* CECT 2779, *Aureobasidium pullulans* CECT 2703, *Coriolus versicolor* LCP 87.3524, *Pleurotus ostreatus* LCP 49.83 and *Dichomitus squalens* from Faculdade de Ciências de Lisboa were the strains used in this study.
Culture conditions - Czapek-Dox mineral salt medium (sodium nitrate 3g/l, dipotassium phosphate 1g/l, magnesium sulphate 0.5g/l, potassium chloride 0.5g/l and ferrous sulphate 0.01g/l) after autoclaving was added of 10g/l saccharose or phenol, catechol and resorcinol to final concentrations of 100, 200, 400, 600, 800 and 1000mg/l, respectively. As organic solidified agent 15g/l agar was used. Each plate was inoculated in the centre with mycelial bits with 5mm of diameter of 10-20 days old cultures of all the selected fungi and incubated at 30°C. Radial growth of mycelium was measured using a millimetric rule. Czapek-Dox with saccharose (Control A) or without carbon source (Control B) agar plates were used as controls. Each experiment was done in triplicate.

RESULTS AND DISCUSSION: The results of the mycelial growth studies for the five white rot fungi with different concentrations of phenol, catechol and resorcinol, after incubation at 30° during 7 days, are shown in Tables 1, 2 and 3, respectively.

Table 1 - Effect of different concentrations of phenol on radial mycelial growth.

Fungus	Control A	Control B	100 mg/l	200 mg/l	400 mg/l	600 mg/l	800 mg/l	1000 mg/l
P. chrysosporium	>85	>85	>85	>85	>85	59	9	0
A. pullulans	22	15	18	15	8	3	0	0
C. versicolor	>85	>85	45	28	13	4	0	0
P. ostreatus	46	64	34	16	0	0	0	0
D. squalens	>85	58	59	45	39	16	0	0

Table 2 - Effect of different concentrations of catechol on radial mycelial growth.

Fungus	Control A	Control B	100 mg/l	200 mg/l	400 mg/l	600 mg/l	800 mg/l	1000 mg/l
P. chrysosporium	>85	>85	>85	61	14	0	0	0
A. pullulans	22	15	15	15	10	3	2	0
C. versicolor	0	>85	0	0	0	0	0	0
P. ostreatus	0	64	0	0	0	0	0	0
D. squalens	>85	58	34	12	0	0	0	0

Table 3 - Effect of different concentrations of resorcinol on radial mycelial growth.

Fungus	Control A	Control B	100 mg/l	200 mg/l	400 mg/l	600 mg/l	800 mg/l	1000 mg/l
P. chrysosporium	>85	>85	>85	>85	>85	>85	59	55
A. pullulans	22	15	16	16	16	18	15	14
C. versicolor	>85	>85	64	51	34	28	15	9
P. ostreatus	46	64	35	32	16	4	0	0
D. squalens	>85	58	74	64	49	40	32	15

The screening of the ability of these five strains to tolerate the phenolic compounds show different patterns. All fungi grew in the presence of phenol and resorcinol, but *C. versicolor* and *P. ostreatus* failed to grow in the presence of catechol. Table 4 summarizes the maximum concentration for different phenolic compounds where mycelia could grow.

Table 4 - Maximum concentrations of phenolic compounds (mg/l) where myceliar growth was observed.

Fungus	Phenol	Catechol	Resorcinol
P. chrysosporium	800	400	1000
A. pullulans	400	400	1000
C. versicolor	400	0	1000
P. ostreatus	200	0	400
D. squalens	600	200	1000

Furthermore, all fungi showed the ability to grow at the expense of agar as sole carbon and energy source (Control B). Taking this observation in mind, it was decided to study other different solid supports. All fungi could utilize the carbon and energy from agarose and gelatine as they did from agar. On the other hand, when an inorganic solid support, like silica-gel, was used it was observed that all fungi were unable to grow when phenol, catechol and resorcinol at 100mg/l were supplied.

CONCLUSIONS: These results permit to conclude that these fungi tolerate the presence of phenolic compounds when metabolize the ordinary microbiological solidified agents. Moreover, the fungi were unable to grow with phenol, catechol or resorcinol as sole carbon and energy source.
Finally, this work indicates that in studies involving filamentous fungi and degradation/metabolization of recalcitrant pollutants in plates special attention should be given to the selection of the solid support, as it can act as carbon and energy source. Alternatively, the introduction of a new control in the experimental design to study the specific response of microorganisms to the solid support should be carried out.

REFERENCE:

Santos, I.M. (1995) Estudo da capacidade de degradação de fenol, catecol e resorcinol por fungos da podridão branca. Tese de Mestrado, Universidade do Minho, Braga.