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PRODUCTION OF PHENOL-OXIDASES AND PEROXIDASES BY FUNGI ISOLATED FROM IRRIGATED RICE

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ABSTRACT

The aim of this work was to study the potential of fungus strains considered as prospective degraders for the herbicides quinclorac and propanil, for ligninolytic enzyme production. Eight fungal strains were grown in King’s B liquid culture medium supplemented with 0.05% Remazol Brilliant Blue R (RBBR) and in liquid culture medium containing wheat bran as substrate. The enzymatic system assessment were: lignin peroxidase, manganese peroxidase and laccases. Results indicated differential patterns of ligninolytic enzyme production; the highest enzymatic activities were related to the production of lignin peroxidase. Among the strains, two (P3SA1F and P11SA2F) showed RBBR discoloration, suggesting the possibility of their application in bioremediation studies.

Key words: ligninolytic enzymes, laccases, Remazol Brilliant Blue R.

INTRODUCTION

From an economic and social standpoints, irrigated rice is one of the most important crop in southern Brazil. Almost all rice growers need to use herbicides to control weeds. Quinclorac and propanil are the herbicides utilized for this purpose. Biodegradation is a viable alternative to reduce the concentration of these compounds in the ecosystem. The enzymatic complex that degrades lignin has been described as responsible for the degradation of several organic pollutants (5). This trait is an advantage when using ligninolytic fungi for bioremediation. A simple and quick method to select fungi with ligninolytic activity is the use of polymeric dyes that are similar to the lignin polymer. Due to its industrial importance, the most utilized dye is Remazol Brilliant Blue R (RBBR).

Considering that the sediment of irrigated rice producing areas may present a mixture of agrochemical and crop residues, this work proposed to study the potential ligninolytic enzyme production by fungal strains obtained from rhizozphere of rice plants. These strains can be considered potential degraders of the herbicides propanil and quinclorac.

MATERIALS AND METHODS

The fungal strains were isolated from the soil rizosphere of rice in farm of rice crops in Santa Catarina. Initially, the fungi were grown in solid culture medium containing malt extract, supplemented with the herbicides propanil and quinclorac, in order to isolate strains that would be degraders and/or resistant to these compounds. Eight strains were selected (Table 1), and transferred (three 9mm discs) to Erlenmeyer flasks containing liquid culture medium, prepared for the following analytical determinations: enzymatic activities and oxidation of the Remazol Brilliant Blue R (RBBR) dye.

Enzymatic analyses

The strains were grown in liquid culture medium (4.5g wheat bran; 1.5g yeast extract; 1g glucose; 0.5g ammonia chloride;

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100mL salts solution; 900mL distilled water), and incubated for 7 days at 30ºC in a static culture system. They were then filtered and submitted to determination of the enzymatic activities of lignin peroxidase, manganese peroxidase and laccases. The lignin peroxidase (LiP) activity was evaluated by UV spectrometry of the veratryl aldehyde produced ($^{TM}_{310}$= 9,300M^{-1} cm^{-1}) during veratryl alcohol oxidation. The manganese peroxidase (MnP) was measured at 610nm ($^{TM}_{610}$= 4,460M^{-1} cm^{-1}) using the methodology described by Kuwahara *et al.* (3). The laccase activity was also measured by spectrophotometry, as o-dianisidine oxidation at 525nm ($^{TM}_{525}$= 65,000M^{-1} cm^{-1}). All analyses utilized boiled culture medium as a control. For all enzymes, one activity unit was defined as the amount of enzyme necessary to oxidize 1µmol of substrate per minute.

Oxidation of the RBBR dye

Strains were grown in King's B liquid culture medium containing proteose-peptone (10g), K2HPO4 (1.5g), Mg.SO4.7H2O (1.5g), glycerin (12.45g), distilled water (1L), and 0.05% Remazol Brilliant Blue R. The flasks were incubated at 30ºC, in the dark, under constant agitation (130rpm), for as long as needed. Decolorization was determined by monitoring the decrease in the absorbance peak at 595nm. The mycelium was separated by filtration and then weighed. The biomass determination was performed by gravimetry, using oven-drying at 70ºC until constant weight was achieved.

RESULTS AND DISCUSSION

The fungi strains presented differentiated ligninolytic enzyme production patterns (Table 1). The highest enzymatic activities detected were related to the production of lignin peroxidase. The maximum detected level was 6.079U L^{-1} (P11SA4F strain), followed by 3.332U L^{-1} (P2SA6F strain). None of the fungi strain was comparable in terms of LiP production to *Ganoderma* sp.strain GASI3.4 (18.851U L^{-1}), used as control.

MnP enzymatic activity was observed in three strains only, and only one of them (P2SA3F) resulted in a higher concentration (2.765U L^{-1}) than that obtained with the control strain (1.084U L^{-1}). The other strains showed very low or none activity. The production of MnP by strain P2SA3F can be considered efficient, especially when compared to activities obtained by Arora *et al.* (1) with white rot fungi. The activities found for laccase ranged from 0.056U L^{-1} to 0.753U L^{-1}. The strain with the highest laccase production was P2SA3F, followed by P2SA5F (0.699U L^{-1}) and P3SA1F (0.609U L^{-1}). The *Ganoderma* sp. control strain GASI3.4 showed a much higher activity (6.057U L^{-1}), thus rendering the other observed activities negligible. However, the values obtained in this work are higher than those obtained for *Coriolus versicolor* (4.406U mL^{-1}) and *Funalia trogii* (4.880U mL^{-1}) (2), or for *Trametes trogii* (0.55U mL^{-1}) when utilizing malt extract as a C source (4).

Some strains of the studied showed an enzymatic complex capable of decolorizing the Remazol Brilliant Blue R dye. Among the eight strains tested (Table 1), two, P3SA1F and P11SA2F, showed 42 and 47% decolorization, respectively, although they showed activity for laccase only. When compared to the standard *Ganoderma* sp. strain GAI3.4, the behavior of these strains was promising, suggesting the possibility of their application in bioremediation studies. Further studies are needed in order to test the efficiency of these strains as degraders, with the objective of exploring the biotechnological potential of such alternative enzyme sources.

RESUMO

Produção de fenol-oxidases e peroxidases por fungos isolados da cultura de arroz irrigado

A proposta deste trabalho foi estudar o potencial das linhagens fúngicas, consideradas potenciais degradadoras dos herbicidas quinclorac e propanil, para produção de enzimas

Table 1. Ligninolytic enzyme production and decolorization of Remazol Brilliant Blue R by fungal strains isolated from a rice crop.

Strains	Enzymatic activity			Remazol degradation (%)	Biomass
	Lignin peroxidase	Manganese peroxidase	Laccase		
P2SA5F	ND	0.192	0.699	6.0	0.90
P2SA3F	3.125	2.765	0.753	4.1	0.82
Q4SR1F	1.136	ND	0.056	25.0	0.83
P3SA1F	ND	ND	0.609	42.1	1.50
P11SA2F	ND	ND	0.299	47.2	0.98
P6SM2F	1.745	0.736	ND	8.2	0.82
P2SA6F	3.332	ND	0.530	1.3	1.13
P11SA4F	6.079	ND	ND	10.3	1.23
GASI3.4 (control)	18.851	1.084	6.057	54.1	1.11

ND= not detected.

ligninolíticas. Oito linhagens fúngicas foram cultivadas em meio de cultura líquido King's B suplementado com 0,05% de Remazol Brilliant Blue R (RBBR) e em meio de cultura líquido contendo farelo de trigo como substrato. Os sistemas enzimáticos avaliados foram: lignina peroxidase, manganês peroxidase e lacases. Os resultados demonstraram padrões diferenciados quanto à produção de enzimas ligninolíticas entre as linhagens, sendo que as maiores atividades enzimáticas estiveram relacionadas à produção de lignina peroxidase. Das oito linhagens, duas (P3SA1F e P11SA2F) apresentaram descoloração do RBBR, sugerindo a possibilidade de sua aplicabilicação em estudos de biorremediação

Palavras-chave: enzimas ligninolíticas, lacases, Remazol Brilliant Blur R.

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