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Textile dyes decolorization and ligninolytic activity by marine-derived *Peniophora* sp. CBMAI 1063

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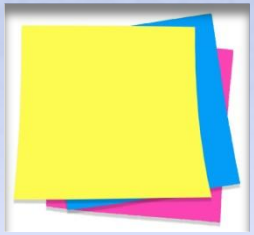
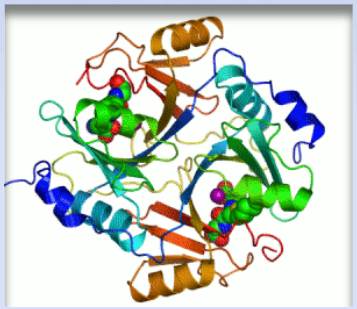
Introduction

- Synthetic dyes are extensively used in different industries:

- The discharge of little amounts of dyes can harm the environment especially the aquatic ecosystem;
- Colored effluents released by different industries may be mutagenic, carcinogenic and toxic;


- Synthetic dyes are usually treated by physical or chemical methods (Fu and Viraraghavan, 2001);



Alternatives for the treatment of dye: ligninolytic fungi, which is able to produce extracellular nonspecific and non-stereoselective enzyme system (Enayatzamir et al., 2009), such as:

- Lignin peroxidases, manganese peroxidases and laccases

Ligninolytic enzymes

- Ability to decompose the heterogeneous plant polymer lignin;
- Potential application in bioremediation of toxic compounds, especially PAHs;

•Enayatzamir, k., Tabandeh, F., Yakhchali, B., Alikhani, H.A., Couto, S.R. J Hazard Mater. 30;169(1-3):176-81, 2009.
•Fu, Y., Viraraghavan, T. Biores. Technol. 79, 251–262, 2001.

- Different groups of fungi have been reported as producers of ligninolytic enzymes;
- The white-rot fungi have received extensive attention due to their powerful production and decolorizing ability (Arora e Sharma, 2010);

- Recent isolation of strains with a better color removal ability different from terrestrial strains, calls worldwide attention towards to the search of fungi belonging to different ecophysiological and taxonomic groups (Hernández-Luna et al. 2008);

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Treatment of Colored Effluents with Lignin-Degrading Enzymes: An Emerging Role of Marine-Derived Fungi

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•Arora D.S., Sharma, R.K. Applied Biochemistry and Biotechnology, 160 (6): 1760-1788, 2010.
•Hernández-Luna, C.E., Gutiérrez-Soto, G., Salcedo-Martínez, S.M. World J. Microbiol. Biotechnol. 24, 465–473, 2008.

Objective

The basidiomycete *Peniophora* sp. CBMAI 1063 isolated from the Brazilian sponge (Menezes et al. 2010), which showed efficient ligninolytic activity in previous studies (Bonugli-Santos et al. 2010), was evaluated in reference to the ability to decolorize two dyes used in the Brazilian textile industries: Remazol Brilliant Blue R – RBBR (also known as Reactive Blue 19) and Indigo dye.

•Bonugli-Santos, R.C., Durrant, L.R., Sette, L.D., Fungal Biology, doi:10.1016/j.funbio.2010.08.003.

•Menezes, C.B., Bonugli-Santos, R.C., Miqueletto, P.B., Passarini, M.R.Z., Silva, C.H.D., Justo, M.R., Leal, R.R., Fantinatti-Garboggini, F., Oliveira, V.M., Berlinck, R.G.S., Sette, L.R. Microbiol. Res. 165 (6): 466-482, 2010.

Methods

Screening of decolorization activity on solid media

Culture media:

- ❖Agar MA2
- ❖Agar MA2+ 3% NaCl
- ❖ Agar MA2ASW (artificial sea water)

200 mg L-1 of RBBR

200 mg L-1 of Indigo

Incubation: 21 days at 28°C

After 7, 14 and 21 days of incubation:

The fungal growth and the decolorization ability on these plates were compared with the controls (RBBR-free inoculated media)

Results

- Fungal mycelia were, in general, not affected by dyes added to the medium, since the diameter growth of colony were similar to the control (RBBR-free) for both dyes;
- After 14 days the dye RBBR was completely decolorized by *Peniophora* sp. CBMAI 1063 in the medium without salt:

Media and Dye concentrations	Time of incubation					
	7 days		14 days		21 days	
	Growth	Decoloriz.	Growth	Decoloriz.	Growth	Decoloriz.
MA2 Control (RBBR-free)	7	0	Total	0	Total	0
MA2 + 200 mg L^{-1} RBBR	7	5.1	Total	Total	Total	Total
MA2ASW Control (RBBR-free)	3	0	5,3	0	6,2	0
MA2ASW 200 mg L^{-1} RBBR	3,3	0	4,6	0	6	0
MA2+3%NaCl Control (RBBR-free)	0	0	2	0	3,5	0
MA2+3%NaCl 200 mg L^{-1} RBBR	0	0	2,3	0	2,8	0

- RBBR decolorization on MA2 medium:

Control (zero time) **7-day-old** **14-day-old** **Control (RBBR–free)**

- No decolorization was observed:
  - in saline conditions
  - for Indigo dye
- To stimulate the decolorization of Indigo the fungus was also inoculated at different concentrations of malt extract:

MA1 (1% malt extract) and MA0,5 (0,5% malt extract)

- There was no decolorization during 21 days of incubation

Methods

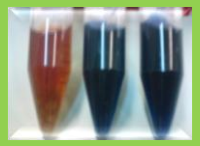
Determination of decolorization ability on liquid medium

Fungal culture plugs were transferred to 50 ml MA2 broth

After 72 h, at 140 rpm and 28°C:

RBBR (500 and 1000 mg L^{-1}) was added

Incubation: 7 days, 28°C and 140 rpm



Aliquots from the cultures were taken after dye addition (zero time) and in each 24 hours

❑ **Color reduction: Decolorizing activity** (López et al. 2006):

$$\text{Decolorization (\%)} = \frac{A_{\lambda\ \text{initial}} - A_{\lambda\ \text{Final}}}{A_{\lambda\ \text{initial}}} \times 100$$

❑ **Ligninolytic activities** (Bonugli-Santos et al. 2010) :

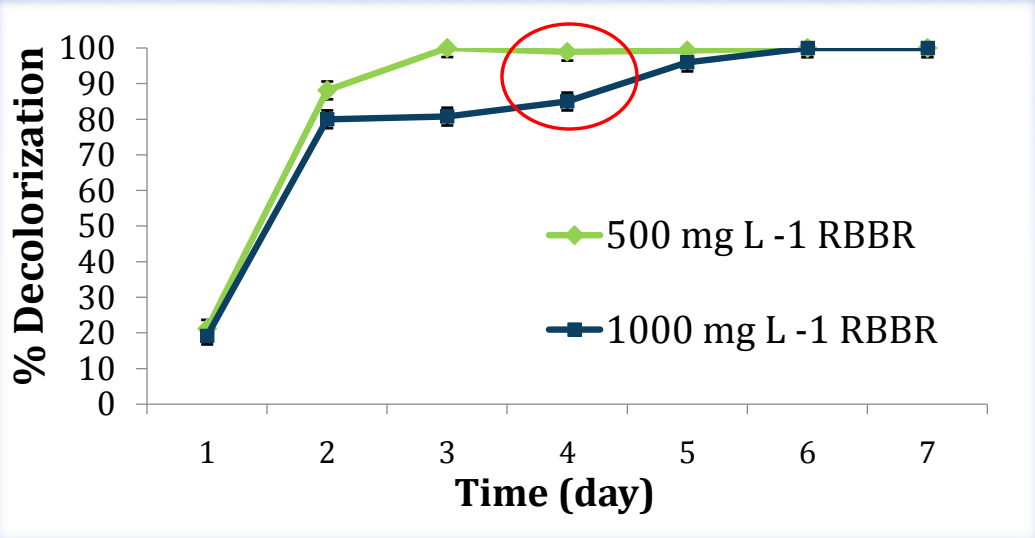
- Laccase: ABTS;
- MnP: phenol red;
- LiP: veratryl alcohol.

Samples were centrifuged (12,074 g, 10 min) and the supernatants were spectrophotometrically evaluated :

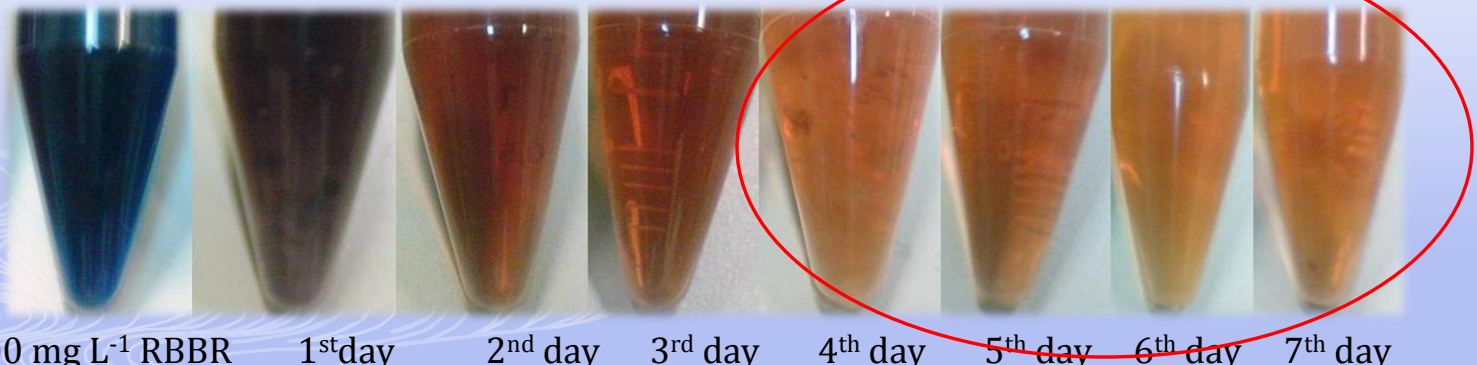
- Bonugli-Santos, R.C., Durrant, L.R., da Silva, M., Sette, L.D. Enzyme Microb. Technol. 46, 32-37, 2010.
- López, M. J., Guisado, G., Vargas-García, M. C., Suárez-Estrella, F. and Moreno, J. Enzyme Microb. Technol. 40, 42–45, 2006.

Results

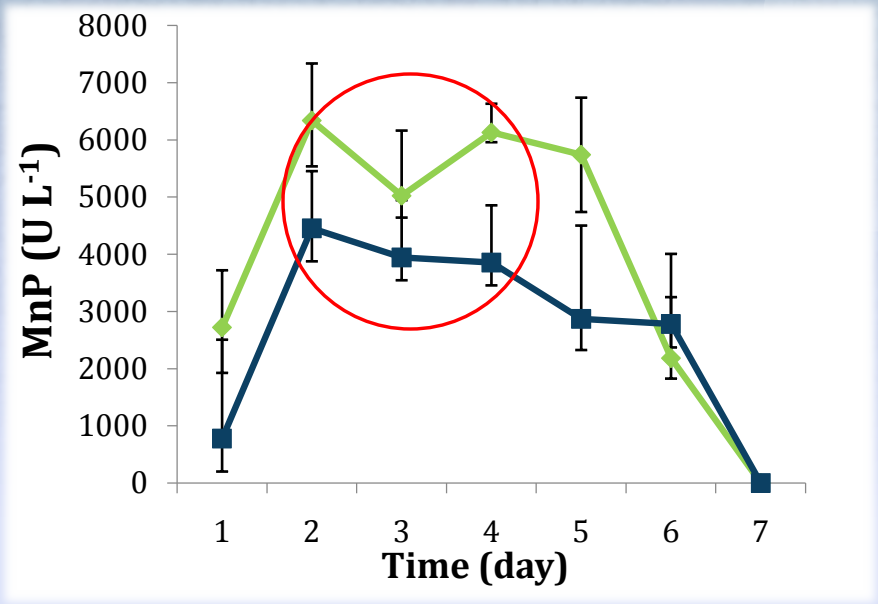
- RRBR was decolorized in the 4th day;



- Range of decolorization:



Control 500 mg L^{-1} RBBR 1st day 2nd day 3rd day 4th day 5th day 6th day 7th day



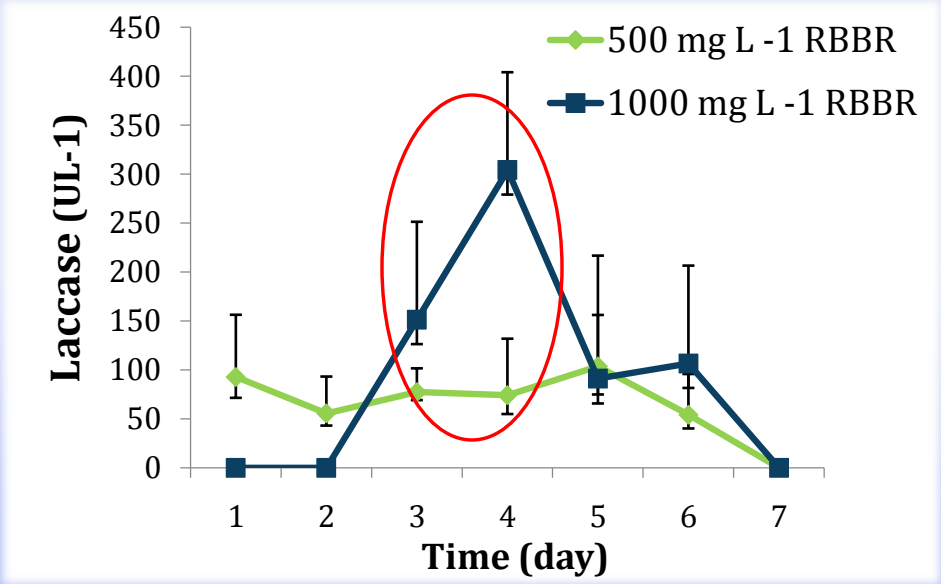
- MnP and laccase were detected during the decolorization process;
- LiP was not producted;
- The highest productions were proportional to the rate of decolorization;

- The activity of MnP increased in the presence of RBBR;

Highest enzymatic activities in control (RBBR-free):

MnP = 1,099 Ul^{-1} (after 21 days)

Lac = 677.5 Ul^{-1} (after 7days)



Methods

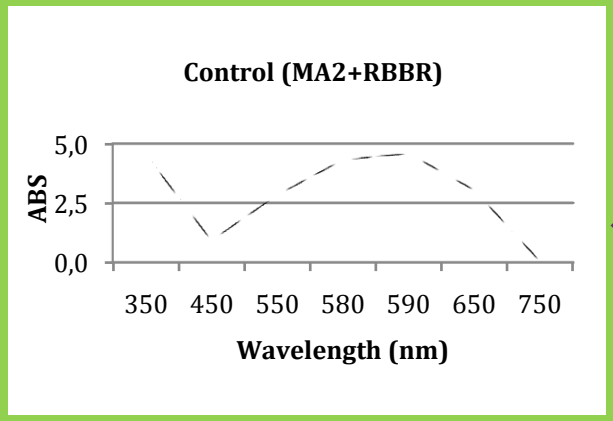
Determination of decolorization ability on crude enzymatic extract

7-day-old cultures (RBBR-free)

Centrifuged (12,074 g, 30 min)

RBBR (500 mg L^{-1}) were added

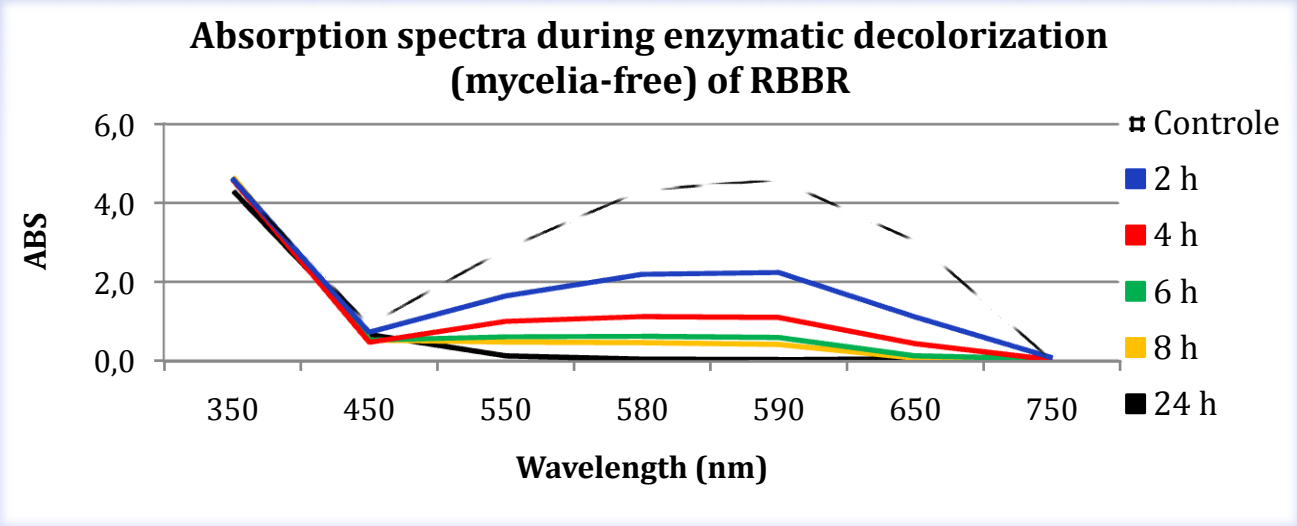
Supernatant samples with ligninolytic activity = crude enzymatic extract



The absorption spectra were read at the range of 200–800 nm in each 2 h from time zero (addition RBBR) during 24 h of incubation at 28 °C

Results

- After 2 h of incubation at 28°C, the crude enzymatic extract showed a decreasing of 50% in the absorption spectrum, reaching 100% after 24 h;



- This result showed that there was a complete removal of the major visible light absorbance peak, suggesting that RBBR decolorization can take place in the absence of mycelia.

Discussion

- Representatives of genus *Peniophora* have been reported as able to decolorize RBBR dye (Barrasa et al. 2009) and to produce ligninolytic enzymes, mainly laccase (Niku-Paavola et al., 2004);

Peniophora cinerea

The evaluation of RBBR decolorization by terrestrial *Peniophora cinerea* showed that MnP is the mainly enzyme in the process (Machado et al., 2005).

•Barrasa, J.M., Martínez, A.T., Martínez, M.J. Folia microbiologica. 54(1): 59-66, 2009.
•Machado, K.M.G., Matheus, D.R., Bononi, V.L.R. Brazilian Journal of Microbiology. 36,246-252, 2005.
•Niku-Paavola, M.-L., Fagerström, R., Kruus, K., Viikari, L. Enzyme Microb. Technol. 35: 100-102, 2004.

- Advantage → Marine-derived fungi

- Marine-derived fungi are being reported as efficient fungi for decolorization of dyes and colored effluents:

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CNIDARIAN-DERIVED FILAMENTOUS FUNGI FROM BRAZIL: ISOLATION, CHARACTERISATION AND RBBR DECOLOURISATION SCREENING

M. DA SILVA[1*], M. R. Z. PASSARINI[2], R. C. BONUGLI[2] AND L. D. SETTE[2*]

Da Silva, M., Passarini, M.R.Z., Bonugli, R.C, Sette, L.D. Environ. Technol. 29, 1331-1339, 2008.

Mar Biotechnol
DOI 10.1007/s10126-009-9187-0

ORIGINAL ARTICLE

A Thermostable Metal-Tolerant Laccase with Bioremediation Potential from a Marine-Derived Fungus

Donna D'Souza-Ticlo · Deepak Sharma · Chandralata Raghukumar

D'Souza-Ticlo, D., Sharma, D., Raghukumar, C. Mar Biotechnol. 11(6):725-37, 2009.

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Treatment of Colored Effluents with Lignin-Degrading Enzymes: An Emerging Role of Marine-Derived Fungi

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Raghukumar ,C., D'Souza-Ticlo, D., Verma, A.K. Crit Rev Microbiol. 34: 189–206, 2008.

- Fungi derived from marine environments have been one of the best alternatives for the bioremediation of environmental pollutants with **alkaline and/or saline conditions**, such as colored industrial effluents:

Marine environment:

- ph: ~ 8
- Salinity: ~ 3,5%

- Although there was no decolorization in saline conditions, *Peniophora* sp., produced significant amounts of Ligninolytic enzymes in the medium with saline conditions:

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Laccase activity and putative laccase genes in marine-derived basidiomycetes

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ABSTRACT

Studies of laccases from marine-derived fungi are limited. In the present work, putative laccase genes from three marine-derived basidiomycetes and their laccase activities were evaluated. High amounts of laccase were produced by the fungal strains *Marasmiellus* sp. CBMAI 1062 (971.2 U L^{-1}) and *Peniophora* sp. CBMAI 1063 (709.03 U L^{-1}) when grown for 21 d at 28 °C in MA2ASW medium prepared with artificial seawater. Marine-derived basidiomycetes produced multiple distinct laccase sequences of about 200 bp with 73–90 % similarity to terrestrial basidiomycete laccases. *Marasmiellus* sp. CBMAI 1062 and *Tinctoporellus* sp.

- Fungi derived from marine environments have been one of the best alternatives for the bioremediation of environmental pollutants with **alkaline and/or saline conditions**, such as colored industrial effluents:

Marine environment:

- ph: ~ 8
- Salinity: ~ 3,5%

- Marine-Derived *Peniophora* sp.

Next steps:

- Decolorization ability in the liquid medium with saline conditions

Conclusion

- RBBR decolorization is a good methods for screening of ligninolytic activity and treatment of environmental pollutants ability;
- Additionally:
  - Probably, MnP is the manly enzyme in this RBBR decolorization;
  - RBBR decolorization using crude enzymatic extract may be used in processes where the fungal cultivation could not be possible;
  - Results are valuable for several biotechnological applications;

Next Steps

Results obtained in the present work stimulate the development of new studies concerning to:

- Decolorization and degradation of synthetic dyes in saline conditions;
- Decolorization and degradation of colored effluents, from the textile industries;
- Degradation of several environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs).

Thanks!

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