Discovery of two low redox potential, novel laccases *Pc*Lac1 and *Pc*Lac2 from *Pleurotus citrinopileatus.*

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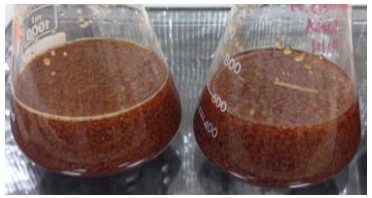
Introduction

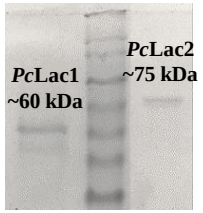
The technological utilization of lignocellulosic biomass and, in particular, its bioconversion towards biofuels and other high added-value products attract the scientific interest in the field of Industrial Biotechnology. Especially in terms of circular economy, bioprocesses of sustainable waste management are even more advantageous. White-rot Basidiomycetes possess an intricate enzymatic system, able to efficiently decompose lignocellulosic biomass. More specifically, laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2), belonging to the family of multicopper oxidases, catalyze the breakdown of covalent carbon-carbon or carbon-oxygen bonds in complex lignin polymers. The aim of the present study is the characterization of two novel laccase-like multicopper oxidases (LMCOs), from the Basidiomycete *Pleurotus citrinopileatus* LGAM 28684, with potential for biotechnological application in the field of phenolic oligomer synthesis.

Olive mill wastewater cultivation

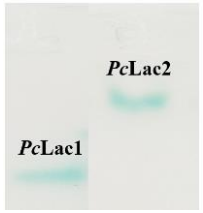
P. citrinopileatus was cultivated in an olive mill wastewater-based liquid medium. *Pc*Lac1 and *Pc*Lac2 were isolated from the supernatant of the culture.



SDS-PAGE analysis



Zymogram



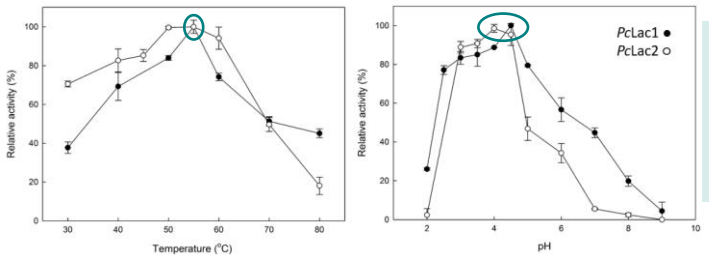
pI for *Pc*Lac1 ~ 3.5

E_0 redox potential vs. NHE (normal hydrogen electrode) at 30 °C
***Pc*Lac1** 453±1.2 mV
***Pc*Lac2** 374±3.9 mV

Purification of isolated LMCOs

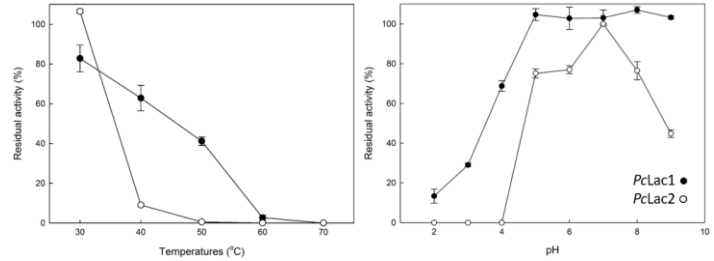
	*Pc*Lac1 U mg^{-1}	*Pc*Lac1 Purification (fold)	*Pc*Lac1 Yield (%)	*Pc*Lac2 U mg^{-1}	*Pc*Lac2 Purification (fold)	*Pc*Lac2 Yield (%)
crude	0.47	1.00	100.00	0.47	1.00	100.00
Q sepharose	10.19	21.76	36.29	18.40	39.30	15.12
DEAE	18.68	39.91	30.37	27.65	59.07	11.79

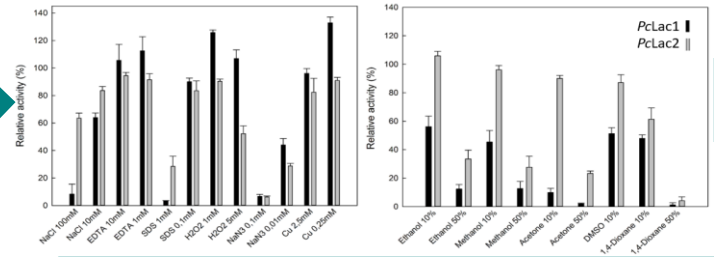
Biochemical Characterization



Optimum reaction conditions
Temperature:55 ˚C
pH: *Pc*Lac1 ~4.5 and *Pc*Lac2 ~4

T stability after 4 h incubation
pH stability after 24 h incubation.





Effect of inhibitors and solvents

***Pc*Lac1**: catecholic structures and hydroxycinnamic acids with hydroxyls at ortho-position, dimethoxy-substituted compounds (not phenolic alcohols, amines, aldehydes or hydroxybenzoic acids)
***Pc*Lac2:** phenolic compounds with catecholic structure

Substrate specificity of *Pc*Lac1 and *Pc*Lac2

Enzyme	ABTS (U mg^{-1})	2,6 DMP (U mg^{-1})	Catechol (U mg^{-1})	Pyrogallol (U mg^{-1})	Guaiacol (U mg^{-1})	Hydroquinone (U mg^{-1})
***Pc*Lac1**	1.48 ± 0.09	0.08 ± 0.00	0.06 ± 0.01	0.020 ± 0.001	0.003 ± 0.000	0.079 ± 0.003
***Pc*Lac2**	4.78 ± 0.33	0.019 ± 0.003	0.04 ± 0.01	0.0390 ± 0.0003	0.0006 ± 0.0002	0.09 ± 0.02

Phenolic oligomer synthesis

*Pc*Lac1 Sinapic Acid	*Pc*Lac1 Ferulic Acid	*Pc*Lac2 Sinapic Acid

	*Pc*Lac1 Sinapic Acid	*Pc*Lac1 Ferulic Acid	*Pc*Lac2 Sinapic Acid
$m_{product}$ / Catalytic activity (mg/Unit)	0,73%	0,14%	1,40%
$m_{product}$ /$m_{substrate}$ (mg/mg)	36,0%	6,8%	81,2%

Highlights

- Two laccase-like multicopper oxidases were isolated from *Pleurotus citrinopileatus*
- *Pc*Lac1 and *Pc*Lac2 were characterized and used for phenolic acids oligomerization
- Both LMCOs were of low redox potential, and similar to known *Pleurotus* laccases
- The synthesis of oligomer products shows the biocatalytic potential of LMCOs

Further studies are necessary to determine the exact structure and bioactivity of the oligomer products

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