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Topic: Wine components with physiological effects.

OTA degradation by bacterial laccases

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Keywords: Ochratoxin A reduction, lactic acid bacteria laccases, polyphenolic compounds, redox mediators.

Laccases from lactic acid bacteria (LAB) are described as multicopper oxidase enzymes with copper union sites. Among their applications, phenolic compounds' oxidation and biogenic amines' degradation have been described. Besides, the role of LAB in the toxicity reduction of ochratoxin A (OTA) has been reported (Fuchs et al., 2008; Luz et al., 2018). Fungal laccases, but not bacterial laccases, have been screened for OTA and mycotoxins' degradation (Loi et al., 2018). OTA is a mycotoxin produced by some fungal species, such as *Penicillium* and *Aspergillus* sp., which infect grape bunches used for winemaking. OTA degradation is paramount given that it has been described as human-health harmful according to EFSA.

The work aimed to evaluate the OTA degrading capacity of three heterologous LAB laccases expressed in *E. coli*. The experimental procedure consisted on testing bacterial laccases from *L. lactis, L. paracasei* and *P. parvulus* in acetate buffer pH 4 with or without $CuSO_4$ and OTA in presence and absence of several concentrations of epicatequin and complete polyphenolic extracts from red and white wine as mediators. Degradation of OTA was followed and quantified by analyzing samples with HPLC-QToF-MS.

According to the results, OTA degradation in the reaction buffer with copper was at least three times higher than without copper. In addition, 0.75 mM epicatequin was the optimum concentration to obtain the highest OTA degradation with *L. paracasei* laccase (78%). Then, *P. parvulus* and *L. lactis* laccases were tested at this concentration, averaging 70% degradation. Finally, mean values of 40% and 10% OTA degradation were revealed when using polyphenolic extracts from red and white wine, respectively, for the three laccases. The application of these LAB laccases on OTA degradation in real wine needs to be further explored.

References:
Fuchs S., et al. (2008). Food Chem Toxicol; 46:1398-1407.
Loi M., et al. (2018). Food Control; 90: 401-406.
Luz C., et al. (2018). Food Chem Toxicol; 112: 60-66.

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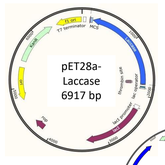
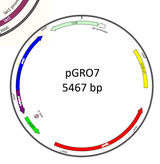
- ✓ Laccases from lactic acid bacteria (LAB) are multicopper oxidase enzymes that have been reported with potential in wine industry as biological tools for degrading toxic and undesirable compounds such as biogenic amines[1]. It has been demonstrated that LAB cells, but not bacterial laccases, reduce the toxicity of ochratoxine A (OTA)[2].
- ✓ OTA is a mycotoxin produced by several filamentous fungus species (*Penicillium* and *Aspergillus* sp.) that infect grape bunches used for winemaking, and is considered nephrotoxic, hepatotoxic, teratogenic and immunotoxic for animals and possible carcinogenic for humans according to the International Agency for Research on Cancer (IARC).
- ✓ This work aimed to evaluate the OTA degrading capacity of three heterologous lactic acid bacteria (LAB) laccases from *L. paracasei, L. lactis and P. parvulus,* expressed in *E. coli*, using ABTS and epicatechin as mediators.

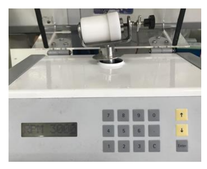
Production of recombinant LAB laccases

Cloning → Laccase induction → Laccase extraction → Lacasse purification





2 L – TB medium

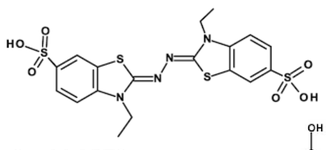


OTA degradation essays

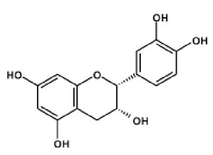
Sodium acetate buffer 50mM
+/- 0,1 mM $CuSO_4$

25 µg purified laccase

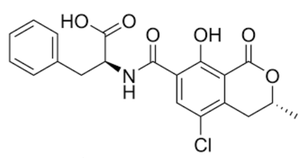
- 1.56 mg/mL *L. lactis* laccase
- 13.64 mg/mL *L. paracasei* laccase
- 2.51 mg/mL *P. parvulus* laccase



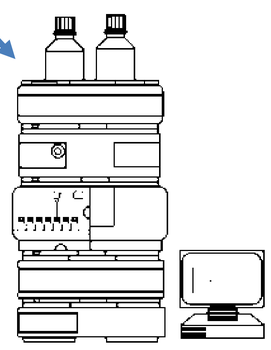
2 mM **ABTS**



2 mM **epicatechin**



500 µg/L OTA



HPLC –QToF-MS

Complete polyphenolic extracts from red or white wine resuspended in sodium acetate buffer 50mM with 0,1 mM $CuSO_4$

25 µg purified laccase

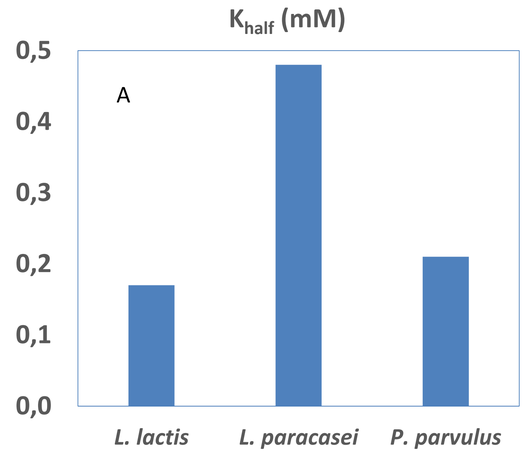


Figure 1. Kinetic parameters of bacterial laccases. A: K_{half} parameter, B: Specific activity of each protein.

Copper does not affect OTA degradation with ABTS, but does it with epicatechin; Cu increases three times the OTA degradation percentage when epicatechin is present (for the three laccases) (Table 1).

Table 1. OTA degradation by bacterial laccases with and without copper using ABTS and epicatechin as mediators.

Lacasse	ABTS 2mM		Epicatechin 2mM	
	+Cu	-Cu	+Cu	-Cu
Commercial laccase	0%	0%	22.0±1.0%	35.0±2.9%
L. lactis	19.7±3.1%	16.2±2.4%	64.6±1.5%	2.3±2.3%
L. paracasei	9.5±0.7%	9.4±1.9%	79.5±2.5%	26.4±1.0%
P. parvulus	-	-	81.4±1.0%	18.2±2.8%

OTA degradation percentages higher than 70% were obtained with 0.5 mM or above epicatechin, and the highest percentage was accomplished at 0.75 mM (Figure 2).

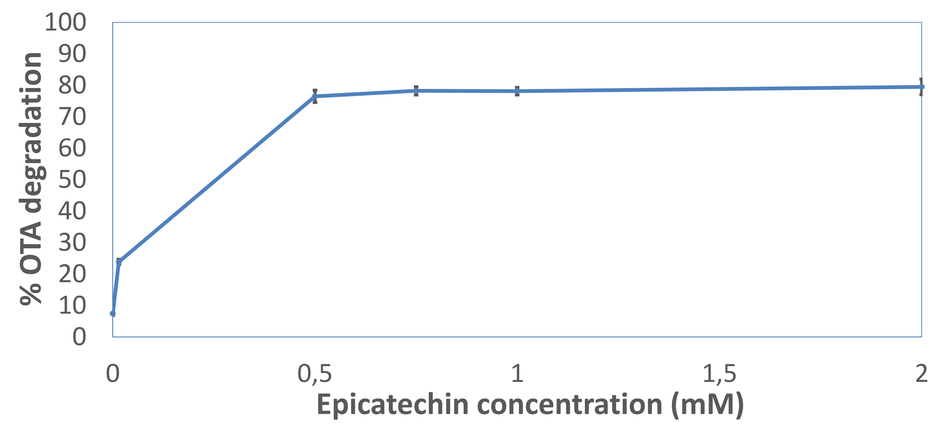


Figure 2. % OTA degradation at different epicatechin concentrations for *L. paracasei* laccase.

The optimum concentration of epicatechin for the three laccases is evidenced as 0.75 mM (Figure 3).

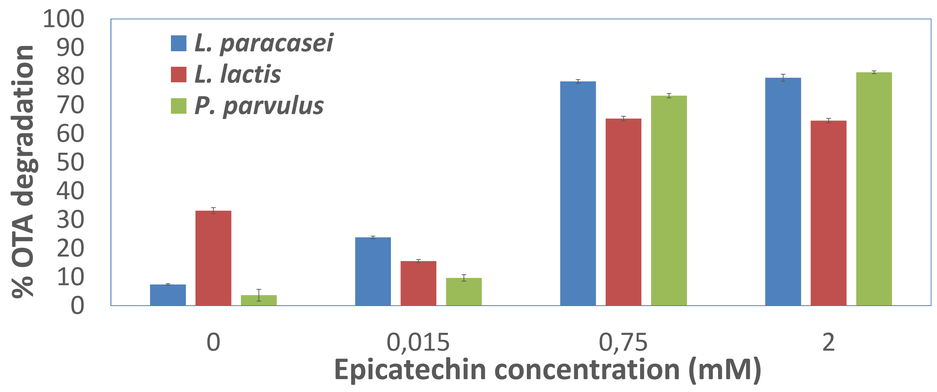


Figure 3. % OTA degradation for the three bacterial laccases in buffer with copper.

The percentage of OTA degradation is three times higher in the red wine polyphenolic extract than in the white wine one (Figure 4).

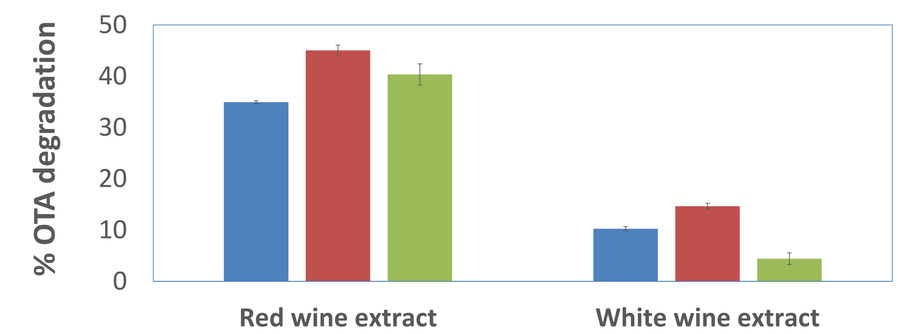


Figure 4. OTA degradation by bacterial laccases in complete polyphenolic extracts from red and white wine resuspended in sodium acetate with copper.

Conclusions

- ➢ Copper does not improve OTA degradation with any laccase in presence of ABTS, but it does with epicatechin.
- ➢ The optimum epicatechin concentration for OTA degradation with *L. paracasei* laccase was 0,75 mM.
- ➢ The three laccases averaging 70% OTA degradation at and above 0.75 mM epicatechin.
- ➢ 40% and 10% OTA degradation for the three recombinant laccases was accomplished in complete polyphenolic extracts from red and white wine respectively.
- ➢ These results are promising in terms of the posible application of these three bacterial laccases on wine.

References

1. Olmeda I., Casino P, Collins R.E, Sendra R, Callejón S, Huesa J, Soares A, Ferrer S, Pardo I. (2021) Structural analysis and biochemical properties of laccase enzymes from two *Pediococcus* species, *Microbial Biotechnology* 14: 1026-1043
2. Luz C, Ferrer J, Mañes J, Meca G. (2018) Toxicity reduction of ochratoxin A by lactic acid bacteria. Food Chem Toxicol. 112:60-66

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