Application of a new recombinant laccase in the biodegradation of antibiotics

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Introduction

Laccases (EC 1.10.3.2, benzenediol: oxygen reductase) are multi-copper oxidases enzymes that are capable of oxidizing a wide range of phenolic and non-phenolic substrates. Those from fungi and bacteria are the most studied, with enzymes from extremophiles currently generating great interest. Laccases present interesting characteristics for biotechnological applications, particularly for bioremediation as their catalysis requires only oxygen as a co-substrate, generating water as only by-product (1). Furthermore, these enzymes can be used in conjunction with redox mediators that allow increasing the range of oxidizable substrates.

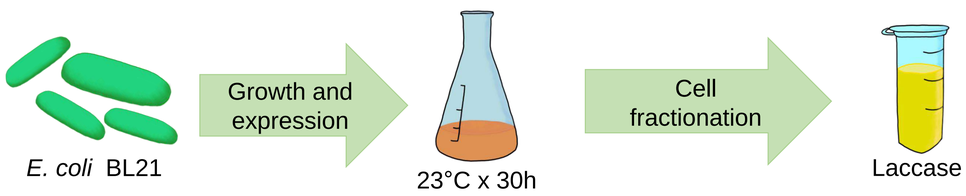
Annually, 200,000 tons of antibiotics are used for human and veterinary consumption. Their indiscriminate usage has led to an increase in their presence in the environment, generating a harmful impact in the biodiversity as well as increased appearance of antibiotic resistant bacteria. Quinolones and macrolides been detected in rivers and even in the Antarctic Sea (2).

In Fundación Biociencia a novel laccase from a thermoalkaliphilic microorganism has been isolated and heterologously produced. The recombinant enzyme exhibits remarkably high specific activity (>450,000 U/mg) at 70°C, pH 6.0, using syringaldazine substrate, and is active in a wide range of temperature (20-90°C) (3).

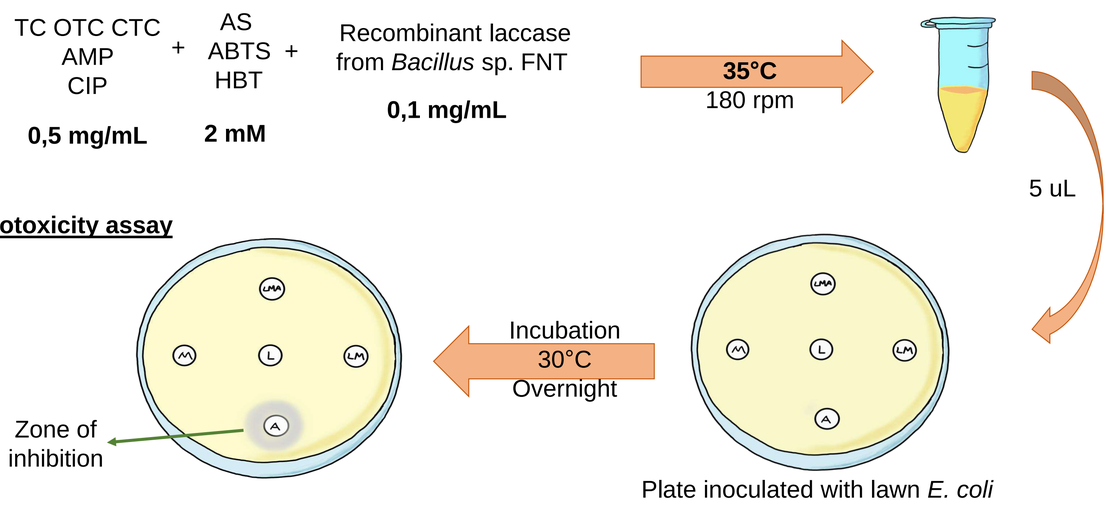
In this work, we evaluate the ability of this thermophilic laccase to biodegrade five different antibiotics from three different families (β-lactam, tetracyclines and quinolones) in presence of three different redox mediators (HBT, ABTS, acetosyringone).

Methodology

Production of recombinant laccase



Biodegradation assay



TC: tetracycline; **OTC**: oxytetracycline; **CTC**: chlortetracycline; **AMP**: ampicillin; **CIP**: ciprofloxacin
AS: acetosyringone; **L**: laccase; **M**: mediator; **A**: antibiotics.

Results

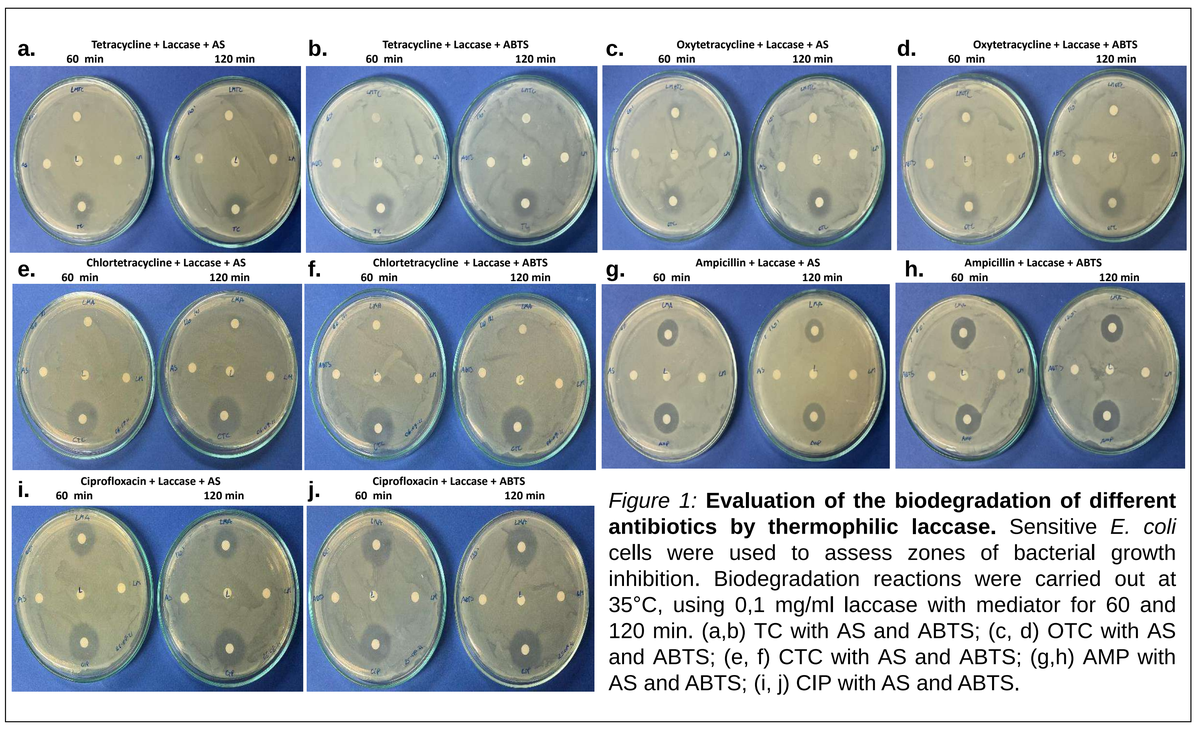


Figure 1: **Evaluation of the biodegradation of different antibiotics by thermophilic laccase.** Sensitive *E. coli* cells were used to assess zones of bacterial growth inhibition. Biodegradation reactions were carried out at 35°C, using 0,1 mg/ml laccase with mediator for 60 and 120 min. (a,b) TC with AS and ABTS; (c, d) OTC with AS and ABTS; (e, f) CTC with AS and ABTS; (g,h) AMP with AS and ABTS; (i, j) CIP with AS and ABTS.

Table 1: **Diameters of bacterial growth inhibition zones.** L: laccase; M: mediator; A: antibiotic. The diameter of filter paper disc is 6 mm. Antibiotics biodegradation reactions were carried out at 35°C, using 0,1 mg/ml laccase with mediator (AS, ABTS or HBT)) for 60 and 120 min.

	AS			
	60'		120'	
	A	L + M + A	A	L + M + A
Tetracycline	16,8 ± 0,6	6,4 ± 0,7	17,0 ± 1,8	7,1 ± 1,9
Oxytetracycline	17,2 ± 2,3	8,8 ± 2,1	17,2 ± 1,1	6,0 ± 0,0
Chlortetracycline	23,5 ± 0,6	7,3 ± 2,3	23,7 ± 1,6	7,9 ± 0,8
Ampicillin	15,4 ± 6,6	12,5 ± 5,6	15,0 ± 5,3	11,6 ± 4,9
Ciprofloxacin	21,2 ± 0,2	16,2 ± 2,6	21,8 ± 0,6	14,5 ± 2,2

	ABTS			
	60'		120 '	
	A	L + M + A	A	L + M + A
Tetracycline	18,0 ± 1,9	7,6 ± 1,5	18,6 ± 1,1	6,5 ± 0,9
Oxytetracycline	16,9 ± 0,5	8,1 ± 2,2	18,6 ± 0,8	6,6 ± 1,0
Chlortetracycline	24,1 ± 0,6	6,0 ± 0,0	24,7 ± 1,0	6,0 ± 0,0
Ampicillin	16,2 ± 5,8	15,4 ± 5,8	15,9 ± 6,0	13,1 ± 5,1
Ciprofloxacin	21,9 ± 0,4	21,5 ± 1,0	22,2 ± 0,7	20,4 ± 1,1

	HBT			
	60'		120'	
	A	L + M + A	A	L + M + A
Tetracycline	16,6 ± 0,6	13,0 ± 2,3	17,8 ± 1,0	11,4 ± 4,3
Oxytetracycline	18,1 ± 0,2	16,1 ± 1,6	17,3 ± 0,8	17,6 ± 3,4
Chlortetracycline	22,8 ± 0,8	15,2 ± 2,1	24,5 ± 0,9	10,9 ± 0,7
Ampicillin	16,0 ± 6,1	15,5 ± 5,6	17,7 ± 4,0	14,8 ± 3,7
Ciprofloxacin	21,2 ± 0,9	20,9 ± 1,4	21,7 ± 0,9	22,2 ± 1,4

No growth inhibition zones were observed in the controls: L, M and L+M

Conclusions

- The results obtained indicate that this thermophilic laccase has the ability to biodegrade antibiotics of three different classes, especially tetracyclines at 35°C. It is worth to note that this degradation occurs 35°C below the optimum temperature determined for the enzyme.
- The use of this novel thermophilic laccase, with the natural mediator acetosyringone, allows the biodegradation of all the structurally different antibiotics tested: tetracycline, oxytetracycline, chlortetracycline, ampicillin and ciprofloxacin, at a concentration of 0,5 mg/ml. Apparently without presenting degradation products that could be ecotoxic for *E. coli* cells.
- There is a clear absence of the inhibition halo generated by the antibiotics TC, OTC and CTC when they are treated with laccase and acetosyringone in 60 minutes, while a small decrease is observed with AMP and CIP.
- The use of laccase in conjunction with ABTS allows the degradation of TC, OTC and CTC at 60 minutes, reducing the zone of inhibition almost totally after 120 minutes of incubation. When evaluating the degradation of AMP and CIP, ABTS did not degrade them. This is observed from the null decrease in the zone of inhibition.
- The use of laccase together with HBT does not allow the degradation of TC, OTC, AMP and CIP, but manages to degrade CTC. The low reduction of the zone of inhibition of antibiotics may be due to the null capacity to degrade this antibiotics by laccase with HBT, or due to the possible generation of some degradation product with antimicrobial activity.
- This new laccase has interesting biotechnological potential based on its high activity even at 35°C below its optimum temperature and the ability to degrade different families of antibiotics in a more efficient manner (higher concentration in shortened reaction times) than other laccases described in the literature. For example, laccase from *Trametes versicolor* requires at least 3h to biodegrade tetracycline at a concentration of 0,01 mg/mL (4).

Future Work

- To evaluate the ability of the thermophilic laccase to degrade amoxicillin, another antibiotic from the β-lactam family.
- To analyze the antibiotics biodegradation products by means of HPLC.
- To evaluate the effect of the antibiotics biodegradation products on *Bacillus subtilis* (ecotoxicity assays).

Acknowledgement

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References

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