Characterization of 2 novel H2O2 producing glyoxal oxidases from Pycnoporus cinnabarinus implicated in the breakdown of lignocelluloses

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Heterologous Production and Characterization of Two Glyoxal Oxidases from *Pycnoporus cinnabarinus*

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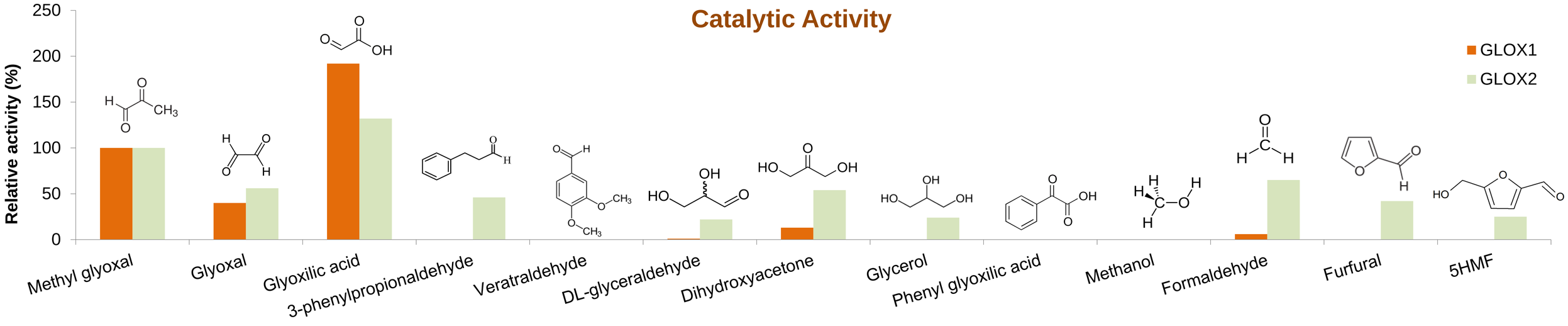
Introduction

Plant derived biomass is the most abundant and renewable material on earth and it is gaining great importance as a sustainable source of energy and valuable molecules. The distinctive ability of wood-decaying filamentous fungi in degrading lignocellulose helped extend the exploitation of this material in biotechnology and industry. Fungi belonging to this group modify or degrade lignocellulose through the production of a wide variety of enzymes including heme-peroxidases (manganese peroxidase, lignin peroxidase and versatile peroxidase), multicopper-containing phenol oxidase (laccase) and hydrogen peroxide-generating oxidoreductases (e.g. aryl alcohol oxidase and glyoxal oxidase).

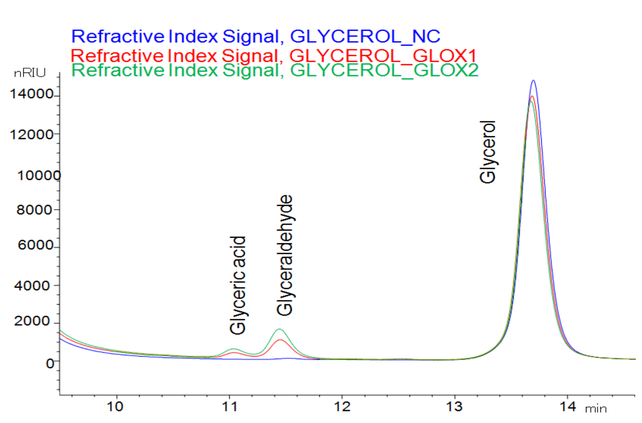
In order to understand the role of these enzymes in the degradation of lignocellulose by the Basidiomycete *Pycnoporus cinnabarinus*, and to find potential applications in biotechnology, targeted enzymes were heterologous expressed and characterized. This poster will present the results of the characterisation of two secreted glyoxal oxidases (GLOX) from *P. cinnabarinus* heterologously produced in *Aspergillus niger* and will focus on certain reactions catalysed by these enzymes.

Catalytic Activity

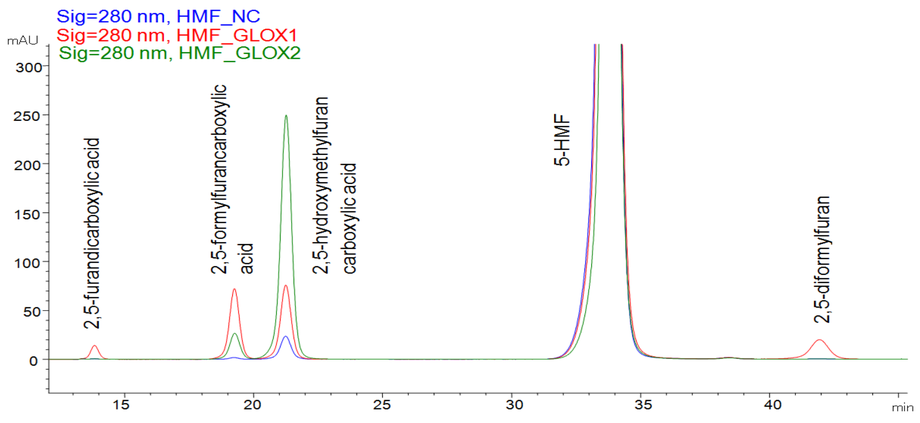


*Relative activity on different substrates calculated as a percentage of the activity on methylglyoxal and tested spectrophotometrically during first 30 seconds of reaction.

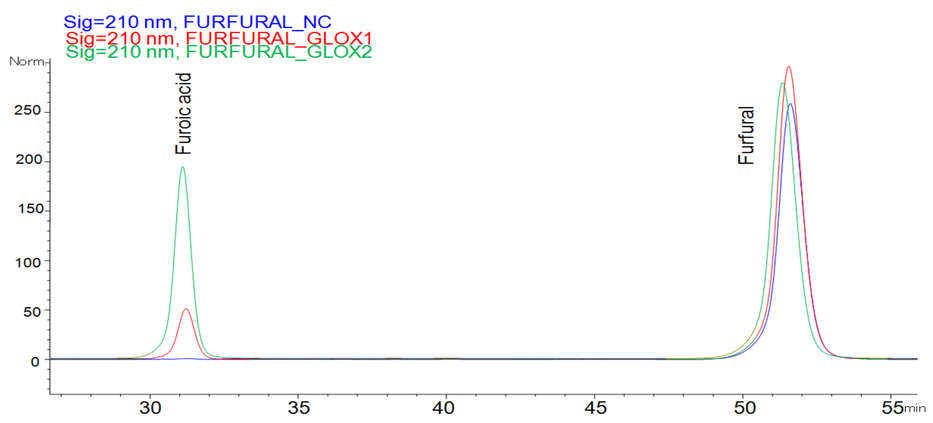
Glycerol



5-HMF



Furfural



Substrate	GLOX1		GLOX2	
	Km (mM)	Catalytic efficiency (s^{-1} mM^{-1})	Km (mM)	Catalytic efficiency (s^{-1} mM^{-1})
Methylglyoxal	1.3	58.46	0.2	7
Glyoxal	13.1	6.33	2.2	0.63
Glyoxylic acid	0.08	2136.36	0.1	17
Glycerol	660.5	0.05	9.4	0.06
Formaldehyde	4.9	0.58	0.6	13.33
Furfural	-	-	3.2	1.21
5-HMF	-	-	8.1	0.54

Conclusion

The genes of two glyoxal oxidases (GLOX) were cloned in *A. niger* and the recombinant proteins were produced, purified and characterized. A significant difference in catalytic efficiency and substrate preference was observed between GLOX1 and GLOX2 suggesting that these two enzymes are involved in different reactions *in vivo.* The results showing the detection of different products in the reactions of the two enzymes on the same substrate further support this hypothesis. The reactions catalysed by GLOX also reflects the importance of these enzymes as candidates for biotechnological applications.

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