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ORIGINAL ARTICLE

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Recombinant expression of an L-amino acid oxidase from the fungus *Hebeloma cylindrosporum* in *Pichia pastoris* including fermentation

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Abstract

L-amino acid oxidases (LAAOs) are flavoenzymes that catalyze the oxidative deamination of L-amino acids to the corresponding α-keto acids, ammonia, and hydrogen peroxide. Here, we show the overexpression, purification, and the characterization of LAAO4 from the fungus *Hebeloma cylindrosporum* in the yeast *Pichia pastoris* with a 9His-tag and compare this with the recently characterized 6His-*hc*LAAO4 expressed in *E. coli*. The expression of the enzyme with an ER-signal sequence in *P. pastoris* resulted in a glycosylated, secreted protein. The enzymatic activity without activation was higher after expression in *P. pastoris* compared to *E. coli*. Due to treatment with acidic pH, a striking increase of activity could be detected for both expression systems resulting in similar specific activities after acid activation. Regarding the substrate spectrum, temperature stability, $K_{m,}$ and v_{max} values, *hc*LAAO4 showed very few differences when produced in these two expression systems. A higher yield of *hc*LAAO4 could be obtained by fermentation.

KEYWORDS

bioreactor, glycosylation, heterologous expression, L-amino acid oxidases, *P. pastoris*

1 | INTRODUCTION

L-amino acid oxidases (LAAOs, EC 1.4.3.2) are oxidoreductases, which catalyze the oxidative deamination of L-amino acids to imino acids. Due to spontaneous hydrolysis, the corresponding α-keto acid and ammonia are formed. As a byproduct, hydrogen peroxide is formed during regeneration of the non-covalently bound cofactor flavin adenine dinucleotide (FAD; Pollegioni, Motta, & Molla, 2013). LAAOs are found in several organisms like mammals, bacteria, algae, and fungi even though the functions differ in different organisms. Snake venom LAAOs (SV-LAAO) are the best-characterized enzymes and can cause apoptosis, edema, or hemolysis (Ali et al., 2000; Du & Clemetson, 2002; Suhr & Kim, 1996; Weia et al., 2007). LAAOs possess antimicrobial or antiparasitic functions in fish, molluscs, fungi, and bacteria due to H_2O_2 formation (Kasai et al., 2010; Tong, Chen, Shi, Qi, & Dong, 2008; Yang et al., 2005, 2011). Furthermore, the formation of ammonia is an important nitrogen source for anabolic reactions in fungi (Nuutinen & Timonen, 2008).

For industry, LAAOs are of great interest because of the formation of α-keto acids or the possibility to obtain enantiomerically pure D-amino acids from racemic mixtures by enzymatic resolution. Unfortunately, recombinant expression of LAAOs with broad substrate spectrum has been difficult to obtain for a biotechnological application (Hossain et al., 2014). Using different bacterial sequences, an ancestral LAAO has been designed and expressed efficiently in *E. coli*, which has a broad substrate specificity but low thermal stability