

Accepted Manuscript

Title: Improved production and characterization of a highly stable laccase from the halophilic bacterium *Chromohalobacter salexigens* for the efficient delignification of almond shell bio-waste

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PII: S0141-8130(17)30273-8
DOI: <http://dx.doi.org/doi:10.1016/j.ijbiomac.2017.07.055>
Reference: BIOMAC 7863

To appear in: *International Journal of Biological Macromolecules*

Received date: 19-1-2017
Revised date: 28-3-2017
Accepted date: 9-7-2017

Please cite this article as: Nasrin Jafari, Shahla Rezaei, Rezvan Rezaie, Haleh Dilmaghani, Mohammad Reza Khoshayand, Mohammad Ali Faramarzi, Improved production and characterization of a highly stable laccase from the halophilic bacterium *Chromohalobacter salexigens* for the efficient delignification of almond shell bio-waste, *International Journal of Biological Macromolecules* <http://dx.doi.org/10.1016/j.ijbiomac.2017.07.055>

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<AT>Improved production and characterization of a highly stable laccase from the halophilic bacterium *Chromohalobacter salexigens* for the efficient delignification of almond shell bio-waste

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<ABS-HEAD>Highlights ► A highly stable laccase from halophilic *Chromohalobacter salexigens* was isolated. ► The enzyme yield increased 80-fold using optimization of the key parameters. ► The crude laccase was stable against salts, chemicals, and pH-temperature ranges. ► Stimulation by organic solvents and an ionic liquid on laccase activity was observed. ► 58% laccase-assisted delignification was achieved in the extreme tested conditions.

<ABS-HEAD>Abstract

<ABS-P>Extremozymes have gained importance for their ability to efficiently develop the processes in rigorous industrial conditions with incidence in the recycling of especially robust natural wastes. The production of an extracellular laccase from the halophilic bacterium *Chromohalobacter salexigens* aided for the bio-delignification of almond shell was optimized using response surface methodology followed by one-factor-at-a-time, resulting in an 80-fold increase in the enzyme yield. Out of 10 different medium components, CuSO₄, ZnSO₄, glucose, and urea were shown to have the greatest effects on the laccase production. The crude laccase was surprisingly stable against the various solvents, salts, chemicals, pH ranges, and temperatures, and it exhibited a high catalytic efficiency to a wide range of phenolic and non-phenolic substrates. Laccase reduced the kappa number of the lignin of almond shell by approximately 27% without the aid of a mediator, and the delignification efficiency strengthened by up to 58% reduction in kappa number in the used harsh conditions. Due to the high potential of the enzyme in delignification, specifically under extreme conditions, laccase from *C. salexigens* can be considered as an ideal alternative for chemical treatment methods in cellulose fibers extraction of lignocellulosic bio-wastes or delignification of the lignin and lignin-derived industrial wastes.

<KWD>Keywords: Laccase; Halophile; Optimization; *Chromohalobacter salexigens*; Almond shell;

Delignification

<H1>1. Introduction

Even though many enzymes have yet to be identified, the present enzymes do not meet all the industrial demands and many of them have not found a role in biotechnological and industrial applications. This is

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