Cloning, recombinant expression and characterization of two cellulases of family GH5, Cel1 and Cel2

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Introduction

This work is in the frame of the “Enhanced bioconversion of agricultural residues through cascading use” BIOrescue project.
BIOrescue aims to develop and demonstrate a new innovative biorefinery concept based on
the cascading use of lignocellulosic biomasses as spent mushroom substrate supplemented by wheat straw.
Industrial conversion of lignocellulosic biomasses into second-generation biofuels or other high-added-value products includes a **saccharification step** for hydrolyzing polysaccharides into fermentable sugars carried out by an **enzymatic cocktail including cellulases.**
The production cost of these enzymes represents the main contributor to the cost of the overall process and a huge amount of enzymes is required for hydrolysis on an industrial scale that makes the production of the biochemicals not competitive yet in comparison to the fossil counterparts.
Therefore, the search for **new cellulolytic enzymes** more efficient in lignocellulose conversion represents one of the main routes to contribute to the **cost reduction of biomass conversion**.

Background

Twenty-four Actinobacteria strains were previously identified and screened for their cellulase activity to select microorganisms producing biocatalysts able to hydrolyse pretreated lignocellulose biomasses [1],[2],[3].
The highest cellulase activity production was exhibited by the strain ***Streptomyces argenteolus* AE58P** both in solid and liquid medium [3].
This strain was selected to identify the proteins responsible for the cellulase activity by proteomic approach. Several proteins were confidently identified in different *Streptomyces spp*., 8 of which belong to the class of Carbohydrate active enzymes. In detail, three proteins were annotated as containing carbohydrate-binding modules (CMB), three as cellulases of family GH5, one as a cellulase of family GH12, and another as a cellulase that is homologous to glycosyl hydrolases of GH109 family.

Aim

The aim of this work was to develop biocatalysts for enhanced hydrolysis of (hemi)cellulose into monosaccharides and in particular the development of improved cellulase variants for Spent Mushroom Substrate (SMS) conversion by directed evolution

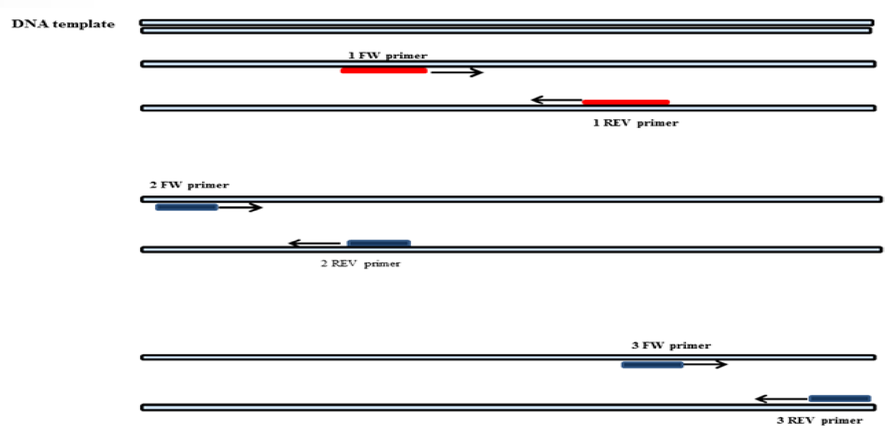
Among the identified proteins, two new cellulases of the family GH5, Cel1 and Cel2, were selected for cloning and recombinant expression in order to develop improved biocatalysts for conversion of spent mushroom substrate (SMS).

Results

1

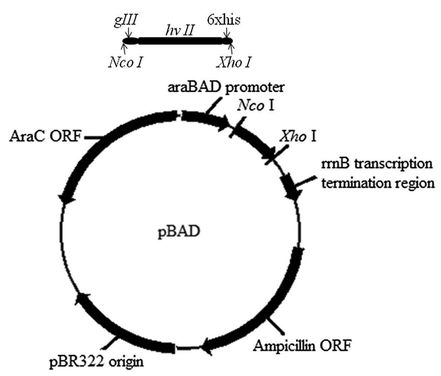
Cloning of the genes coding for the two new GH5 cellulases, Cel1 and Cel2, by **PCR** (Polymerase chain reaction) on *Streptomyces argenteolus* AE58P’s genomic DNA with degenerate oligonucleotides whose design was based on the sequences of identified peptides.

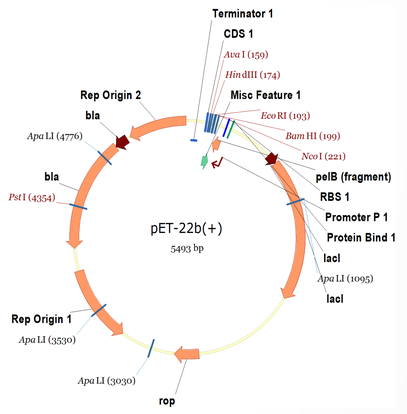
The central region was amplified with the oligonucleotides 1 FW/1 REV, the 5’ region with the oligonucleotides 2 FW/2 REV and the 3’ terminal region with the oligonucleotides 3 FW/3 REV



2

Cloning of the genes coding for Cel1 and Cel2 into the **expression vectors pBAD and pET22b** and **recombinant expression** of Cel1 and Cel2 in ***Escherichia coli.***





3

Functional characterization of Cel1 and Cel2 to select the best enzyme for the creation of the directed evolution library

In order to select the cellulase to be subjected to directed evolution experiments to improve the performance in the conversion of lignocellulosic substrates, the experiments **of thermoresistance and pH resistance** were carried out.
The choice of these experiments for the enzyme selection is due to the fact that the bioconversions of lignocellulosic substrates generally take 72 hours and it is important that the enzyme maintains its activity as long as possible **to allow a higher conversion yield of the substrate**.

Thermoresistance	
Ratio of the residual activity (%) of Cel2/Cel1 after 72h of incubation at different temperatures	
37°C	1.05
45°C	1.11
50°C	1.13
55°C	1.15
60°C	1.11

pH resistance	
Ratio of the residual activity (%) Cel2/Cel1 after 72h of incubation at different pH	
pH4	51.5
pH5	1.76
pH6	7.12
pH7	15.5

Cel2 exhibited a relevant thermoresitance and pH resistance compared to Cel1 increasing its potential for cellulose conversion. Furthermore its thermoresitance and pH resistance would make this cellulase an appropriate candidate as a scaffold for directed evolution experiments aimed at developing better biocatalysts for cellulose conversion.

References &* Acknowledgments *:
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[1] Montella et al Sci Rep. 15, 7-42623, 2017;
[2] Ventorino et al., Sci Rep. 5, 8161, 2015;
[3] Ventorino et al., Front. Microbiol. 7, 2061, 2016.