

# An efficient Azorean thermophilic consortium for lignocellulosic biomass degradation

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Lignocellulosic plant biomass is being envisioned by biorefinery industry as an alternative to current petroleum platform because of the large scale availability, low cost and environmentally benign production. The industrial bioprocessing designed to transform lignocellulosic biomass into biofuels are harsh and the enzymatic reactions may be severely compromised reducing the production of fermentable sugars from lignocellulosic biomass. Thermophilic bacteria consortium are a potential source of cellulases and hemicellulases adapted to extreme environmental conditions, which can be exploited as a new source for the development of more robust enzymatic cocktails.

Therefore, in this study a thermophilic bacteria consortium, isolated from compost plant materials in boilers and steam vents of Azores, was adapted to wheat straw at 60°C under aerobic stirring conditions, in order to evaluate and better understand the enzymatic performance on this lignocellulosic substrate.

Several analyzes were performed including the efficiency of substrate degradation by dry weigh, identification of bacterial community by 16S rRNA sequencing, determination of enzymatic activities and reducing sugars by 2,4-dinitrosalicylic acid (DNS) assay and HPLC and evaluation of secreted proteins by SDS-PAGE and zymography.

The preliminary data showed that the consortium include many bacterial populations, including the genus *Geobacillus*, which were also possible to be isolated in the laboratory. The consortium had the ability to hydrolyze  $\approx$  50% of wheat straw after fermenting 3 to 4 days. The crude culture supernatants presented the reducing sugars glucose, cellobiose and xylose and exhibited lignocellulolytic enzyme activities on xylan, carboxymethylcellulose (CMC) and cellobiose substrates under neutral pH 7 conditions, but the activities on xylan, followed by cellobiose, are much higher than on CMC. In fact, this consortium demonstrated much higher enzymatic activity on xylan ( $\approx$  0,300 IU/mL) than any other consortia found in literature. In addition, SDS-PAGE analysis demonstrate the presence of 4 distinct bands, where one of them was already verified to have homology with a *Geobacillus* hydrolase. These results demonstrate the potential for successful future improvement of microbial and enzymatic processes on lignocellulosic biomass degradation supporting the development of research in sustainable "green" biotechnology.