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HETEROLOGOUS EXPRESSION IN PICHIA PASTORIS OF BACTERIAL CELLULASES FOR IMPROVED BIOGAS PRODUCTION

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Aim of this project is the development of heterologous expression systems for production of cellulolytic and hemicellulolytic enzymes to be used in biogas production. The first practical application should be pre-treatment of cellulose- and hemicellulose-containing biomass used to feed biogas digestors, but also wastes from pulp and paper industry and any other IOW (industrial organic waste) that could be used for production of biofuels, either biogas (by anaerobic digestion) or ethanol (by yeast fermentation), according to the principles of second generation biofuels.Biogas has become an important renewable energy. In Italy there are more than 1400 biogas digestors with an average power of 1 MW/h. Organic wastes may be converted into biogas, which is produced by anaerobic digestion or fermentation of biodegradable materials (Kamali, et al., 2016). The major limitation of this process is the poor conversion of biomass into energy by the methanogenic bacteria, from 40% to 60%: the low conversion rate is due to the presence in the substrates of complex biopolymers that are poorly degraded.A pretreatment of biomass with either microbial consortia or pools of enzymes particularly efficient in de-polymerization of these molecules could increase the production of biogas. Therefore we focused on the heterologous expression of cellulolytic and hemicellulolytic enzymes that catalyze de-polymerization of plant cell wall components, facilitating their conversion into biogas by methanogenic microbial consortia. Among the enzymes suitable for this application are those that degrade the plant cell wall, such as enzymes degrading cellulose (cellulases, cellobiohydrolases and beta-glucosidases), hemicellulases (xylanases) and related accessory enzymes, pectinases and polygalacturonases. Considering the abundance of cellulose and its high energy content, we focused primarily on cellulose-degrading enzymes and in particular (i) β-glucosidase, that attacks cellobiose relasing glucose monomers, (ii) cellobiohydrolase, that attacks the cellulose chain end releasing cellobiose, and (iii) glycosyl-hydrolase, that breaks internal glycosides bonds of cellulose chain randomly.The first heterologous expression of three cellulolytic enzymes-encoding genes was performed in E.coli. The enzymes and related donor organisms were:

(i) - endocellulase from Bacillus pumilus (ii) - cellobiohydrolase from Xanthomonas axonopodis pv glycines (iii) - beta-glucosidase from Bacillus amyloliquefaciens

The first two microorganisms are soil bacteria, the third one is a bacterial plant pathogen, all well known for their ability to attack plant cell wall, although Bacilli usually do it mainly in consortia. We obtained expression and excellent yields in E.coli but all the proteins expressed formed inclusion bodies, making necessary to consider the change of expression system.

Although it has been used already to express many cellulolytic enzymes, Pichia pastoris was never used for expressing the cellulases of the organisms of our choice. This eukaryotic system has several advantages: first, its respiratory growth enables it to be cultured to high cell densities; second, it is capable of post-translational modifications (i.e., proteolytic processing, glycosylation, disulphide bridge formation). It is cost-effective and less time-consuming: it is easy to scale-up and easy to handle. It brings to high protein yields and high levels of secretion (Kalidas, 1999).

Two major problems to be solved when these enzymes are heterologously expressed in high amount are:

(i) the possible lack of solubility in the heterologous expression system (Baneyx & Mujacic, 2004), and (ii) the need to have them secreted into the medium in order to be able to recover only the cell-free supernatant and overcome the regulatory and industrial constraints of the use of a genetically modified microorganism (Chatani et al., 2000). For reaching the higher expression as possible, we studied and improved the codon usage of the sequences codifying for the three enzymes of interest. We synthesized the sequences with optimized codons, cloned them in the shuttle expression vector pPick9k and transformed Pichia pastoris. The positive clones are currently under evaluation and the results will be presented in the poster.

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