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Employment of *Zymomonas mobilis* as a cellulolytic microbial factory for bioethanol production

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PURPOSE OF THE ABSTRACT

The industrialized world faces progressive depletion of its energetic resources, mainly based on non-renewable fuels.[1] As the world population and its energy demand increases, there is an urgent need to develop environmentally sustainable alternatives. In this context, the employment of microbial cell factories, powered by waste products, is an attractive means for large and sustainable biofuel production.[2]

In the present project, we aim at utilizing cellulose waste as feedstock to produce bioethanol in the Gram-negative fermentative bacterium *Zymomonas mobilis*. Its natural ethanol production coupled to a high sugar uptake make *Z. mobilis* an appealing platform for a large-scale industrial application.

To fully convert *Z. mobilis* into a cellulolytic microbial factory, it is required the production and secretion of three enzymes: exoglucanases, endoglucanases and β -glucosidases.[3] These enzymes are not present in *Z. mobilis*; hence, we have developed a tailor-made molecular toolbox to finely tune their heterologous expression. This includes a library of synthetic constitutive promoters and transcriptional terminators.

Besides the production of these enzymes, it is fundamental to ensure their secretion extracellularly, where the cellulose biodegradation occurs. Presently, there is a lack of robust secretion tags for use in *Z. mobilis* and related organisms. To address this, we aim at combining gene knockouts and machine learning to identify which proteins are secreted through the native Type I secretion system and isolate their secretion tags. These can then be repurposed for the secretion of heterologous enzymes.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

bioethanol | cellulose biodegradation | *Zymomonas mobilis* | molecular toolbox

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