Optimization of γ-PGA biosynthesis supported by synthetic biology and metabolic engineering strategies

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Poly-γ-glutamate (γ-PGA) is a natural polymer composed by glutamic acid residues, synthesized by the *pgs* operon of *Bacillus subtilis*. γ-PGA has a wide range of applications as food, cosmetic and pharmaceutical additive. However, to increase its industrial attractiveness, it is necessary to cut production costs utilizing cost-competitive feedstocks for fermentation. A low-cost by-product that can be used as feedstock is raw glycerol, that accounts for 10% (w/w) of the total biodiesel production.

To achieve cost-competitive γ-PGA production from glycerol a multifaceted approach has been set up that includes:

1) improvement of *pgs* expression;
2) accumulation of γ-PGA precursors by metabolic engineering;
3) enhancement of glycerol metabolism.

1) The strength of the *pgs* operon regulatory elements has been analysed both by a synthetic biology approach, exploiting the well-characterized *expression operating unit* (EOU) inserted in *amyE* [1], and by a classical in-locus transcriptional fusion. Results from the two settings will be compared. These data will be then used to finely tune *pgs* expression and optimize γ-PGA yield. To this end, an inducible *pgs* operon has been constructed.

2) A genome-scale metabolic model [2] was used to identify suitable targets for enhancing central carbon pathway flux toward γ-PGA synthesis. The first two *B. subtilis* strains, engineered following this analysis, showed enhanced polymer production. Other target genes are currently under investigation.

3) *B. subtilis* tolerance to raw glycerol obtained from a biodiesel plant (from both vegetable and animal origin) was verified. Further investigations are underway to improve glycerol uptake and consumption.

References

[1] Guiziou et al., 2016. *Nucleic Acids Res.* 44: 7495-508

[2] Oh et al., 2007. *J. Biol. Chem.* 282: 28791–28799.