

Improved characteristics of an industrial biocatalyst expressed in engineered *E. coli*

The constantly expanding field of biocatalysis is in search of novel enzymes to meet its new needs. The global market for enzyme biocatalysts in industrial applications was about \$4.8 billion in 2014 and it is expected to reach \$7.1 billion by 2018 and \$10 billion in 2020. Specifically, in the food/beverage industry and detergent markets, it is expected to reach a value of \$1.7 billion and \$1.8 billion by 2018, respectively.

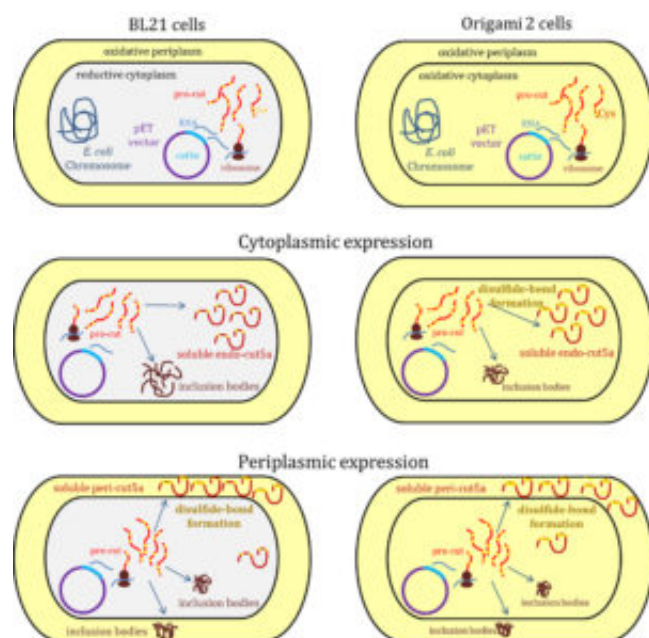


Fig. 1. Heterologous expression of *cut5a* in *E. coli*.

Cutinase took its name from cutin –a polyester produced by plants as a protective cover– because in the 1970s it was found that this was the enzyme plant pathogens used to penetrate the outer layer of their hosts. Since then, cutinase has been referred to in over 1000 publications with an average of 62 references per year since 2010 and continues to be a hot topic with 24 references in papers already published in the first 15 days of 2017 (according to sciencedirect.com).

Cutinase's natural activity on cutin can be used by the Food Industry for the increase of water permeability of vegetables and by Textile Industry for the removal of wax from natural fibers. Furthermore, cutinases can be used for the surface modification of synthetic fabrics increasing their wettability, which facilitates uniform dyeing. Another potential application of these enzymes is the Detergent Industry, utilizing their ability to hydrolyze fats. Cutinases also possess synthetic activity in the absence of water and have been used to produce biodiesel and other esters used as flavor

ingredients.

Phytopathogenic fungi are a great source for discovering new cutinase genes to be applied in the aforementioned fields. *Escherichia coli* is the most commonly used host for heterologous expression of enzymes, due to its many advantages (low-cost cultivation procedures, fast growth rate, ability for high-cell density cultures, quick and efficient transformation methods, availability of advanced molecular tools and genetic expertise). Nonetheless, *E. coli* system engages several drawbacks, for example their incapability of post-translational modifications (e.g. disulfide-bond formation, glycosylation, phosphorylation) and inclusion body formation. Inclusion bodies are protein aggregates formed from misfolded or incompletely folded polypeptides.

Proteins that contain disulfide bonds are challenging to produce in prokaryotic hosts and are more likely to be aggregated, due to misfolding. Disulfide bonds are formed from the oxidation of sulfhydryl (–SH) groups on two cysteines of the protein. In the bacterial cytoplasm reductive conditions prevail, so the oxidation of cysteines cannot occur. Periplasmic folding could be a solution to this problem, since this environment is oxidative and there are several enzymes located in the cell envelope facilitating correctly folded protein formation. *E. coli* Origami™ is an engineered strain that has an oxidative cytoplasm. (Fig. 1).

In the present work, we studied the heterologous expression of a cutinase gene from the fungus *Fusarium oxysporum* aiming in the production of a thermostable enzyme in a soluble form, for use in polymer modification. For that purpose, we used two *E. coli* hosts, BL21 and Origami 2 strains, in which we expressed the recombinant cutinase either in the cytoplasm or in the periplasm (Fig. 1), with the aim of correct formation of disulfide bonds.

Increase of thermostability occurred when the enzyme was expressed in the oxidative cytoplasm of Origami 2. All expression products showed maximum enzyme activity at 40 °C, while thermostability increased by 73% when expressed in the Origami strain compared to the cytoplasmic expression in BL21 cells. The melting temperature of each protein construct was determined by fluorescence spectroscopy showing an additional transition at about 63 °C for enzymes expressed in Origami cells, indicating the co-presence of a different thermostable species.

Efstratios Nikolaivits, Evangelos Topakas

*IndBioCat, Biotechnology Laboratory, School of Chemical Engineering,
National Technical University of Athens, Athens, Greece*

Publication

[Microbial host selection and periplasmic folding in *Escherichia coli* affect the biochemical](#)

[characteristics of a cutinase from *Fusarium oxysporum*.](#)

Nikolaivits E, Kokkinou A, Karpusas M, Topakas E

Protein Expr Purif. 2016 Nov