

## OPTIMISING A CELL FACTORY SYSTEM FOR THE BIOPRODUCTION OF SILK-ELASTIN-LIKE POLYMERS

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### ABSTRACT

Silk-elastin-like proteins (SELPs) combining the physicochemical and biological properties of silk and elastin have a high potential for use in the pharmaceutical, regenerative medicine and materials fields. Their development for use is however restrained by their production levels. Here we describe the production optimisation for a novel recently described SELP in the pET-*E. coli* BL21(DE3) expression system. Both batch production in shake flasks and fed-batch production in fermenters were investigated and optimised. A comprehensive empirical approach optimising all process variables for both processes, in addition to molecular biology approaches for improving performance of the production plasmid were used to maximise production levels. Typical volumetric productivities reported for SELPs are approximately 30 mg/L but here we have increased production levels up to approximately 4 g/L, representing the highest reported SELP productivity to date.

### INTRODUCTION

Natural fibrous proteins such as silk, elastin, collagen and keratin are characterized by quite remarkable mechanical and biological properties which can include a high strength, elasticity, biodegradability and/or biocompatibility. The primary structures of these proteins has been found to be composed of highly repetitive amino acid sequence blocks which dictate the structure and properties of the proteins and are of much interest in the fields of polymer and materials sciences as they offer an exceptional opportunity for the production of polymeric materials in which structure and function are precisely controlled. We have recently synthesized a series of diblock polymers based on silk and elastin in which multiple blocks of the silkworm silk consensus amino acid sequence (GAGAGS) have been fused in various combinations with a variant (VPAVG) of the natural mammalian elastin consensus amino acid sequence block (VPGVG). This incorporation of the high tensile strength silk blocks with the highly resilient elastin-like blocks in various combinations allows for the controlled manufacture of a range of novel structures of diverse mechanical and biological properties.

SELPs have potential for use in the pharmaceutical, regenerative medicine and materials fields but one of the principle constraints to their commercial viability is their low production levels as productivities of only 20 – 30 mg/L have been typically reported. SELPs can be produced in an ecologically friendly manner via recombinant protein production and *E. coli* is currently one of the most commonly used hosts for this. Here we undertook to optimize the production levels of one of our novel SELPs with the pET-*E. coli* BL21(DE3) expression system by investigating both batch production in shake flasks and fed batch production in fermenters. In both cases a comprehensive empirical approach examining all process variables (media, medium composition, inducer, induction time and period, temperature, pH, aeration,

agitation, pre- and post-induction growth rates) and a detailed characterization of the bioprocesses were carried out in an attempt to maximize production and to identify the factors limiting higher production levels. Furthermore, genetic engineering approaches were used to investigate the use of toxin/antitoxin postsegregational suicide systems for improved plasmid stability and SELP productivity.

## RESULTS AND CONCLUSIONS

Optimum process conditions for production of the novel SELP by both batch and fed-batch approaches have been determined and the major factors limiting SELP yields have been identified as a heightened host cell metabolic burden as well as acetate accumulation and plasmid instability. Using the optimized conditions developed in this study, approximately 0.5 g/L of purified SELP was obtained in shake flasks and approximately 4 g/L was obtained when using the fed-batch approach. These represent, respectively, approximately 10 and 100-fold increases on that previously reported for SELPs

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## REFERENCES

- Collins T., Azevedo-Silva J., da Costa A., Branca F., Machado R. and Casal M. (2013). Batch production of a silk-elastin-like protein in *E. coli* BL21(DE3): key stress factors and parameters for optimisation. *Microb. Cell Fact.*, Feb 27; 12:21. doi: 10.1186/1475-2859-12-21
- Machado M., Correia C., Azevedo-Silva J, Collins T, Arias J, Rodríguez-Cabello J.C. and Casal M. (2013). High level expression and facile purification of recombinant silk-elastin-like polymers in auto induction shake flask cultures. *AMB Express*, Feb 5; 3(1):11. doi: 10.1186/2191-0855-3-11