Optimising the Production of a Silk-Elastin-Like Protein in *E. coli*: Overcoming Acetate Accumulation and Plasmid Instability.

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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 approaches were investigated and optimised. 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 characterisation of the bioprocesses were used in an attempt to maximise production and identify the factors limiting higher production levels. The major factors limiting SELP yields have been identified as acetate accumulation, plasmid instability on induction and a heightened host cell metabolic burden during SELP production. To circumvent these limitations we have optimised the fed-batch production approach and engineered the production plasmid for an improved stability. Using the optimised conditions, approximately 0.5 g/l of purified SELP was obtained in shake flasks and as much as 4.3 g/L was obtained when using the fed-batch approach. These are the highest reported SELP productivities to date and represent, respectively, approximately 10- and 150-fold increases on that previously reported.

Keywords: protein based polymers, silk-elastin-like polymers, production optimisation, batch and fed-batch production, pET-*E. coli* BL21(DE3).

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