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Use of physiological information and process optimisation enhances production of extracellular nuclease by a marine strain of *Bacillus licheniformis*

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HIGHLIGHTS

- Production of an antibiofilm nuclease from *Bacillus licheniformis* was achieved.
- Use of manganese as a secretory stimulus increased NucB production 5-fold.
- Statistical optimisation gave a 10-fold improvement in NucB secretion.
- NucB production was enhanced using a unique physiology driven optimisation approach.

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ABSTRACT

The extracellular nuclease, NucB, from *Bacillus licheniformis*, can digest extracellular DNA in biofilms, causing biofilm dispersal, and may therefore be used commercially to remove biofilms. However, producing quantities of this secreted peptide is difficult and our aim was therefore to improve its laboratory scale production. This study builds on our understanding of *B. licheniformis* physiology to enhance NucB production. The addition of manganese, which triggers sporulation and enhances NucB expression, lead to a 5-fold increase in NucB production. Optimisation via Placket–Burman design of experiments identified 3 significant medium components and a subsequent Central Composite Design, to determine the optimum levels of these components, resulted in a 10-fold increase to 471 U/ml. The optimal phosphate concentration was less than 0.3 mM as this is known to inhibit nuclease production. The use of physiologically relevant information combined with optimisation represents a promising approach to increased enzyme production, which may also be widely applicable.

1. Introduction

Biofilms are composed of extracellular polymeric substances (EPS) such as polysaccharides, lipids, proteins and extracellular DNA (eDNA) (Flemming et al., 2007). Biofilms can occur on medical devices and represent a major cause of biofouling in industrial settings (Gunduz and Tuncel, 2006; Vertes et al., 2012). In the last two decades, eDNA has been reported widely to be present in abundance as a crucial structural component of both single and mixed species biofilms (Dominiak et al., 2011). Earlier studies demonstrated that eDNA originates during cell lysis, produced during various biochemical pathways as excretory double stranded DNA from living cells and/or through vesicles and also in some case during prophage mediated lysis of a sub-population of living cells forming complex biofilm structures (Dominiak et al., 2011). In a wide variety of cases, hydrolysis of eDNA by a nuclease does lead to biofilm breakdown which was demonstrated in increasing number of studies (Nijland et al., 2010). This suggests eDNA has a broad functionality in the structural integrity of biofilms that could be degraded ubiquitously and can be considered as a novel target in the removal of harmful biofilms. DNase 1 was first reported to be able to dissolve bacterial eDNA and this treatment resulted in complete biofilm breakdown (Whitchurch et al., 2002). Recently we reported a secreted bacterial nuclease, NucB, as an alternative novel biofilm dispersing enzyme. NucB was hypothesised to be released

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