A New Tool to optimize Sporulation of Probiotic *Bacillus sp* for an Industrial use

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Abstract

The use of *Bacillus subtilis* and *Bacillus licheniformis* as probiotic agents offers numerous benefits for both health and industrial applications. Both species are endospore-forming bacteria, making them highly resistant to extreme chemical and physical conditions, such as heat, gastric acidity, and bile salts, which ensures their survival through the gastrointestinal tract. From an industrial standpoint, the stability and longevity of endospores during manufacturing, storage, and transportation reduce costs associated with maintaining cold chains. However, the conversion of vegetative bacterial cells to endospores in *Bacillus* species poses several industrial and economic challenges. Achieving high spore yields necessitates optimizing fermentation, which can be resource-intensive and complex. Using a tool to monitor *Bacillus* sporulation is of significant interest for optimizing the conversion of vegetative cells into endospores. This paper reports on the selection and application of a plasmid carrying the *gfp-mut3* gene, which codes for green fluorescent protein (GFP), under the control of a sporulation-specific promoter. Among the three selected promoters regulating the *spo0A*, *spoIIE*, and *spoIIIAA* genes, only the *spoIIE* promoter effectively monitors the initiation of sporulation and the transient expression of *spoIIE* by measuring GFP fluorescence during cell growth. The equivalent construct applied to *B. licheniformis* produced similar results. The tool developed in this study is effective for identifying optimal growth conditions to achieve high-yield production of *Bacillus* endospores, reducing variability, and enhancing product quality.