

Metabolic Engineering of *Vibrio natriegens* for Biosynthesis of BETA-Nicotinamide Mononucleotide

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Nicotinamide mononucleotide (NMN), is a promising nutraceutical attracting much attention for its pharmacological and anti-aging efficacies. While NMN biosynthesis in *Escherichia coli* by metabolic engineering has been successful, *Vibrio natriegens* has recently emerged as an attractive alternative host owing to its rapid growth and broad substrate utilization. This study aims to evaluate the capability of NMN biosynthesis in *V. natriegens*. To achieve this goal, firstly, a mutant *V. natriegens* strain, namely V54-33, was generated via multiplex genome editing by natural transformation in order to insert NMN synthase from *Francisella tularensis* (FtNadE) and to inactivate two endogenous NMN-degrading enzymes (NMN amidohydrolase, encoded by *pncC*, and NMN adenyltransferase, encoded by *nadR*). Subsequently, Nampt genes encoding nicotinamide phosphoribosyltransferase from *Chitinophaga pinensis*, *Sphingopyxis* sp. C-1, *Haemophilus ducreyi*, and *Vibrio* phage KVP40 were codon-optimized, cloned into pACYCDuet™-1 and expressed in V54-33 strain. SDS-PAGE analysis demonstrated that all NAMPTs were strongly expressed in the V54-33 strain. HPLC analysis revealed that the highest intracellular NMN concentration was obtained with NAMPT from *C. pinensis* (44.5 μ M). Based on these results, three essential genes in the pentose phosphate pathway from *E. coli* encoding ribose-phosphate diphosphokinase, glucose 6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were co-expressed with NAMPT from *C. pinensis* in V54-33 strain. TLC analysis showed that the NMN titer of this recombinant strain reached 1.34 g/L after only 14 hours of fermentation. This study demonstrated for the first time the capability of efficient production of NMN in *Vibrio natriegens*.

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