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 adenylyltransferase, 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 μM). 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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