

## Enhanced cyanophycin accumulation in diazotrophic cyanobacterium through random mutagenesis and tailored selection under varying phosphorus availability

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This study explored a sustainable alternative to the Haber-Bosch process by enhancing the production of the nitrogen-rich polymer cyanophycin (CGP) in the diazotrophic cyanobacterium *Nostoc* sp. PCC 7120. Applying UV-mutagenesis followed by canavanine selection, we isolate an initial mutant with enhanced CGP accumulation. Subsequently, a secondary selection under phosphorus-limited conditions was employed to decrease cellular ploidy, yielding stable mutants. Among these, strain 44 exhibited an improved CGP accumulation, achieving up to 34 % of cellular dry weight in batch cultures. Under continuous phosphorus-limited cultivation, this mutant demonstrated a CGP productivity of  $63 \text{ mg L}^{-1} \text{ day}^{-1}$ , approximately a fourfold improvement over the wild type. Genomic analysis of the mutants revealed mutations unrelated to known CGP biosynthetic pathways, suggesting that the observed enhancement in CGP may arise from complex, synergistic effects of multiple genetic changes. This integrative approach—combining mutagenesis, screening, and cultivation techniques—successfully increased CGP accumulation from atmospheric nitrogen over threefold compared to the wild-type.