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Obtaining high yield recombinant Enterococcus faecium nicotinate nucleotide adenylyltransferase for X-ray crystallography

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The enzyme nicotinate nucleotide adenylyltransferase (NNAT) has proven to be a potential drug target for the design of new antibacterial agents because of its indispensability in the biosynthesis of NAD+, a metabolite crucial to the survival of pathogens. However, no information is available on the structure-function of E. faecium NNAT (EfNNAT). To provide this missing information while validating EfNNAT as a potential druggable target, the availability of a highly purified recombinant EfNNAT is a significant step and a pipeline to accessing this knowledge. This study established how to obtain high-yield recombinant EfNNAT using the Escherichia coli expression system and a single-step IMAC purification method. We further solved the three-dimensional structure of EfNNAT by X-ray crystallography. Two high-resolution crystal structures of EfNNAT in its native and adenine-bound forms were determined at 1.90 Å and 1.82 Å, respectively. The presence of phosphate and sulfate ions occupying and interacting with conserved amino acid residues within the putative substrate binding site aided better insight into the enzyme's probable substrate preference. With the accessibility to high-resolution structures of EfNNAT, further structural evaluation and drug-based screening can now be achieved to aid the discovery of structure-based inhibitors against this enzyme.

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