Metabolic engineering of *Escherichia coli* for optimized biosynthesis of nicotinamide mononucleotide, a noncanonical redox cofactor

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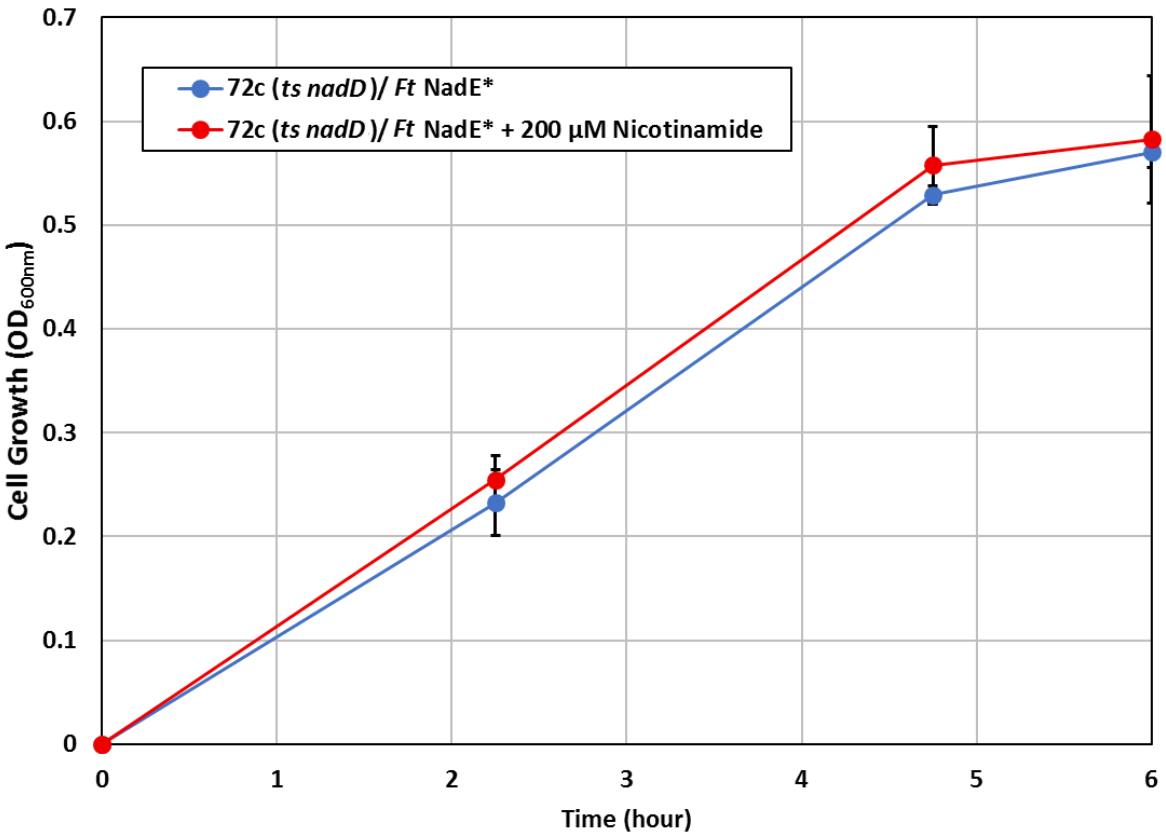
Supplementary information

Supplementary Figure 1: *Francisella tularensis* NadE*-based Growth Restoration is Not Nicotinamide Feeding Dependent.

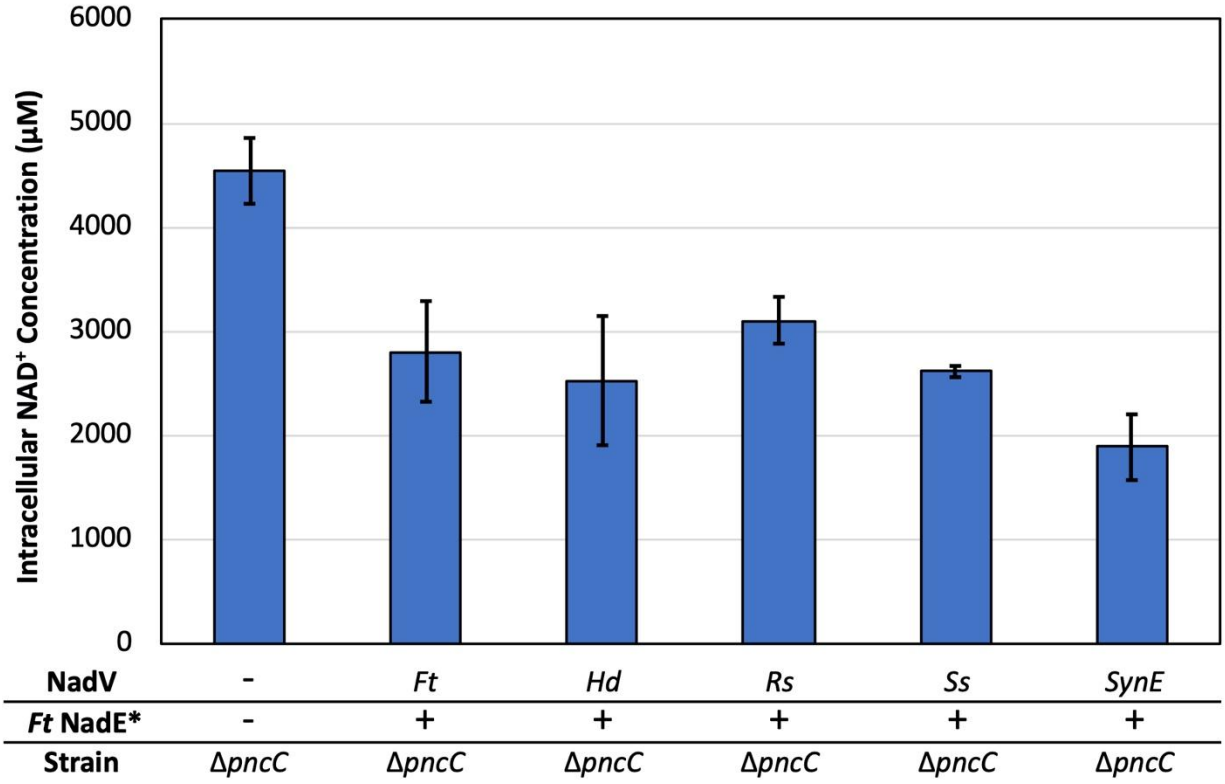
Supplementary Figure 2: Intracellular NAD^+ Decreases in NMN^+ Accumulating Strains.

DNA sequences of genes used in this study



Supplementary Figure 1: *Francisella tularensis* NadE*-based Growth Restoration is Not Nicotinamide Feeding Dependent.

A growth restoration platform was used to screen pathways for the efficient generation of nicotinamide mononucleotide (NMN^+). The *Escherichia coli* strain 72c [1] contains a temperature sensitive allele of *nadD* (*ts nadD*). As a result, this strain cannot grow at 42 ℃. By overexpressing *Francisella tularensis nadE**, cells are able to produce NMN^+, which can then be converted to NAD^+, and thus restoring growth. We observed no dependence of growth restoration with feeding 200 µM nicotinamide (NA). This indicates either efficient NMN^+ generation can be achieved through channeling the intermediate nicotinic acid mononucleotide ($NaMN^+$) from *E. coli* 's native *de novo* NAD^+ biosynthesis pathway, or LB medium used in this experiment already contains sufficient precursors for this pathway. Screening was performed in a deep-well 96-well plate containing 1 mL of LB medium supplemented with 2 g/L D-glucose and 200 µM of NA if applicable. Detailed conditions are described in the Methods section.



Supplementary Figure 2: Intracellular NAD^+ Decreases in NMN^+ Accumulating Strains
From Figure 3, co-overexpression of NMN^+ generating *Francisella tularensis* NadE* and NadVs increases intracellular NMN^+ when the NMN^+ degrading PncC is disrupted. However, as shown here, NAD^+ levels decreased in cells expressing *F. tularensis* NadE* and NadV compared to cells without overexpression. This potentially indicates NMN^+ plays a regulatory role in NAD^+ biosynthesis. Cells were cultured in 2xYT medium supplemented with 1 mM nicotinamide at 30 ℃ for four hours. Intracellular NAD^+ concentrations were determined by UPLC-MS/MS. Detailed conditions and analytical techniques are described in the Methods section.

DNA sequences of genes used in this study

Escherichia coli* BL21 *yqhD

ATGAACAACTTTAATCTGCACACCCCAACCCGCATTCTGTTTGGTAAAGGCGCAATC
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Francisella tularensis nadE* **(Codon optimized for *E. coli*)**

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CAAACTTTtaa

Salmonella enterica pnuC* KA, red text indicates the KA insertion made to the DNA sequence

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Escherichia coli* BL21 *pnuC

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Salmonella enterica nadR

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Saccharomyces cerevisiae* BY4741 *NRK1

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***Francisella tularensis nadV* (Codon optimized for *E. coli*)**

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***Ralstonia solanacearum nadV* (Codon optimized for *E. coli*)**

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Synechocystis sp.* PCC 6803 *nadV

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Synechococcus elongatus* PCC 7942 *nadV

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Haemophilus ducreyi nadV **(Codon optimized for** ***E. coli*****)**

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GTAA

References

[1] Stancek M, Isaksson LA, Rydén-Aulin M. fusB is an Allele of nadD, Encoding Nicotinate Mononucleotide Andeylyltransferase in *Escherichia coli*. Microbiology. 2003;149(9):2427-33.