Cloning, Expression and Characterization of NAD Kinase from *Staphylococcus aureus* Involved in the Formation of NADP (H): A Key Molecule in the Maintaining of Redox Status and Biofilm Formation

Abstract

**Background:** *Staphylococcus aureus* has the ability to form biofilms on any niches, a key pathogenic factor of this organism and this phenomenon is directly related to the concentration of NADPH. The formation of NADP is catalyzed by NAD kinase (NADK) and this gene of *S. aureus* ATCC 12600 was cloned, sequenced, expressed and characterized. **Materials and Methods:** The NADK gene was polymerase chain reaction amplified from the chromosomal DNA of *S. aureus* ATCC 12600 and cloned in pQE 30 vector, sequenced and expressed in *Escherichia coli* DH5α. The pure protein was obtained by passing through nickel metal chelate agarose column. The enzyme kinetics of the enzyme and biofilm assay of the *S. aureus* was carried out in both aerobic and anaerobic conditions. The kinetics was further confirmed by the ability of the substrates to dock to the NADK structure.

**Results:** The recombinant NADK exhibited single band with a molecular weight of 31kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the gene sequence (GenBank: JN645814) revealed presence of only one kind of NADK in all *S. aureus* strains. The enzyme exhibited very high affinity for NAD compared to adenosine triphosphate concurring with the docking results. A root-mean-square deviation value 14.039Å observed when NADK structure was superimposed with its human counterpart suggesting very low homology. In anaerobic conditions, higher biofilm units were found with decreased NADK activity. **Conclusion:** The results of this study suggest increased NADPH concentration in *S. aureus* plays a vital role in the biofilm formation and survival of this pathogen in any environmental conditions.

**Keywords:** Adenosine triphosphate, biofilms, NAD kinase, NADPH, root-mean-square deviation

Introduction

*Staphylococcus aureus* is a Gram-positive coccocci and an important cause of nosocomial infections. Appearance of multidrug resistance strains of *S. aureus* including resistant to vancomycin all over the world in such strains conspicuous variations are observed in colony morphology, physiology and growth characteristics due to high reductive conditions with poor acetate metabolism. Studies have shown that high anaerobic conditions favor accumulation of NADPH and NADH in bacteria and these molecules inhibit NAD kinase (NADK). The numerous pivotal functions of NAD(P) in metabolism, transcription, signaling pathways and detoxification reactions makes nicotinamide dinucleotide a central molecule for cell viability, which means that its concentration is firmly regulated. The biosynthesis of NAD(P) have been elucidated in detail in *Escherichia coli*, *Salmonella enterica* serovar and *Salmonella typhimurium*, a multifunctional protein NADR is reported as NAD-dependent repressor of transcription of genes implicated in NAD biosynthesis thus explains this pathway is regulated at the transcriptional level.

NAD is synthesized through *de novo* or pyridine salvage pathway and there are many differences exists between prokaryotes and eukaryotes. The main metabolite in *de novo* NAD biosynthesis in all living organisms is quinolinic acid (QA). Eukaryotes synthesize QA via tryptophan degradation while prokaryotes obtain QA through the condensation of imino aspartate with dihydroxyacetone phosphate which is catalyzed by quinolinate synthetase system. QA is involved in the formation of NADP (H): A Key Molecule in the Maintaining of Redox Status and Biofilm Formation.

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transformed into nicotinic acid mononucleotide (NaMN) by QA phosphoribosyltransferase afterward NaMN adenyltransferase catalyzes adenylation of NaMN to nicotinic acid adenine dinucleotide.[6,7,10] The nadD gene encoding NaMN adenyltransferase was shown to be essential for survival in *S. aureus* and *Streptococcus pneumonia* that are fully dependent on niacin salvage pathway.[11] Finally, nicotinic acid adenine dinucleotide is changed into NAD through the reaction catalyzed by NAD synthetase encoded by gene nadE, this reaction is followed by the synthesis of NADP catalyzed by magnesium dependent ubiquitous enzyme NADK[6,7] - encoded by gene NADK. Since this reaction is the only biochemical step in the synthesis of NADP from NAD, NADK is therefore, key enzyme for NADP synthesis and for the NADP-dependent anabolic and biosynthetic pathways in the cell. NADKs show homo oligomer structures but differ in the molecular size and number of subunits.[12] The molecular size of subunit from prokaryotes is approximately 30–35 kDa, almost all known NADKs are oligomeric proteins consisting of 2-8 identical subunits of 30–60 kDa.[12]

NADK is a ubiquitous, allosteric enzyme, catalyzes the formation of NADP using adenosine triphosphate (ATP) as phosphoryl donor and plays a central role in coupling oxidative - reductive conditions. It dictates whether the system is in oxidative or reductive conditions based on the concentration of [NAD]/[NADH] ratio near 1000, favors the oxidative conditions whereas [NADP]/[NADPH] ratio near 0.01, favors the reductive conditions[13] and defense against oxidative stress[14] by providing electrons for reductive repair. This could be one of the most crucial growth-limiting stimuli to control the pathogenesis.[15] As, NAD(H) and NADP(H) participate in more than 300 different oxidative-reductive reactions[16] their importance in substance metabolism and energy metabolism has long been known in tricarboxylic acid (TCA) cycle and *de novo* biosynthesis. NAD(H) is primarily involved in oxidative catabolic reactions, whereas NADP(H) participates in reductive anabolic reactions.[13] Thus, owing to the importance of NADK which is essential for the survival of microorganisms[17] the present study is focused on cloning, expression and characterization of NADK of *S. aureus* ATCC 12600.

**Materials and Methods**

**Bacterial strains and conditions**

*S. aureus* ATCC 12600 and *E. coli* DH5α were obtained from Merck Biosciences. Pvt Ltd. *S. aureus* was grown on modified Baird Parker media at 37°C. A clear isolated colony exhibiting distinct zone and shiny black color was picked and inoculated in brain heart infusion (BHI) broth and grown for overnight at 37°C and this grown *S. aureus* ATCC 12600 culture was used to characterize NADK. *E. coli* DH5α were used in the expression of *S. aureus* NADK gene cloned in pQE 30 vector.[14]

**Biofilm assay**

The biofilm assay was carried out for *S. aureus* ATCC 12600 grown in Luria-Bertani and BHI broths following earlier explained method.[19]

**Kinetic study of NAD kinase**

The cytosolic fraction was collected from the *S. aureus* to perform the enzyme assay and kinetics for NADK. The reaction mixture contains 10 mM MgCl₂, 5 mM NAD, 10 mM ATP, 5 mM glucose-6-phosphate, 10 mM Tris-HCl pH 7.5, pure glucose-6-phosphate dehydrogenase, crude or pure NADK of *S. aureus*. The reaction mixture was incubated for 4-5 min at 37°C and absorbance was taken at 340 nm. The maximum velocity of the enzyme catalyzed reaction was calculated by taking varying concentrations of substrate NAD from 0.25 to 10 mM. Kₘ and Vₘₐₓ for NADK were determined using Hanes–Woolf plot by taking [S₀] on X-axis and [S₀/V₀] on Y-axis. From Y-intercept value obtained in the graph, Kₘ was calculated.[10] Bradford method was applied to estimate the concentration of proteins.[21]

**NAD kinase gene amplification and sequencing from Staphylococcus aureus ATCC 12600**

Chromosomal DNA was extracted from late log phase culture of *S. aureus* and NADK gene was amplified by using forward primer: 5'-CATGCGTTATACAATTT-3' and reverse primer: 5'-TCATCGTTCTTCAACAC-3' which were designed from the NADK gene sequence of *S. aureus* Mu 50 strain.[22] The cocktail reaction mixture of 50 µl contained 0.5 µg of chromosomal DNA, 100 µM of dNTPs mixture, 100 picomoles of forward primer, and reverse primer, 1 unit of Taq DNA polymerase (Merck Biosciences, Pvt Ltd). Amplification parameters included an initial denaturation step for 10 min at 94°C; 40 cycles of each having denaturation at 94°C for 60 s, annealing at 37.35°C for 90 s and amplification at 72°C for 120 s which was followed by a final extension step at 72°C for 5 min in a master cycler gradient Thermocycler (Eppendorf), Nanoparticle-polymerase chain reaction (PCR) cleansing kit (Taurus Scientific, USA) was used to purify the PCR products which were then subjected to sequencing using dye terminating method at MWG Biotech India Ltd. Thus, obtained NADK gene sequence was deposited at GenBank (www.ncbi.nlm.nih.gov/genbank).

**Cloning, expression and purification of NAD kinase**

NADK gene was cloned in the Sma I site of pQE 30 and followed by transformation in *E. coli* DH5α. Thus formed clone was called as NADK-1. The insert in the clone was sequenced and on confirming the sequencing in the clone the gene was over-expressed with 1mM IPTG. The recombinant NADK [rNADK] was purified from the cytosolic fraction of clones by passing through nickel metal chelate agarose column (by following QIA express expression system.
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The three-dimensional structure of S. aureus NADK was built by using modeler 9v8 tool. The stereochemistry of the final model was verified by submitting to PROCHECK and ProSA-web servers. The structural alignment of S. aureus NADK and human NADK structures were carried out using PyMol software. ATP and NAD docking to NADK of S. aureus and human was performed to find out the mode of binding and affinity variations using Molecular Operating Environment MOE version 2011.10 software (Chemical Computing Group, Canada).

Results

Characterization of NAD kinase

NADK is the most prominent enzyme whose products detects whether the system is in oxidative or reductive conditions. In the present study, we have cloned, sequenced, expressed, and characterized NADK gene from S. aureus ATCC 12600. The sequence of NADK (GenBank accession number: JN645814) showed complete homology with NADK gene of all the strains of S. aureus reported in the database. The NADK gene expressed from NADK-1 clone was purified by passing through nickel metal chelate affinity column showed a molecular weight of 31 kD on 10% SDS-PAGE (Figure 1a and b).

The NADK identified in the cytoplasm of S. aureus ATCC 12600 demonstrated an enzyme activity of 1.65 ± 0.05 mM/ml/min and K_m 0.64 ± 0.5 mM [Table 1] for NADP substrate and for ATP as substrate the enzyme exhibited 1.2 ± 0.04 μM/ml/min as enzyme activity with K_m 1.01 ± 0.4 mM. Similar results were observed with pure rNADK [Table 2], signifying the presence of only one NADK gene in S. aureus corroborating with the basic local alignment search tool and docking results. NADK docking of S. aureus results showed highest docking score with NAD (−13.9069 kcal) compared to ATP (−13.7903 Kcal) [Supplementary Table 1 and Supplementary Figure 1a and b]; however, human NADK showed highest docking score with ATP (−12.7409) compared to NAD (−9.7059) [Supplementary Table 2].

Figure 1: (a) Amplification of NAD kinase gene using NAD kinase 1 and NAD kinase 2 primers from the chromosomal DNA of Staphylococcus aureus. M Lane showing DNA ladder L1 lane showing amplified product of NAD kinase gene, (b) electrophoretogram showing the expression of recombinant NAD kinase clone in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lane M: Molecular weight marker. Lane 1 and 2: Uninduced cell lysate of NAD kinase 1 clone. Lane 3: Induced cell lysate of NAD kinase 1 clone Lane 4: Purified recombinant NAD kinase. (c) Multiple sequence alignment of NAD kinase. (d) Phylogenetic tree based on NAD kinase sequence of Staphylococcus aureus with other bacterial NAD kinase sequences and Human NAD kinase.
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2 and Supplementary Figure 1c-d]. The buildup of NADPH in the bacteria allosterically inhibits NADK[5] and in the present study the pure rNADK activity was inhibited by the NADPH [Supplementary Figure 2]. The enzyme kinetics when compared with other organisms such as Saccharomyces cerevisiae,[27] Mycobacterium tuberculosis,[28-30] E. coli[29-31] and human[32] showed significant differences [Supplementary Table 3] correlating with the differences observed in the multiple sequence alignment of NADK gene sequence [Figure 1c and d].

Increased NADK activity was observed in S. aureus ATCC 12600 grown in aerobic broth compared with anaerobic conditions indicating increased buildup of NADPH in the organism corroborating the high reductive conditions favoring high rate of biofilm formation [Table 3].

Table 1: The enzyme kinetics of NADK and rNADK for NAD substrate

| Cytosolic fraction | Enzyme activity (NADPH mM/mg/min) | V<sub>max</sub> (NADPH mM/mg/min) | K<sub>M</sub> (mM) |
|--------------------|----------------------------------|----------------------------------|------------------|
| S. aureus ATCC 12600 | 1.65±0.05                        | 331±0.7                          | 0.64±0.5         |
| rNADK              | 1.7±0.03                         | 329.49±0.6                       | 0.66±0.2         |

Values±SD from three determinations. NADK: NAD kinase, V<sub>max</sub>: Maximum velocity, SD: Standard deviation, S. aureus: Staphylococcus aureus, rNADK: Recombinant NADK

Table 2: The enzyme kinetics of NADK and rNADK for ATP substrate

| Cytosolic fraction | Enzyme activity (ATP mM/mg/min) | V<sub>max</sub> (ATP mM/mg/min) | K<sub>M</sub> (mM) |
|--------------------|---------------------------------|---------------------------------|------------------|
| S. aureus ATCC 12600 | 1.2±0.04                        | 147.4±0.8                       | 1.01±0.4         |
| rNADK              | 1.1±0.03                        | 169.49±0.6                      | 0.99±0.2         |

Values±SD from three determinations. SD: Standard deviation, NADK: NAD kinase, V<sub>max</sub>: Maximum velocity, ATP: Adenosine triphosphate, S. aureus: Staphylococcus aureus, rNADK: Recombinant NADK

2 and Supplementary Figure 1c-d]. The buildup of NADPH in the bacteria allosterically inhibits NADK[5] and in the present study the pure rNADK activity was inhibited by the NADPH [Supplementary Figure 2]. The enzyme kinetics when compared with other organisms such as Saccharomyces cerevisiae,[27] Mycobacterium tuberculosis,[28-30] E. coli[29-31] and human[32] showed significant differences [Supplementary Table 3] correlating with the differences observed in the multiple sequence alignment of NADK gene sequence [Figure 1c and d].

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Comparative structural analysis of S. aureus NAD kinase with human NAD kinase

The structural alignment of S. aureus ATCC 12600 NADK [Figure 2a] and human NADK [Figure 2b] structures revealed an identity of 9.1% that is, distributed randomly throughout the conformation and when both structures were superimposed [Figure 2c], the root-mean-square deviation (RMSD) value 14.039Å indicates the extensive structural variations in both domain and nondomain regions. This value also implies distantly aligned variation between the C<sub>α</sub> atoms of the backbones of two aligned structures.

Discussion

There is a growing recognition that NADP (H) is the crucial coenzyme for many cellular processes in living organisms, such as NADPH-dependent reductive anabolic pathways; signal transduction, cellular defense against stress.[33,34] Studies have indicated that NADK is an essential enzyme for the survival of microorganisms in varied environmental conditions;[17] therefore, the present study is focused on cloning, expression and characterization of NADK from S. aureus ATCC 12600. In S. aureus and in M. tuberculosis, the activity of NADK is inhibited intensively by NADP+[29,31] whereas in E. coli[35] and S. enteric[5] NADH and NADPH are potent allosteric negative modulators of NADK.

NADK mainly concerned with these reactions and dictates whether the system is in oxidative or reductive conditions. This could be one of the most crucial growth-limiting stimuli to control the pathogenesis.[15] The ratio of NAD/NADP+ and NADH/NADPH controls many pathways in S. aureus such as TCA cycle, de novo biosynthesis and this regulation has profound effect on the redox status which is a key factor in S. aureus for the production of toxins, virulence factors and biofilm formation.[19,26,36,37] In the present study, the anaerobic conditions favored biofilm formation with decreased NADK activity which explains there is a greater build-up of NADPH in the bacteria leading to increased synthetic phase [Table 3].

The comparison of amino acid sequences of several known prokaryotic and eukaryotic NADKs revealed a general structural organization consisting of a conserved catalytic domain within the C-terminus and variable N-terminal parts[12] but the regulatory patterns of NADKs differ distinctively among microbes. These conspicuous differences throw light on the pathogenesis of S. aureus in the human host as compared with other bacteria.

The structural analysis of NADK revealed that it has a very low homology with human NADK as indicated from
the RMSD value 14.039Å. The results correlated with the enzyme kinetic data [28-32] which indicated that human NADK has very high affinity for the substrate compared to S. aureus NADK [32] [Supplementary Tables 1-3 and Supplementary Figures 1 and 2]; which is understandable owing to the fact that high oxidative conditions prevails in human tissues compared with S. aureus. However, it is very well known that this human pathogen can colonize in any anatomical locales in the host [38] for which S. aureus must be influencing the cellular redox state thus regulating metabolic, signaling and transcriptional processes in the cell. [2,19,33,34,37] this probably facilitates in the colonization and biofilm formation.

Conclusion
In the present study, NADK which catalyzes the synthesis of NADP in S. aureus was cloned, sequenced, expressed and characterized. The results of the present study indicated the buildup of NADPH in S. aureus in reductive conditions favors higher biofilm formation and this phenomenon is vital in the survival and spread of its infection in both hospital settings and community-acquired conditions.

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Conflicts of interest
There are no conflicts of interest.

References
1. Somerville GA, Chaussee MS, Morgan CI, Fitzgerald JR, Dorward DW, Reitzer LJ, et al. Staphylococcus aureus aconitase inactivation unexpectedly inhibits post-exponential-phase growth and enhances stationary-phase survival. Infect Immun 2002;70:6373-82.
2. Nelson JL, Rice KC, Slater SR, Fox PM, Archer GL, Bayles KW, et al. Vancomycin-intermediate Staphylococcus aureus strains have impaired acetate catabolism: Implications for polysaccharide intercellular adhesin synthesis and autolysis. Antimicrob Agents Chemother 2007;51:616-22.
3. Cui L, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, et al. Cell wall thickening is a common feature of vancomycin resistance in Staphylococcus aureus. J Clin Microbiol 2003;41:5-14.
4. Sifri CD, Baresh-Bernal A, Calderwood SB, von Eiff C. Virulence of Staphylococcus aureus small colony variants in the Caenorhabditis elegans infection model. Infect Immun 2006;74:1091-6.
5. Grose JH, Joss L, Velick SF, Roth JR. Evidence that feedback inhibition of NAD kinase controls responses to oxidative stress. Proc Natl Acad Sci U S A 2006;103:7601-6.
6. Begley TP, Kinsland C, Mehrl RA, Osterman A, Dorrestein P. The biosynthesis of nicotinamide adenine dinucleotides in bacteria. Vitam Horm 2001;61:103-19.
7. Magni G, Amici A, Emanuelli M, Raffaelli N, Ruggieri S. Enzymology of NAD+ synthesis. Adv Enzymol Relat Areas Mol Biol 1999;73:135-82, xi.
8. Penfound T, Foster JW. NAD-dependent DNA-binding activity of the bifunctional NadR regulator of Salmonella typhimurium. J Bacteriol 1999;181:648-55.
9. Tritz GJ, Chandler JL. Recognition of a gene involved in the regulation of nicotinamide adenine dinucleotide biosynthesis. J Bacteriol 1973;114:128-36.
10. Stone TW, Addae JJ. The pharmacological manipulation of glutamate receptors and neuroprotection. Eur J Pharmacol 2002;447:285-96.
11. Sorci L, Pan Y, Eyobo Y, Rodionova I, Huang N, Kurnasov O, et al. Targeting NAD biosynthesis in bacterial pathogens: Structure-based development of inhibitors of nicotinate mononucleotide adenylyltransferase NadD. Chem Biol 2009;16:849-61.
12. Feng S, Yongfu L, Ye L, Xiaoyuan W. Molecular properties, functions, and potential applications of NAD kinases. Acta Biochim Biophys Sin 2009;41:352-61.
13. Voet D, Voet JG. Other pathways of carbohydrate metabolism. Biochemistry. 2nd ed. New York: John Wiley and Sons; 1995.
14. Pomposiello PJ, Bennik MH, Demple B. Genome-wide transcriptional profiling of the Escherichia coli responses to superoxide stress and sodium salicylate. J Bacteriol 2001;183:3890-902.
15. Coleman G, Garbutt IT, Demmitz U. Ability of a Staphylococcus aureus isolate from a chronic osteomyelitis lesion to survive in the absence of air. Eur J Clin Microbiol 1983;2:595-7.
16. Foster JW, Moat AG. Nicotinamide adenine dinucleotide biosynthesis and pyridine nucleotide cycle metabolism in microbial systems. Microbiol Rev 1980;44:83-105.
17. Shi F, Li Y, Li Y, Wang X. Molecular properties, functions, and potential applications of NAD kinases. Acta Biochim Biophys...
Prasad, et al.: Characterization of S. aureus NAD kinase

Sin (Shanghai) 2009;41:352-61.

18. Prasad UV, Vasu D, Kumar YN, Kumar PS, Yeswanth S, Swarupa V, et al. Cloning, expression and characterization of NADP-dependent isocitrate dehydrogenase from Staphylococcus aureus. Appl Biochem Biotechnol 2013;169:862-9.

19. Yeswanth S, Nanda Kumar Y, Venkateswara Prasad U, Swarupa V, Koteswara rao V, Venkata Gurunadha Krishna Sarma P Cloning and characterization of l-lactate dehydrogenase gene of Staphylococcus aureus. Anaerobe 2013;24:43-8.

20. Palmer T. Enzymes Biochemistry, Biotechnology and Clinical Chemistry. 1st ed. Chichester, West Sussex, England: Horwood Publishing Ltd.; 2001.

21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.

22. Ohta T, Hirakawa H, Morikawa K, Maruyama A, Inose Y, Yamashita A, et al. Nucleotide substitutions in Staphylococcus aureus strains, Mu50, Mu3, and N315. DNA Res 2004;11:51-6.

23. Sambrook J, Russell DW. Molecular Cloning, A Laboratory Manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press; 2001.

24. Bramucci E, Piaiordini A, Bossa F, Pascarella S. PyMod: Sequence similarity searches, multiple sequence-structure alignments, and homology modeling within PyMOL. BMC Bioinformatics 2012;13 Suppl 4:S2.

25. Kumar PS, Kumar YN, Prasad UV, Yeswanth S, Swarupa V, Sovijenya G, et al. In silico designing and molecular docking of a potent analog against Staphylococcus aureus porphobilinogen synthase. J Pharm Bioallied Sci 2014;6:158-66.

26. Prasad UV, Swarupa V, Yeswanth S, Kumar PS, Kumar ES, Reddy KM, et al. Structural and Functional analysis of Staphylococcus aureus NADP-dependent IDH and its comparison with Bacterial and Human NADP-dependent IDH. Bioinformation 2014;10:81-6.

27. Apps DK. The NAD kinases of Saccharomyces cerevisiae. Eur J Biochem 1970;13:223-30.

28. Kawai S, Mori S, Mukai T, Suzuki S, Yamada T, Hashimoto W, et al. Inorganic polyphosphate/ATP-NAD kinase of Micrococcus flauus and Mycobacterium tuberculosis H37Rv. Biochem Biophys Res Commun 2000;276:57-63.

29. Raffaelli N, Finaurini L, Mazzola F, Pucci L, Sorci L, Amici A, et al. Characterization of Mycobacterium tuberculosis NAD kinase: Functional analysis of the full-length enzyme by site-directed mutagenesis. Biochemistry 2004;43:7610-7.

30. Mori S, Kawai S, Shi F, Mikami B, Murata K. Molecular conversion of NAD kinase to NADH kinase through single amino acid residue substitution. J Biol Chem 2005;280:24104-12.

31. Venkateswara Prasad U, Vasu D, Yeswanth S, Swarupa V, Sunitha MM, Choudhary A, et al. Phosphorylation controls the functioning of Staphylococcus aureus isocitrate dehydrogenase – Favours biofilm formation. J Enzyme Inhib Med Chem 2015;30:655-61.

32. Lowy FD. Staphylococcus aureus infections. N Engl J Med 1998;339:520-32.
Supplementary Table 1: Molecular docking of S. aureus NADK

| Compound | Docking score (kcal/mol) | Number of hydrogen bonds | Interacting residues | Hydrogen bond length in Å |
|----------|--------------------------|--------------------------|----------------------|--------------------------|
| NAD      | −13.9069                 | 2                        | Val 201              | 2.2                      |
|          |                          |                          | His 206              | 2.4                      |
| ATP      | −13.7903                 | 1                        | Arg 141              | 2.8                      |

NADK: NAD kinase, ATP: Adenosine triphosphate, S. aureus: Staphylococcus aureus

Supplementary Table 2: Molecular docking of human NADK

| Ligand | Docking score | Interacting residues | Number of hydrogen bonds | Distance in Å |
|--------|---------------|----------------------|--------------------------|---------------|
| NAD    | −9.7059       | Asp (304)            | 1                        | 2.2           |
|        |               | Gly (293)            | 1                        | 2.4           |
|        |               | Asp (304)            | 1                        | 2.6           |
| ATP    | −12.7409      | Pro (93)             | 1                        | 2.3           |
|        |               | Asn (339)            | 1                        | 2.5           |
|        |               | His (337)            | 1                        | 2.6           |
|        |               | Pro (413)            | 1                        | 2.2           |
|        |               | Val (414)            | 1                        | 2.1           |
|        |               | His (306)            | 1                        | 2             |
|        |               | ASP (304)            | 1                        | 2.1           |

NADK: NAD kinase, ATP: Adenosine triphosphate

Supplementary Table 3: The comparative NADK kinetics with other bacteria and human

| Organism | $K_M$         | References       |
|----------|---------------|------------------|
| S. aureus | 0.66 mM      | In the current study |
| S. cerevisiae | 0.32 mM | [27]             |
| Human    | 0.54 mM      | [32]             |
| E. coli  | 2 mM         | [30]             |
| M. tuberculosis | 0.9-3.3 mM | [31]             |

NADK: NAD kinase, S. aureus: Staphylococcus aureus, S. cerevisiae: Saccharomyces cerevisiae, E. coli: Escherichia coli, M. tuberculosis: Mycobacterium tuberculosis

Supplementary Figure 1: (a) Docking of NAD with Staphylococcus aureus NAD kinase. (b) Docking of adenosine triphosphate with Staphylococcus aureus NAD kinase. (c) Docking of NAD with human NAD kinase (d) Docking of NAD with human NAD kinase

Supplementary Figure 2: Graph showing the inhibition of NAD kinase with NADPH