

Immucillin-H, a purine nucleoside phosphorylase transition state analog, causes non-lethal attenuation of growth in *Staphylococcus aureus*

Christopher F Stratton & Vern L Schramm*

Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461; Vern L Schramm - Email: vern.schramm@einstein.yu.edu; Phone: +17184302813; *Corresponding author

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Abstract:

Purine nucleoside phosphorylase (PNP; EC: 2.4.2.1) is a key enzyme involved in the purine salvage pathway. A recent bioinformatic study by Yadav, P. K. *et al.* (*Bioinformatics* 2012, 8(14), 664–672) reports PNP as an essential enzyme and potential drug target in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). We conducted an analysis using the methodology outlined by the authors, but were unable to identify PNP as an essential gene product in CA-MRSA. In addition, the treatment of *Staphylococcus aureus* cultures with immucillin-H, a powerful inhibitor of PNP, resulted in the non-lethal attenuation of growth, suggesting that PNP activity is not essential for cell viability.

Background:

A recent study by Yadav, P. K. *et al.* used a bioinformatic approach to identify potential drug targets in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) [1]. In this work, the BLASTp program was used to search metabolically relevant gene products in CA-MRSA against the proteome of *Homo sapiens*. A collection of non-homologous gene products was generated and further refined by the identification of essential genes in the DEG 6.8 database [2]. Using this approach, Yadav, P. K. *et al.* identified purine nucleoside phosphorylase (PNP; EC: 2.4.2.1) as an essential enzyme and potential drug target in CA-MRSA [1].

PNP catalyzes the reversible phosphorolysis of 6-oxypurine ribonucleosides to the corresponding purine nucleobases and ribose 1-phosphate (Figure 1A) [3]. PNP is a key enzyme involved in the purine salvage pathway and is essential in humans for the formation of uric acid [3]. Our laboratory has previously investigated the catalytic mechanism and transition state structure of PNP (Figure 1B) using kinetic isotope effect (KIE) analysis [4, 5]. Transition state analysis was used in the design of transition state analogs for PNP, including immucillin-H (ImmH; Figure 1C), which is a potent inhibitor of bovine PNP ($K_i = 23$ pM) [6], human PNP ($K_i = 58$ pM) [5], and

bacterial PNP ($K_i = 28$ pM; *M. tuberculosis*) [7]. ImmH is currently in clinical trials for the treatment of leukemia [8, 9].

Yadav, P. K. *et al.* used non-homology between gene products in CA-MRSA and *Homo sapiens* as a key parameter to select potential drug targets. We were interested to find the authors identified PNP as a candidate despite the presence of PNP homologs in both CA-MRSA (NCBI Gene ID: 1004861) and *Homo sapiens* (NCBI Gene ID: 4860). An independent analysis of the DEG 6.8 database following the methodology outlined by Yadav, P. K. *et al.* was unable to identify PNP as an essential enzyme in CA-MRSA. Moreover, we conducted a series of cellular growth assays using a laboratory strain of *Staphylococcus aureus* and ImmH, a potent inhibitor of PNP developed by our laboratory. These assays indicate that ImmH causes non-lethal attenuation of growth in *Staphylococcus aureus*, suggesting that PNP activity is not essential to the organism.

Methodology:

Database searches

The DEG 6.8 database was accessed at URL: <http://www.essentialgene.org> and searches were executed for DEG accession number: DEG10020139.

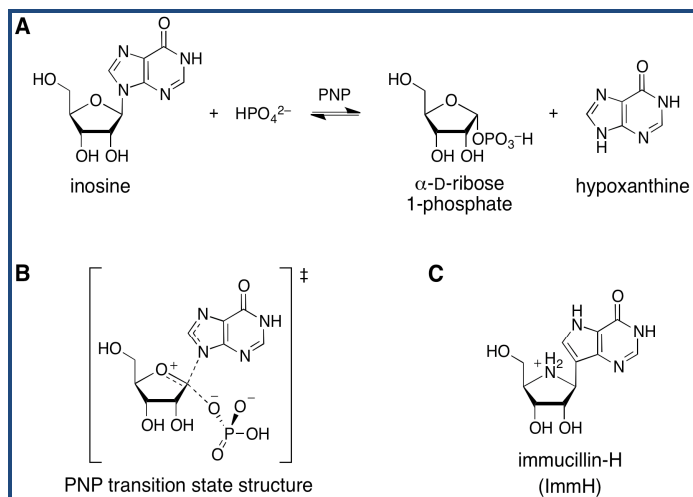


Figure 1: PNP-catalyzed Phosphorolysis of Inosine and PNP Transition State Structure. **(A)** PNP catalyzes the reversible phosphorolysis of purine nucleosides to form ribose 1-phosphate and a purine nucleobase. Here, the PNP-catalyzed phosphorolysis of inosine is shown. **(B)** Transition state structure of the PNP-catalyzed phosphorolysis of inosine. **(C)** Immucillin H (ImmH) is a transition state analog and powerful inhibitor of PNP.

Staphylococcus aureus growth assays with ImmH

The effect of ImmH on *Staphylococcus aureus* subsp. *aureus* Rosenbach growth was assessed by comparison of bacterial cultures grown on solid media (LB agar) in the presence and absence of inhibitor. Control plates (no inhibitor) were prepared using an LB agar mix (Fisher Scientific) with no additional additives; plates containing inhibitor were prepared by adding ImmH to molten LB agar (final concentration of ImmH = 100 μ M) just prior to pouring. Each plate of solid media was inoculated with *Staphylococcus aureus* using 30 μ L of a culture in liquid LB. The inoculated plates were incubated at 37 $^{\circ}$ C for 18 h and then removed from the incubator for analysis (**Figure 2A, B**). The plates were returned to the incubator for an additional 24 h (42 h total) and then removed for analysis (**Figure 2C, D**). All experiments on solid media were performed in triplicate and cell culture work was carried out under aseptic conditions in a laminar flow hood.

Discussion:

Yadav, P. K. *et al.* report PNP (EC: 2.4.2.1) is an essential enzyme in *Staphylococcus aureus* N315 based on identification of the gene *pnp* (DEG10020139) in the DEG 6.8 database [1]. A search of the DEG database for accession number DEG10020139 returned an entry for the gene *pnpA* (NCBI Gene ID: 1123948), which codes for polyribonucleotide nucleotidyltransferase (EC: 2.7.7.8), an enzyme that exhibits 3'-to-5' exonuclease activity. A subsequent search of the DEG database for essential genes in *Staphylococcus aureus* N315 returned 302 entries (see **supplementary material**). Examination of these genes failed to identify an entry for PNP (EC: 2.4.2.1). Based on these findings, we believe PNP (EC: 2.4.2.1) was incorrectly reported as an essential gene product in *Staphylococcus aureus* N315.

To investigate further the essentiality of PNP, we used ImmH to probe the effect of PNP inhibition on *Staphylococcus aureus* growth. It was found that the growth of *Staphylococcus aureus* cultures after 18 h at 37 $^{\circ}$ C was attenuated (relative to controls)

in the presence of 100 μ M ImmH (**Figure 2A, B**). This observation is consistent with ImmH acting to inhibit PNP and block the purine salvage pathway. When the same cultures were incubated for an additional 24 h at 37 $^{\circ}$ C (42 h total), bacterial growth recovered and appeared indistinguishable from the controls (**Figure 2C, D**). These data suggest the inhibition of PNP is non-lethal to *Staphylococcus aureus*, but rather only slows bacterial growth. Based on these results, we propose that PNP is not an essential enzyme in *Staphylococcus aureus* and that the inhibition of PNP activity can be compensated for by *de novo* nucleoside biosynthesis.

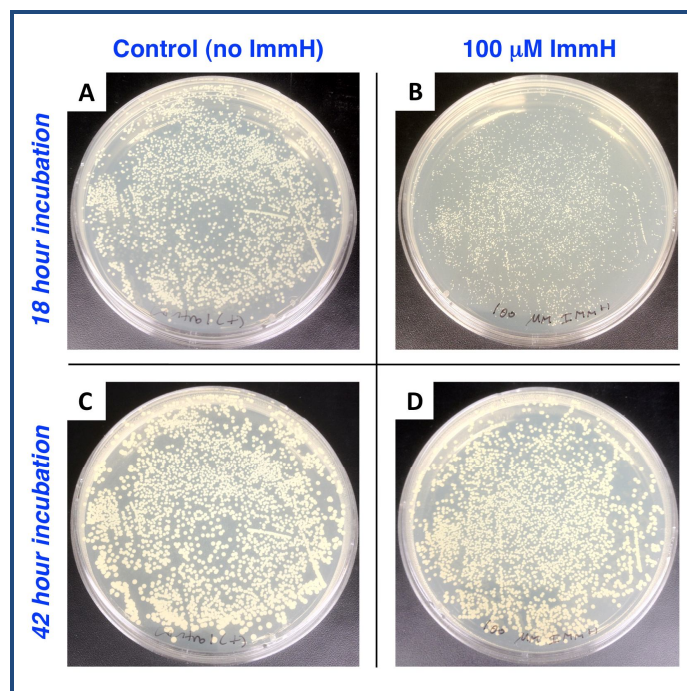


Figure 2: Effect of ImmH on *Staphylococcus aureus* Growth. **(A)** Growth of *Staphylococcus aureus* after 18 h incubation at 37 $^{\circ}$ C (Control / no ImmH). **(B)** Growth of *Staphylococcus aureus* in the presence of 100 μ M ImmH after 18 h incubation at 37 $^{\circ}$ C. **(C)** Growth of *Staphylococcus aureus* after 42 h incubation at 37 $^{\circ}$ C (Control/no ImmH). **(D)** Growth of *Staphylococcus aureus* in the presence of 100 μ M ImmH after 42 h incubation at 37 $^{\circ}$ C.

Conclusion:

In conclusion, we believe PNP (EC: 2.4.2.1) was incorrectly cited as an essential enzyme in CA-MRSA by Yadav, P. K. *et al.* A follow-up analysis using the methodology outlined by the authors was unable to identify PNP as an essential enzyme in CA-MRSA, but instead found the correct citation should be for polyribonucleotide nucleotidyltransferase (EC: 2.7.7.8). In addition, cellular growth assays using ImmH, a potent inhibitor of PNP, suggest that inhibition of PNP activity is not lethal to *Staphylococcus aureus*. These data further suggest that PNP is not an essential enzyme in *Staphylococcus aureus* and thus, may be of limited use as a therapeutic target in CA-MRSA.

References:

- [1] Yadav PK *et al.* *Bioinformatics*. 2012 **8**: 664 [PMID: 23055607]
- [2] Zhang R & Lin Y, *Nucleic Acids Res*. 2009 **37**: D455 [PMID: 18974178]
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[8] Homminga I *et al. Blood.* 2011 **118**: 2184 [PMID: 21730354]
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Supplementary material:

Table 1: Results from Search of the DEG 6.8 Database for Essential Genes in *Staphylococcus aureus* N315.

#	Accession No.	Gene Name	Function
1	DEG10020001	<i>dnaA</i>	chromosomal replication initiator protein
2	DEG10020002	<i>recF</i>	Recombinational DNA repair ATPase (RecF pathway)
3	DEG10020003	<i>gyrB</i>	DNA gyrase subunit B
4	DEG10020004	<i>gyrA</i>	DNA gyrase subunit A
5	DEG10020005	<i>serS</i>	seryl-tRNA synthetase
6	DEG10020006	<i>rplI</i>	50S ribosomal protein L9
7	DEG10020007	<i>dnaC</i>	replicative DNA helicase
8	DEG10020008	SA0021	Conserved hypothetical protein
9	DEG10020009	SA0085	Conserved hypothetical protein
10	DEG10020010	<i>drm</i>	Phosphopentomutase
11	DEG10020011	SA0179	Ornithine aminotransferase
12	DEG10020012	SA0181	Hypothetical protein
13	DEG10020013	<i>oppF</i>	oligopeptide transport ATP-binding protein
14	DEG10020014	<i>msmX</i>	Multiple sugar-binding transport ATP-binding protein
15	DEG10020015	SA0230	Conserved hypothetical protein
16	DEG10020016	SA0272	similar to transmembrane protein Tmp7
17	DEG10020017	SA0348	Hypothetical protein
18	DEG10020018	SA0351	hypothetical protein, similar to GTP-binding protein
19	DEG10020019	<i>rpsF</i>	30S ribosomal protein S6
20	DEG10020020	<i>ssb</i>	Single-strand DNA-binding protein
21	DEG10020021	<i>rpsR</i>	30S ribosomal protein S18
22	DEG10020022	<i>pubX/pbuX</i>	Xanthine permease
23	DEG10020023	<i>guaB</i>	Inositol-monophosphate dehydrogenase
24	DEG10020024	<i>guaA</i>	GMP synthase
25	DEG10020025	SA0422	Hypothetical protein
26	DEG10020026	<i>dnaX</i>	DNA polymerase III gamma and tau subunits
27	DEG10020027	SA0437	conserved hypothetical protein
28	DEG10020028	<i>tmk</i>	Thymidylate kinase homolog
29	DEG10020029	SA0446	Conserved hypothetical protein
30	DEG10020030	SA0447	conserved hypothetical protein
31	DEG10020031	<i>metS</i>	methionyl-tRNA synthetase
32	DEG10020032	SA0449	conserved hypothetical protein
33	DEG10020033	<i>gcaD</i>	UDP-N-acetylglucosamine pyrophosphorylase
34	DEG10020034	<i>prs</i>	Ribose-phosphate pyrophosphokinase
35	DEG10020035	<i>pth</i>	Peptidyl-tRNA hydrolase
36	DEG10020036	SA0464	conserved hypothetical protein
37	DEG10020037	SA0467	conserved hypothetical protein
38	DEG10020038	<i>ftsH</i>	cell-division protein
39	DEG10020039	<i>lysS</i>	lysyl-tRNA synthetase
40	DEG10020040	<i>clpC</i>	endopeptidase
41	DEG10020041	<i>gltX</i>	Glutamyl-tRNA synthetase (glutamate--tRNA ligase)
42	DEG10020042	<i>cysS</i>	cysteinyl-tRNA synthetase
43	DEG10020043	<i>rplK</i>	50S ribosomal protein L11
44	DEG10020044	<i>rplA</i>	50S ribosomal protein L1
45	DEG10020045	<i>rplJ</i>	50S ribosomal protein L10
46	DEG10020046	<i>rplL</i>	50S ribosomal protein L7/L12
47	DEG10020047	<i>rpoB</i>	DNA-directed RNA polymerase subunit beta
48	DEG10020048	<i>rpoC</i>	RNA polymerase beta-prime chain
49	DEG10020049	<i>rpsL</i>	30S ribosomal protein S12
50	DEG10020050	<i>rpsG</i>	30S ribosomal protein S7
51	DEG10020051	<i>fus</i>	translational elongation factor G
52	DEG10020052	<i>tufA/tuf</i>	translational elongation factor TU
53	DEG10020053	<i>pta/eutD</i>	phosphotransacetylase
54	DEG10020054	SA0560	Conserved hypothetical protein
55	DEG10020055	<i>adh1</i>	alcohol dehydrogenase
56	DEG10020056	<i>argS</i>	arginyl-tRNA synthetase

57	DEG10020057	<i>vraF</i>	ABC transporter ATP-binding protein
58	DEG10020058	<i>bacA/uppP</i>	Bacitracin resistance protein
59	DEG10020059	<i>fruB</i>	fructose 1-phosphate kinase
60	DEG10020060	<i>fruA</i>	fructose specific permease
61	DEG10020061	SA0675	similar to ABC transporter ATP-binding protein
62	DEG10020062	<i>nrdE</i>	Ribonucleoside-diphosphate reductase (major subunit)
63	DEG10020063	SA0703	Conserved hypothetical protein
64	DEG10020064	<i>secA</i>	preprotein translocase subunit
65	DEG10020065	<i>prfB</i>	Peptide chain release factor 2, authentic frameshift
66	DEG10020066	<i>uvrB</i>	Exonuclease ABC subunit B
67	DEG10020067	<i>uvrA</i>	exinuclease ABC subunit A
68	DEG10020068	<i>hprK</i>	HPr kinase/phosphatase
69	DEG10020069	SA0722	conserved hypothetical protein
70	DEG10020070	<i>gap</i>	glyceraldehyde-3-phosphate dehydrogenase
71	DEG10020071	<i>pgk</i>	Phosphoglycerate kinase
72	DEG10020072	<i>tpi</i>	Triose phosphate isomerase
73	DEG10020073	<i>eno</i>	enolase
74	DEG10020074	SA0732	hypothetical protein
75	DEG10020075	<i>ssrP/smpB</i>	ssrA-binding protein
76	DEG10020076	SA0771	Conserved hypothetical protein
77	DEG10020077	SA0772	conserved hypothetical protein
78	DEG10020078	SA0774	ABC transporter ATP-binding protein homologue
79	DEG10020079	<i>nifS</i>	Pyridoxal-phosphate-dependent aminotransferase
80	DEG10020080	<i>lipA</i>	Lipoic acid synthetase
81	DEG10020081	<i>pgi</i>	glucose-6-phosphate isomerase A
82	DEG10020082	<i>clpB</i>	ClpB chaperone homologue
83	DEG10020083	<i>oppD</i>	Oligopeptide transport system ATP-binding protein OppD homolog
84	DEG10020084	<i>trpS</i>	tryptophanyl-tRNA synthetase
85	DEG10020085	<i>fabI</i>	Trans-2-enoyl-ACP reductase
86	DEG10020086	<i>prfC</i>	Peptide chain release factor 3
87	DEG10020087	<i>menB</i>	Naphthoate synthase
88	DEG10020088	<i>qoxB</i>	Quinol oxidase polypeptide I QoxB
89	DEG10020089	<i>purC</i>	phosphoribosylaminoimidazole-succinocarboxamide synthase
90	DEG10020090	<i>purN</i>	5-Phosphoribosylglycinamide transformylase 1
91	DEG10020091	<i>ptsH</i>	phophocarrier protein HPR
92	DEG10020092	<i>ptsI</i>	phosphoenolpyruvate-protein phosphatase
93	DEG10020093	SA0940	conserved hypothetical protein
94	DEG10020094	<i>phdB/pdhB</i>	pyruvate dehydrogenase E1 component beta subunit
95	DEG10020095	<i>pdhC</i>	dihydrolipoamide S-acetyltransferase component of pyruvate dehydrogenase E2
96	DEG10020096	<i>pdhD</i>	dihydrolipoamide dehydrogenase component of pyruvate dehydrogenase E3
97	DEG10020097	<i>potA</i>	Spermidine/putrescine ABC transporter, ATP-binding protein homolog
98	DEG10020098	SA0956	Hypothetical protein
99	DEG10020099	SA0959	GTP-binding elongation factor
100	DEG10020100	<i>rpmF</i>	Ribosomal protein L32
101	DEG10020101	<i>pheS</i>	Phe-tRNA synthetase alpha chain
102	DEG10020102	<i>pheT</i>	Phe-tRNA synthetase beta chain
103	DEG10020103	<i>mutS2</i>	MutS-like protein
104	DEG10020104	<i>uvrC</i>	Exonuclease ABC subunit C
105	DEG10020105	<i>murI</i>	Glutamate racemase
106	DEG10020106	SA0998	Conserved hypothetical protein
107	DEG10020107	<i>mraY</i>	Phospho-N-muramic acid-pentapeptide translocase
108	DEG10020108	<i>murD</i>	UDP-N-acetylmuramoyl-L-alanine--D-glutamate ligase
109	DEG10020109	<i>ftsA</i>	Cell division protein FtsA
110	DEG10020110	<i>ftsZ</i>	cell division protein
111	DEG10020111	SA1031	conserved hypothetical protein
112	DEG10020112	<i>ileS</i>	Ile-tRNA synthetase

113	DEG10020113	<i>pyrC</i>	dihydroorotase	
114	DEG10020114	<i>SA1054</i>	Pantothenate metabolism flavoprotein homolog	
115	DEG10020115	<i>priA</i>	Primosomal replication factor Y	
116	DEG10020116	<i>SA1063</i>	protein kinase	
117	DEG10020117	<i>rpmB</i>	Ribosomal protein L28	
118	DEG10020118	<i>pIsX</i>	Fatty acid/phospholipid synthesis protein	
119	DEG10020119	<i>fabD</i>	Malonyl CoA-acyl carrier protein transacylase	
120	DEG10020120	<i>fabG</i>	3-Oxoacyl-[acyl-carrier protein] reductase	
121	DEG10020121	<i>rnc</i>	RNase III	
122	DEG10020122	<i>smc</i>	chromosome segregation SMC protein	
123	DEG10020123	<i>SA1078</i>	signal recognition particle	
124	DEG10020124	<i>ffh</i>	signal recognition particle homolog	
125	DEG10020125	<i>rpsP</i>	30S ribosomal protein S16	
126	DEG10020126	<i>trmD</i>	tRNA	
127	DEG10020127	<i>rplS</i>	50S ribosomal protein L19	
128	DEG10020128	<i>rbgA</i>	conserved hypothetical protein	
129	DEG10020129	<i>topA</i>	DNA topoisomerase I	
130	DEG10020130	<i>gid</i>	Glucose inhibited division protein gid	
131	DEG10020131	<i>rpsB</i>	30S ribosomal protein S2	
132	DEG10020132	<i>tsf</i>	homolog elongation factor TS	
133	DEG10020133	<i>cdsA</i>	phosphatidate cytidyltransferase	
134	DEG10020134	<i>proS</i>	prolyl-tRNA synthetase	
135	DEG10020135	<i>polC</i>	DNA polymerase III, alpha chain PolC-type	
136	DEG10020136	<i>nusA</i>	Transcription termination	
137	DEG10020137	<i>infB</i>	translation initiation factor IF-2	
138	DEG10020138	<i>rbfA</i>	Ribosome-binding factor A	
139	DEG10020139	<i>pnpA</i>	Polyribonucleotide nucleotidyltransferase	
140	DEG10020140	<i>SA1118</i>	conserved hypotehtical protein	
141	DEG10020141	<i>pgsA</i>	Phosphotidylglycerophosphate synthase	
142	DEG10020142	<i>recA</i>	RecA/RadA recombinase	
143	DEG10020143	<i>glpP</i>	glycerol uptake operon antiterminator regulatory protein	
144	DEG10020144	<i>glpK</i>	glycerol kinase	
145	DEG10020145	<i>SA1147</i>	Hypothetical protein	
146	DEG10020146	<i>SA1176</i>	Conserved hypothetical protein	
147	DEG10020147	<i>tkt</i>	transketolase	
148	DEG10020148	<i>SA1187</i>	conserved hypothetical protein	
149	DEG10020149	<i>parE</i>	topoisomerase IV subunit B	
150	DEG10020150	<i>parC</i>	topoisomerase IV subunit A	
151	DEG10020151	<i>glcT</i>	transcription antiterminator	
152	DEG10020152	<i>trpB</i>	Tryptophan synthase beta chain	
153	DEG10020153	<i>odhB</i>	Dihydrolipoamide succinyltransferase	
154	DEG10020154	<i>murG</i>	Undecaprenyl-PP-MurNAc-pentapeptide-UDPGlcNAc transferase	GlcNAc
155	DEG10020155	<i>SA1252</i>	conserved hypothetical protein	
156	DEG10020156	<i>dfrA</i>	dihydrofolate reductase	
157	DEG10020157	<i>thyA</i>	Thymidylate synthase	
158	DEG10020158	<i>SA1277</i>	Conserved hypothetical protein	
159	DEG10020159	<i>asnS/asnC</i>	Asparaginyl-tRNA synthetase	
160	DEG10020160	<i>gerCD/ubiE</i>	Menaquinone biosynthesis methyltransferase	
161	DEG10020161	<i>engA</i>	hypothetical protein, similar to GTP binding protein	
162	DEG10020162	<i>cmk</i>	Cytidylate kinase	
163	DEG10020163	<i>bmfBB</i>	branched-chain alpha-keto acid dehydrogenase E2	
164	DEG10020164	<i>bmfBAB</i>	branched-chain alpha-keto acid dehydrogenase E1	
165	DEG10020165	<i>bmfBAA</i>	branched-chain alpha-keto acid dehydrogenase E1	
166	DEG10020166	<i>SA1349</i>	dihydrolipoamide dehydrogenase	
167	DEG10020167	<i>SA1359</i>	Translation elongation factor P	
168	DEG10020168	<i>rpmG</i>	50S ribosomal protein L33	
169	DEG10020169	<i>sigA</i>	RNA polymerase major rho factor	

170	DEG10020170	<i>dnaG</i>	DNA primase
171	DEG10020171	<i>rpsU</i>	30S ribosomal protein S21
172	DEG10020172	<i>dnaJ</i>	Molecular chaperones (contain C-terminal Zn finger domain)
173	DEG10020173	<i>dnaK</i>	Molecular chaperone
174	DEG10020174	<i>lepA</i>	GTP-binding protein
175	DEG10020175	<i>rpsT</i>	30S ribosomal protein S20
176	DEG10020176	<i>greA</i>	Transcription elongation factor GreA
177	DEG10020177	SA1445	conserved hypothetical protein
178	DEG10020178	<i>alaS</i>	alanyl-tRNA synthetase
179	DEG10020179	SA1449	(5-methylaminomethyl-2-thiouridylate)-methyltransferase
180	DEG10020180	SA1450	Iron-sulfur cofactor synthesis protein homolog
181	DEG10020181	<i>aspS</i>	aspartyl-tRNA synthetase
182	DEG10020182	<i>hisS</i>	histidyl-tRNA synthetase
183	DEG10020183	<i>obg/obgE</i>	Spo0B-associated GTP-binding protein
184	DEG10020184	<i>rpmA</i>	50S ribosomal protein L27
185	DEG10020185	<i>rplU</i>	50S ribosomal protein L21
186	DEG10020186	<i>valS</i>	valine-tRNA ligase
187	DEG10020187	<i>hemB</i>	Delta-aminolevulinic acid dehydratase
188	DEG10020188	<i>hemC</i>	porphobilinogen deaminase
189	DEG10020189	<i>rplT</i>	50S ribosomal protein L20
190	DEG10020190	<i>rpml</i>	50S ribosomal protein L35
191	DEG10020191	<i>infC</i>	translation initiation factor IF-3 infC
192	DEG10020192	<i>thrS</i>	threonyl-tRNA synthetase 1
193	DEG10020193	SA1509	Conserved hypothetical protein
194	DEG10020194	<i>gapB</i>	Glyceraldehyde-3-phosphate dehydrogenase 2
195	DEG10020195	<i>polA</i>	DNA polymerase I
196	DEG10020196	<i>pykA</i>	pyruvate kinase
197	DEG10020197	<i>pfk/pfkA</i>	6-phosphofructokinase
198	DEG10020198	<i>accA</i>	Acetyl-CoA carboxylase, carboxyl transferase, alpha subunit
199	DEG10020199	SA1523	acetyl-CoA carboxylase transferase beta subunit
200	DEG10020200	SA1524	Malate dehydrogenase homolog
201	DEG10020201	<i>dnaE</i>	DNA polymerase III, alpha chain
202	DEG10020202	<i>ackA</i>	Acetate kinase homolog
203	DEG10020203	<i>rpsD</i>	30S ribosomal protein S4
204	DEG10020204	<i>serA</i>	Phosphoglycerate dehydrogenase
205	DEG10020205	<i>tyrS</i>	tyrosyl-tRNA synthetase
206	DEG10020206	<i>acsA</i>	Acetyl-CoA synthetase
207	DEG10020207	<i>ccpA</i>	Catabolite control protein A
208	DEG10020208	<i>murC</i>	UDP-N-acetylmuramate-alanine ligase
209	DEG10020209	SA1563	phenylalanyl-tRNA synthetase (beta subunit) homolog
210	DEG10020210	<i>leuS</i>	leucyl-rRNA synthetase
211	DEG10020211	<i>metK</i>	S-adenosylmethionine synthetase
212	DEG10020212	<i>ampS</i>	aminopeptidase ampS
213	DEG10020213	<i>vraS</i>	two-component sensor histidine kinase
214	DEG10020214	<i>map</i>	Methionine aminopeptidase
215	DEG10020215	SA1713	RNA methyltransferase homolog
216	DEG10020216	<i>gatB</i>	glutamyl-tRNAGln amidotransferase subunit B
217	DEG10020217	SA1716	glutamyl-tRNAGln amidotransferase subunit A
218	DEG10020218	<i>lig</i>	DNA ligase
219	DEG10020219	<i>pcrA</i>	ATP-dependent DNA helicase
220	DEG10020220	<i>purB</i>	adenylosuccinate lyase
221	DEG10020221	<i>nadE</i>	NAD synthetase, prefers NH ₃ over glutamine
222	DEG10020222	SA1747	similar to ABC transporter, ATP-binding protein
223	DEG10020223	SA1792	Single-strand DNA-binding protein
224	DEG10020224	<i>groES</i>	Protein fate: Protein folding and stabilization
225	DEG10020225	<i>vga</i>	Hypothetical ABC transporter ATP-binding protein
226	DEG10020226	SA1854	similar to O-sialoglycoprotein
227	DEG10020227	<i>kdpA</i>	potassium-transporting ATPase subunit A

228	DEG10020228	SA1885	hypothetical protein, similar to ATP-dependent RNA helicase UDP-N-acetylmuramoylalanine-D-glutamyl-lysine-D-alanyl-D-alanine
229	DEG10020229	<i>murF</i>	ligase
230	DEG10020230	<i>ddlA/ddl</i>	D-Alanine-D-alanine ligase
231	DEG10020231	<i>murA</i>	UDP-N-acetylglucosamine 1-carboxyvinyl transferase 1
232	DEG10020232	<i>atpF</i>	ATP synthase B chain
233	DEG10020233	<i>upp</i>	uracil phosphoribosyl transferase
234	DEG10020234	<i>glyA</i>	serine hydroxymethyl transferase
235	DEG10020235	<i>prfA</i>	peptide chain release factor 1
236	DEG10020236	<i>tdk</i>	thymidine kinase
237	DEG10020237	<i>rpmE/rpmE2</i>	Ribosomal protein L31
238	DEG10020238	<i>rho</i>	Transcription termination factor Rho
239	DEG10020239	<i>fbaA</i>	fructose-bisphosphate aldolase
240	DEG10020240	SA1939	deoxyribose-phosphate aldolase
241	DEG10020241	SA1957	conserved hypothetical protein
242	DEG10020242	<i>glmS</i>	Glucosamine-fructose-6-phosphate aminotransferase
243	DEG10020243	<i>mtIA</i>	Mannitol PTS EII
244	DEG10020244	<i>mtID</i>	mannitol-1-phosphate 5-dehydrogenase
245	DEG10020245	SA1966	conserved hypothetical protein
246	DEG10020246	<i>lacA</i>	galactose-6-phosphate isomerase LacA subunit
247	DEG10020247	<i>rpsL</i>	30S ribosomal protein S9
248	DEG10020248	<i>rplM</i>	50S ribosomal protein L13
249	DEG10020249	SA2019	Conserved hypothetical protein
250	DEG10020250	SA2020	Hypothetical protein
251	DEG10020251	<i>rplQ</i>	50S ribosomal protein L17
252	DEG10020252	<i>rpoA</i>	DNA-directed RNA polymerase alpha chain
253	DEG10020253	<i>rpsK</i>	30S ribosomal protein S11
254	DEG10020254	<i>rpsM</i>	30S ribosomal protein S13
255	DEG10020255	<i>rpmJ</i>	50S ribosomal protein L36
256	DEG10020256	<i>infA</i>	translation initiation factor IF-1
257	DEG10020257	<i>adk</i>	adenylate kinase
258	DEG10020258	<i>secY</i>	preprotein translocase SecY subunit
259	DEG10020259	<i>rplO</i>	50S ribosomal protein L15
260	DEG10020260	<i>rpmD</i>	50S ribosomal protein L30
261	DEG10020261	<i>rpsE</i>	30S ribosomal protein S5
262	DEG10020262	<i>rplR</i>	50S ribosomal protein L18
263	DEG10020263	<i>rplF</i>	50S ribosomal protein L6
264	DEG10020264	<i>rpsH</i>	30S ribosomal protein S8
265	DEG10020265	<i>rpsN</i>	30S ribosomal protein S14
266	DEG10020266	<i>rplE</i>	50S ribosomal protein L5
267	DEG10020267	<i>rplX</i>	50S ribosomal protein L24
268	DEG10020268	<i>rplN</i>	50S ribosomal protein L14
269	DEG10020269	<i>rpsQ</i>	30S ribosomal protein S17
270	DEG10020270	<i>rpmC</i>	50S ribosomal protein L29
271	DEG10020271	<i>rplP</i>	50S ribosomal protein L16
272	DEG10020272	<i>rpsC</i>	30S ribosomal protein S3
273	DEG10020273	<i>rplV</i>	50S ribosomal protein L22
274	DEG10020274	<i>rpsS</i>	30S ribosomal protein S19
275	DEG10020275	<i>rplB</i>	50S ribosomal protein L2
276	DEG10020276	<i>rplW</i>	50S ribosomal protein L23
277	DEG10020277	<i>rplD</i>	50S ribosomal protein L4
278	DEG10020278	<i>rplC</i>	50S ribosomal protein L3
279	DEG10020279	<i>rpsJ</i>	30S ribosomal protein S10
280	DEG10020280	<i>ureA</i>	Urease gamma subunit
281	DEG10020281	<i>ureB</i>	Urease beta subunit
282	DEG10020282	<i>ureC</i>	Urease alpha subunit
283	DEG10020283	SA2156	L-Lactate permease lctP homolog
284	DEG10020284	<i>narG</i>	Respiratory nitrate reductase alpha chain
285	DEG10020285	<i>fmhA</i>	fmhA protein
286	DEG10020286	<i>opuCA</i>	glycine betaine/carnitine/choline ABC transporter
287	DEG10020287	<i>opp-1D</i>	oligopeptide transporter putative ATPase domain

288	DEG10020288	SA2279	hypothetical protein, similar to phosphomannomutase
289	DEG10020289	<i>gntK</i>	gluconokinase
290	DEG10020290	<i>ptsG</i>	PTS system, glucose-specific IIABC component
291	DEG10020291	<i>clpL</i>	ATP-dependent Clp proteinase chain ClpL
292	DEG10020292	<i>copA</i>	Copper-transporting ATPase <i>copA</i>
293	DEG10020293	<i>ldh</i>	L-Lactate dehydrogenase
294	DEG10020294	<i>gbsA</i>	Glycine betaine aldehyde dehydrogenase <i>gbsA</i>
295	DEG10020295	SA2434	fructose phosphotransferase system enzyme fruA homolog
296	DEG10020296	<i>pmi</i>	Mannose-6-phosphate isomerase
297	DEG10020297	SA2442	preprotein translocase SecA homolog
298	DEG10020298	<i>vraD</i>	hypothetical protein, similar to ABC transporter
299	DEG10020299	<i>gidB</i>	Glucose inhibited division protein B
300	DEG10020300	<i>gidA</i>	glucose inhibited division protein A
301	DEG10020301	<i>rnpA</i>	Ribonuclease P - protein component
302	DEG10020302	<i>rpmH</i>	50S ribosomal protein L34
