Regulation and Consequence of Serine Catabolism in *Streptococcus pyogenes*

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SUMMARY

The Gram-positive bacterium *Streptococcus pyogenes* (Group A Streptococcus, GAS), is found strictly in humans and is capable of causing a wide variety of infections. Here we demonstrate that serine catabolism in GAS is controlled by the transcriptional regulator, Spy49_0126c. We have designated this regulator SerR (serine catabolism regulator). Microarray and transcriptional reporter data show that SerR acts as a transcriptional repressor of multiple operons, including *sloR* and *sdhBA*. Purified recombinant SerR binds to promoters of both *sloR* and *sdhB*, demonstrating that this regulation is direct. Deletion of *serR* results in reduced culture yield of the mutant compared to wild-type when grown in defined medium unless additional serine is provided, suggesting that regulation of serine metabolism is important for maximizing bacterial growth. Deletion of *sloR* or *sdhB* in the Δ*serR* mutant background restores growth to wild-type levels, suggesting that both operons have roles in serine catabolism. While reports have linked *sloR* function to streptolysin O expression, transport experiments with radiolabeled L-serine reveal that the *sloR* operon is required for rapid acquisition of serine, implicating a novel role for this operon in amino acid metabolism.

INTRODUCTION

The Gram-positive bacterium *Streptococcus pyogenes*, also known as Group A Streptococcus (GAS), is a strictly human pathogen that poses large health and economic burdens worldwide (2). The most familiar illness associated with GAS is acute pharyngitis, commonly known as “strep throat”, but *S. pyogenes* also causes a wide range of other non-invasive and invasive infections, including pyoderma, toxic shock syndrome, and necrotizing fasciitis, as well as post-infection sequelae, such as acute rheumatic fever, rheumatic heart disease, and glomerulonephritis (9). The wide range of infections caused by GAS requires the bacterium to survive at various locations within the human host, including

on the skin, in the mucosal lining of the nasopharynx, and in the blood, with each site posing different physiological difficulties that GAS must overcome in order to survive. *In vitro* cultures in saliva, blood, and amniotic fluid have demonstrated high plasticity of the GAS transcriptome in response to various host environments (16, 39, 43), suggesting that GAS uses adaption to changing environments via differential expression of stress and nutrient-acquisition genes to ensure survival.

The GAS genome is predicted to encode an average of 13 two-component systems and over 100 transcription factors whose activity depend on environmental and bacterial factors yet to be discovered (34). It has become increasingly clear that the regulatory system of *S. pyogenes* is extremely complex, consisting of numerous parallel and converging regulatory networks partaking in regulation of both virulence and metabolic genes (21). For example, CcpA is a transcriptional regulator that mediates carbon catabolite repression (CCR) in Gram-positive bacteria (48), while the stand-alone regulator CodY responds to branched-chain amino acid (BCAA) concentrations, as well as GTP levels in at least one species, to modulate BCAA biosynthesis accordingly (27). Importantly, in addition to regulating genes involved in carbon source utilization and BCAA biosynthesis, both CcpA and CodY alter virulence factor expression in GAS (20, 26, 40, 41), attesting to the importance of coordinated regulation of metabolism and virulence in this organism.

Amino acids play various roles within the cell, contributing to both bacterial growth and survival. As the building blocks of peptides and proteins in all organisms, amino acids are essential nutrients that must be produced by the cell or scavenged from the environment in order to permit growth. Group A Streptococcus is auxotrophic for 15 of the 20 essential amino acids (10) , including L-serine, and thus is highly dependent on amino acids obtained from the host organism in the form of free amino acids or peptides (35). Apart from allowing for protein synthesis, amino acid availability has substantial effects on gene expression in *S. pyogenes* through modulation of transcriptional regulator

activity (27) and riboswitch formation (45, 49). Some amino acids, such as L-arginine, also play an important role in pH resistance in many bacteria, including GAS (8). Despite the importance of amino acids for various cellular processes and the polyauxotrophy for most amino acids in *S. pyogenes*, little is understood regarding the transport of free amino acids or the regulation of genes involved in free amino acid uptake in GAS. In fact, we have been unable to find any reports experimentally identifying a transporter responsible for uptake of any free amino acid in *S. pyogenes*. A previous report provided evidence that a common transporter may exist for L-isomers of alanine, serine, threonine, and glycine in GAS (36), however the transporter itself was never identified. By the same token, information regarding the direct regulation of amino acid degradation in general is lacking in this organism.

In this study we aimed to characterize the role of the *sloR* operon in *S. pyogenes*, especially with regards to the upstream putative transcriptional regulator. The *sloR* gene (*spy49_0128*) was previously linked to the expression of streptolysin O (SLO), an important GAS virulence factor (13, 23, 42, 46), however the exact nature of this effect was not elucidated. Our studies reveal a novel role for this operon in regulation of L-serine catabolism. Our data demonstrate that the SloR operon and the divergently transcribed transcriptional regulator, which we have designated SerR, are directly involved in L-serine utilization in GAS. Furthermore, regulation of L-serine catabolism is demonstrated to be important under conditions where L-serine is not overly abundant, as increased degradation of L-serine resulting from the absence of SerR can result in restricted cell growth.

MATERIALS AND METHODS

Bacterial strains and media. All *S. pyogenes* strains used in this study were derivatives of the sequenced serotype M49 strain NZ131 (Table 1) (31). All GAS strains were maintained on Todd Hewitt with 2% (w/v) yeast extract (THY, Difco) plates with antibiotics as needed at the following

concentrations: chloramphenicol, 3 µg/mL; erythromycin, 0.5 µg/mL; and spectinomycin, 100 µg/mL. GAS liquid cultures were grown statically in one of the following types of media: THY, C-medium (25), or chemically-defined media [CDM; (47)] containing 0.5% glucose and supplemented with L-asparagine to 100 mg/L. For all experiments, overnight cultures were grown at 30°C in indicated media, diluted to $OD_{600} \approx 0.01$ into fresh media of the same type, and incubated at 37°C for the duration of the experiment. All plasmids were constructed using *E. coli* DH10β (Invitrogen) or BH10C (18) as the host, and all *E. coli* were grown in Luria broth at 30°C with shaking with antibiotics as needed at the following concentrations: chloramphenicol, 10 µg/mL; erythromycin, 500 µg/mL; and spectinomycin, 100 µg/mL.

Construction of *serR, sdhB, and sloR* mutants and complementation vectors. All plasmids and oligonucleotide primers (Integrated DNA Technologies) used in this study are listed in Table 1 and Table 2, respectively. Construction of mutants was accomplished by allelic exchange using the method previously described (11). This method uses a temperature-sensitive pG^{+}host9 plasmid (pFed760) (29) to facilitate deletion of target genes via homologous recombination. 500 bp regions upstream and downstream of the targeted gene(s) were amplified by PCR (Phusion DNA Polymerase, Finnzymes) using *S. pyogenes* NZ131 DNA as the template and the following primer pairs: *serR*, BL1/BL2 and BL3/BL4; *sloR*, BL5/BL6 and BL7/BL8; *sdhB*, BL9/BL10 and BL11/BL12. Restriction sites included within these primers created a *PstI* site at the 5' end and *XhoI* site at the 3' end of the upstream flanking regions (UFR) and a *BglII* site at the 5' end and *PstI* site at the 3' end of the downstream flanking regions (DFR). Primers used for amplification of a spectinomycin resistance cassette (*aad9*), BL13/BL14, from pLZ12Spec (19), and a chloramphenicol resistance cassette (*cat*), BL15/BL16, amplified from pEVP3 (7), included restriction enzyme sites that created *XhoI* and *BglII* sites at the ends of the resistance cassettes. PCR fragments digested with *XhoI* and/or *BglII* (all restriction enzymes

purchased from New England Biolabs) were ligated *in vitro* (T4 DNA Ligase, New England Biolabs), and the desired UFR-*aad9*/*cat*-DFR fusions were amplified using outside primer pairs BL1/BL4, BL5/BL8, or BL9/BL12. The amplified fusions were digested with *PstI* and ligated into pFed760 at the *PstI* site by *in vitro* ligation to generate the deletion plasmids (pBL100-103). Following allelic exchange, Em-sensitive, Cm- or Spc-resistant colonies were confirmed for deletion of the targeted gene(s) by PCR using mutant chromosomal DNA as template.

Construction of complementation vectors was accomplished as follows. *serR* with its native promoter was amplified using primer pair BL29/BL42 and cloned into p7INT between the *BamHI* and *EcoRI* sites to generate pBL109. The *sloR* promoter and *sloR* gene were amplified using primer pairs BL38/BL39 and BL40/BL41, respectively. Primers BL39 and BL40 contain complementary sequence to each other, allowing for overlap extension PCR between the two amplification products resulting in an in-frame fusion between *sloR* and its promoter while eliminating the *spy49_0127* gene. The *sloR*-promoter fusion was ligated into p7INT between the *BamHI* and *XhoI* sites to generate pBL110. p7INT integrates into a neutral site in the GAS chromosome (*attB*) (32), thus the plasmids were transformed into GAS by electroporation and transformants were selected for on THY/Erm plates. Chromosomal DNA was purified from all complemented strains for use in PCR confirmation of vector integration.

Construction of *luxAB* transcriptional fusions. Transcriptional fusions of the *sloR* and *sdhBA* promoter regions to *luxAB* were generated as follows. The *sloR* and *sdhBA* promoter regions were amplified by PCR from *S. pyogenes* NZ131 DNA using primer sets BL17/BL18 and BL19/BL20, respectively. The *luxAB* genes were amplified from pCN59 (3) using primers BL25 and BL27. These primers include restriction enzyme sites that create *NotI* sites at the 3' end of the promoter regions and at the 5' end of the *luxAB* operon. Following digestion of all products with NotI, the promoter fragments were fused to *luxAB* by *in vitro* ligation. The desired fusion products were amplified using the ligation

reactions as template and primers BL17 or BL19 and BL27. These primers include restriction enzyme sites that create *EcoRI* sites at both ends of the P_{sloR}-*luxAB* fusion product or *BamHI* and *EcoRI* sites at the 5' and 3' ends of the P_{sdh}-*luxAB* fusion product, respectively. Following digestion with *EcoRI* and/or *BamHI*, the desired fusion products were gel purified and ligated into likewise digested p7INT to generate pBL105 and pBL106. All plasmid-generating ligation reactions were transformed into DH10β or BH10C *E. coli* and cells were plated on Luria Broth plates with erythromycin to select for transformants. Following transformation of reporter constructs into GAS, and integration of the reporters into the chromosome was confirmed by PCR.

Luciferase reporter assay. Overnight cultures of GAS strains carrying transcriptional fusions were diluted to OD_{600} = 0.01 in fresh media of the same type and grown at 37°C without agitation. Throughout growth, culture optical transmittance at 600 nm was measured using a Spectronic 20D spectrophotometer (Milton Roy). Concurrently, 100 µL aliquots of cultures were placed in a 96-well dish and exposed to decyl aldehyde vapors for 35 seconds (Acros Organics). Prior to measuring luminescence, the plate lid was removed and luminescence readings were performed within 1 minute. All luminescence measurements were performed using a 1450 Microbeta Plus Liquid Scintillation Counter (Wallac). All luciferase data shown is representative of experiments performed in triplicate.

Purification of RNA and Microarray Analysis. NZ131 and the isogenic *serR* deletion mutant (BNL100) were grown to mid-logarithmic phase in C-medium at 37°C without shaking. RNA was harvested from three replicate cultures using RiboPure-Bacteria Kit (Ambion) according to the manufacturer's instructions, and the quality of the RNA was confirmed by agarose gel electrophoresis. RNA concentrations were determined using a NanoDrop 1000 spectrophotometer (Thermo Scientifiic). Reverse transcription using Superscript II RT (Life Technologies) and random hexamer primers (Life Technologies) was performed with 5 µg total bacterial RNA incorporating an amino-allyl dUTP

(Sigma). RNA was degraded using sodium hydroxide and cDNA was purified using PCR Purification Kit (Qiagen). cDNA was then incubated with Cyanine-3 ester (AmershamPharmacia), washed again using the PCR Purification Kit, and dye incorporation was measured using a NanoDrop. All hybridization to *S. pyogenes* whole genome microarrays (Nimbelgen) was performed in accordance with the Nimbelgen Hyrbidization protocol. Nimblescan was used to create raw data files and Partek was used for RMA normalization. Labeling, array hybridization, and quantitation were conducted as a service by The W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois. All data has been deposited in the NCBI Gene Expression Ominibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE24860.

Expression and purification of recombinant SerR. The *serR* gene was amplified from NZ131 genomic DNA using primers BL29 and BL30 containing *BamHI* and *NdeI* restriction sites, respectively. Following BamHI/NdeI digest, the PCR product was cloned into the pET15b vector (Novagen), which adds a 6-histidine tag to the amino-terminus of the protein, to generate pBL104. pBL104 was then transformed into BL21(DE3) *E. coli* (Novagen) by electroporation and grown in Luria broth with 100 µg/mL ampicillin at 30°C with shaking. At an OD_{600} of 0.8, protein expression was induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 16°C with shaking for 16 hours. Following induction, cells were pelleted by centrifugation and then resupsended in cold Buffer A [20 mM sodium phosphate buffer pH 7.6, 0.5 M NaCl, 20 mM imidazole, 20 mM beta-mercaptoethanol (BME), 1 µg/mL DNase I, and protease inhibitor cocktail]. Cells were lysed by sonication and the soluble and insoluble fractions of the cell lysates were separated by centrifugation at 14,000 rpm in a Sorvall SA-600 rotor. Solubility studies indicated that recombinant SerR was found in inclusion bodies, thus the insoluble pellet was resuspended in cold Buffer B [8 M urea, 20 mM sodium phosphate buffer pH 7.6, 0.5 M NaCl, 20 mM imidazole, and 20 mM BME] to denature $SerR_{His}$. After a 30 minute incubation at 30°C

with periodic vortexing, the remaining insoluble fraction was removed by centrifugation. $SerR_{His}$ was purified from the denatured, soluble fraction by Ni^{2+}-affinity chromatography using a 1 mL HisTrap HP column (GE Healthcare) and eluted in cold Buffer C [8 M urea, 20 mM sodium phosphate buffer pH 7.6, 0.5 M NaCl, 500 mM imidazole]. Purity of $SerR_{His}$ was estimated to be >95% as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining. Denatured $SerR_{His}$ was refolded *in vitro* by buffer exchange using a Slide-A-Lyzer cassette (Thermo Scientific) and dialyzed against cold Buffer D [20 mM Tris-HCl pH 8.0, 100 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM EDTA pH 8.0, 1 mM DTT, protease inhibitor cocktail] at 4°C for 3.5 hours twice in succession. The sample was then dialyzed against Buffer E [20 mM Tris-HCl pH 8.0, 100 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM EDTA pH 8.0, 1 mM DTT, 25% glycerol, protease inhibitor cocktail] at 4°C overnight. Following buffer exchange, all precipitated $SerR_{His}$ was removed by centrifugation and the final soluble $SerR_{His}$ concentration was determined by Bradford Assay using Coomassie Protein Assay Reagent and Albumin Standards (Thermo Sceintific). Protein aliquots were stored at -80°C until use.

Electrophoretic mobility shift assays (EMSAs). All DNA fragments for EMSAs were amplified from chromosomal NZ131 DNA by PCR using primers outlined in Table 2 and purified by gel purification. Select primers included 5'-FAM fluorescent tags (Integrated DNA Technologies). The following EMSA probes were amplified with indicated primers: P_{sloR} (BL17/BL32); P_{sdh} (BL19 and BL33); P_{serS} (BL21 and BL34); and P_{ssRNA} (BL35/BL37). Unlabeled probes were amplified using primers BL31, BL20, and BL36 in place of BL32, BL33, and BL37, respectively. For EMSAs, varying concentrations of $SerR_{His}$ (in Buffer E) was incubated with 20 mM HEPES, pH 7.9; 100 mM KCl; 12.5 mM $MgCl_2$; 0.2 mM EDTA, pH 8.0; 0.5 mM dithiothreitol; 50 µg/mL salmon sperm DNA; .001 U/µL poly(dI•dC); 100 µg/mL bovine serum albumin; 0.5mM $CaCl_2$; and 12% (v/v) glycerol, at room temperature for 15 minutes prior to addition of 10 nM probe, and competitor DNA if applicable. Following the addition of

185 the probe, the reactions were incubated at room temperature for an additional 30 minutes and then
186 fractionated on 5% native polyacrylamide gels buffered with 20 mM potassium phosphate, pH 7.5, for
187 90min at 110V at 4°C. If competitor DNA was included in the reaction, it was added simultaneously
188 with the labeled probe at 10X molar excess. All gel shifts were detected by fluorescence imaging using a
189 *Typhoon PhosphorImager* (GE Life Sciences).
190 **Metabolite analysis.** NZ131 and isogenic mutant strains were growth in THY at 37°C for 3.5 hours
191 until mid-log phase. Cells were treated with hyaluronidase and immediately pelletted by centrifugation,
192 washed once with 20 mM Na_2HPO_4/NaH_2PO_4, 1 mM $MgCl_2$ (pH 7.0) buffer, and then resuspended at
193 approximate concentrations of 10^8 CFU/mL in 20 mM Na_2HPO_4/NaH_2PO_4, 1 mM $MgCl_2$ (pH 7.0)
194 buffer containing 50 mM L-serine. Cells were incubated statically at 37°C for 4 hours, at which point
195 cells were pelletted by centrifugation and the cell-free supernatant was collected for metabolite analysis.
196 Viable CFUs were determined at 0 hours and 4 hours by plating 10-fold dilutions of cells on THY to
197 ensure equivalent numbers of cells between cultures and confirm that cells remained viable over the
198 course of the experiment. The concentrations of ammonia in supernatants were determined using an
199 ammonia assay kit (R-Biopharm, Cat. 11 112 732 035) according to manufacturer's instructions but
200 scaled down for use in a 96-well plate. Analysis was performed using a *Synergy 2* microplate reader
201 (BioTek).
202 **L-serine transport analysis.** NZ131 and isogenic mutant strains were grown in CDM at 37°C until
203 OD_{600} reached approximately 0.3. Linezolid was added to all cultures at a final concentration of 5
204 μg/mL and incubated for 20 minutes at 37°C to allow protein synthesis to subside. 0.5 μCi L-[^{14}C(U)]-
205 serine (60 mCi/mmol; MP Radiochemicals) was added to 1 mL aliquots of each culture, and starting 4
206 minutes after the addition of L-[^{14}C(U)]-serine, 200 μL samples were removed every 10 minutes for a
207 total of 30 minutes and filtered on 0.45 μm membrane filters (Millipore) by vacuum filtration. Cells

were immediately washed with 5 mL PBS at room temperature. Filters were air dried at 37°C and placed in scintillation vials containing 5 mL scintillation fluid cocktail (Ultima Gold, Perkin Elmer). Radioactivity was assessed using a Beckman LS 6000 IC scintillation counter.

RESULTS

Deletion of *spy49_0126c* results in aberrant growth compared to wild-type GAS. In this study, we aimed to investigate more thoroughly the role of the *sloR* operon in GAS due to its previously reported effect on streptolysin O expression (33, 37). *sloR* is the second gene in a an operon that also contains a predicted member of the YjgF family (*spy49_0127*), as well as a divergently transcribed putative transcriptional regulator (*spy49_0126c*) whose function was briefly examined with regard to SLO expression but has never been investigated directly (Figure 1A) (33, 37). A temperature sensitive plasmid containing the upstream and downstream regions of the *spy49_0126c* gene flanking a spectinomycin resistance cassette (plasmid pBL100; Table 1) was used to delete this putative regulator from the chromosome by allelic exchange, generating strain BNL100 (Figure 1A). Initial characterization of BNL100 included examination of growth compared to the parental strain in a variety of growth mediums. No difference in growth was detected when strains were grown in glucose- and peptide-rich Todd-Hewitt broth with yeast extract (THY) (Figure 1B), in agreement with previous reports (33). However, when BNL100 was grown in more nutrient-poor mediums, growth defects became apparent. In the peptide-rich, glucose-low C-medium, BNL100 grew at a similar rate to wild-type until the optical density of the culture (OD_{600}) reached approximately 0.3, at which point the growth rate of the mutant slowed. The growth of BNL100 remained slower than wild-type for the remainder of the growth curve but similar culture yields were reached with both strains (Figure 1C). In contrast, in chemically-defined media (CDM), which is rich in glucose but has only free amino acids and no

peptides, BNL100 showed a sizeable growth yield defect. Despite having similar growth rates compared to wild-type GAS, BNL100 cultures never reached as high of cell densities as did wild-type (Figure 1D). A single copy of the *spy49_0126c* gene at a neutral location in the chromosome fully complemented this growth defect (Figure 1D).

Deletion of *spy49_0126c* results in the enhanced expression of multiple operons, some associated with L-serine utilization. Given the annotation of *spy49_0126c* as a putative transcriptional regulator and the aberrant growth profiles of BNL100, we sought to identify putative targets of regulation by the *spy49_0126c* gene product. Microarray analysis was carried out using RNA harvested from wild-type and BNL100 strains at mid-log phase grown in C-medium. This medium was used in order to enhance the likelihood that genes responsible for aberrant growth would be identified while allowing for sufficient growth of both strains without the risk of BNL100 entering stationary phase, as might occur had CDM been used. Microarray analysis revealed higher expression of four annotated operons by more than 5-fold in BNL100 compared to the parental strain (Table 3). Two of these operons consisted of genes encoded divergently from *spy49_0126c*, including *sloR* (*spy49_0128*) and subunits of a putative V-type Na^+-ATP synthase (*spy49_0130-0137*). The other two operons were comprised of three genes total, all linked to utilization of L-serine. *serS* (*spy49_1358*) encodes a putative seryl-tRNA synthetase responsible for loading L-serine to cognate uncharged tRNAs. Expression of *serS* has been published to be under the control of T-box regulation in *S. pyogenes* (49), a mechanism in which the concentration of charged tRNAs determines the structure of a riboswitch present in the leader region of the regulated gene, resulting in the formation of a terminator or anti-terminator structure that alters the gene expression. *sdhBA* (*spy49_1794-1795*) encode the putative α- and β-subunits of L-serine dehydratase, a catabolic enzyme that converts L-serine to ammonium and pyruvate (E.C. 4.3.1.17). These enzymes are widespread in bacteria (14) and sequence homology is highly variable (17). *sdhBA*

from GAS are 38% and 41% identical, respectively, to the L-serine dehydratase enzyme subunits from *Peptostreptococcus asaccharolyticus* which have demonstrated enzymatic activity (17). Sequence analysis indicates that the GAS β-subunit contains the C-terminal ACT domain including the putative L-serine binding site of the enzyme (Conserved Domain Database, NCBI) (28). Additionally, an increase in *sdhBA* expression in a *ropB* (*rgg)* mutant was accompanied by increased degradation of L-serine with concomitant ammonia production (4, 5). Given this information, in addition to the results presented herein, we find it reasonable to conclude that the *sdhBA* genes encode an L-serine dehydratase that is functional in GAS. No regulator has been shown to directly control expression of *sdhAB* in this organism.

Expression of *sloR* and *sdhAB* is increased in the absence of *spy49_0126c*. To validate the microarray results (Table 3), reporter fusions linking the promoter regions of either *sloR* or *sdhBA* to the *luxAB* genes that encode for luciferase were integrated in single copy into the GAS chromosome. Strains carrying the reporter fusions were grown in THY and *luxAB* expression was assessed over the entire growth curve of the bacteria. THY was used in these experiments in order to certify that differences in gene expression levels resulted from the lack of *spy49_0126c* rather than as a consequence of variances in growth between strains. Light expression profiles revealed that both *sloR* and *sdhAB* expression was increased between 5- and 10-fold over the entire course of growth in BNL100 compared to in the parental strain (Figure 2A, 2B). A luciferase reporter to the promoter of the *recA* gene, a housekeeping gene expressed during exponential phase (30) but not predicted to be regulated by SerR, showed no difference in expression between strains, demonstrating that the up-regulation of *sloR* and *sdhAB* was not due to a general increase in gene expression in BNL100 (data not shown). These data, in addition to the microarray results, indicate that the *spy49_1026c* gene product represses transcription of both the *sloR* and *sdhAB* operons throughout exponential growth of *S. pyogenes*. Given that the *spy49_0126c*

277 gene product represses genes involved in L-serine utilization, we have termed the gene serine catabolism
278 regulator (*serR*).

279 **SerR binds directly to the *sloR* and *sdhAB* promoters.** To determine if the repression of the
280 *sloR*, *sdhBA,* and *serS* operons was due to direct or indirect regulation by SerR, His_6-tagged SerR
281 ($SerR_{His}$) was purified from *E. coli* and incubated with fluorescently-labeled DNA fragments of the *sloR*,
282 *sdhBA,* and *serS* promoter regions. A fluorescently-labeled DNA fragment of a ribosomal RNA
283 promoter was used as a negative control. Electrophoretic mobility shift assay (EMSA) analysis showed a
284 shift of both the *sloR* and *sdhBA* probes in the presence of $SerR_{His}$ (Figure 3A, 3B), indicating that SerR
285 bound directly to the promoter regions of these operons. These shifts could be interrupted by the
286 addition of cold *sloR* (Figure 3A, lane 5) or cold *sdhBA* (Figure 3B, lane 5) competitor, but not by cold
287 competitor consisting of the ribosomal RNA promoter (Figure 3A and 3B, lane 6), demonstrating
288 specificity of the binding interactions. Negligible binding was seen when $SerR_{His}$ was incubated with the
289 ribosomal RNA probe, even at the highest protein concentrations used (Figure 3C). Interestingly, no
290 shift was detected when $SerR_{His}$ was incubated with a *serS*-promoter probe (Figure 3D), suggesting that
291 the upregulation of *serS* expression in BNL100 is due to indirect regulation by SerR. This would be
292 consistent with the idea that *serS* is under control of T-box regulation. Increased transcription of *serS*
293 would be observed if levels of L-serine in the cell are decreased, which is the expected result of
294 increased L-serine dehydratase (*sdhBA*) expression in BNL100. Thus, transcription of the *sloR* and
295 *sdhBA* operons are directly repressed by SerR while repression of *serS* is likely an indirect effect of
296 SerR via inhibition of L-serine degradation.

297 **The growth culture yield defect of BNL100 in CDM can be rescued by the addition of extra**
298 **L-serine.** As mentioned previously, SdhBA is an L-serine dehydratase that catalyzes the degradation of
299 L-serine into pyruvate and ammonia. Based on the upregulation of *sdhBA* in BNL100, it seemed likely

300 that the growth yield defect of BNL100 in CDM could be the result of increased L-serine degradation
301 and subsequent faster depletion from the medium. Given the polyauxotrophy of GAS for 15 amino acids
302 (10), including L-serine, depletion of L-serine from the medium would inhibit further growth as none
303 would be available for protein synthesis. Thus, in essence, the BNL100 strain could have an increased
304 nutritional requirement for L-serine due to increased SdhBA expression, and the traditional CDM recipe
305 (47) may not contain enough L-serine to satisfy this requirement. To test this hypothesis, extra L-serine
306 and other L-amino acids tested individually were added to CDM to a final concentration 6-times that
307 normally included in the medium (final concentration of 600mg/L), and growth of wild-type GAS and
308 BNL100 (Δ*serR*) was monitored. Whereas the addition of L-serine and other L-amino acids had no
309 effect on the growth of the parental strain NZ131 (data not shown), addition of L-serine complemented
310 the growth yield defect of BNL100, nearly doubling the final yield of the mutant culture (Figure 4A). L-
311 amino acids other than L-serine had no effect on BNL100 growth (Figure 4A). Furthermore,
312 supplementation of CDM with L-serine complemented the culture yield defect of BNL100 in a
313 concentration dependent manner (data not shown). These data are consistent with the hypothesis that L-
314 serine is limiting in CDM due to upregulation of *sdhBA* and consequent L-serine depletion from the
315 media in BNL100 cultures, resulting in the growth yield defect. The addition of extra L-serine to CDM
316 is likely able to more fully satisfy the nutritional requirement of BNL100 for L-serine, allowing the
317 culture to reach greater cell densities. This is also consistent with the lack of growth defect in THY
318 (Figure 1B), as the peptides present in this medium could provide sufficient serine to allow for greater
319 culture growth.

320 **Deletion of *sdhB* in BNL100 can complement the culture yield defect in CDM by preventing**
321 **L-serine degradation.** If the increased activity of SdhBA was indeed responsible for early depletion of
322 L-serine from the medium and the subsequent early cessation of growth of BNL100, we reasoned that

deletion of *sdhBA* should fully restore culture yields of BNL100 grown in CDM to wild-type levels. We deleted *sdhB* from the chromosome by allelic exchange in NZ131 and BNL100 to generate BNL116 and BNL117, respectively. In the *sdhBA* operon, the *sdhB* gene is located proximal to the promoter, followed by *sdhA*. The chloramphenicol resistance cassette used to replace the *sdhB* gene included a transcriptional terminator, thereby likely disrupting expression of *sdhA* as well. NZ131 (wild-type), BNL100 (Δ*serR*), BNL116 (Δ*sdhB*), and BNL117 (Δ*serR*, Δ*sdhB*) were grown in CDM and culture growth was assessed. Deletion of *sdhB* in NZ131 had no effect on growth yield of the culture (NZ131 compared to BNL116, Figure 5A). However, deletion of *sdhB* in BNL100 was sufficient to fully restore growth culture yields to wild-type levels without the addition of extra L-serine (BNL100 compared to BNL117, Figure 5A). These results support the hypothesis that increased *sdhBA* expression in BNL100 contributes to the reduced culture yield in CDM, likely by increasing the nutritional requirement for L-serine.

To further test our hypothesis, we examined if *sdhBA* expression correlated with ammonia excretion, as L-serine degradation by SdhBA should produce pyruvate and ammonia. We hypothesized that BNL100 would produce more ammonia than wild-type NZ131 due to increased expression of *sdhBA*, whereas BNL117 should produce less ammonia due to the lack of SdhBA. Results of our experiment supported this hypothesis. NZ131 (wild-type), BNL100 (Δ*serR*), and BNL117 (Δ*serR,*Δ*sdhB*) were harvested from mid-log cultures, resuspended in a neutral buffer conatining 50 mM L-serine, and incubated at 37°C to allow for the enzymatic degradation of L-serine and excretion of ammonia. After 4 hours, NZ131 supernatants contained 0.797 +/- 0.078 mM ammonia, whereas BNL100 supernatants contained over 3-fold more ammonia after the same amount of time (2.726 +/- 0.0116 mM) (Figure 5B). This indicated that increased expression of *sdhBA* correlated with increased ammonia production in the presence of L-serine, consistent with L-serine degradation by SdhBA.

Additionally, deletion of *sdhB* (BNL117) resulted in the production of only 24.66 +/- 0.59 μM ammonia after 4 hours, over 100-times less than that produced by BNL100, demonstrating that enhanced ammonia production by BNL100 in the presence of L-serine depended on expression of *sdhBA* (Figure 5B). These data provide further evidence that BNL100 has an increased nutritional requirement for L-serine due to increased expression of *sdhBA* and consequent L-serine degradation.

The SloR operon modulates the rate of L-serine consumption in GAS. Unexpectedly, strain BNL161 (Δ*serR,* Δ*sloR*), generated by deletion of *sloR* in BNL100, did not have a growth yield defect in CDM (Figure 6A). Previous publications have indicated roles for SloR activity in SLO regulation at both transcriptional and secretory steps (33, 37) but have never linked this gene to any metabolic role in the cell. The ability of the *sloR* deletion to complement the growth yield defect of BNL100 in CDM suggested that SloR plays a role in L-serine catabolism (BNL161 versus BNL100, Figure 6A). Single-copy complementation of *sloR* (BNL164) resulted in a statistically significant decrease in culture yield in the Δ*serR* Δ*sloR* double mutant, but only partially complemented the phenotype compared with BNL100 (Δ*serR*) (Figure 6A), bringing into question the polarity of the *sloR* mutation. We confirmed that *sloR* and downstream genes are co-expressed on a polycistronic message by amplifying the contiguous region spanning from *sloR* to *spy0148* using PCR where cDNA was the template source (results not shown). As such, we expect that Δ*sloR* mutations have polar effects on the downstream ATP synthase genes. Consequently, these downstream genes may also contribute to serine catabolism in GAS.

We reasoned that the SloR operon may be acting to alter L-serine catabolism by transcriptional or post-transcriptional means. One possibility was that the SloR operon acts to enhance *sdhBA* transcription and that deletion of *sloR* would reduce SdhBA levels and thereby decrease the L-serine nutritional requirement in BNL114, allowing for higher culture yields. Using our *sdhBA* reporter fusion,

we found that this was not the case. Light expression profiles revealed that expression from the *sdhBA* promoter is equivalently high in BNL114 and BNL100 (data not shown), indicating that SloR does not modulate L-serine catabolism via regulation of *sdhBA* transcription.

SloR is predicted to have nine trans-membrane domains (TopPred, Mobyle Server, Pasteur Institute) (33), and thus we next hypothesized that the SloR operon was affecting L-serine catabolism by increasing the rate of L-serine transport into the cell. If this were the case, deletion of *sloR* in BNL100 would decrease the rate at which L-serine was transported into the cell, thereby disrupting the enhanced L-serine turnover we hypothesized to be present in strain BNL100. To test this hypothesis, we measured the ability of GAS strains to take up radiolabeled L-serine at a point in exponential growth prior to amino-acid depletion, with the prediction that inactivation of the *sloR* operon would suppress the serine deficiency caused by the deletion of *serR*. L-[^{14}C(U)]-serine transport experiments demonstrated that BNL100 (Δ*serR*) does indeed transport L-serine at a faster rate than does wild-type NZ131 (Figure 6B). After 30 minutes, BNL100 cells contained 19,675 +/- 376 CPM whereas NZ131 cells contained 1,260 +/- 27 CPM, an approximately 15-fold difference between the strains (Figure 6B). In agreement with our hypothesis, BNL114 (Δ*serR,* Δ*sloR*) transported L-serine into cells at rates comparable to that of NZ131, containing only 925 +/- 80 CPM after 30 minutes (Figure 6B). These data provide evidence that the SloR operon is involved in modulating L-serine consumption in *S. pyogenes*, a novel function for this operon.

DISCUSSION

Complex regulatory networks have been shown to coordinate expression of both virulence- and metabolism-associated genes in *S. pyogenes*. In this report, we characterize a transcriptional regulator which we have named SerR (serine catabolism regulator). The *serR* gene (*spy49_0126c*) is divergently

transcribed from a polycistronic operon containing the gene *sloR*, which has previously been shown to influence expression of the virulence factor streptolysin O (33, 37). In this report, we describe a novel role for the SloR operon in L-serine utilization in GAS. Our data provide evidence that the *sloR* operon and *sdhBA*, the latter encoding an L-serine dehydratase, are repressed by SerR. Deletion of *serR* from the chromosome results in increased transcription of the *sloR* and *sdhBA* operons and an increased nutritional requirement for L-serine, resulting in decreased culture yield in chemically-defined media (CDM) unless additional L-serine is provided. The negative transcriptional regulation of these genes by SerR is direct in nature, as purified SerR binds specifically to the *sloR* and *sdhBA* promoters *in vitro*. The SerR protein is predicted to have an N-terminal PER-ARNT-SIM (PAS) domain and C-terminal helix-turn-helix that could facilitate this DNA binding (Pfam protein families database, Sanger Institute). Additionally, the decreased culture yield of the *serR* mutant in CDM can be rescued by the deletion of *sdhB* or *sloR*, presumably by preventing the rapid degradation of L-serine. To our knowledge, this is the first report identifying a transcriptional regulator that directly regulates expression of genes involved in L-serine catabolism in *S. pyogenes* or in any other Gram-positive bacterium.

The physiological importance of regulation by SerR when L-serine is not overly abundant is clear in this study, as increased L-serine catabolism due to the absence of SerR results in restricted cell growth (Figures 1B, 4, 5, and 6). However, the importance of L-serine catabolism to GAS physiology remains unclear. It seems paradoxical that *S. pyogenes* would maintain a mechanism for degrading L-serine considering that it is auxotrophic for this amino acid, however *serR*, *sloR*, and *sdhBA* are conserved in all sequenced GAS genomes (http://www.microbesonline.org) (12). We hypothesize that L-serine degradation may contribute in yet to be defined ways to GAS survival and pathogenesis. During our investigation, we observed that NZ131 cells harvested at mid-log phase are less sensitive to low pH in the presence of L-serine than in its absence, while the presence of L-serine had no effect on

415 the pH tolerance of a Δ*sdhB* mutant (our unpublished results), suggesting that L-serine catabolism may
416 contribute to pH tolerance in GAS. We also observed that the *sdhB* mutant fails to aggregate and does
417 not pellet readily as compared to its wild-type counterpart (our unpublished data), suggesting that
418 surface components, such as extracellular polysaccharide or membrane and cell wall-associated proteins,
419 are altered in the *sdhB* mutant. The mechanism(s) behind these physiological changes resulting from the
420 deletion of *sdhB* remain to be elucidated, but it is clear that additional work will be needed to determine
421 how L-serine catabolism and its regulation affect GAS physiology and pathogenicity.

422 The ability of the *sloR* mutation to rescue the growth phenotype of the *serR* mutant came as a
423 surprise, as the SloR operon had not previously been linked to L-serine catabolism. However, uptake
424 studies presented here using radiolabeled L-serine provide evidence that the SloR operon influences the
425 rate at which L-serine can be consumed by GAS. Two mechanisms by which SloR and the ATP
426 synthase genes could be acting is modulation of L-serine uptake, potentially being directly involved in
427 serine transport, or modulation of SdhBA activity at a post-transcriptional level. Further experiments
428 will be needed to precisely define the role of SloR and the downstream ATP synthase in L-serine
429 catabolism. It has previously been suggested that SloR acts as an environmental sensor (33). If this
430 hypothesis is true, given its involvement in L-serine catabolism, we speculate that L-serine availability
431 may be the signal for SloR, ultimately influencing SLO expression through an indirect mechanism.
432 Furthermore, a previous study in *S. pyogenes* concluded that L-serine shares a common transporter with
433 other neutral amino acids and that uptake may be driven via symport with protons (36). Thus, the
434 putative ATP synthase comprised by these downstream gene products might be involved in generation
435 and maintenance of gradients required for L-serine uptake.

436 Given that *sloR* is the second gene in a polycistronic operon, our *sloR* expression data
437 concurrently demonstrate that the first gene in the operon, *spy49_0127*, is also negatively regulated by

438 SerR. *Spy49_0127* is predicted to encode a YjgF family member. The YjgF family includes proteins
439 found in all kingdoms of life, however no definitive function for any of its members has been
440 experimentally demonstrated (1). Interestingly, various reports have implicated roles for YjgF family
441 members in amino acid metabolism, including in isoleucine biosynthesis and L-threonine deamination
442 (6, 38). Crystalization of TdcF, a YjgF family member from *E. coli*, revealed that L-serine, in addition to
443 L-threonine and 2-ketobutyrate, can bind in the cavity of TdcF, although L-serine did not fully occupy
444 all of the binding sites (1). Whether the *spy49_0127* gene product can in fact bind L-serine, as can TdcF,
445 or perhaps binds an intermediate of L-serine catabolism remains to be determined. However, the co-
446 transcription of *spy49_0127* and *sloR*, negative regulation by SerR, and the suggested roles for
447 homologous family members in amino acid metabolism advocate for the future evaluation of the role of
448 this YjgF family member in L-serine catabolism in GAS.

449 The distance between the *sdhBA* operon relative to *serR* in the genome initially seemed odd,
450 given that the other genes regulated by SerR, with the exception of SerS, are in direct proximity to SerR.
451 However, regulation of these genes by a common regulator may occur in at least one other bacterial
452 species. *Enterococcus faecalis* has a gene homologous to SerR, EF0096, that is divergently transcribed
453 from an operon consisting of four putative genes: a *sloR* homologue, *serS*, and *sdhBA*
454 (http://www.microbesonline.org) (12). These genes have been shown to be regulated by CcpA in *E.*
455 *faecalis* (22) but regulation by the EF0096 gene product was not investigated. In many organisms,
456 multiple regulators contribute to the expression of amino acid catabolic genes, thus regulation by both
457 CcpA and the SerR homologue may coordinate expression of L-serine utilization genes in *E. faecalis*.
458 This type of coordinated regulation likely occurs in GAS as well. Many different studies have identified
459 various transcriptional regulators that affect expression of genes identified herein as being involved in L-
460 serine catabolism, although it should be noted that none of these regulators have been shown to directly

bind the promoters of these genes. Expression of *sdhB* was increased in a *ccpA covR* double mutant in GAS, although no difference was seen in the either single mutant (41), and deletion of the stand-alone regulator *ropB* (*rgg*) results in increased expression of *sdhBA* as well as genes involved in the arginine demininase pathway (4, 5). *Spy49_0127* and *sloR* were upregulated in both a *covR* mutant (15) and a *vicR* mutant (24), and *sloR* expression was upregulated at 29°C relative to 37°C (44). Interestingly, *S. agalactiae* and *S. dysgalactiae* have homologues of SerR that are divergently transcribed from homologues of SloR (http://www.microbesonline.org) (12). *S. dysgalactiae* also has a putative YjgF family member upstream of the *sloR* homologue, as is seen in GAS. Furthermore, the *sdhBA* operons in these species are located in comparable regions of the genome as in GAS, just downstream of *trmU* in all species. Other streptococcal species do not have SerR or SloR homologues that we have been able to identify by BLAST analysis. However, for species that do have SerR and SloR homologues, including *E. faecalis*, we put forward the possibility that SerR and its homologues, in coordination with other regulatory proteins, act as a negative transcriptional regulators in multiple species to modulate L-serine acquisition and catabolism.

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FIGURE LEGENDS

Figure 1. Deletion of *spy49_0126c* affects GAS growth in culture media that differ in nutrient availablitiy. **(A)** Schematic of the *sloR* operon in wild-type NZ131 and the BNL100 mutant in which *spy49_0126c* (light gray arrow) is replaced with the *aad9* cassette (white arrow) under the spc^R promoter, providing spectinomycin resistance. NZ131 chromosomal DNA and spectinomycin cassette DNA are represented by black and striped bars, respectively. **(B,C)** Growth curves of NZ131 (▲) and BNL100 (■) in THY (B) and C-medium (C). **(D)** Growth curves of BNL158 (WT with empty vector) (◆), BNL159 (Δ*serR* with empty vector) (●), and BNL162 (Δ*serR* with pBL109) (▽) in CDM. Results shown are representative of three independent experiments.

Figure 2. Deletion of *spy49_0126c* results in increased transcription from promoters of target genes. Growth curves (open symbols, dashed lines) and relative light unit (RLU; CPM/OD_{600}) expression

curves (closed symbols and solid lines) of WT (▲) and Δ*spy49_0126c* (■) grown in THY. **(A)** Growth and expression curves of strains carrying the P_{sloR}-*luxAB* reporter (strains BNL105 and BNL106). **(B)** Growth and expression curves of strains carrying the P_{sdh}-*luxAB* reporter (strains BNL141 and BNL142). Results shown are representative of three independent experiments.

Figure 3. $SerR_{His}$ binds to promoter regions of *sloR* and *sdhBA*. Electrophoretic mobility shift assay (EMSA) analysis of $SerR_{His}$ binding to labeled promoter regions of *sloR* **(A)**, *sdhBA* **(B)**, ribosomal RNA (rRNA) **(C)**, and *serS* **(D)**. Amount of $SerR_{His}$ is indicated above each lane. All reactions included 10 nM labeled probe. Where indicated, 10-fold molar excess of unlabeled probe (specific) or unlabeled rRNA probe (nonspecific) was included. The rRNA promoter was included as a negative control.

Figure 4. Supplementation of CDM with L-serine rescues the reduced culture yield of BNL100. All strains were grown in CDM + 0.5% glucose at 37°C. Selected amino acids were supplemented at a final concentration of 600 mg/L. Strains and amino acid supplementation are as follows: NZ131 (▲), BNL100 (□), and BNL100 with extra L-serine (○), L-alanine (△), L-arginine (◇), L-histidine (▽) and L-threonine (✱). Results shown are representative of three independent experiments.

Figure 5. Deletion of *sdhB* rescues the reduced culture yield of BNL100 in CDM by preventing degradation of L-serine. **(A)** Deletion of *sdhB* has no effect on NZ131 growth but restores culture yield of BNL100 to wild-type levels when grown in CDM + 0.5% glucose at 37°C. Strains shown are NZ131 (WT) (black bar), BNL100 (Δ*serR*) (light gray bar), BNL116 (Δ*sdhB*) (white bar), and BNL117 (Δ*serR,* Δ*sdhB*) (dark gray bar). **(B)** Deletion of *sdhB* prevents the degradation of L-serine to pyruvate and ammonia. NZ131 (WT) (black bar), BNL100 (Δ*serR*) (gray bar), and BNL117 (Δ*serR,* Δ*sdhB*) (white

bar) were harvested during mid-log phase and resuspended to equal cell densities in 20 mM Na_2HPO_4/NaH_2PO_4, 1 mM $MgCl_2$ (pH 7.0) buffer containing 50 mM L-serine. Ammonia concentrations in cell-free supernatants were determined after 4 hours. Results shown are averages and SEM (error bars) of three independent experiments. ** $P < 0.001$ using one-way analysis of variance with Tukey's multiple comparison test (Graph Pad v5.0).

Figure 6. Deletion of *sloR* rescues the reduced culture yield of BNL100 in CDM by altering the rate of L-serine consumption in GAS. **(A)** Deletion of *sloR* rescues the reduced culture yield of BNL100 while not affecting growth of NZ131, while single-copy complementation with *sloR* partially restores the growth defect when *serR* is absent. Stains shown are NZ131 (WT) (black bar), BNL100 (Δ*serR*) (dark gray bar), BNL160 (Δ*sloR* with empty vector) (white bar), BNL163 (Δ*sloR* with pBL110) (striped bar), BNL161 (Δ*serR,* Δ*sloR* with empty vector) (light gray bar), and BNL114 (Δ*serR,* Δ*sloR* with pBL110) (checkered bar). **(B)** The SloR operon alters the rate of L-serine consumption in GAS. All strains were grown in CDM +0.5% glucose at 37°C until mid-log phase, at which point L-[^{14}C(U)]-serine was added to all cultures and radioactive counts contained within cells from 200 µL culture aliquots were measured at indicated time points. Strains shown are NZ131 (WT) (▲), BNL100 (Δ*serR*) (■), and BNL114 (Δ*serR,*Δ*sloR)* (◇). Results shown are averages and SEM (error bars) of three independent experiments. ** $P < 0.001$ using one-way analysis of variance with Tukey's multiple comparison test (Graph Pad v5.0).

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Table 1. Bacterial strains and plasmids used in this study

Strain	Genotype; phenotype	Reference
E. coli		
BL21(DE3)	protein expression *E. coli* host	Invitrogen
DH10B	F– *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 *deo*R Δ(*ara leu*)7697 *gal*U *gal*K *rps*L *nup*G λ–	Invitrogen
BH10C	Δ*pncB* variant of DH10B	Howell-Adams & Seifert, 2000
S. pyogenes		
NZ131	wild-type M49 *S. pyogenes* isolate	Simon and Ferretti, 1991
BNL100	NZ131 but Δ*serR::aad9;* Spc^R	This study
BNL102	NZ131 but Δ*sloR::aad9*; Spc^R	This study
BNL105	NZ131::pBL105; Em^R	This study
BNL106	BNL100::pBL105; Spc^R Em^R	This study
BNL114	NZ131 but Δ*serR::aad9* ΔsloR*::cat*; $Spc^R Cm^R$	This study
BNL116	NZ131 but Δ*sdhB::cat*; Cm^R	This study
BNL117	NZ131 but Δ*serR::aad9* Δ*sdhB::cat*; $Spc^R Cm^R$	This study
BNL141	NZ131::pBL106; Em^R	This study
BNL142	BNL100::pBL106; Spc^R Em^R	This study
BNL143	BNL102::pBL106; Spc^R Em^R	This study
BNL144	BNL114::pBL106; Spc^R Cm^R Em^R	This study
BNL158	NZ131::p7Int; Em^R	This study
BNL159	BNL100::p7Int; Spc^R Em^R	This study
BNL160	BNL102::p7Int; Spc^R Em^R	This study
BNL161	BNL114::p7Int; Spc^R Cm^R Em^R	This study
BNL162	BNL100::pBL109; Spc^R Em^R	This study
BNL163	BNL102::pBL110; Spc^R Em^R	This study
BNL164	BNL114::pBL110; Spc^R Cm^R Em^R	This study

Plasmid	Description	Source
pET-15b	Protein expression vector; Amp^R	Novagen
pFED760	Temperature sensitive pG^+host9 plasmid (ISS1 deleted); Em^R	Mashburn-Warren et al., 2010
p7INT	pUC18-derived streptococcal integration vector; Em^R	McShan et al., 1998
pBL100	pFED760 containing the upstream and downstream regions of *serR* flankning *aad9* at the *PstI* site; Em^R Spc^R	This study
pBL101	pFED760 containing the upstream and downstream regions of *sloR* flankning *aad9* at the *PstI* site; Em^R Spc^R	This study
pBL102	pFED760 containing the upstream and downstream regions of *sloR* flankning *cat* at the *PstI* site; Em^R Cm^R	This study
pBL103	pFED760 containing the upstream and downstream regions of *sdhB* flankning *cat* at the *PstI* site; Em^R Cm^R	This study
pBL104	pET-15b vector containing *serR* between the *BamHI* and *NdeI* sites; Amp^R	This study
pBL105	p7INT containing the P_{sloR}-luxAB fusion at the *EcoRI* site; Em^R	This study
pBL106	p7INT containing the P_{sdhAB}-luxAB fusion between the *BamHI* and *EcoRI* sites; Em^R	This study
pBL109	p7INT comtaining *serR* under its native promoter between the *BamHI* and *EcoRI* sites; Em^R	This study
pBL110	p7INT containing *sloR* under its native promoter between the *BamHI* and *XhoI* sites; Em^R	This study

Abbreviations: Cm, chloramphenicol; Em, erythromycin; Spc, spectinomycin; Amp, ampicillin

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Table 2. Oligonucleotide primers used in this study

Name	Sequence (5'-3')	Usage
Gene Deletions		
BL1	GCGTGCTGCAGTAATAGTGCAGATTCTTTTG	UFR of *serR*
BL2	GCGTGCTCGAGCCAAATCACCATAAAA	UFR of *serR*
BL3	GCGTGAGATCTGTCAAACTTCCTATATCTATCTTC	DFR of *serR*
BL4	GCGTGCTGCAGTCGACTTCAATCAAAGCTTGCAAAG	DFR of *serR*
BL5	GCGTGCTGCAGAATCACCTTAATAATAATTT	UFR of *sloR*
BL6	GCGTGCTCGAGAATCTATTCCACCAATCTAT	UFR of *sloR*
BL7	GCGTGAGATCTTAAACATCTTTTTGACAGGAC	DFR of *sloR*
BL8	GCGTGCTGCAGCATAAATTGTTCTAGCCTCTTGTTC	DFR of *sloR*
BL9	GCGTGCTGCAGGACACCAATAACATCATAACCTTGC	UFR of *sdhB*
BL10	GCGTGCTCGAGAGGCAACTCCGTTTTTTATTTCTATGT	UFR of *sdhB*
BL11	GCGTGAGATCTAAAGGAAAGCTATGTTTTATACTATTGAAGAACTTGT	DFR of *sdhB*
BL12	GCGTGCTGCAGATTTTAGCATTTAATTCGTTTACAGCTATAGCATTTC	DFR of *sdhB*
BL13	GCGTGAGATCTTCGATTTTCGTTCGT	*aad9*
BL14	GCGTGCTCGAGTTAGAATGAATATTT	*aad9*
BL15	GCGTGCTCGAGGCGAAAAAGGAGAAGTCGGTTCAGAAA	*cat*
BL16	GCGTGAGATCTCGGTATCGATAAGCTTGATGAAAATTTGTTTG	*cat*
Reporter Fusions		
BL17	GCGTGGAATTCGTCAAACTTCCTATATCTATCTTCTTG	*sloR* promoter
BL18	GCGTGGCGGCCGCATACGAACCTCCTCATTGATAATAT	*sloR promoter*
BL19	GCGTGGGATCCAAAAATTCTCCCATCAATAATAAGATAGAAAAAGA	*sdhBA* promoter
BL20	GCGTGGCGGCCGCAGGCAACTCCGTTTTTTATTTCTATGTTTATTATA	*sdhBA* promoter
BL25	GCGTGGCGGCCGCATTAATCACCAAAAAGGAATAGAGT	*luxAB*
BL27	GCGTGGAATTCGCCTTTAATTTTATTATGGT	*luxAB*
His-SerR Purification		
BL29	GCGTGGGATCCTTATTGATCGGCTTCAATTTTTTTAAGG	*serR*
BL30	GCGTGCATATGGATAAAGAAACGCTAAACTACTGGA	*serR*
EMSA		
BL31	GCGTGGAATTCACAATTGACCTGTCACTGGATTAA	*sloR* promoter
BL32	/56-FAM/ACAATTGACCTGTCACTGGATTAA	*sloR* promoter
BL33	/56-FAM/AGGCAACTCCGTTTTTTATTTCTATGTTTATTATA	*sdhBA* promoter
BL34	/56-FAM/ACAGAAGCTCCTTTAAGATAGTTATTAGTAGCTGTC	*serS* promoter
BL35	GTGTTTAAAGACCTCTCATGGGCAAAT	*rRNA* promoter
BL36	CAGGTTTCTCATAGCCTGTCAACTACTTTT	*rRNA* promoter
BL37	/56-FAM/CAGGTTTCTCATAGCCTGTCAACTACTTTT	*rRNA* promoter
Complementation Constructs		
BL38	GCGTGGGATCCGTCAAACTTCCTATATCTATCTTCTTG	*sloR promoter*
BL39	**AATAATAATATCCATATACGAACCTCCTCA**TTGATAATATAGTTAAATTT	*sloR promoter*
BL40	**TGAGGAGGTTCGTATATGGATATTATTATT**GGAACAAGTCTTTTGATTCTT	*sloR*
BL41	GCGTGCTCGAGATCTTGTTAAAAGTCCTGTCAAAAA	*sloR*
BL42	GCGTGGAATTCATACGAACCTCCTCATTGATAATATA	*serR*

Restriction sites are underlined

Sequence complementary between *sloR* complementation primers in bold

Table 3. Genes up-regulated > 5-fold in Δ*spy49_0126c* vs. NZ131 by microarray analysis

Gene ID	Operon Name	Putative Function	Fold Change[1]
Spy49_0127-0128	Spy49_0127, *sloR*	Putative endoribonuclease, SLO regulator	14.1, 16.8
Spy49_0129-0137	Spy49_0129, *ntpI-ntpD*	Hypothetical protein, V-type Na^+-dependent ATP synthase	15.3-24.5
Spy49_1358	*serS*	Seryl-tRNA synthetase	6.5,
Spy49_1794-1795	*sdhBA*	L-serine dehydratase	7.6, 9.11

[1] Values for operons containing more than two genes are given as a range for the entire operon

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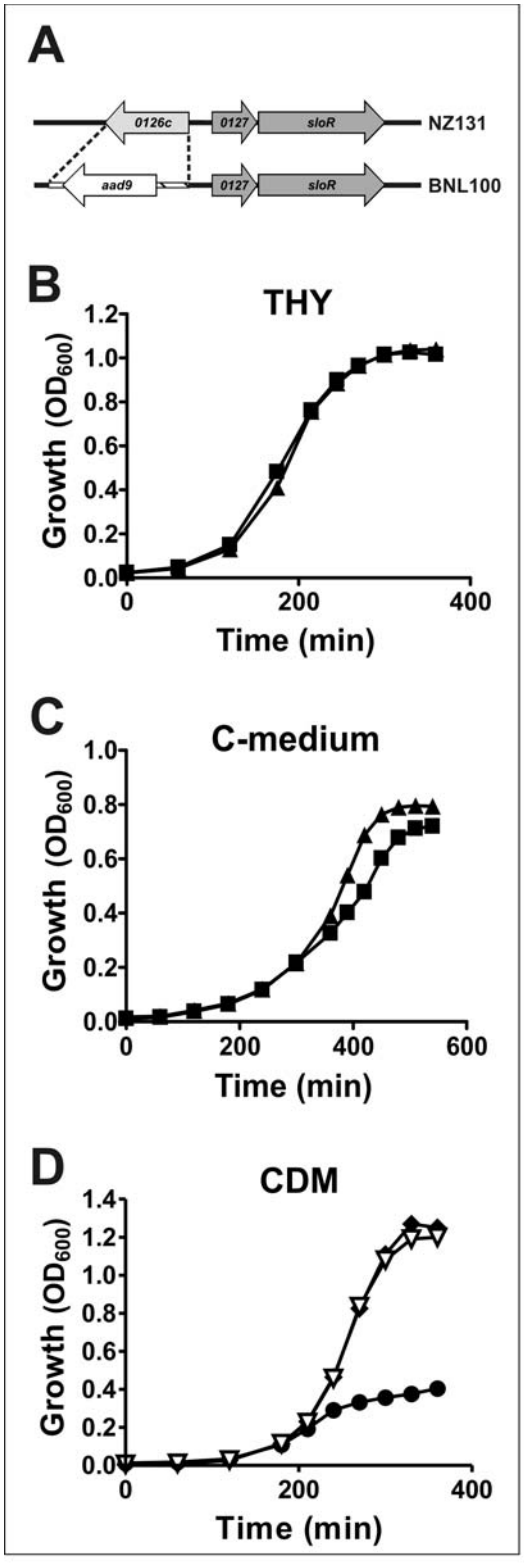
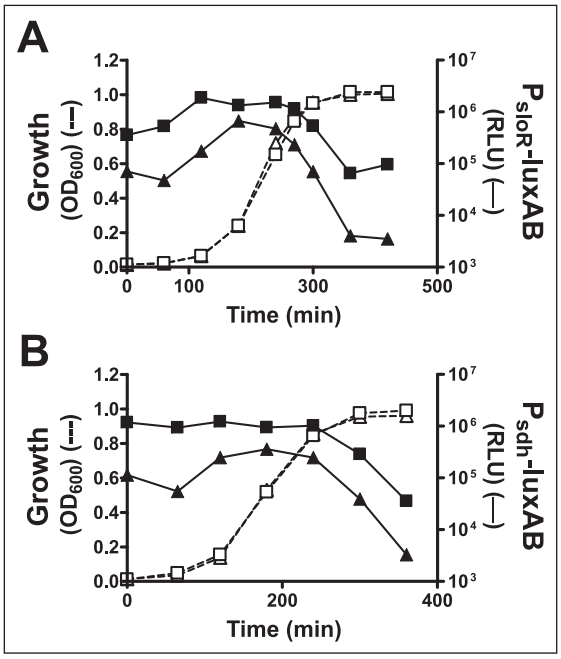
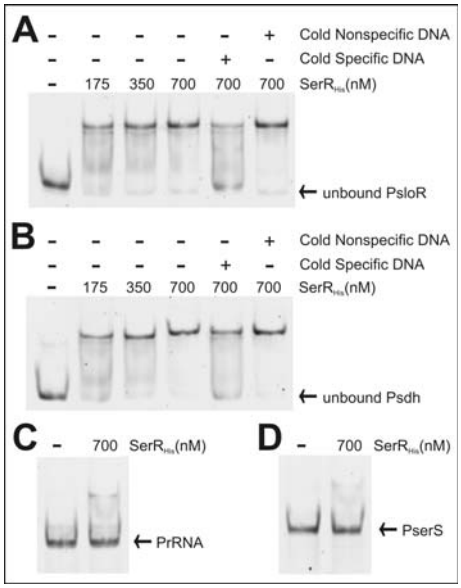
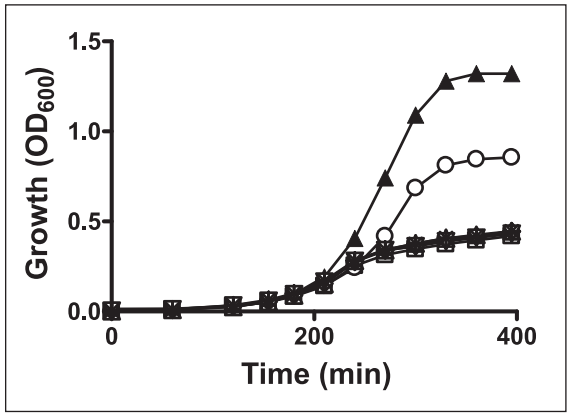
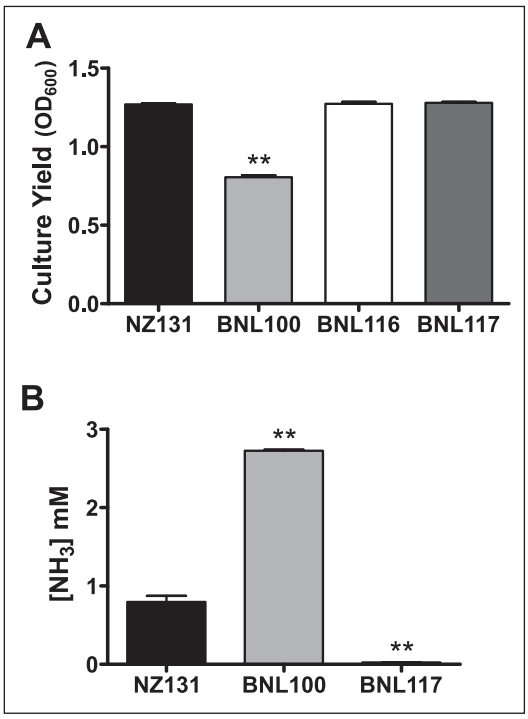
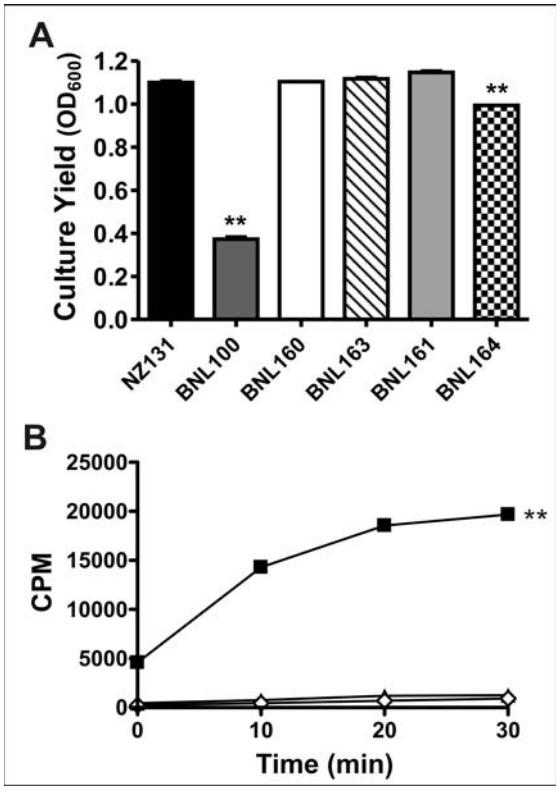

A
0126c
0127
sloR
NZ131
aad9
0127
sloR
BNL100
B
THY
Growth (OD600)
Time (min)
C
C-medium
Growth (OD600)
Time (min)
D
CDM
Growth (OD600)
Time (min)


A
Growth
(OD600) (---)
PsloR-luxAB
(RLU) (—)
Time (min)
B
Growth
(OD600) (---)
Psdh-luxAB
(RLU) (—)
Time (min)


A
- - - - - + Cold Nonspecific DNA
- - - - + - Cold Specific DNA
- 175 350 700 700 700 SerR$_{His}$(nM)
← unbound PsloR
B
- - - - - + Cold Nonspecific DNA
- - - - + - Cold Specific DNA
- 175 350 700 700 700 SerR$_{His}$(nM)
← unbound Psdh
C
- 700 SerR$_{His}$(nM)
← PrRNA
D
- 700 SerR$_{His}$(nM)
← PserS


1.5
1.0
0.5
0.0
Growth (OD600)
0
200
400
Time (min)


A
Culture Yield (OD_{600})
1.5
1.0
0.5
0.0
**
NZ131
BNL100
BNL116
BNL117
B
$[NH_3]$ mM
3
2
1
0
**
**
NZ131
BNL100
BNL117


A
Culture Yield (OD_{600})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
NZ131
BNL100
BNL160
BNL163
BNL161
BNL164
B
CPM
25000
20000
15000
10000
5000
0
**
0
10
20
30
Time (min)