REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
28-03-2017	Article	-

4. TITLE AND SUBTITLE
Spermine and spermidine alter gene expression and antigenic profile of Borrelia burgdorferi.

5a. CONTRACT NUMBER
W911NF-11-1-0136

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
206022

6. AUTHORS
Ying-Han Lin, Jesus A, Romo, Ann N. Reyes, Trever C. Smith II, S. L. Rajasekhar Karna, Christine L.Miller, Tricia A. Van Laar, Raghunandan Yendapally, James P. Chambers, and J. Seshu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Texas at San Antonio
One UTSA Circle

San Antonio, TX 78249 -1644

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
59027-LS-REP.156

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
Borrelia burgdorferi, the agent of Lyme disease, responds to numerous host-derived signals to alter adaptive capabilities during its enzootic cycle in an arthropod vector and mammalian host. Bioinformatic analysis of the borrelial genome revealed the presence of polyamine transport components (PotA, B, C and D) while homologs for polyamine biosynthesis were conspicuously absent. Although potABCD is co-transcribed, the level of PotA was elevated under in vitro growth conditions mimicking unfed-ticks compared to fed-ticks while the levels of PotD were similar under the aforementioned conditions in B. burgdorferi. Among several polyamines and polyamine

15. SUBJECT TERMS
Polyamines, Borrelia burgdorferi, Lyme disease,

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Bernard Arulanandam
UU	UU	UU	UU		19b. TELEPHONE NUMBER 210-458-5492

REPORT DOCUMENTATION PAGE (SF298)
(Continuation Sheet)

Continuation for Block 13

Proposal/Report Number: 59027.156-LS-REP

Report Title: Spermine and spermidine alter gene expression and antigenic profile of Borrelia burgdorferi.
Report Type: Article

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Infection and Immunity
Publication Identifier Type: Publication Identifier: 10.1128/IAI.00684-16
Volume: Issue: First Page #:
Date Submitted: 3/28/17 12:00AM Date Published: 1/4/17 6:00AM
Publication Location:
Article Title: Spermine and spermidine alter gene expression and antigenic profile of Borrelia burgdorferi.
Authors: Ying-Han Lin, Jesus A, Romo, Ann N. Reyes, Trever C. Smith II, S. L. Rajasekhar Karna, Christine L.Mi
Keywords: Polyamines, Borrelia burgdorferi, Lyme disease,

Abstract: Borrelia burgdorferi, the agent of Lyme disease, responds to numerous host-derived signals to alter adaptive capabilities during its enzootic cycle in an arthropod vector and mammalian host. Bioinformatic analysis of the borrelial genome revealed the presence of polyamine transport components (PotA, B, C and D) while homologs for polyamine biosynthesis were conspicuously absent. Although potABCD is co-transcribed, the level of PotA was elevated under in vitro growth conditions mimicking unfed-ticks compared to fed-ticks while the levels of PotD were similar under the aforementioned conditions in B. burgdorferi. Among several polyamines and polyamine precursors, supplementation of spermine or spermidine in the borrelial growth medium induced synthesis of major regulators of gene expression in Bb such as RpoS and BosR with a concomitant increase in proteins that contribute to colonization and survival of B. burgdorferi in the mammalian host.
Distribution Statement: 1
Acknowledged Federal Support: **Y**

Spermine and spermidine alter gene expression and antigenic profile of *Borrelia burgdorferi*.

Running title: Polyamines and *Borrelia burgdorferi*

Ying-Han Lin[1], Jesus A, Romo[1], Ann N. Reyes, Trever C. Smith II, S. L. Rajasekhar Karna, Christine L. Miller, Tricia A. Van Laar[2], Raghunandan Yendapally[3], James P. Chambers, and J. Seshu*

South Texas Center for Emerging Infectious Diseases (STCEID), Center of Excellence in Infection Genomics (CEIG) and Department of Biology, The University of Texas at San Antonio, San Antonio, TX-78249;

[2] Department of Biology, California State University Fresno, Fresno, CA

[3]Feik School of Pharmacy, University of The Incarnate Word, San Antonio, TX-78209

[1]Contributed equally to the work and should be considered as co-first authors

*Corresponding Author:

BSE3.230, Department of Biology,

The University of Texas at San Antonio,

One UTSA Circle, San Antonio, TX-78249.

Phone: (210) 458-6578.

Fax: (210) 458-5658

Email: j.seshu@utsa.edu

ABSTRACT

Borrelia burgdorferi, the agent of Lyme disease, responds to numerous host-derived signals to alter adaptive capabilities during its enzootic cycle in an arthropod vector and mammalian host. Molecular mechanisms that enable *B. burgdorferi* to detect, channel, and respond to these signals have become an intense area of study for developing strategies to limit transmission/infection. Bioinformatic analysis of the borrelial genome revealed the presence of polyamine transport components (PotA, B, C and D) while homologs for polyamine biosynthesis were conspicuously absent. Although *potABCD* is co-transcribed, the level of PotA was elevated under *in vitro* growth conditions mimicking unfed-ticks compared to fed-ticks while the levels of PotD were similar under the aforementioned conditions in *B. burgdorferi*. Among several polyamines and polyamine precursors, supplementation of spermine or spermidine in the borrelial growth medium induced synthesis of major regulators of gene expression in *Bb* such as RpoS and BosR with a concomitant increase in proteins that contribute to colonization and survival of *B. burgdorferi* in the mammalian host. Short transcripts of *rpoS* were elevated in response to spermidine which was correlated with increased protein levels of RpoS. Transcriptional analysis of *rpoZ* and rel_{Bbu} (BB0198) in the presence of spermidine revealed interplay of multiple regulatory factors in *B. burgdorferi* gene expression. The effect of spermidine was also influenced by serum factors while addition of arginine, a polyamine precursor, induced minimal changes in key borrelial proteins. These studies suggest that multiple host-derived signals/nutrients and their transport systems contribute to *B.burgdorferi* adaptation during the vector and vertebrate-host phases of infection.

INTRODUCTION

Lyme disease is the most prevalent arthropod-borne infectious disease in the US with an estimated 300,000 cases in 2013 by Centers for Disease Control and Prevention (CDC). The causative agent of Lyme disease is the spirochetal pathogen, *Borrelia burgdorferi (Bb)* which is transmitted to humans and other vertebrate hosts following the bite of infected *Ixodes scapularis* ticks (1, 26). The ability of this spirochete to recognize and respond to environmental signals and nutrients present in the vertebrate host or the tick vector plays a crucial role in the survival, transmission, and colonization capability, leading to Lyme disease (16, 34, 37). Numerous studies have established the significance of temperature (33, 38, 42), pH (4), dissolved gases (17, 39), host-specific stressors (3), and select nutrients (2, 10, 20) playing a role in a concerted fashion for expression and synthesis of key borrelial determinants that enable spirochete survival and colonization in different hosts. However, there remain additional host signals and molecular transduction mechanisms that are yet to be defined in understanding the dynamics of colonization of the vector and vertebrate hosts by Lyme spirochetes.

Since *Bb* has a limited genome and is incapable of synthesis of amino acids, fatty acids, nucleotides and other critical biomolecules, many of these substances are acquired from the host (9, 14). Therefore, analysis of the mechanisms of transport of these key host-derived molecules will not only extend our understanding of host-specific adaptation of spirochetes in general but also provide novel strategies to subvert and/or interdict these survival processes in hosts; thus potentially leading to a reduction in the incidence of Lyme disease. Moreover, host-derived components that are abundant in select sites of colonization in different hosts could potentially contribute to tissue-specific modulation of borrelial physiology resulting in favorable or unfavorable conditions for adaptation.

Among the many host-derived components, polyamines are organic bases that are prevalent in millimolar range in many mammalian tissues (41). Polyamines, such as spermine, spermidine, cadaverine, and putrescine are cationic, exhibiting a net positive charge ranging from

+2 to +4 while polyamine precursors such as ornithine and arginine carry a charge of +1. Both the structure and charge of polyamines facilitate their interaction with DNA, RNA, proteins, and phospholipids, thereby affect a wide variety of biological functions such as increasing resistance to oxidative stress and regulating transcription/translation, among others (7, 19, 32). Moreover, polyamines are known to alter both bacterial response to antibiotics and via structure/charge alterations, functional efficacy of antimicrobial agents (23-25). There is a renewed interest in polyamine biogenesis, and the role that polyamines play in regulating adaptive gene expression in many pathogenic bacteria and their ability to confer bacterial cell protection against oxidative and other host-derived physiological stressors. In *Escherichia coli*, polyamines increase the levels of RpoS (σ^{38} subunit of the RNA polymerase), leading to increased levels of the glutamate decarboxylase-dependent acid response system (6, 8).

Bioinformatic analysis of the *B. burgdorferi* genome revealed the presence of homologs that could be involved in polyamine transport, e.g., spermine, spermidine, putrescine, and cadaverine, but no apparent homologs were found for biogenesis of polyamines suggesting that *B. burgdorferi* acquires polyamines from the host (14). Moreover, a few enzymes involved in the conversion of precursors of polyamines such as arginine and ornithine are also apparent in the borrelial genome. While many bacteria have more than one transport system for polyamine transport, the absence of multiple polyamine binding and transport homologs suggested the presence of one polyamine transport (PotABCD) system within the genome of *B. burgdorferi* (14). Although recent transcriptional analyses have revealed altered levels of members of the polyamine transport system the contribution(s) of polyamines in the pathogenesis of *B. burgdorferi* are yet to be uncovered. (2).

In this study, we determined that the members of the polyamine transport system are co-transcribed and that supplementation of Spermine (Spm) and Spermidine (Spd) drastically altered the *B. burgdoferi* protein profile, especially key proteins critical for colonization in the vertebrate host. Regulators of gene expression in *B. burgdorferi* such as RpoS and BosR were elevated in

response to increased levels of spm and spd. Effects of these polyamines on transcription of *rpoZ* and the bifunctional synthetase/hydrolase homolog (*bb0198*, rel_{Bbu}) suggest that the polyamines impact key borrelial regulatory proteins. Characterizing the role of polyamines, including Spm and Spd, in regulating multiple components of *B. burgdorferi* will pave the way for identifying new and novel strategies to reduce borrelial burden in the vector/vertebrate hosts, and thereby reduce transmission of this vector-borne pathogen, leading to a lowered incidence of Lyme disease.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and plasmids. *B. burgdorferi* strain B31-A3 was cultured in BSK-II medium supplemented with 6% (v/v) normal rabbit serum (Pel-Freez, AK) at 32ºC/pH7.6, 1% CO_2 in a CO_2 incubator. Borrelial cultures grown in BSKII media at pH7.6/23˚C were shifted to pH6.8/37°C to mimic conditions within the tick-midgut before and after a blood-meal respectively as described previously (28, 29). Growth of *Bb* in the presence of 4mM Spermine or Spermidine at pH7.6/32˚C was monitored every24hrs for 168 hrs or 120 hrs respectively. Cultures grown to a density of less than 1-2 $x10^7$ or 1-2 $x10^8$ spirochetes/ml were considered as low- and high-density cultures, respectively. pCR2.1 cloning vector (Invitrogen) was routinely used for products and transformed into *E. coli* Top10 cells followed by blue/white screening. Recombinant PotA (BB0642) and PotD (BB0639) were obtained by generating the respective amplicons using primers (Table 1) with appropriate engineered restriction enzyme sites that facilitated cloning into pET23a (Novagen) followed by overexpression using *E. coli* Rossetta strain as reported previously (31, 45). Histidine tagged (6X C-terminus) PotA and PotD were purified using nickel affinity column chromatography and was used as a source of protein for generating specific antisera (11).

Generation of antisera against PotA and PotD. Six to eight week old mice (*n*=5) were immunized subcutaneously with 50 μg of recombinant PotA or PotD in phosphate buffered saline (PBS) with an equal volume of adjuvant TiterMax (Sigma). Booster immunizations were given at days 14 and 21 and immune serum was collected on day 28 (11). Serum was checked for reactivity to PotA or PotD in *B. burgdorferi* by immunoblot analysis as described below.

Preparation of *B. burgdorferi* lysates. Spirochetes were grown in the presence or absence various polyamines (4mM) and monitored for growth. When the density of the culture reached $1x10^8$ bacteria /ml, spirochetes were washed three times in Hanks Balanced Salt Solution (HBSS)

and processed for SDS-PAGE analysis. *B. burgdorferi* was grown in the absence or presence of spermine (4mM) in BSKII growth media supplemented with 6% (v/v) normal or heat inactivated rabbit (56˚C for 1 hr) serum. Protease sensitivity was determined to assess PotD is surface exposed by washing the spirochetes in PBS (pH7.4) supplemented with 5mM $MgCl_2$ and 50 mM sucrose as described previously (35). Samples were split in two and incubated with either 50 μl sterile water (Proteinase K negative control) or with Proteinase K (final concentration 200μg/ml) at 20˚C for 40 minutes. The protease activity was stopped by adding phenylmethylsulfonyl fluoride (PMSF, 1mM final concentration) and the spirochetes were examined by dark-field microscopy for motility as a measure of viability. Samples were washed and processed for SDS-PAGE and immunoblot analysis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis. *B. burgdorferi* whole-cell lysates were prepared and separated on SDS-12.5% PAGE as described previously (45). The separated proteins were either visualized by Coomassie brilliant blue staining or transferred onto a PVDF membrane (Amersham Hybond™-P; GE Healthcare, Buckinghamshire, UK), and subjected to immunoblot analysis as described previously (22). Membranes were probed with monoclonal antibodies or monospecific serum against a variety of borrelial proteins. Blots were developed following incubation with appropriate dilutions of HRP conjugated anti-mouse, anti-rabbit or anti-rat secondary antibodies using ECL™ Western blotting reagents (GE Healthcare).

RNA extraction and quantitative real-time PCR analysis. Transcriptional analysis of key genes relevant to this study was carried out using quantitative real-time PCR analysis as described previously (12, 21). Total RNA was extracted as previously described from *B. burgdorferi* cultures grown in the presence or absence of 4 mM spermine to low density 2-3x10^7 or high

density, $1x10^8$ spirochetes per ml (22). Total RNA was treated twice at 37˚C for 45 minutes with DNaseI to remove any contaminating DNA, and quantified spectrophotometrically. RNA purity was determined using real-time PCR with *recA* primers (recAFq and recARq) to rule out the presence of contaminating DNA. RNA samples were reverse transcribed to cDNA using BIO-RAD iScript Select cDNA synthesis kit (BIO-RAD Laboratories, Hercules, CA). Real-time PCR was carried out using iTaq Universal SYBR Green Supermix with various oligonucleotide primers (Table S2) at a final concentration of 100 nM, and quantitative real-time PCR was done using ABI Prism 7300 system (Applied Biosystems) as described previously (22). The cycle threshold (C_T) values of each of the genes were averaged following normalization. Levels of induction were determined usingthe $\Delta\Delta C_T$ method where the quantity of each transcript was determined by the equation $2^{-\Delta\Delta CT}$, as described previously (21, 22). Normalized C_T values were subjected to unpaired Student's *t*-test implemented in GraphPad PRISM software. *P* values less than 0.05 were considered significant.

RESULTS

Polyamine transport system of *B. burgdorferi*. Bioinformatic analysis of the *B. burgdorferi* genome revealed the presence of a transport system for polyamines and no apparent homologs associated with biogenesis of polyamines (14). Since *B. burgdorferi* has limited metabolic capabilities and is intimately associated with its hosts, analysis of the key components of the polyamine transport system should afford a greater understanding of the adaptive capabilities of this spirochetal pathogen. Based on the organization of the polyamine transport system in other bacteria (19), *B. burgdorferi* has homologs of polyamine binding protein PotD (*bb0639*) located in the periplasm, and two channel forming transmembrane proteins PotC (*bb0640*) and PotB (*bb0641*) that interact with PotA (ATPase, *bb0642*), a membrane associated ATPase that facilitates transport of polyamines into the bacterial cytoplasm (Fig 1A). The open reading frames encoding *potABCD* are located adjacent to each other in the borrelial genome (Fig 1B). Analysis of cDNA generated from total RNA using primers specific to *potA/potB* (Primer set 1 and 2), *potB/potC* (Primer set 3 and 4) and *potC/potD* (Primer set 5 and 6) revealed the members of the *potABCD* in *B. burgdorferi* to be transcriptionally linked (Fig 1C). Amplicon size was similar using either cDNA (Fig 1C, Lane 3) or total genomic DNA (Fig 1C, Lane 4) as the template. No amplification was observed using total RNA (Fig 1C, Lane 2) or PCR mix without template (Fig 1C, Lane 1) indicating no DNA contamination in the RNA sample used for generating cDNA. Next, we focused on determining the role of PotD and PotA in modulating the adaptive capabilities of *B. burgdorferi* in response to varying levels of polyamines.

Levels of PotA and PotD in *B. burgdorferi*. Immunoblot analysis of total lysates from *B. burgdorferi* grown under *in vitro* conditions mimicking the tick midgut before a blood meal (Fig 2A, Lane 1 pH7.6/32˚C) compared to that after a blood meal (Fig 2A, Lane 2, pH6.8/37˚C) was carried out using antisera specific to PotA or PotD. Increased levels of PotA was observed in *B. burgdorferi* propagated under unfed tick-midgut conditions compared to fed-midgut conditions

(Fig 2B, α-PotA). However, levels of PotD were similar in *B. burgdorferi* under both *in vitro* growth conditions (Fig 2B, α-PotD). These findings suggested that the increased levels of PotA under conditions mimicking unfed ticks might reflect the kinetics of polyamine transport during the vector-phase of borrelial infection (Fig 2).

Localization of PotD. The location of PotD, the substrate binding protein in the polyamine transport system, is critical for its role in responding to extracellular levels of different polyamines. In order to determine if PotD is localized to the outer surface, we performed Proteinase K sensitivity assays as described previously (35). Immunoblot analysis revealed that PotD levels were similar in *B. burgdorferi* treated with or without treatment with Proteinase K (Fig 3B, +PK, α-PotD). As expected, there was no difference between treated and untreated samples in the levels of PotA due to its predicted localization in the inner membrane. The levels of another periplasmic protein, FlaB, were also similar in treated and untreated spirochetes while P66, an outer membrane protein, exhibited a reduction in size only in Proteinase K treated samples (Fig 3B, +PK, α-FlaB, α-P66). Coomassie blue staining of the lysates from *B. burgdorferi* (Fig 3A) before and after treatment with Proteinase K exhibited similar levels of proteins loading for each sample. This analysis revealed that PotD is a periplasmic protein, and that the transport of polyamines presumably occurs from the periplasmic space to the cytoplasm of the spirochetes.

Effects of polyamines on the levels of key borrelial proteins. We determined the effects of various polyamines on *B. burgdorferi* by supplementing the borrelial growth medium (BSKII) with various concentrations of polyamines, i.e., Spermidine (Spd), Spermine (Spm), Arginine (Arg), Cadaverine (Cad), Ornithine (Ort), and Putrescine (Put). We found that 4 mM concentration of various polyamines supported borrelial growth at pH7.6/32˚C (data not shown).

We then analyzed the levels of key borrelial proteins that reflect the physiological status of *B. burgdorferi* in the presence of 4 mM concentration of the indicated polyamines. Immunoblot analysis revealed that the levels of Outer Surface Protein C (OspC) were elevated in *B. burgdorferi* propagated in presence of Spd and Spm compared to growth in conventional BSKII medium with no polyamine supplementation (control) or in the presence of Arg, Cad, Ort, and Put. Coincidentally, the levels of RpoS, a key regulator of the gene expression for the adaptation of *B. burgdorferi* to the mammalian host (34), were significantly elevated in Spm supplemented samples, and to a lesser extent in Spd-treated samples. The levels of RpoS were minimal in the control lysates, and from lysates of *B. burgdorferi* grown in the presence of Arg, Cad, Ort and Put. While the levels of PotA were elevated in *B. burgdorferi* grown in the presence of Cad, Ort, and Put, minimal changes were observed in the levels of PotA in Spd, Spm, and Arg treated samples. PotD was decreased marginally in the Spd and Spm treated samples compared to the control lysate or lysates from *B. burgdorferi* treated with other polyamines. The levels of Rho were similar in all borrelial lysates while levels of Rel_{Bbu} exhibited a modest reduction in *B. burgdorferi* supplemented with Arg compared to other polyamines. These observations indicate that increased levels of Spd and Spm altered the levels of a major regulator of gene expression, RpoS, and a key pathogenesis-associated protein, OspC, in *B. burgdorferi*. Therefore, we focused on determining the effects of these two polyamines to further assess their contributions towards modulating host-adaptation of *B. burgdorferi* to the mammalian host.

Effect of spermidine and spermine on expression of pathogenesis related proteins of *B. burgdorferi*. Compared to lysates from *B. burgdorferi* grown in BSKII medium at pH7.6/32˚C (Fig 5, Lane 3), there were increased levels of RpoS and OspC in lysates of *B. burgdorferi* grown in BSKII medium supplemented with 4 mM Spd (Fig 5B, Lane 4) or Spm (Fig 5B, Lane 5). Moreover, the levels of other *rpoS*-regulated proteins such as DbpA and BBK32 (40) were also elevated in the presence of Spd and Spm (Fig 5C, α–DbpA, α-BBK32). The increase in RpoS

284 and OspC in the presence of Spd or Spm was similar to that when *B. burgdorferi* was grown
285 under conditions mimicking the midgut of fed ticks (Fig 5, Lane 1, pH6.8/37˚C) compared to
286 unfed ticks (Fig 5, Lane 2, pH7.6/23˚C). No significant differences were observed in the levels of
287 FlaB, P66 and NapA in lysates of *B. burgdorferi* with or without Spd or Spm (Fig 5C). These
288 observations suggest that increasing levels of select polyamines, notably Spd and Spm, result in
289 increased levels of proteins involved in the adaptation of *B. burgdorferi* to the mammalian host.
290 Among all polyamines, Spm consistently induced higher levels of RpoS and other pathogenesis-
291 related proteins supporting its significance in contributing to the adaptive capabilities of *B.*
292 *burgdorferi.*
293
294 **Effect of polyamine transport inhibitors on *B. burgdorferi.*** We determined the effect of a
295 hydrophobic 1, 3-dicyclohexylcarbodi-imide (DCC) and a polar 1-ethyl-3-(3-
296 dimethylaminopropyl) carbodi-imide (EDC) inhibitor of polyamine transport that has been shown
297 to be effective with eukaryotic cells for their inhibitory effects on spirochetes (44). *B. burgdorferi*
298 was grown in the presence of 4mM Spm and 5 mM EDC or 5mM DCC, and observed the levels
299 of PotA, RpoS, BosR, DbpA, and OspC were not altered in the presence of either polyamine
300 transport inhibitor (Fig 6B, Lanes 3 and 4). However, consistent previous observations (Fig 4 and
301 5) except for PotA, the levels of RpoS and other *rpoS*-regulated gene products in *B. burgdorferi*
302 were elevated following growth in spermine supplemented media (Fig 6B, Lane 2) compared to
303 the control (Fig 6B, Lane 1). Total protein staining of SDS-PAGE gels on protein samples from
304 different lysates indicated 1) loading was comparable and 2) the increased levels of OspC are
305 apparent in spermine treated samples (Fig 6A).
306
307 **Growth rates of *B. burgdorferi* in the presence of Spermine and Spermidine**. Growth rates
308 of *B. burgdorferi* strain B31-A3 in the presence of 4 mM Spm and Spd was performed over 168
309 or 120 hrs, respectively, at pH 7.6/32˚C until untreated control samples reached a density of ~ 4

310 x10^8 cells per ml. As shown in Fig 7, a significant reduction in the *B. burgdorferi* growth in the
311 presence of Spm (Fig 7B) compared to growth in the presence of Spd (Fig 7A). Although the
312 growth curve was extended for 168 hrs for cultures grown in the presence of Spm, there was no
313 significant difference between growth rates in the presence or absence of Spd (Fig 7B). It should
314 be noted that the motility of the spirochetes was comparable indicating viability in both
315 supplemented and control media although the rates of growth over a period of time in the
316 presence of Spm was significantly lower compared to that of control media. While no significant
317 difference in the growth of *B. burgdorferi* at pH7.6/23˚C (conditions mimicking unfed ticks) was
318 observed in the presence or absence of 4 mM Spd (Fig S1), significant differences in growth rates
319 in the presence of spermidine when cultures were grown at pH6.8/37˚C (conditions mimicking
320 fed ticks). These studies indicate that the effects of polyamines overlap with other environmental
321 signals encountered during the vector or mammalian phases of borrelial infection.
322
323 **Effects of spermidine and spermine on transcriptional rates of select borrelial determinants**.
324 Transcriptional analysis of cDNA generated from total *B. burgdorferi* RNA grown in the
325 presence of Spd or Spm showed that *ospC* levels were significantly higher than in the control (Fig
326 8), corresponding to protein expression levels (Fig 4). We also saw higher levels of *rpoS* with
327 Spm treatment (also corresponding to protein expression levels, Fig 4), while levels of *flaB* were
328 lower than the control (Fig 8). *ospA* levels were decreased with both Spd and Spm treatment.
329 These observations suggest that select polyamines can mediate changes in *B. burgdorferi* at the
330 transcriptional level by potentially stabilizing select specific transcripts with minimal effects on
331 their induction.
332
333 **Effects of cell density and polyamine on transcriptional levels in *B. burgdorferi.*** Since there
334 is an impact of the density of the spirochetes on borrelial gene expression, we determined the
335 transcriptional levels of select borrelial determinants at low and high density in the presence or

absence of Spd. We focused on effects of Spd supplementation to discern whether there are other factors that contribute to the levels of *rpoS,* which was relatively lower in the Spd-treated sample and also to determine if there are differences in the long or short transcripts of *rpoS* induced in response to Spd (27). As shown in Fig 9A, when the media was supplemented with Spd, the levels of *rpoS* were significantly decreased in spirochetes grown to low density (1-2 $x10^7$ cells/ml) compared to levels from spirochetes grown to high density (1-2 $x10^8$ cells/ml). The levels of *ospC* were also lower when Spd-treated cells were harvested at low density, while there was a significant increase in *ospC* expression when cells were grown to high density with Spd addition. These transcriptional analyses underscore the importance of the role of polyamines and other growth-dependent conditions such as cell density on gene expression in *B. burgdorferi.* Additionally, we determined the levels of short and long *rpoS* transcripts induced in response to different environmental cues (27). As shown in Fig 9B, the short *rpoS* transcript was up-regulated in Spd treated samples, while the long transcript was significantly down-regulated. In combination with density dependent signals, these data reflect the role of RpoS in Spd treated spirochetes. Both *rpoZ* and rel_{Bbu} (*bb0198*) have been implicated in the metabolic response observed when spirochetes are shifted from minimal growth medium to nutritionally replete conditions that reflect the conditions in the mid-gut of tick before and after a blood meal (2, 10). The transcript levels of rel_{Bbu} were significantly elevated in *B. burgdorferi* grown to low density in the presence of Spd, while the gene was down-regulated in the high-density, Spd-treated samples (Fig 9C). Levels of *rpoZ* were similar in treated and untreated low density samples, while the addition of Spd lead to downregulation of *rpoZ* when cells were grown to high density (Fig 9C). Consistent with our transcriptional data, immunoblot analysis revealed increased levels of Rel_{Bbu} (BB0198) in Spd-treated low density samples compared to untreated controls (Fig 9D). These observations indicate that the overall effects of polyamines on borrelial gene expression could also be influenced by other environmental cues and open new avenues for regulating the adaptive responses of *B. burgdorferi* in response to host-specific signals.

362 **Effect of polyamines in the presence of serum.** It has been previously shown that Gram-
363 negative bacteria such as *Neisseria gonorrhoeae* withstand the effects of cationic antimicrobial
364 peptides, and complement-mediated killing in the presence of polyamines (15). As shown in Fig
365 10B, *B. burgdorferi* grown in medium supplemented with heat inactivated rabbit serum (IS, 6%
366 v/v) plus 4mM Spd had increased levels of OspC (IS, +Spd) compared to spirochetes grown in
367 the presence of normal rabbit serum (NS, 6% v/v, +Spd). Levels of OspC in absence of Spd were
368 comparable in *B. burgdorferi* grown in either IS (Fig 10B, IS, -Spd) or NS (Fig 10B, NS, -Spd).
369 Immunoblot analysis also revealed increased levels of DbpA in lysates from *B. burgdorferi*
370 grown with IS and Spd compared to those grown with NS and Spd. The levels of OspA and
371 Oligopeptide permease A1 (OppA1) were similar in borrelial cultures grown with IS or NS with
372 or without spermidine. These observations indicate that *in vitro* growth of *B. burgdorferi* in media
373 supplemented with IS and Spd leads to higher levels of OspC and DbpA compared to growth with
374 NS and Spd, suggesting that the presence of heat labile components in the serum potentially limit
375 the effect of Spd. Although lipoproteins involved in the pathogenesis of *B. burgdorferi* such as
376 OspC and DbpA are elevated in the presence of IS and Spd, the levels of another key borrelial
377 lipoprotein, OspA, remained similar in the presence of either IS or NS plus Spd. The levels of
378 OppA1 were similar in all samples examined, serving as a control reflecting the physiological
379 status of the spirochetes grown in media supplemented with IS or NS with Spd.
380
381 **Effect of supplementation with arginine.** In our initial analysis, addition of 4mM arginine (Arg)
382 resulted in no detectable increase in the levels of RpoS and OspC compared to cultures grown
383 with Spd or Spm (Fig 4). However, the levels of PotA were lower with Arg supplementation and
384 therefore, we determined the effects of higher concentrations of Arg (up to 20mM) on expression
385 of select *B. burgdorferi* proteins. Levels of PotA were increased, while levels of PotD were
386 relatively unchanged when *B. burgdorferi* was grown with increasing concentrations of L-
387 Arginine (Fig 11B, α-PotA, -PotD). The levels of arginine deiminase (ArcA), an enzyme that

participates in arginine metabolism, were elevated in lysates of *B. burgdorferi* grown in the presence of higher concentrations of arginine compared to untreated controls (Fig 11B, α-ArcA). The levels of P66, a porin, remained unchanged in *B. burgdorferi* with increasing concentrations of arginine (Fig 11B, α-P66). Bioinformatic analysis of the arginine utilization in *B. burgdorferi* is schematically described in Fig 11C and the implications of this pathway in borrelial physiology are discussed .

DISCUSSION

B. burgdorferi has limited metabolic capabilities which imposes several limitations on its growth, metabolism, and virulence (34). The spirochetes, however, utilize a variety of transporters to acquire many host-derived components to overcome their metabolic insufficiency and achieve successful colonization and survival in different compartments of the tick vector or vertebrate hosts (9). Thus investigating the role played by key host-derived components in borrelial physiology are likely to provide new avenues to alter the uptake of these biomolecules, limiting the survival and virulence potential. Among the multitude of host-derived molecules, polycations, polyamines, inorganic cations such as magnesium and calcium and metals (among others) known to contribute to functional conformations of nucleic acids, facilitating DNA replication, transcription, and translation (43). Notably, polyamines with a uniform distribution of positive charge serve as electrostatic bridges between the negatively charged phosphate backbone of nucleic acids, and other biologically-relevant negatively charged polymers affecting multiple cellular processes (23). Several studies have shown that biogenesis and degradation of oracquisition of polyamines via active transport from the host, modulate multiple biological processes such as nucleic acid and protein synthesis as well as cell growth, protection against oxidative stressors, and virulence (23, 30). However, the role of polyamines in regulating the adaptive response of the agent of Lyme disease, a host-adapted pathogen lacking multiple metabolic pathways, has remained unexplored.

Bioinformatic analysis of the borrelial genome revealed the presence of an ATP binding cassette (ABC) transport system comprised of a periplasmic substrate binding protein (PotD), two channel-forming transmembrane proteins (PotB and PotC), and a membrane-associated ATPase (PotA) (Fig 1A). The genes encoding these proteins are adjacent to each other in the borrelial chromosome, and are co-transcribed, suggesting the possibility of the transport system being regulated in response to the availability of the binding substrate/cargo (Fig 1B and 1C). The absence of apparent homologs of a polyamine biosynthetic pathway suggests that differences in

439 the transport of polyamines in different microenvironments could potentially influence the
440 adaptive capabilities of *B. burgdorferi*. Moreover, *B. burgdorferi* is exposed to variations in
441 signals, nutrients, and mediators of host immunity in different microenvironments, notably in the
442 mammalian hosts and during transmission from a tick vector to the vertebrate host and vice versa.
443 Polyamine transport and utilization could therefore serve to enhance the survival of the
444 spirochetes by altering a variety of physiological responses pertinent to the host-specific
445 conditions. For example, we noted that *B. burgdorferi* propagated under *in vitro* growth
446 conditions mimicking the tick midgut before a blood meal had higher levels of PotA, reflecting
447 differences in the levels of polyamine transport under temperature/pH parameters mimicking
448 conditions of unfed- (pH7.6/23˚C) and fed-ticks (pH6.8/37˚C). It is possible that increased level
449 of PotA in *B. burgdorferi* coincide with increased polyamine transport, which exists as a
450 membrane permeant weak base when the pH of the medium is basic (pH7.6/23˚C). Furthermore,
451 the borrelial PotA homolog has several conserved residues that have been shown to play a role in
452 ATPase nucleotide binding and spermidine uptake in *E. coli*. These include Walker motif A
453 (G^{40}PSGC^{44}GKT), and Walker motif B (L^{158}LLLD^{162}E) with C^{44} and D^{162} potentially involved in
454 ATPase activity (18). As shown in Fig 6, the levels of PotA were lower when *B. burgdorferi* was
455 propagated with increased Spd compared to untreated spirochetes suggesting that the Spd levels
456 modulate levels and presumably the ATPase activity of PotA. The changes in the levels of PotA
457 in *B. burgdorferi* were similar to that observed for *E. coli* where the ATPase activity of PotA was
458 shown to be lower with increasing concentrations of Spd (18).
459 While PotA was elevated under unfed tick-midgut conditions, PotD, (the periplasmically-
460 located polyamine binding protein) was similar under both *in vitro* growth conditions mimicking
461 the fed and unfed ticks. Although, the borrelial PotD homolog possesses S and D residues at
462 positions 226 and 29, respectively and is similar to the PotD homolog of *E. coli*. However, these
463 residues have been shown to be not critical for Put binding coupled with limited conservation in
464 this region of PotD known to facilitate binding of Putrescine in *E. coli* (18). It is possible that the

borrelial PotD homolog binds to Put with less affinity compared to binding of Spd or Spm, two polyamines leading to changes in the levels of several borrelial determinants (Fig 4). Supplementation with 4 mM Spd resulted in lower levels of PotD, while the same level of Put exhibited no effect in the untreated control (Fig 4B, α-PotD). Since *potABCD* is co-transcribed, it is possible that higher concentrations of Spd could regulate the levels of transcription of *potABCD*, as is the case previously demonstrated in *E. coli*, inhibitory effects of PotD were increased by Spd. Although we have not directly evaluated the binding constants with PotD, but the addition of exogenous Spd and Spm exhibited most dramatic effect on the borrelial proteins compared to other polyamines such as Cad or Put as well as polyamine precursors such as Arg and Ort (Fig 4B). Supplementation with Spd and Spm had the most dramatic effect on *B. burgdorferi* strain B31-A3 at pH7.6/32˚C (*in vitro* growth conditions not associated with increased levels of RpoS or *rpoS*-regulated proteins) with increased levels of RpoS and *rpoS*-regulated borrelial lipoproteins that are known to contribute to the colonization of Lyme spirochetes in the mammalian hosts such as OspC, DbpA, and BBK32 (Fig 5 B and C). The increase in the levels of OspC and RpoS with addition of Spd and Spm was similar to the effects of pH and temperature mimicking the fed tick mid-gut (Fig 5B, Lane 1, pH6.8/37˚C). This is in contrast to *B. burgdorferi* grown under conditions mimicking an unfed tick (Fig 5B Lane 2, pH7.6/23˚C) as well as under laboratory growth conditions (Fig 5B, Lane 3, pH7.6/32˚C) without Spd and Spm supplementation. Importantly, it should be pointed out that the levels of polyamines present in the conventional BSKII growth medium containing 6% normal rabbit serum were not sufficient to alter the levels of key borrelial proteins at pH7.6/32˚C while supplementation with Spd and Spm resulted in increased levels of the aforementioned proteins. However, the levels of FlaB (loading control), NapA, and P66 were similar in absence or presence of Spd or Spm and were similar to borrelial lysates from cultures propagated under conditions mimicking fed or unfed ticks indicating that polyamines exert effects only on specific borrelial determinants. These observations demonstrate that the supplementation with 4mM Spd or Spm increased levels of

RpoS and *rpoS*-regulated proteins in *B. burgdorferi* under *in vitro* growth conditions where the levels of these proteins are minimal. In summary, polyamines, and their transport serve to alter the levels of borrelial proteins in different microenvironments supporting the metabolic needs of the spirochetes in tick and mammalian hosts.

The effect of polyamines in response to growth medium pH has been studied extensively in *E. coli* (6-8, 47). When pH is low, a reduction in the entry of exogenous amines, and an increase in the production of membrane permeant amines via arginine/ornithine/lysine decarboxylases results in generation and efflux of putrescine and cadaverine to neutralize the acidity in the external environment is observed. This mechanism has been attributed to survival of enteric pathogens during their transit through the stomach. However, at high pH, deprotonated polyamines transit the cell membrane as weak bases, resulting in enhanced translation of RpoS, a critical sigma factor contributing to survival during stationary phase in *E. coli* (48-50). Although, there are significant pathogen-specific differences in the role of RpoS regulating gene expression between *E. coli* and *B. burgdorferi*, increased levels of polyamines resulted in elevated levels of RpoS and *rpoS*-regulated proteins in *B. burgdorferi.* One possible mechanism for this regulation is the acid stress response in *B. burgdorferi*, although the borrelial genome does not encode a variety of genes contributing to polyamine-dependent modulation of the acid-stress response as observed in *E. coli* (8). For example, 5 genes, glutamate decarboxylase B (*gadB*), glutamate-GABA antiporter (*gadC*), outer membrane lipoprotein (*slp*), periplasmic chaperone of acid-denatured proteins A (*hdeA*), and glutamate decarboxylase (*gadA*), involved in glutamate decarboxylase-dependent acid stress response were induced in *E. coli* following the addition of polyamine (13, 36). Since there are no apparent homologs of these genes in *B. burgdorferi* except for a glutamate transporter (GltP, BB0401),it is possible that the spirochetes possess enzymes that carry out similar functions but share little to no similarity to characterized homologs. Among three types of acid resistance systems (AR), the presence of known and unknown RpoS-dependent response (AR1), and the absence of apparent homologs of glutamate decarboxylase

517 (AR2) and arginine decarboxylase (AR3) indicate that *B. burgdorferi* presumably have limited
518 acid stress response mechanisms likely due to the near neutral pH they encounter, unlike the
519 dramatic pH changes encountered by enteric pathogens. The specific ability of Spd and Spm to
520 induce increased levels of RpoS and proteins of *rpoS*-regulated genes is indicative of a AR1-type
521 acid stress response in *B. burgdorferi*; although, all the key players of this system are yet to be
522 functionally characterized. Alternatively, it is possible that there exists functional redundancies
523 distributed over a large array of hypothetical proteins that could mediate the acid stress response
524 in *B. burgdorferi*.

525 Since supplementation of Spd or Spm induced levels of RpoS and *rpoS*
526 dependent/independent pathogenesis-related proteins of *B. burgdorferi*, we determined if addition
527 of hydrophobic 1, 3-dicyclohexylcarbodi-imide (DCC) or polar 1-ethyl-3-(3-
528 dimethylaminopropyl)carbodi-imide (EDC), both of which are known polyamine transport
529 inhibitors, alters the effects of 4mM Spd on the borrelial protein profile. Both these carbodi-
530 imides readily interact with carboxy groups of aspartate or glutamate residues that stabilize
531 carrier-substrate interactions during polyamine transport (44). We did not observe any major
532 alterations in the levels of the RpoS, OspC, DbpA, and BosR following addition of either DCC or
533 EDC in conjunction with added 4 mM Spd (Fig 6). Consistent with previous observations,
534 addition of Spd induced increased levels of the above proteins compared to untreated control. The
535 levels of PotA were higher in the control samples, but lower in the presence of Spd independent
536 of addition of DCC or EDC. It is possible that the lack of an effect seen when using these
537 compounds is due to the inaccessibility of carboxy groups of residues involved in stabilizing the
538 substrate interactions within the polyamine transport system in *B. burgdorferi*. Pursuit of
539 synthetic compounds interfering with polyamine uptake is used therapeutically in cancer
540 treatment. Non-metabolizable analogs of polyamines are an ideal group of drug candidates to test
541 against borrelial infection, as regular polyamines are able to modulate the expression of key
542 virulence-related proteins in *B. burgdorferi*. These therapeutic pursuits could potentially

543 reduce/prevent the colonization capabilities of spirochetes in infected hosts. Structural mimics of
544 polyamines offers a rational strategy to reduce or eliminate *B. burgdorferi* burden from infected
545 vertebrate hosts, and help in reducing the incidence of Lyme disease.

546 Lower levels of the long-form of *rpoS* was observed in *B. burgdorferi* exposed to Spd
547 compared to untreated or Spm-treated spirochetes; although the mRNA levels for *ospC* were
548 significantly higher following treatment with both polyamines (Fig 8). The short *rpoS* transcript,
549 however, was significantly higher in the Spd-treated spirochetes indicating differences in the
550 transcriptional levels of *rpoS* (Fig 9B). Significant differences were also noted in the levels of
551 *rpoS* transcripts in spirochetes grown to low or high densities in the presence of polyamines.
552 Interestingly, an increase in transcript levels specific to *ospA* was observed at low density while
553 both *rpoS* and *ospC* levels were higher in cultures grown to higher density in the presence of Spd.
554 The growth rates of spirochetes in the presence of 4 mM Spm and Spd also reflected the
555 differences noted in the levels of RpoS, OspC, with Spm treated spirochetes showing a
556 significantly lower cell density after 96 hrs (Fig 7). These observations indicate that the effects of
557 polyamines on borrelial gene expression/growth rates are affected by additional culture
558 conditions reflecting the complex regulatory nature of the adaptive gene expression in Lyme
559 spirochetes.

560 Previous studies have shown that polyamines increase the viability of *E. coli* in the
561 stationary phase by increasing the levels of ppGpp regulatory protein (SpoT) and the omega (ω)
562 subunit of the RNA polymerase (RpoZ) (5). We found that the transcript levels of *rpoZ* were
563 similar at low density with and without Spd, while the addition of Spd and growth to high density
564 led to a significant decrease in transcription. On the other hand, rel_{Bbu} (*bb0198*) was significantly
565 upregulated in the lower density culture with the addition of Spd (Fig 9C). Moreover, protein
566 levels of Rel_{Bbu} were higher in Spd-treated spirochetes grown to low density consistent with what
567 was observed at the transcriptional level (Fig 9D). Rel_{Bbu} is a bifunctional synthetase/hydrolase
568 controlling the levels of guanosine tetra/penta phosphate [(p)ppGpp]. (p)ppGpp has been shown

569 to play a role in the regulation of the stringent response during the survival of *B. burgdorferi* in
570 the tick vector or during starvation (2, 10). While temperature, growth phase, or pH of the media
571 did not affect the transcriptional level of rel_{Bbu}, shifting the spirochetes from nutritionally starved
572 media to that supplemented with rabbit serum significantly induced its transcription (10).
573 Although the culture conditions used in this study are different, we did observe that the addition
574 of Spd in low-density cultures significantly induced the levels of Rel_{Bbu} at both the
575 transcriptional and translational level (Fig 9 C&D). These observations indicate that polyamines,
576 specifically Spd and Spm, may contribute *B. burgdorferi* adaptation as it transits from nutrient
577 limited to nutrient enriched conditions within the midgut of the tick or during various stages of
578 dissemination through different tissues.

579 A key *in vitro* growth component that allows the spirochetes to recover from starvation is
580 rabbit serum, and this was reflected with increased transcription of rel_{Bbu} (2, 10). Importantly,
581 polyamines affect the surface composition, notably porins, in many bacterial species.
582 Furthermore, polyamines increase the ability of *Neisseria gonorrhoeae* to withstand the effects of
583 cationic antimicrobial peptides and complement-mediated killing (15). While the protein profile
584 of spirochetes grown in media with heat inactivated or normal rabbit serum were similar (Fig 10),
585 supplementation with 4 mM Spd with heat inactivated serum resulted in a noticeable increase in
586 the levels of OspC and DbpA compared levels with normal serum, although the levels of OspA
587 and OppA1 were similar under all conditions (Fig 10B). It is interesting to speculate that the
588 presence of heat-labile serum components interfering with the availability of Spd or alternatively,
589 the heat inactivated serum containing components that contribute to increased induction of levels
590 of OspC and DbpA in the presence of 4mM Spd.

591 Lyme spirochetes lack the full spectrum of homologs of 4 different pathways that have
592 been characterized in other bacterial systems for catabolism of arginine such as 1) arginase, 2)
593 arginine deiminase (ADI), 3) arginine succinyltransferase (AST), and 4) arginine transaminase
594 pathways except for the presence of some members of the ADI pathway: ArcA (BB_0841,

arginine deiminase), ArgF (BB_0842, ornithine carbamoyltransferase), and ArcD (BB_0843, arginine/ornithine antiporter) (46). ArcA is the first enzyme that converts arginine to citrulline with the release of NH_3 molecule (Fig 11). Citrulline then converted to carbamoyl phosphate by ArcB or to ornithine by ArgF. Since *B. burgdorferi* has an ORF annotated as ArgF, it is possible that increased amounts of arginine lead to increased levels of ornithine which would presumably be effluxed out via ArcD (arginine/ornithine antiporter). Since there is no apparent homolog of ArcC (carbamate kinase) in *B. burgdorferi*, it is unknown if carbamoyl-phosphate is indeed generated via the effects of ArcB and in turn converted to ammonia and carbon dioxide with concommitant generation of ATP by an ArcC-like homolog (Fig 11C). Taken together, these observations indicate that *B. burgdorferi* presumably possesses a truncated arginine deiminase pathway for metabolism of arginine, and therefore cannot utilize this polyamine precursor for the synthesis of polyamines. Moreover, there are no apparent homologs of the arginase pathway in *B. burgdorferi* such as RocF, RocD, and RocA, it is likely that *B. burgdorferi* is dependent on the host for polyamines. These bioinformatic-based observations indicate that *B. burgdorferi* has limited or truncated metabolic pathways underscoring unique pathogen-host interactions. In summary, understanding the mechanisms of transport of polyamines and their role in connecting the physiology of *B. burgdorferi* to virulence potential is certain to provide novel tools to reduce the borrelial burden in infected reservoir hosts; thereby, interfering with the transmission kinetics leading to a reduction in the incidence of Lyme disease.

ACKNOWLEDGEMENTS

This study was partly supported by Public Health Service grant SC1-AI-078559 from the National Institute of Allergy and Infectious Diseases, the Army Research Office of the Department of Defense under Contract No. W911NF-11-1-0136, pre-doctoral fellowships from the South Texas Center for Emerging Infectious Diseases (YHL, TAV, SLRK), and the Center For Excellence in Infection Genomics (TCS, CLM, TAV). TCS is supported by a pre-doctoral fellowship from The Brown Foundation. JAR and ANR were supported by fellowships from NIGMS- MBRS-RISE GM060655.

TABLES

Table 1. Oligonucleotides used in this study.

recAFq	ATGCTCTTGATCCTGTTTATGCAA
recARq	GGTATCAGGCTGACTAAGCCAAA
flaBFq	CAGCTAATGTTGCAAATCTTTTCTCT
flaBRq	TTCCTGTTGAACACCCTCTTGA
rpoSFq	AGATATGCGGGTAAAGGGTTAAAA
rpoSRq	CAGCAGCTCTTATTAATCCCAAGTT
ospCFq	AATCAGTAGAGGTCTTGTCAAAAGCA
ospCRq	CCACAACAGGGCTTGTAAGCT
ospAFq	CCAAAGACAAGTCATCAACAGAAGAA
ospARq	GGTTCCGTCTGCTCTTGTTATTATTT
PotA-B_int_F	TGTAATACGCCCAGAAGATG
PotA-B_int_R	TTCTCATCCAGGCATAAGTTC
PotB-C_int_F	TGAAGCATCACAAGATCTTGG
PotB-C_int_R	AGATTATGGCTATTAAAATGGTG
PotC-D_int_F	ATCCGGAAATCGCAGGAAG
PotC-D_int_R	GCCTTCATCGATTAATTCTTG
BB0639F/NdeI	ACGCCATATGAAAAAAATTTTTATATTAATAG
BB0639R/XhoI	ACGCCTCGAGAGAAGACAGAATTAAATATATT
BB0642F/NheI	TCGCCGCTAGCTTGGATAATTGTATCCTAGAG
BB0642R/XhoI	TGCGGCTCGAGTTCCTTATGCATAACATGAATATC
BB0198 qRT F	TGTTTACACCCCAGAAGGAGAAG
BB0198 qRT Rv	GCATAAAGAGCTTGATCTCCAATATCT
RpoZ q RT Fw	GCGCACTGAGCAAATTATAGACA
RpoZ q RT Rv	CGTCATTAAAAGCTTGCCCTAATAT

Table 2. Plasmids and strains used in this study.

Plasmids	**Description**	**Reference or source**
pCR2.1-TOPO	PCR cloning vector	Invitrogen
pET23a	Expression vector with a C-terminal 6-His tag	Novagen
pJR-100	*bb0639* cloned into pCR2.1	This study
pJR-101	*bb0639* cloned into pET23a	This study
pJR-102	*bb0642* cloned into pCR2.1	This study
pJR-103	*bb0642* cloned into pET23a	This study
***B. burgdorferi* strains**	**Description**	**Reference or source**
B31-A3 (wt)	B31, low passage, virulent isolate	(1)

Reference:

1. Elias AF, Stewart PE, Grimm D, Caimano MJ, Eggers CH, Tilly K, et al. Clonal polymorphism of *Borrelia burgdorferi* strain B31 MI: implications for mutagenesis in an infectious strain background. Infect Immun. 2002;**70**(4):2139-50. PMCID: 127854.

FIGURE LEGENDS

Fig 1 Polyamine transport system of *B. burgdorferi*. (A) Schematic representation of the polyamine transport system. PotD is localized in the periplasmic space, PotB and PotC make up a channel across the plasma membrane for polyamine access. PotA exhibits ATPase activity that facilitates polyamine transport. The model is based upon a polyamine transport system found in *E. coli* (19). (B) Organization of *potABCD* transport system in the chromosome of *B. burgdorferi*. The arrows with numbers refer to the primers used in the RT-PCR depicted below. (C) Transcriptional analysis of *potABCD* genes in *B. burgdorferi* strain B31-A3. The RT-PCR products were separated on a 1% agarose gel. Lanes 1 to 4, PCR using primer sets 1 and 2. ; lanes 5 to 8, PCR using primer sets 3 and 4; lanes 9 to 12, PCR using primer sets 5 and 6 . Templates used in all PCR amplifications are from B31-A3, and are as follows: lanes 1, 5, and 9: PCR master mix with no template (double-distilled water control); lanes 2, 6, and 10: total RNA (RT control); lanes 3, 7, and 11: cDNA (RT); lanes 4, 8, and 12: total genomic DNA.

Fig 2. Levels of PotA and PotD in *B. burgdorferi*. (A) Protein profile for *B. burgdorferi* grown under conditions mimicking the tick vector before (pH7.6/23˚C) and after (pH6.8/37˚C), a blood meal. (B) Immunoblot analysis with anti-PotD or -PotA antibodies reveals increased levels of PotA under unfed-tick mimicking conditions as described above. Molecular weight markers in kilodaltons (kDa) are indicated to the left.

Fig 3. PotD is a periplasmic protein of *B. burgdorferi*. Proteinase K treated (+PK) intact *B. burgdorferi* or untreated (-PK) were subjected to immunoblot analysis. (A) Coomassie blue-stained 12.5% SDS PAGE gel of *B. burgdorferi* depicting the protein profile before and after PK treatment. (B) Immunoblot analysis using monoclonal antibodies against FlaB or monospecific serum against PotD, PotA, and P66. Molecular weight markers in kilodaltons (kDa) are indicated to the left.

731

732 **Fig 4. Effect of polyamines or their precursors on *B. burgdorferi* protein profile.** *B.*
733 *burgdorferi* was grown in the absence (Ctrl) or presence of 4 mM spermidine (Spd), spermine
734 (Spm), arginine (Arg), cadaverine (Cad), ornithine (Ort), and putrescine (Put) at 32°C/pH 7.6. (A)
735 Total cell lysates were separated on a 12.5% SDS-PAGE gel, and stained with Coomassie
736 brilliant blue. (B) Proteins were transferred to a PVDF membrane, and probed with specific
737 antisera indicated to the right. Several proteins critical for the colonization of the vertebrate host
738 were up-regulated in the presence of Spd and Spm while the effect of other
739 polyamines/polyamine precursors were less dramatic. Molecular weight markers in kilodaltons
740 (kDa) are indicated to the left. Blot probed with RpoS, exposed for a longer period of time, is
741 indicated by an asterisk.

742

743 **Fig 5. Effect of spermidine and spermine on virulence associated proteins of *B. burgdorferi.***
744 Spirochetes were grown under different *in vitro* growth conditions mimicking the tick vector
745 before (pH7.6/23˚C), after (pH 6.8/37˚C) blood meal or under laboratory growth conditions
746 (pH7.6/32˚C) supplemented with 4mM Spd or Spm. (A) Total cell lysates were separated on a
747 12.5% SDS-PAGE gel and stained with Coomassie brilliant blue. (B) Immunoblot analysis of
748 borrelial samples grown under conditions mimicking the fed state (Lane 1), unfed state (Lane 2)
749 of tick midgut, laboratory growth conditions (Lane 3) and laboratory growth conditions plus
750 4mM Spd (Lane 4) or 4 mM Spm (Lane 5). (C) Immunoblot analysis of *B. burgdorferi* grown
751 under laboratory growth condition alone (Lane 3) and with either 4 mM Spd (Lane 4) or Spm
752 (Lane 5). Molecular weight markers in kilodaltons (kDa) are indicated to the left.

753

754 **Fig 6. Effects polyamine transport inhibitors on *B. burgdorferi.*** *B. burgdorferi* grown under
755 laboratory growth conditions (Lane 1, pH7.6/32˚C) and in the presence of 4mM Spm (Lane 2)
756 was treated with 5mM DCC (Lane 3) or 5mM EDC (Lane 4) . (A) Total cell lysates were

757 separated on a 12.5% SDS-PAGE gel and stained with Coomassie brilliant blue. (B) Immunoblot
758 analysis with serum generated against PotA, RpoS, BosR, DbpA, and OspC. The inhibitors do not
759 appear to lead to alterations in protein expression levels. Molecular weight markers in
760 kilodaltons (kDa) indicated to the right.
761
762 **Fig 7. Growth rates of *B. burgdorferi* in the presence of spermidine or spermine**. *B.*
763 *burgdorferi* was grown in triplicate in the absence or presence of 4 mM spermine or spermidine
764 at 32˚C/pH7.6. Growth was monitored every 24 hrs for 168 (Spm) or 120 (Spd) hrs by examining
765 the motility of spirochetes as a measure of viability by dark field microscopy. A representative
766 experiment is shown.
767
768 **Fig 8. Effect of spermine and spermidine on borrelial gene expression.** Spirochetes were
769 grown at pH 7.6/32°C in BSK-II growth medium (white bars), and in the presence of 4mM Spd
770 (checkered bar) or 4mM Spm (hatched bar). Spirochetes were collected at 100 spirochetes per
771 field. RNA was isolated and subjected to reverse transcription-PCR using primers specific for
772 *rpoS, flaB, ospC,* and *ospA*. Primers specific for *recA* were used for normalization of the
773 transcript levels between samples under different growth conditions. Normalized C_T values were
774 subjected to unpaired Student's *t*-test using PRISM software. Statistical significance was accepted
775 when *P* values were less than 0.05. Asterisks indicate samples whose C_T values are statistically
776 significantly different between untreated control and treated sample. (***, $P < 0.001$).
777
778 **Fig 9. Effect of spermidine on density-dependent borrelial gene expression.** Spirochetes were
779 propagated at pH 7.6/32°C in BSK-II growth medium in the absence (white bars) or presence
780 (black bars) of 4mM spermidine, and collected at 40 cells (LD; low density) or 100 cells (HD,
781 high density) per field. RNA was isolated and subjected to reverse transcription-PCR using
782 primers specific for *ospA*, *rpoS*, and *ospC (*A); short or long *rpoS* (B); *rpoZ* and rel_{Bbu} (C).

783 Samples were normalized relative to the value for *recA*. C_T values were collected and subjected to
784 unpaired Student's *t* test using Prism software. The asterisks indicate samples whose C_T values
785 are statistically significantly different between untreated control and treated sample. (***, P <
786 0.001). (D) Immunoblot analysis of lysates of *B. burgdorferi* grown in the absence (-) or presence
787 (+) of 4 mM Spd with anti-BB0198 (Rel_{Bbu}) serum. Molecular weight markers in kilodaltons
788 (kDa) indicated to the right.
789
790 **Figure 10. Effect of serum and polyamines on *B. burgdorferi.*** Spirochetes were grown
791 (pH7.6/32˚C) in the presence of heat inactivated (IS) or normal rabbit serum (NS) either without
792 (-) or with (+) Spd. (A) Total cell lysates were separated on a 12.5% SDS-PAGE gel and stained
793 with Coomassie brilliant blue. (B) Immunoblot analysis with antibodies against OspC, DbpA,
794 OspA, and OppA1. Molecular weight markers in kilodaltons (kDa) are indicated to the right.
795
796 **Fig 11. Effect of arginine on select borrelial proteins.** *B. burgdorferi* were grown in the
797 absence or presence (up to 20 mM) of arginine until the spirochete density reached $1x10^8$ cells
798 per ml. (A) Total cell lysates were separated on a 12.5% SDS-PAGE gel, and stained with
799 Coomassie brilliant blue. (B) Immunoblot analysis with antibodies against P66, PotA, PotD, and
800 ArcA. Molecular weight markers in kilodaltons (kDa) are indicated to the right. (C). A
801 bioinformatic based model for the uptake and conversion of arginine to citrulline and ornithine.
802
803 **Supplementary Figures S1 and S2. Growth rates of *B. burgdorferi* in the presence of**
804 **spermidine or spermine**. *B. burgdorferi* was grown in triplicate in the absence or presence 4
805 mM spermidine under *in vitro* growth conditions mimicking unfed (pH7.6/23˚C) or fed ticks
806 (pH6.8/37˚C) . Growth was monitored every 24 hrs for 192 hrs by examining the motility of
807 spirochetes as a measure of viability by dark field microscopy. A representative experiment is
808 shown.

REFERENCES

1. **Barbour, A. G.** 1989. The molecular biology of Borrelia. Rev Infect Dis **11 Suppl 6:**S1470-4.
2. **Bugrysheva, J. V., C. J. Pappas, D. A. Terekhova, R. Iyer, H. P. Godfrey, I. Schwartz, and F. C. Cabello.** 2015. Characterization of the RelBbu Regulon in Borrelia burgdorferi Reveals Modulation of Glycerol Metabolism by (p)ppGpp. PLoS One **10:**e0118063.
3. **Caimano, M. J., D. Drecktrah, F. Kung, and D. S. Samuels.** 2016. Interaction of the Lyme disease spirochete with its tick vector. Cell Microbiol **18:**919-27.
4. **Carroll, J. A., R. M. Cordova, and C. F. Garon.** 2000. Identification of 11 pH-regulated genes in Borrelia burgdorferi localizing to linear plasmids. Infect Immun **68:**6677-84.
5. **Chatterji, D., Y. Ogawa, T. Shimada, and A. Ishihama.** 2007. The role of the omega subunit of RNA polymerase in expression of the relA gene in Escherichia coli. FEMS Microbiol Lett **267:**51-5.
6. **Chattopadhyay, M. K., C. N. Keembiyehetty, W. Chen, and H. Tabor.** 2015. Polyamines Stimulate the Level of the sigma38 Subunit (RpoS) of Escherichia coli RNA Polymerase, Resulting in the Induction of the Glutamate Decarboxylase-dependent Acid Response System via the gadE Regulon. J Biol Chem **290:**17809-21.
7. **Chattopadhyay, M. K., C. W. Tabor, and H. Tabor.** 2003. Polyamines protect Escherichia coli cells from the toxic effect of oxygen. Proc Natl Acad Sci U S A **100:**2261-5.
8. **Chattopadhyay, M. K., and H. Tabor.** 2013. Polyamines are critical for the induction of the glutamate decarboxylase-dependent acid resistance system in Escherichia coli. J Biol Chem **288:**33559-70.
9. **Corona, A., and I. Schwartz.** 2015. Borrelia burgdorferi: Carbon Metabolism and the Tick-Mammal Enzootic Cycle. Microbiol Spectr **3**.
10. **Drecktrah, D., M. Lybecker, N. Popitsch, P. Rescheneder, L. S. Hall, and D. S. Samuels.** 2015. The Borrelia burgdorferi RelA/SpoT Homolog and Stringent Response Regulate Survival in the Tick Vector and Global Gene Expression during Starvation. PLoS Pathog **11:**e1005160.
11. **Esteve-Gassent, M. D., N. L. Elliott, and J. Seshu.** 2009. sodA is essential for virulence of Borrelia burgdorferi in the murine model of Lyme disease. Mol Microbiol **71:**594-612.
12. **Esteve-Gassent, M. D., T. C. Smith, 2nd, C. M. Small, D. P. Thomas, and J. Seshu.** 2015. Absence of sodA Increases the Levels of Oxidation of Key Metabolic Determinants of Borrelia burgdorferi. PLoS One **10:**e0136707.
13. **Foster, J. W.** 2004. Escherichia coli acid resistance: tales of an amateur acidophile. Nat Rev Microbiol **2:**898-907.
14. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. Weidman, T. Utterback, L. Watthey, L. McDonald, P. Artiach, C. Bowman, S. Garland, C. Fuji, M. D. Cotton, K.**

Horst, K. Roberts, B. Hatch, H. O. Smith, and J. C. Venter. 1997. Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi. Nature **390:**580-6.

15. **Goytia, M., and W. M. Shafer.** 2010. Polyamines can increase resistance of Neisseria gonorrhoeae to mediators of the innate human host defense. Infect Immun **78:**3187-95.

16. **Groshong, A. M., and J. S. Blevins.** 2014. Insights into the biology of Borrelia burgdorferi gained through the application of molecular genetics. Adv Appl Microbiol **86:**41-143.

17. **Hyde, J. A., J. P. Trzeciakowski, and J. T. Skare.** 2007. Borrelia burgdorferi alters its gene expression and antigenic profile in response to CO2 levels. J Bacteriol **189:**437-45.

18. **Igarashi, K., K. Ito, and K. Kashiwagi.** 2001. Polyamine uptake systems in Escherichia coli. Res Microbiol **152:**271-8.

19. **Igarashi, K., and K. Kashiwagi.** 1999. Polyamine transport in bacteria and yeast. Biochem J **344 Pt 3:**633-42.

20. **Iyer, R., M. J. Caimano, A. Luthra, D. Axline, Jr., A. Corona, D. A. Iacobas, J. D. Radolf, and I. Schwartz.** 2015. Stage-specific global alterations in the transcriptomes of Lyme disease spirochetes during tick feeding and following mammalian host adaptation. Mol Microbiol **95:**509-38.

21. **Karna, S. L., R. G. Prabhu, Y. H. Lin, C. L. Miller, and J. Seshu.** 2013. Contributions of environmental signals and conserved residues to the functions of carbon storage regulator A of Borrelia burgdorferi. Infect Immun **81:**2972-85.

22. **Karna, S. L., E. Sanjuan, M. D. Esteve-Gassent, C. L. Miller, M. Maruskova, and J. Seshu.** 2011. CsrA modulates levels of lipoproteins and key regulators of gene expression critical for pathogenic mechanisms of Borrelia burgdorferi. Infect Immun **79:**732-44.

23. **Kwon, D. H., and C. D. Lu.** 2007. Polyamine effects on antibiotic susceptibility in bacteria. Antimicrob Agents Chemother **51:**2070-7.

24. **Kwon, D. H., and C. D. Lu.** 2006. Polyamines increase antibiotic susceptibility in Pseudomonas aeruginosa. Antimicrob Agents Chemother **50:**1623-7.

25. **Kwon, D. H., and C. D. Lu.** 2006. Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in Pseudomonas aeruginosa PAO1. Antimicrob Agents Chemother **50:**1615-22.

26. **Lane, R. S., J. Piesman, and W. Burgdorfer.** 1991. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annu Rev Entomol **36:**587-609.

27. **Lybecker, M. C., and D. S. Samuels.** 2007. Temperature-induced regulation of RpoS by a small RNA in Borrelia burgdorferi. Mol Microbiol **64:**1075-89.

28. **Maruskova, M., M. D. Esteve-Gassent, V. L. Sexton, and J. Seshu.** 2008. Role of the BBA64 locus of Borrelia burgdorferi in early stages of infectivity in a murine model of Lyme disease. Infect Immun **76:**391-402.

29. **Maruskova, M., and J. Seshu.** 2008. Deletion of BBA64, BBA65, and BBA66 loci does not alter the infectivity of Borrelia burgdorferi in the murine model of Lyme disease. Infect Immun **76:**5274-84.

30. **Michael, A. J.** 2016. Polyamines in Eukaryotes, Bacteria and Archaea. J Biol Chem.

31. **Miller, C. L., S. L. Karna, and J. Seshu.** 2013. Borrelia host adaptation Regulator (BadR) regulates rpoS to modulate host adaptation and virulence factors in Borrelia burgdorferi. Mol Microbiol **88:**105-24.
32. **Minton, K. W., H. Tabor, and C. W. Tabor.** 1990. Paraquat toxicity is increased in Escherichia coli defective in the synthesis of polyamines. Proc Natl Acad Sci U S A **87:**2851-5.
33. **Ojaimi, C., C. Brooks, S. Casjens, P. Rosa, A. Elias, A. Barbour, A. Jasinskas, J. Benach, L. Katona, J. Radolf, M. Caimano, J. Skare, K. Swingle, D. Akins, and I. Schwartz.** 2003. Profiling of temperature-induced changes in Borrelia burgdorferi gene expression by using whole genome arrays. Infect Immun **71:**1689-705.
34. **Radolf, J. D., M. J. Caimano, B. Stevenson, and L. T. Hu.** 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. Nat Rev Microbiol **10:**87-99.
35. **Raju, B. V., M. D. Esteve-Gassent, S. L. Karna, C. L. Miller, T. A. Van Laar, and J. Seshu.** 2011. Oligopeptide permease A5 modulates vertebrate host-specific adaptation of Borrelia burgdorferi. Infect Immun **79:**3407-20.
36. **Richard, H. T., and J. W. Foster.** 2003. Acid resistance in Escherichia coli. Adv Appl Microbiol **52:**167-86.
37. **Samuels, D. S.** 2011. Gene regulation in Borrelia burgdorferi. Annu Rev Microbiol **65:**479-99.
38. **Schwan, T. G., J. Piesman, W. T. Golde, M. C. Dolan, and P. A. Rosa.** 1995. Induction of an outer surface protein on Borrelia burgdorferi during tick feeding. Proc Natl Acad Sci U S A **92:**2909-13.
39. **Seshu, J., J. A. Boylan, F. C. Gherardini, and J. T. Skare.** 2004. Dissolved oxygen levels alter gene expression and antigen profiles in Borrelia burgdorferi. Infect Immun **72:**1580-6.
40. **Seshu, J., M. D. Esteve-Gassent, M. Labandeira-Rey, J. H. Kim, J. P. Trzeciakowski, M. Hook, and J. T. Skare.** 2006. Inactivation of the fibronectin-binding adhesin gene bbk32 significantly attenuates the infectivity potential of Borrelia burgdorferi. Mol Microbiol **59:**1591-601.
41. **Shah, P., and E. Swiatlo.** 2008. A multifaceted role for polyamines in bacterial pathogens. Mol Microbiol **68:**4-16.
42. **Stevenson, B., T. G. Schwan, and P. A. Rosa.** 1995. Temperature-related differential expression of antigens in the Lyme disease spirochete, Borrelia burgdorferi. Infect Immun **63:**4535-9.
43. **Tabor, C. W., and H. Tabor.** 1985. Polyamines in microorganisms. Microbiol Rev **49:**81-99.
44. **Torossian, K., M. Audette, and R. Poulin.** 1996. Substrate protection against inactivation of the mammalian polyamine-transport system by 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide. Biochem J **319 (Pt 1):**21-6.
45. **Van Laar, T. A., Y. H. Lin, C. L. Miller, S. L. Karna, J. P. Chambers, and J. Seshu.** 2012. Effect of levels of acetate on the mevalonate pathway of Borrelia burgdorferi. PLoS One **7:**e38171.

46. **Xiong, L., J. L. Teng, M. G. Botelho, R. C. Lo, S. K. Lau, and P. C. Woo.** 2016. Arginine Metabolism in Bacterial Pathogenesis and Cancer Therapy. Int J Mol Sci **17**.
47. **Yohannes, E., A. E. Thurber, J. C. Wilks, D. P. Tate, and J. L. Slonczewski.** 2005. Polyamine stress at high pH in Escherichia coli K-12. BMC Microbiol **5:**59.
48. **Yoshida, M., K. Kashiwagi, G. Kawai, A. Ishihama, and K. Igarashi.** 2002. Polyamines enhance synthesis of the RNA polymerase sigma 38 subunit by suppression of an amber termination codon in the open reading frame. J Biol Chem **277:**37139-46.
49. **Yoshida, M., K. Kashiwagi, A. Shigemasa, S. Taniguchi, K. Yamamoto, H. Makinoshima, A. Ishihama, and K. Igarashi.** 2004. A unifying model for the role of polyamines in bacterial cell growth, the polyamine modulon. J Biol Chem **279:**46008-13.
50. **Yoshida, M., D. Meksuriyen, K. Kashiwagi, G. Kawai, and K. Igarashi.** 1999. Polyamine stimulation of the synthesis of oligopeptide-binding protein (OppA). Involvement of a structural change of the Shine-Dalgarno sequence and the initiation codon aug in oppa mRNA. J Biol Chem **274:**22723-8.

Fig 1

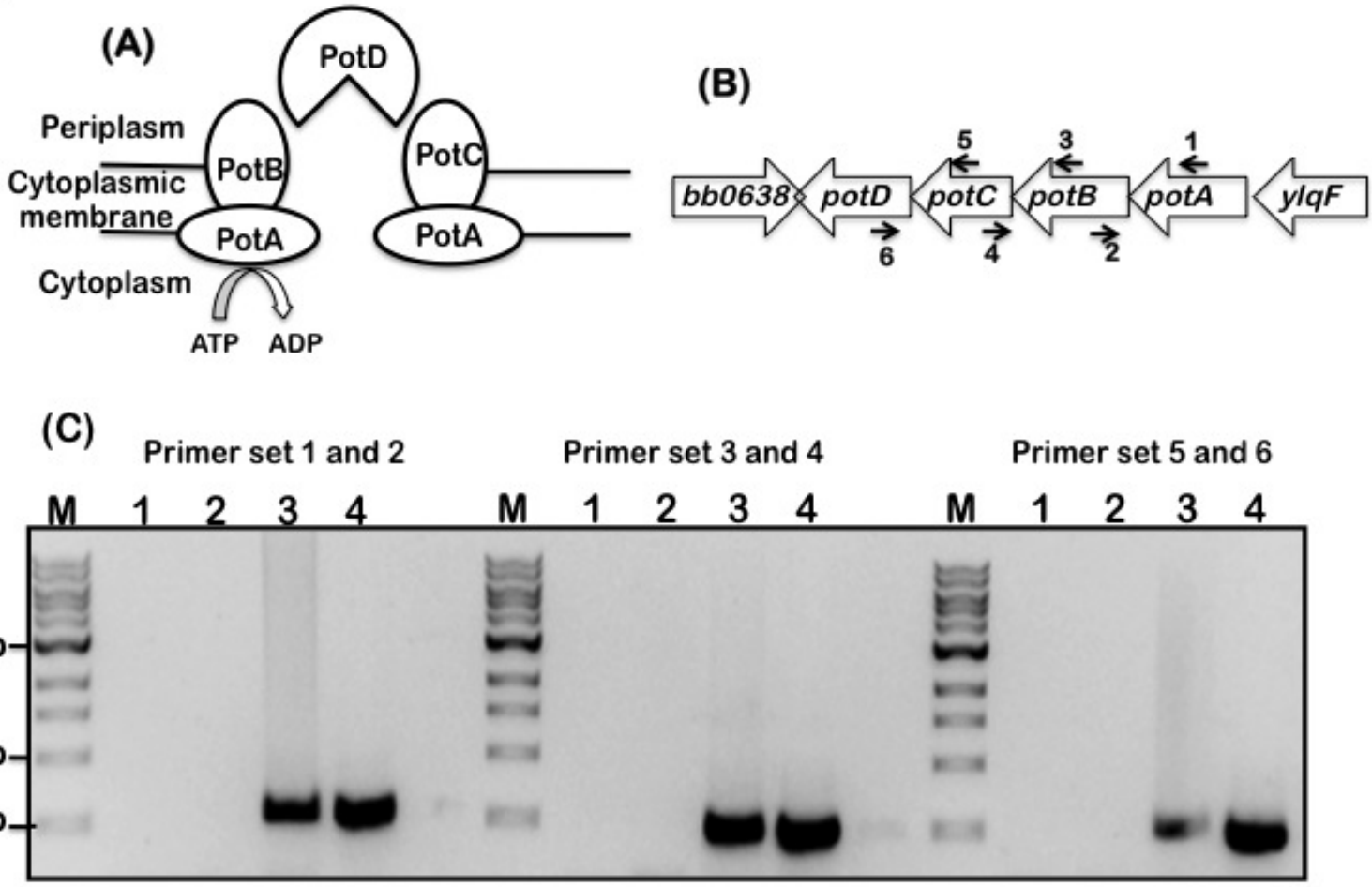

(A)
PotD
Periplasm
PotB
PotC
Cytoplasmic membrane
PotA
PotA
Cytoplasm
ATP
ADP
(B)
bb0638
potD
potC
potB
potA
ylqF
5
3
1
6
4
2
(C)
Primer set 1 and 2
Primer set 3 and 4
Primer set 5 and 6
M 1 2 3 4
M 1 2 3 4
M 1 2 3 4
3kb
1kb
0.5kb

Fig 2

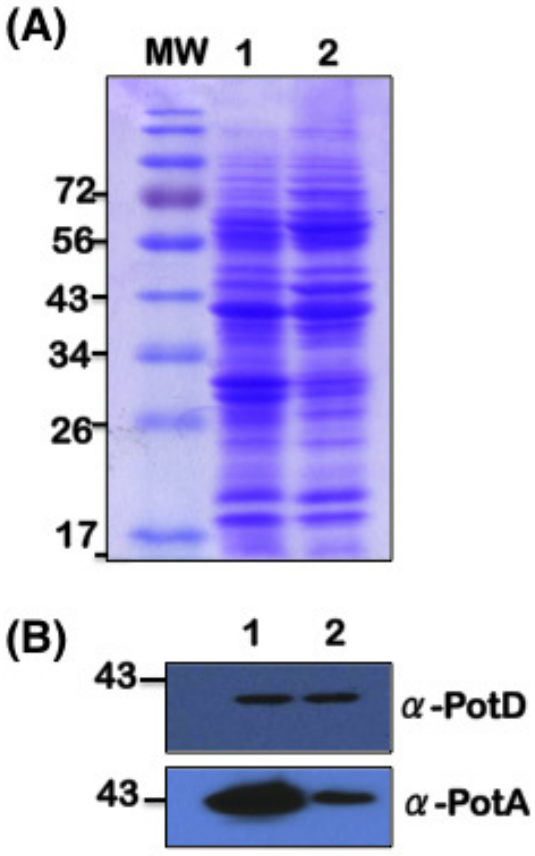


Fig 3

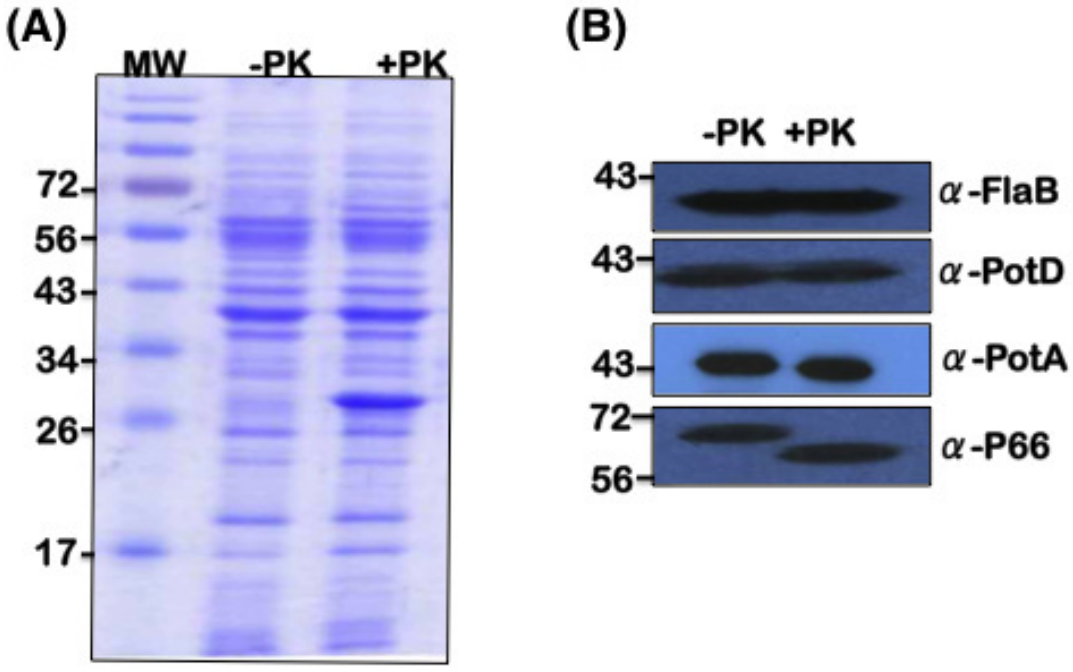

(A)
MW
-PK
+PK
72
56
43
34
26
17
(B)
-PK
+PK
43
α-FlaB
43
α-PotD
43
α-PotA
72
56
α-P66

Fig 4

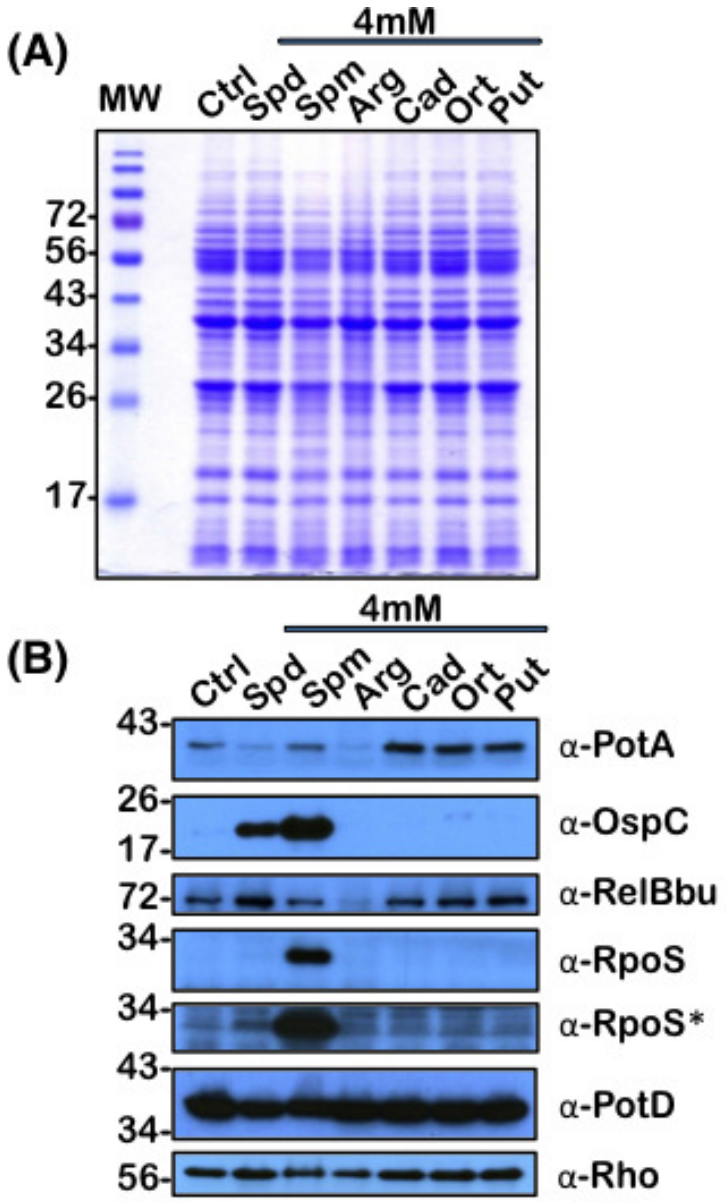

(A)
4mM
MW
Ctrl
Spd
Spm
Arg
Cad
Ort
Put
72
56
43
34
26
17
(B)
4mM
Ctrl
Spd
Spm
Arg
Cad
Ort
Put
43
α-PotA
26
17
α-OspC
72
α-RelBbu
34
α-RpoS
34
α-RpoS*
43
34
α-PotD
56
α-Rho

Fig 5

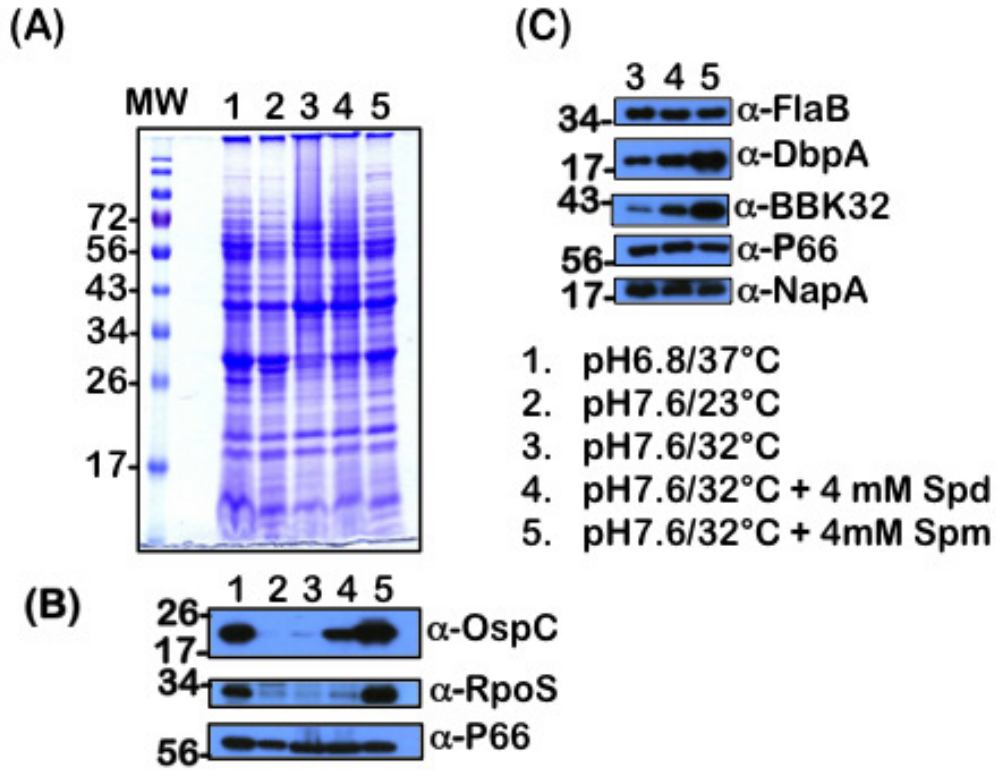
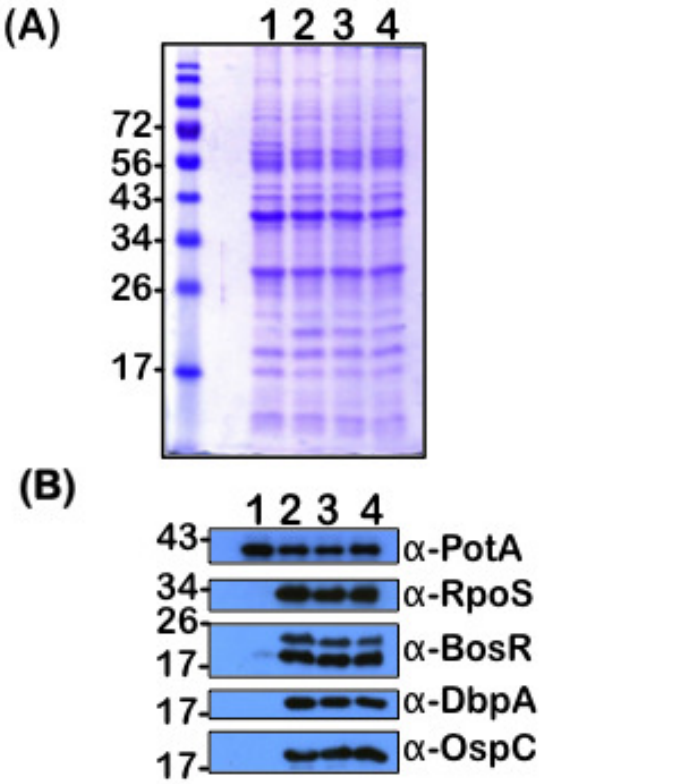
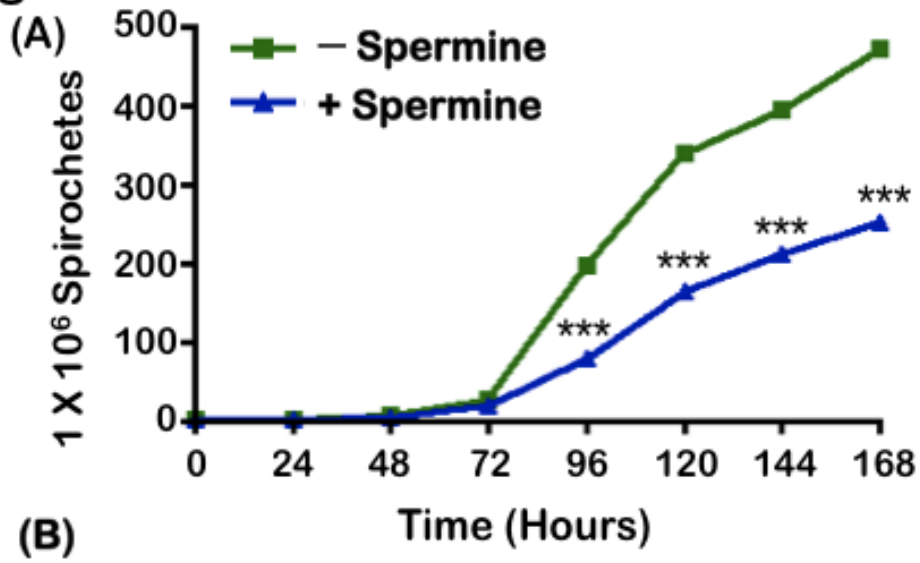

(A)
MW 1 2 3 4 5
72
56
43
34
26
17
(B)
1 2 3 4 5
26
17
34
56
α-OspC
α-RpoS
α-P66
(C)
3 4 5
34
17
43
56
17
α-FlaB
α-DbpA
α-BBK32
α-P66
α-NapA
1. pH6.8/37°C
2. pH7.6/23°C
3. pH7.6/32°C
4. pH7.6/32°C + 4 mM Spd
5. pH7.6/32°C + 4mM Spm

Fig 6



1. A3 Untreated
2. A3 + 4mM SPM
3. A3 + 4mM SPM +5mM EDC
4. A3 + 4mM SPM +5mM DCC

Fig 7


(A)
500
400
300
200
100
0
1 X 10⁶ Spirochetes
– Spermine
+ Spermine
***
***
***
***
0
24
48
72
96
120
144
168
Time (Hours)

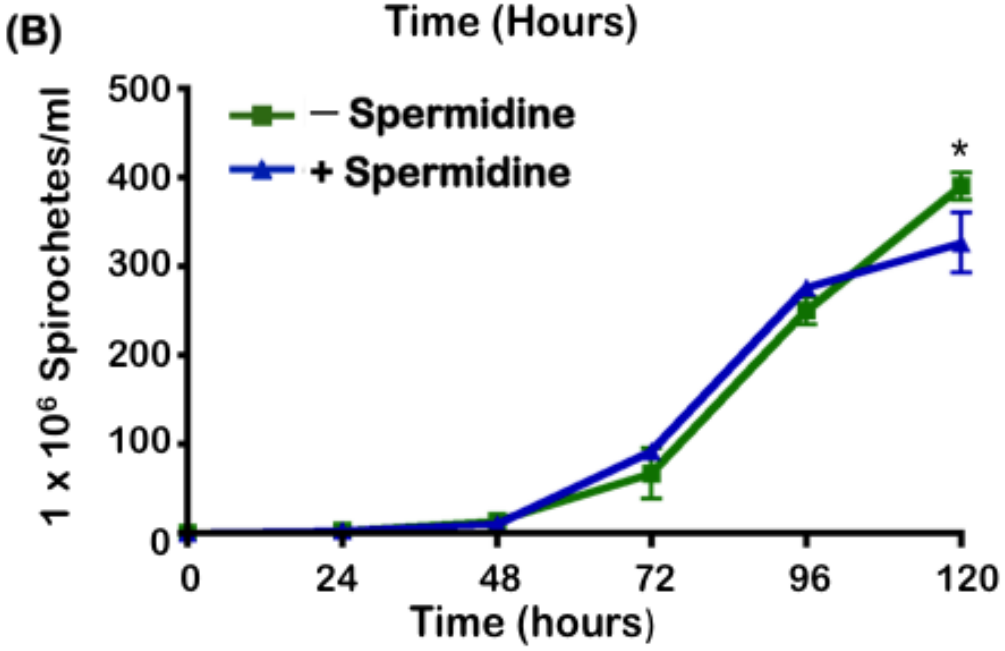

(B)
500
400
300
200
100
0
1 x 10⁶ Spirochetes/ml
– Spermidine
+ Spermidine
*
0
24
48
72
96
120
Time (hours)

Fig 8

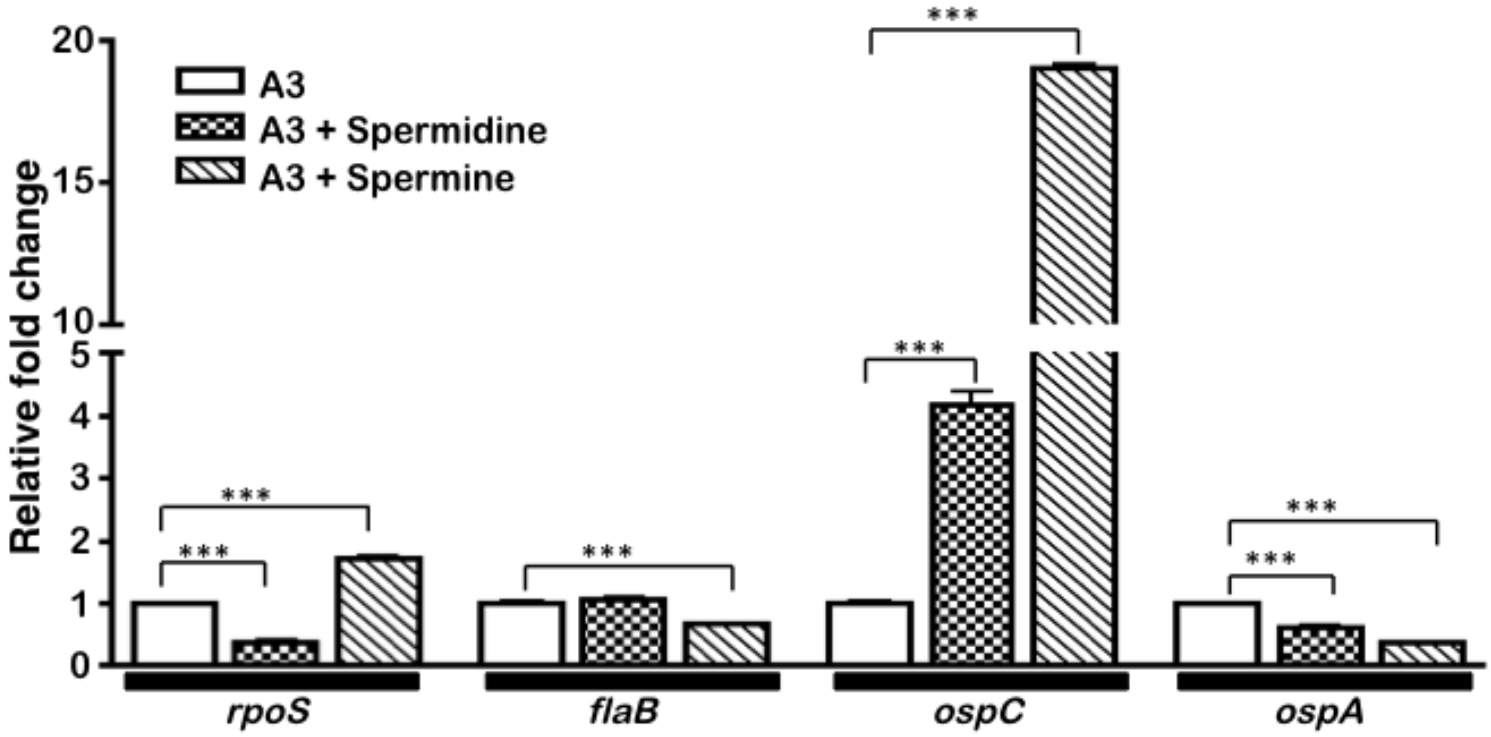

Relative fold change
20
15
10
5
4
3
2
1
0
A3
A3 + Spermidine
A3 + Spermine
***
***
***
***
***
***
rpoS
flaB
ospC
ospA

Fig 9

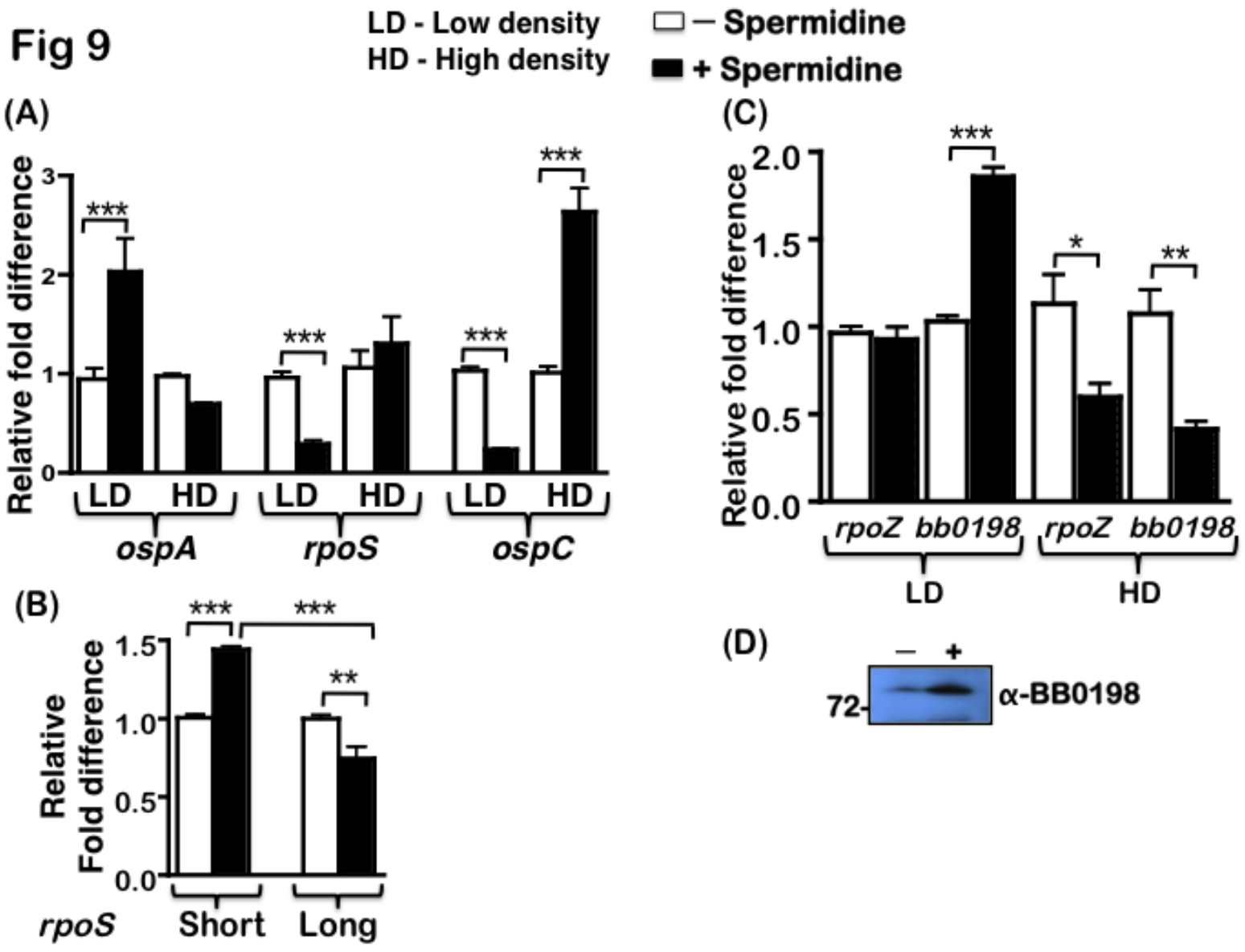

LD - Low density
HD - High density
– Spermidine
+ Spermidine
(A)
Relative fold difference
***
LD
HD
ospA
rpoS
ospC
(B)
Relative Fold difference
**
rpoS
Short
Long
(C)
rpoZ
bb0198
*
(D)
72-
α-BB0198

Fig 10

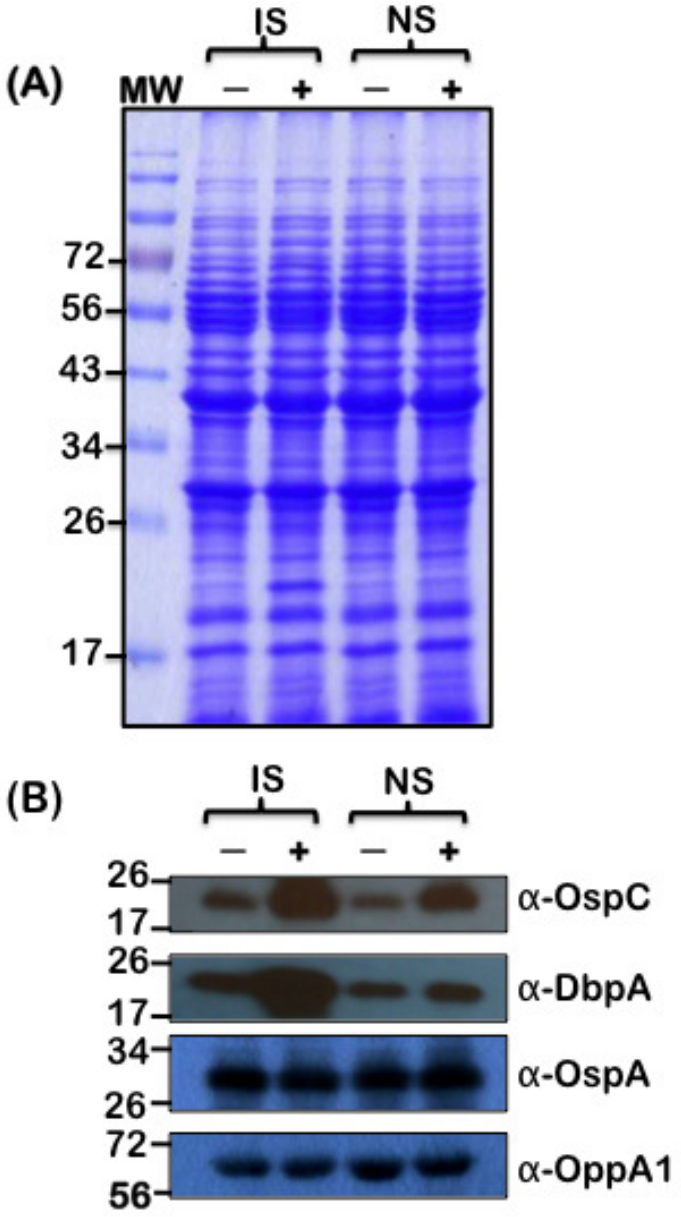
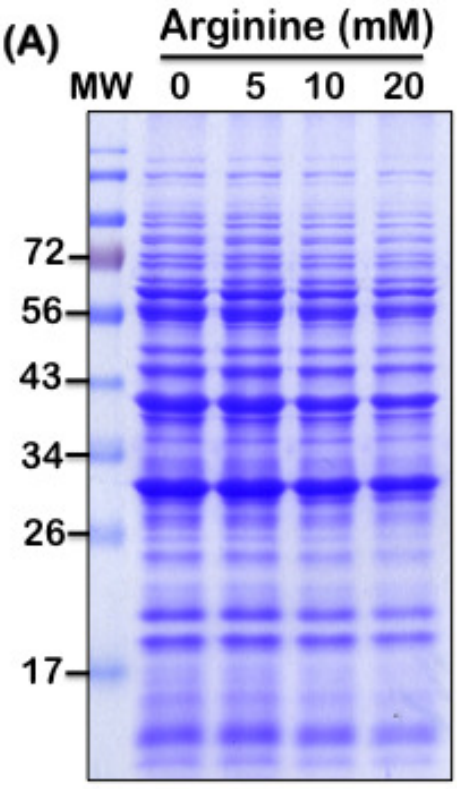
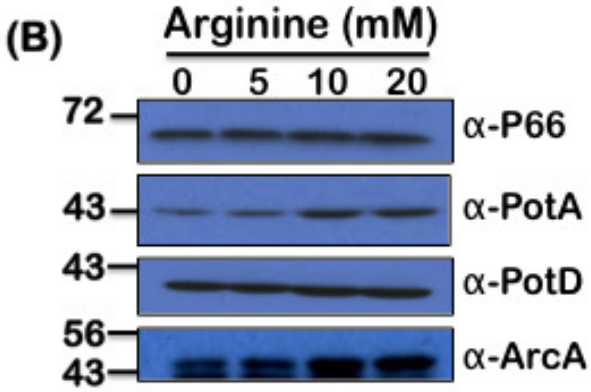
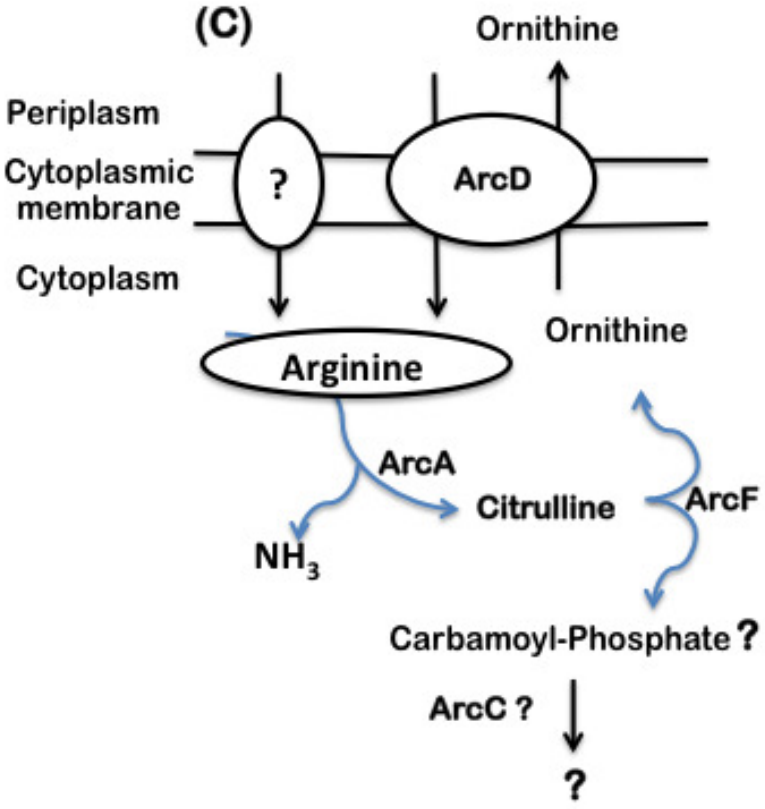

(A)
IS
NS
MW
–
+
–
+
72
56
43
34
26
17
(B)
IS
NS
–
+
–
+
26
17
α-OspC
26
17
α-DbpA
34
26
α-OspA
72
56
α-OppA1

Fig 11


(A)
Arginine (mM)
MW
0
5
10
20
72
56
43
34
26
17


(B)
Arginine (mM)
0
5
10
20
72
α-P66
43
α-PotA
43
α-PotD
56
43
α-ArcA


(C)
Ornithine
Periplasm
Cytoplasmic membrane
Cytoplasm
?
ArcD
Ornithine
Arginine
ArcA
Citrulline
ArcF
NH3
Carbamoyl-Phosphate ?
ArcC ?
?