



a putative putrescine/spermidine transport system PotABCD that is spermidine specific and essential for cell survival

Sébastien Bontemps-Gallo, Kevin A Lawrence, Crystal L Richards, Frank C
Gherardini

► To cite this version:

Sébastien Bontemps-Gallo, Kevin A Lawrence, Crystal L Richards, Frank C Gherardini. a putative putrescine/spermidine transport system PotABCD that is spermidine specific and essential for cell survival. *Molecular Microbiology*, 2018, 108 (4), pp.639 - 0642. 10.1111/mmi.13940 . hal-02947481

HAL Id: hal-02947481

<https://hal.science/hal-02947481>

Submitted on 24 Sep 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Borrelia burgdorferi genes, *bb0639-0642*, encode a putative putrescine/spermidine transport system PotABCD that is spermidine specific and essential for cell survival

Sébastien Bontemps-Gallo, Kevin A. Lawrence, Crystal L. Richards, Frank C. Gherardini*

Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

Running Head: PotABCD is essential for *Borrelia burgdorferi*

*Corresponding author:

FGherardini@nih.gov, Laboratory of Bacteriology, Rocky Mountain Laboratories, Hamilton, 59840, MT, USA Phone: +1 406-363-9474, Fax: +1 406-375-9681

Keywords: Lyme disease / polyamine / *potABCD* / tick / regulation

Summary

Polyamines are an essential class of metabolites found throughout all kingdoms in life. *Borrelia burgdorferi* harbors no enzymes to synthesize or degrade polyamines yet does contain a polyamine uptake system, *potABCD*. In this report, we describe the initial characterization of this putative transport system. After several unsuccessful attempts to inactivate *potABCD*, we placed the operon under the control of an inducible LacI promoter expression system. Analyses of this construct confirmed that *potABCD* was required for *in vitro* survival. Additionally, we demonstrated that the *potABCD* operon were upregulated *in vitro* by low osmolarity. Previously, we had shown that low osmolarity triggers the activation of the Rrp2/RpoN/RpoS regulatory cascade which regulates genes essential for the transmission of spirochetes from ticks to mammalian hosts. Interestingly, induction of the *pot* operon was only affected in an *rpoS* mutant but not in a *rpoN* mutant, suggesting that the genes were RpoS-dependent, RpoN-independent. Furthermore, *potABCD* was upregulated during tick feeding concomitant with the initiation of spirochete replication. Finally, uptake experiments determined the specificity of *B. burgdorferi*'s PotABCD for spermidine. Our studies demonstrate that *potABCD* was essential for survival, regulated in an RpoS-dependent, RpoN-independent manner, and regulated concurrent with the drop in osmolarity observed in the midgut of feeding ticks.

Introduction

Borrelia burgdorferi, one of the causative agents of Lyme disease (Pritt *et al.*, 2016), is maintained in a life cycle that involves a vertebrate host (*e.g.* rodent) and a hard tick: *Ixodes scapularis* (Stewart & Rosa, 2017). During its infectious cycle, *B. burgdorferi* adapts its physiology and metabolism in response to changing environmental conditions in the mammal or the arthropod vector (Caimano *et al.*, 2016, Gherardini *et al.*, 2010). The mammalian host offers this spirochete a nutrient-rich, homeostatic environment (temperature, pH, osmolarity, etc.) (Waymouth, 1970, The staff of the Jackson Laboratory, 1966). In addition, *B. burgdorferi* uses several mechanisms for evading the aggressive host immune system, allowing it to successfully colonize various mammalian species, including humans (Norris, 2014, Petzke & Schwartz, 2015, Radolf *et al.*, 2012). In contrast, environmental parameters inside the arthropod vector are quite dynamic over the course of its life cycle. Studies have shown that the *I. scapularis* midgut has shifting physio-chemical parameters (temperature: 28°C → 37°C; osmolarity: 600 → 300 → 600 mOsM) (Bontemps-Gallo *et al.*, 2016, Schwan *et al.*, 1995, Stevenson *et al.*, 1995) and, nitrosative and oxidative bursts that peak during the feeding process (Bourret *et al.*, 2016, Sonenshine & Anderson, 2014). In response to fluctuating environmental conditions, *B. burgdorferi* modulates its gene expression program to allow it to successfully colonize both host and vector.

A small set of regulators allow *B. burgdorferi* to adapt to changing conditions throughout the infectious process. Upon colonization of *I. scapularis*, the spirochetes upregulate the expression of genes involved in carbohydrate utilization, cell envelope biosynthesis and long-term survival through the two-component system, Hk1-Rrp1 (Caimano *et al.*, 2011, He *et al.*, 2011, Kostick *et al.*, 2011). This two-component system regulates c-di-GMP turnover through the diguanylate cyclase activity on Rrp1 (Caimano *et al.*, 2011, He *et al.*, 2011). *In vitro*, Hk1-Rrp1 is stimulated by high osmolarity (600 mOsM) (Bontemps-Gallo *et al.*, 2016). Long-term survival of the spirochetes, after digestion of the blood meal and subsequent molt, is coordinated via Rel_{Bbu} (RelA/SpoT homolog) (Drecktrah *et al.*, 2015), BosR and RpoS (Samuels, 2011, Skare *et al.*, 2010). After the molt, *I. scapularis* begins its next feeding which provides an influx of nutrients and affects multiple environmental parameters inside the midgut, including temperature

and osmolarity (Blevins *et al.*, 2009, Bontemps-Gallo *et al.*, 2016, Caimano *et al.*, 2004, Caimano *et al.*, 2007, Elias *et al.*, 2000, Fisher *et al.*, 2005, Hubner *et al.*, 2001). Growth and changes in environmental conditions activate the Rrp2/RpoN/RpoS regulatory cascade which is required for successful transmission from a tick to a mammal (Caimano *et al.*, 2016). Over the last two decades, several signals responsible for activating this pathway have been identified including a temperature shift (23 → 34°C) (Schwan *et al.*, 1995, Stevenson *et al.*, 1995), cell density (3×10^8 cells ml⁻¹) (Indest *et al.*, 1997, Ramamoorthy & Philipp, 1998, Yang *et al.*, 2000), low osmolarity (250 mOsM) (Bontemps-Gallo *et al.*, 2016) and acid stress (Carroll *et al.*, 1999, Yang *et al.*, 2000, Dulebohn *et al.*, 2017).

In other bacteria, polyamines (*e.g.* putrescine, spermidine, spermine) are involved in both the oxidative and osmotic stress responses (Altendorf *et al.*, 2009, Chattopadhyay *et al.*, 2003, Cohen, 1998, Munro *et al.*, 1972, Shah & Swiatlo, 2008), both of which are encountered by *B. burgdorferi* during tick feeding. In general, the polyamine content in bacterial cells is regulated by both biosynthesis and transport (Igarashi & Kashiwagi, 1999). In *E. coli*, the most commonly used bacterial model for polyamine research, the intracellular concentration of putrescine (22.1 nmol/10⁹ cells) is higher than spermidine (3.8 nmol/10⁹ cells) (Morris & Jorstad, 1970). Uptake experiments in *E. coli* demonstrate that spermidine/putrescine transport (via PotABCD) is a spermidine-preferential uptake system (Kashiwagi *et al.*, 1993) that also transports putrescine but with lower affinity. Both molecules can bind nucleic acids and proteins to stabilize their interactions (Childs *et al.*, 2003), modulate the rate of transcription (Thomas *et al.*, 1995) and stimulate growth (Igarashi & Kashiwagi, 2010). Despite extensive research efforts, the role of putrescine and spermidine in bacterial cells remains unclear (Miller-Fleming *et al.*, 2015).

Since *B. burgdorferi*'s genome does not appear to contain the genes encoding enzymes with the capacity to synthesize, modify or degrade either putrescine, spermidine or spermine, the bacteria is thought to be a spermidine auxotroph (Fraser *et al.*, 1997, Wyss & Ermert, 1996). Only one putative spermidine/putrescine ABC-transporter, called PotABCD (encoded by *bb0639-642*) is annotated in *B. burgdorferi*'s genome (Fraser *et al.*, 1997, Lin *et al.*, 2017). *bb0642* (*potA*) encodes a potential ATP-binding protein while *bb0641* (*potB*) and *bb0640* (*potC*) encode proteins with 6 transmembrane domains

suggesting a role in membrane translocation. Finally, *bb0639* (*potD*) encodes a putative spermidine/putrescine periplasmic binding protein which could bind polyamines facilitating transport from the periplasm space into the cytosol. It has been shown that these four genes are expressed as an operon in a polycistronic mRNA (Lin *et al.*, 2017).

In this study, we further characterize PotABCD, the spermidine/putrescine transport system from *B. burgdorferi*, using an inducible expression system (*lacUV5* p/o) to regulate *potABCD* expression. Using this genetic construct, we were able to demonstrate that this system was essential for survival *in vitro*. Interestingly, analyses of specific RpoS, BosR and RpoN mutants indicated that *potABCD* was regulated in an RpoS-dependent, RpoN-independent manner, and that regulation was concurrent with the drop in osmolarity measured in the midgut of feeding ticks (600 – 250 mOsM). Finally, uptake experiments strongly suggested that PotABCD was specific for spermidine in *B. burgdorferi*.

Results

The putative PotABCD transport system is essential for B. burgdorferi growth.

Spermidine and spermine have been shown to trigger the expression and synthesis of proteins required for mammalian invasion through RpoS and BosR (Lin *et al.*, 2017). To better understand the significance of this transport system, we attempted to inactivate the *potABCD* operon. Unfortunately, multiple attempts were unsuccessful. In addition, the comprehensive STM mutant library did not detect any transposon insertions in any of the genes in the operon (Lin *et al.*, 2012) supporting the assumption that the *potABCD* system could be essential to *B. burgdorferi*. Therefore, we generated an inducible *pot* operon using the inducible *lac* promoter expression system developed by Gilbert *et al.* (Gilbert *et al.*, 2007). In this system, *lacI* under control of the *flgB* promoter leads to constitutive synthesis of the LacI repressor which binds the *flacp* promoter and represses the expression of the *potABCD* operon. When added, IPTG will bind LacI in a dose-dependent manner, allowing controlled expression of the *potABCD* operon. Using *B. burgdorferi* strain B31-68-LS (Chu *et al.* 2016), we obtained three clones harboring the

flacp promoter fused to *potABCD* and confirmed these putative mutants by sequencing. One clone, designated BBi-*potABCD*, was selected for further characterization.

To confirm that polyamine utilization and the *pot* operon were essential, the wild-type (B31-68-LS) parent and BBi-*potABCD* strains were grown in BSK-II, pH 7.5 with 0.5 mM IPTG to 5×10^7 cells/ml. Cells from the cultures were harvested, washed, sub-cultured (to $\sim 5 \times 10^5$) and grown in BSK-II with or without IPTG. Growth of each culture was monitored every 24 h by dark-field microscopy and aliquots were removed and the cells were grown and quantified on BSK-II plates with IPTG (Fig. 1). In the presence of IPTG, the cells exhibited a normal growth rate and reached 10^8 cells/ml, while without IPTG, growth completely stopped. More importantly, the number of cells that could be recovered from cultures without IPTG decreased dramatically with time suggesting that the cells lost viability without the expression of *potABCD*. These data indicated that *potABCD* is an essential operon in *B. burgdorferi*.

Spermidine is specifically transported by B. burgdorferi

Previous studies of *potABCD* systems in other bacteria have demonstrated that these transporters are capable of a broad range of uptake selectivity for polyamines (Kashiwagi *et al.*, 1993, Yao & Lu, 2014). Some transport spermine, spermidine and/or putrescine with equal efficiency while others demonstrate a selective preference. To determine if *potABCD* in *B. burgdorferi* demonstrated preferential uptake of polyamines, we performed kinetic uptake assays with tritium (^3H) labelled polyamines. *B. burgdorferi* cells were grown to a density of $\sim 5 \times 10^7$ cells/ml, harvested, washed thoroughly and resuspended to a cell density of 6×10^8 cells/ml. Cells were incubated with either ^3H -spermidine or ^3H -putrescine and cell-associated radioactivity was analyzed at 1, 2, 6 and 10 min (Fig. 2). Uptake of ^3H -spermidine increased linearly 2.4-fold between 1 and 10 min, while no ^3H -putrescine uptake was observed during the same time interval. Because the inducible strain was unable to survive without IPTG, we were unable to assay the uptake selected polyamines in BBi-*potABCD*. These data indicated that, unlike the characterized polyamine transport systems in other bacteria (Kashiwagi *et al.*, 1993, Yao & Lu, 2014), *potABCD* in *B. burgdorferi* seemed to be specific for the uptake of

spermidine. Previous studies have shown that spermidine is more abundant in human blood (9 uM) than putrescine (0.13 uM) (Wishart *et al.*, 2007, Wishart *et al.*, 2009, Wishart *et al.*, 2013) and is the only polyamine available in the midgut of nymphal ticks. The availability of spermidine in the various hosts and vectors harboring *B. burgdorferi* and its selective transport strongly suggests that spermidine is the essential polyamine required by *B. burgdorferi* (Narasimhan *et al.*, 2017).

potABCD is up-regulated during nymph tick feeding

To shed light on the role of *potABCD* during tick feeding, we analyzed the expression of the four genes in the operon by RT-qPCR during nymph feeding. Additionally, *rpoS* and *ospC* were assayed as markers for the activation of the Rrp2/RpoN/RpoS pathway (Fig. 3). Ticks were removed daily from mice infected with wild-type *B. burgdorferi* (B31-A3) and the scutal index was measured to standardize feeding progress (Bontemps-Gallo *et al.*, 2016, Falco *et al.*, 1996) before pooling for RNA extraction. For each time-point examined, RNA was extracted from 3 groups of 5 *I. scapularis* ticks. The expression level of *enoS* was using as a housekeeping gene

As expected, *rpoS* transcript increased (6.1-fold) at the first day post attachment (scutal index 2.09) and stayed at a similar level during the feeding process (Fig. 3). Activation of the Rrp2-RpoN-RpoS pathway increases expression of RpoS-dependant genes (e.g. *ospC*) (Caimano *et al.*, 2004). Expression of *ospC* increased 5.6-fold on day one post-attachment and 5.9-fold more on the second day post-attachment after which the expression slowly decreased. The four genes of the *potABCD* operon followed the same pattern of transcription (Fig. 3). On the second day of tick feeding (scutal index of 2.9), the expression of *potABCD* increased 1.9-fold, 2.3-fold, 3.1-fold and 1.8-fold, respectively. In contrast, the expression of the *potABCD* operon decreased about 7-fold in the replete ticks (scutal index of 5.1) compared to the value measured on the second day of feeding (scutal index of 2.9). These data showed that during tick feeding *B. burgdorferi* upregulated the expression of genes encoding the polyamine transport system (*potABCD*) and this expression corresponded with the increase of RpoS-dependent genes.

potABCD is regulated by low osmolarity

During the feeding process of the tick vector, *B. burgdorferi* encounters and must survive two significant stresses in the midgut: oxidative and osmotic (Bontemps-Gallo *et al.*, 2016, Sonenshine & Anderson, 2014). Furthermore, putrescine and spermidine have been shown to be involved in protecting bacteria from both stresses (Chattopadhyay *et al.*, 2003, Cohen, 1998, Munro *et al.*, 1972, Shah & Swiatlo, 2008). We hypothesized that one or both stresses was responsible for the activation of the *potABCD* operon during tick feeding. To test this hypothesis, we followed the expression of each gene by RT-qPCR at 250, 450, and 650 mOsM and after exposure to *tert*-butyl hydroperoxide (Fig. 4). Expression of the *potA*, *potB* and *potC* genes encoding the putative ATP-binding protein and the two membrane transport proteins increased 10-fold at low osmolality compared to medium or high osmolality (Fig. 4A). Surprisingly, expression of the *potD* gene, encoding the periplasmic binding protein, increased 19-fold (Fig. 4A). The higher expression of *potD* compared to the three other genes suggests an internal promoter for *potD*. Recently, Arnold *et al.* demonstrated by RNA-seq that the *potABCD* operon had only one promoter regardless of the phase of growth (Arnold *et al.*, 2016). However, Adams *et al.* showed by using an *in vivo* expression technology-based approach that the *potABCD* operon had one primary promoter and multiple secondary promoters (Adams *et al.*, 2017). These data together with our data from feeding ticks (Fig. 3), strongly suggested that the *potABCD* operon could be expressed as one polycistronic mRNA from a single promoter or as multiple transcripts from secondary promoters to control the ratio of the mRNA of each gene and the stoichiometry of the different proteins. These data may also suggest that increased expression of the putative periplasmic binding protein (PotD) was required to increase the uptake of polyamines.

No induction of the *potABCD* operon was observed under oxidative stress while *coADR* and *napA*, both known to be involved in the oxidative stress response (Boylan *et al.*, 2003, Hyde *et al.*, 2009), were induced (Fig. 4B). Additionally, we tested the potential of spermidine and putrescine to function as a protective molecule against osmotic or oxidative stress (Fig. S1). No effects on the growth or on the susceptibility to both osmotic and oxidative stresses were observed. We were unable to use our *BBi*-

potABCD mutant to assay if the PotABCD system was required for protection against oxidative or osmotic stresses, because the strain did not grow without the IPTG (Fig. 1). It seems very likely that the *in vitro* conditions tested do not accurately reflect the combination of stresses and changing conditions that challenge *B. burgdorferi* *in vivo*.

potABCD is regulated by *RpoS* independent of *RpoN*

Because *potABCD* expression followed a similar pattern to genes regulated by the Rrp2/RpoN/RpoS regulon in a feeding tick under low osmolarity, we hypothesized that this regulatory pathway controlled *potABCD* expression. To test this hypothesis, we assayed the expression levels of *potABCD* in mutant strains deficient in the key regulatory factors ($\Delta rpoN$, $\Delta rpoS$, and $\Delta bosR$) known to directly or indirectly modulate the Rrp2/RpoN/RpoS cascade (Caimano *et al.*, 2016, Samuels, 2011). Wild-type *B. burgdorferi*, $\Delta rpoN$, $\Delta rpoS$, and $\Delta bosR$ mutant strains were grown in BSK-II at 250 or 450 mOsM to approximately $\sim 5 \times 10^7$ cells/ml and RNA was harvested for RT-qPCR analyses. As observed previously, the *potABCD* operon was induced in wild-type cells at low osmolarity (Fig. 4 and 5). *potABCD* induction in the $\Delta rpoN$ mutant was similar to levels observed in WT cells, while the $\Delta rpoS$ mutant exhibited significantly reduced transcript levels under low osmolarity (Fig 5). These data were surprising since the current understanding is that inactivation of either *rpoN* or *rpoS* results in the same phenotype (Caimano *et al.*, 2016, Samuels, 2011). Fisher *et al.* previously demonstrated using microarray analyses that a small set of genes in the Rrp2/RpoN/RpoS regulon were RpoS-dependent, RpoN-independent (Fisher *et al.*, 2005). In their study, they found that *potD* expression was decreased in an *rpoS* mutant but not in an *rpoN* mutant. Those previous observations and the data presented here demonstrate that *potABCD* is regulated in an RpoS-dependent, RpoN-independent manner.

It has been shown that *rpoS* expression can be modulated by BosR, a transcription factor that is activated by oxidative stress (Boylan *et al.*, 2003, Ouyang *et al.*, 2011). We found that inactivation of *bosR* had no effect on the induction of *potABCD* at low osmolarity. This result was expected since oxidative stress had no effect on *potABCD* gene expression (Fig. 4). Taken together, these data suggest that *potABCD* is RpoS-

dependent and BosR- and RpoN-independent. These results also demonstrate that various *in vivo* stresses can be regulated independently [e.g. oxidative stress via BosR (Boylan *et al.*, 2003, Hyde *et al.*, 2009) and acid stress via RpoS (Dulebohn *et al.*, 2017)] from the Rrp2/RpoN/RpoS cascade.

Discussion

Polyamines such as putrescine, spermidine, spermine and cadaverine are found in both prokaryotes and eukaryotes and are essential for normal cell growth, modulating the rate of transcription (Childs *et al.*, 2003), promoting efficient translation, altering and stabilizing the structure of RNA (Thomas *et al.*, 1995) and protecting the cell from deleterious effects of acid, oxidative and nitrosative stresses (Miller-Fleming *et al.*, 2015). Typically, bacterial cells transport, synthesize and/or modify polyamines to maintain an intracellular concentration that is optimal to promote maximum function and these processes are coordinately regulated (Miller-Fleming *et al.*, 2015). Most bacteria maintain higher intercellular levels of spermidine than putrescine. *E. coli* is a notable exception in that higher levels of putrescine (22.1 nmol/10⁹ cells) are detected compared to spermidine (3.8 nmol/10⁹ cells) (Morris & Jorstad, 1970). The synthesis of putrescine and spermidine is accomplished by the decarboxylation of precursor amino acids such as ornithine (via ornithine decarboxylase, encoded by *speC*) or arginine (arginine decarboxylase, encoded by *speA*), while the synthesis of spermine or cadaverine are rare (Miller-Fleming *et al.*, 2015).

The genome of *B. burgdorferi* lacks the genes encoding proteins for the *de novo* synthesis of putrescine or spermidine and it has been proposed that *B. burgdorferi* is a spermidine auxotroph (Wyss & Ermert, 1996). However, it does harbor genes encoding a single putative putrescine/spermidine transport system, *potABCD*, suggesting that this putrescine/spermidine system may be responsible for polyamine utilization in *B. burgdorferi*. Recently, Lin *et al.* showed that high concentration of spermidine (4 mM) *in vitro* triggers the activation of the Rrp2/RpoN/RpoS cascade (Lin *et al.*, 2017), suggesting that this polyamine may affect the regulation of key virulence factors during the infectious cycle. During the infectious process, *B. burgdorferi* switches between an

arthropod vector and a mammalian host. To be successful during this transition, the bacteria needs to adapt to take advantage of the resources available, optimize growth, correctly alter gene expression and protect itself from deleterious environmental conditions. The purpose of this study is to determine the role of *potABCD* and polyamines in these progressions.

To determine the role of *potABCD* in *B. burgdorferi*, we initially attempted to generate a mutant by deleting the entire operon. However, multiple attempts to inactivate the putative polyamine transport system were unsuccessful. Therefore, an inducible *potABCD* (strain BBi-*potABCD*) was generated. Growth studies indicated that without inducer, cell division ceased, and the cells lost viability after 24 h, suggesting that *potABCD* was required for bacterial replication and survival (Fig. 1) *in vitro*. Compared to other bacteria which can synthesize and/or transport putrescine, spermidine or spermine, *B. burgdorferi* is quite metabolically limited. Uptake experiments indicate that the bacteria use the PotABCD transport system to specifically transport spermidine and suggests that this polyamine is an essential metabolite for *B. burgdorferi* (Fig. 2).

Since we could not introduce strain BBi-*potABCD* directly into ticks, we evaluated the expression patterns of *potABCD* in ticks colonized with strain B31-A3. In the tick, spirochetes up-regulated the *potABCD* operon during the early stage of feeding concomitant with the activation of RpoS-dependent genes (Fig. 3). Additionally, we demonstrated that the *potABCD* operon is a part of a small subset of genes that are RpoS-dependent, RpoN-independent (Fig. 5). This result agrees with previous observations by Fisher and colleagues (Fisher *et al.*, 2005). Our understanding of the regulation of this small set of genes by RpoS, independent of RpoN, is incomplete. More investigation will be required to evaluate the importance of this regulation beyond Rrp2/RpoN/RpoS regulation (Samuels, 2011).

Finally, we investigated the potential biological roles of polyamines in *B. burgdorferi* as well as the cues that trigger the activation of the *potABCD* in *B. burgdorferi*. Polyamines, such as putrescine or spermidine, are required for cell growth and, in some bacteria, for protecting cells from oxidative or osmotic stress (Altendorf *et al.*, 2009, Igarashi & Kashiwagi, 2010, Miller-Fleming *et al.*, 2015). First, we tested the ability of polyamines to protect *B. burgdorferi* in protecting the bacterium from the adverse effects

of osmolarity or oxidative stress (Fig. S1). Under the conditions tested, spermidine was not effective at expanding the survivability of cells from osmotic conditions outside the range of those which have been shown to exist in the tick midgut before, during or after feeding (250-600 mOsM) (Bontemps-Gallo *et al.*, 2016). Additionally, it did not increase the resistance of cells to ROS.

However, *potABCD* was up-regulated when the cells were exposed to low osmolarity but not when cells were exposed to ROS (Fig. 4). Also, *potABCD* was upregulated in the tick midgut during feeding, paralleling the drop in osmolarity that has been measured in ticks at the initial stages of feeding (Bontemps-Gallo *et al.*, 2016). While these data suggest a role for *potABCD* in the tick, we cannot assign a specific function for spermidine in *B. burgdorferi*. It is interesting to note that the increase in expression of *potABCD* occurs at a time during tick feeding which corresponds with maximum cell growth. Because of the complexity of the environment in the tick midgut, it seems likely that the conditions tested *in vitro* do not accurately reflect the multiple parameters (osmolarity, ROS, RNS) to which *B. burgdorferi* is exposed in the tick midgut. While spermidine is essential for *B. burgdorferi* survival and growth *in vitro*, its exact biochemical role remains to be determined.

Experimental Procedures

Bacterial strains, media and growth conditions

The bacterial strains used in this study are described in the Table 1. *B. burgdorferi* strains were grown in BSK-II medium (Barbour, 1984) at 34°C under microaerobic conditions (3-5% O₂, 5% CO₂, 90% N₂). Cell densities were determined by dark-field microscopy. The osmolality of BSK-II medium is 450 mOsM. High and low-osmolality media, were generated as previously described (Bontemps-Gallo *et al.*, 2016). Every 24 h, an aliquot from each culture was examined by dark-field microscopy and plated on BSK-II. Plates were incubated at 34°C under a microaerobic atmosphere for 7-14 d to determine CFU. Spermidine and putrescine (Sigma-Aldrich, St. Louis, MO, USA) were added to BSK-II as indicated. Osmolarity of the growth media (mOsM) was measured with a vapor pressure osmometer (Model 3320, Advanced Instruments, Inc., Norwood, MA, USA).

Construction of the B*Bi*-*potABCD* (*B. burgdorferi* *inducible* – *pot* operon) strain

Plasmids and primers designed for PCR are listed in Tables 1 and 2, respectively. A fragment containing *bb0643-flaBp-Kan-flacp-potA* was synthesized by Genscript USA and cloned into pUC57 and was named p*BBi-potABCD*. The plasmid was transformed into *B. burgdorferi* strain B31-68-LS (Chu *et al.*, 2016) as described previously (Samuels, 1995) and kanamycin-resistant colonies were analyzed by PCR and sequenced to confirm the mutation (Table 2). The mutant strain was designated as B*Bi-potABCD*.

Plasmid profile of the wild-type and the B*Bi-potABCD* were performed by using the multiplex PCR system from Bergström laboratory (Bunikis *et al.*, 2011). Wild-type B31-68-LS was missing cp9 and lp5. B*Bi-potABCD* was missing cp9, lp28-1 and lp5.

Uptake of ³H-Spermidine and ³H-Putrescine

Polyamine transport was determined using a previously described assay by Tilly *et al.* (Tilly *et al.*, 2004). Briefly, cells were grown in BSK-II to mid log phase (~5 x 10⁷ cells/ml), harvested by centrifugation, washed three times with an equal volume of HN buffer (50 mM Hepes, 20 mM NaCl, pH 7.6) supplemented with 6 mM glucose and resuspended to a cell density of 6x10⁸ cells/ml in HN buffer supplemented with 6 mM glucose. A 50 µl aliquot was reserved for protein quantification. Tritium labeled polyamine (5 µCi) was added to 1.0 ml cell suspensions and incubated at 34°C for the duration of the experiment. 100 µl aliquots were removed at each time point and applied to a 0.22 µm AcetatePlus filter (Osmonics, Inc., Minnetonka, MN) using a vacuum manifold and washed three times with 5 ml HN buffer before measuring scintillation counts. Spermidine [terminal methylene-³H] trihydrochloride (ART1749) and Putrescine [2,3-³H] dihydrochloride (ART0279) were purchased from American Radiolabeled Chemicals, Inc, (St. Louis, MO). Polyamine amounts (pmol) in samples were determined against a calibration performed with authentic labeled substrate. The protein concentrations were determined using the PierceTM BCA Protein Assay with bovine serum albumin as a standard (ThermoFisher Scientific, Rockford, IL, USA).

Tick rearing and feeding

I. scapularis egg masses (Oklahoma State University) were allowed to hatch and mature in a controlled temperature, humidity and photoperiod environment. RML mice, an outbred strain of Swiss-Webster mice reared at the Rocky Mountain Laboratories breeding facility, were needle inoculated by intradermal injection with 100 µl of BSK-II containing 1×10^5 *B. burgdorferi* B31-A3 and after three weeks, infection was confirmed by re-isolation of spirochetes from ear punch biopsies. Larval ticks were fed to repletion on infected mice, collected and allowed to molt into nymphs in a controlled environment. Nymphs were then fed on naïve RML mice, mechanically removed during the feeding and the scutal index was determined to standardize the feeding progression (Bontemps-Gallo *et al.*, 2016, Falco *et al.*, 1996).

RNA purification and RT-qPCR

RNA samples were extracted from *B. burgdorferi* cultures using the RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Three independent culture samples were used for each condition. RNA samples were also extracted from *B. burgdorferi* infected ticks during feeding. Ticks were frozen at -80°C, crushed and resuspended in TRIzol (Life technologies, Carlsbad, CA) with chloroform. After centrifugation, the upper phase was mixed with 70% ethanol (1:1) and loaded onto RNeasy column (Qiagen) according to the manufacturer's protocol. The removal of contaminating DNA was accomplished using TURBO DNA-free DNase I (Life technologies, Carlsbad, CA). The cDNA was synthesized from the purified RNA using Superscript IV reverse transcriptase with random primers (Invitrogen, Carlsbad, CA). To compare gene expression, a relative quantification method was employed using *enoS* as a reference gene (Pfaffl, 2001, Bontemps-Gallo *et al.*, 2016). All samples were analyzed in triplicate on a Roche LightCycler 480 System (Indianapolis, IN) using *Power SYBR*[®] Green PCR Master Mix according to the manufacturer's instructions (Life technologies, Carlsbad, CA). The primers used for the qPCR are listed in Table 2.

ROS susceptibility and protection assays.

Strains were grown in pyruvate-free BSK-II medium under microaerobic conditions at 34°C to a cell density of 2×10^7 cells/ml and cells were exposed to the different

concentrations of *tert*-Butyl hydroperoxide (Luperox[®] TBH70X, Sigma-Aldrich, St. Louis, MO, USA) for 3 h in pyruvate-free BSK-II. Samples were plated in BSK-II and incubated at 34°C under microaerobic conditions for 7-14 d to allow enumeration of CFU. When indicated, Spermidine (Sigma-Aldrich, St. Louis, MO, USA) was added to the BSK-II.

Ethics statement

Mouse infection studies were carried out in accordance with the Animal Welfare Act (AWA1990) and followed the guidelines of the National Institutes of Health, Public Health Service Policy on Humane Care (PHS 2002) and Use of Laboratory Animals and the United States Institute of Laboratory Animal Resources, National Research Council, Guide for the Care and Use of Laboratory Animals. All animal work was done according to protocols approved by the Rocky Mountain Laboratories, NIAID, NIH Animal Care and Use Committee. The Rocky Mountain Laboratories are accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All efforts were made to minimize animal suffering.

Funding

This study was supported by the Intramural Research Program of the National Institute for Allergy and Infectious Diseases, National Institutes of Health. The funders were not involved in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We thank Drs. Jorge Benach and Laura Katona (Stony Brook University) for the B31-A3Δ*bosR* strain. We would like to acknowledge Drs. Patricia Rosa and Philip Stewart (RML/NIH) for providing the B31-68-LS strain and their critical review of the manuscript, Dr. Mollie Jewett (University of Central Florida - College of Medicine) for discussions and sharing data and Taylor Robinson (RML/NIH) for plagiarism checking. We are also grateful to Anita Mora and The Visual Medical Arts section (NIH) for help with figure preparation.

Author Contributions

S.B-G. designed and performed experiments, interpreted data and wrote manuscript.
K. L. and C. L. R. performed experiments and edited manuscript.
F. C. G. contributed to experimental design, data analysis, and wrote manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest with the contents of this article.

References

- Adams, P.P., C. Flores Avile, N. Popitsch, I. Bilusic, R. Schroeder, M. Lybecker & M.W. Jewett, (2017) In vivo expression technology and 5' end mapping of the *Borrelia burgdorferi* transcriptome identify novel RNAs expressed during mammalian infection. *Nucleic Acids Res* **45**: 775-792.
- Altendorf, K., I.R. Booth, J. Gralla, J.C. Greie, A.Z. Rosenthal & J.M. Wood, (2009) Osmotic Stress. *EcoSal Plus* **3**.
- Arnold, W.K., C.R. Savage, C.A. Brissette, J. Seshu, J. Livny & B. Stevenson, (2016) RNA-Seq of *Borrelia burgdorferi* in Multiple Phases of Growth Reveals Insights into the Dynamics of Gene Expression, Transcriptome Architecture, and Noncoding RNAs. *PLoS One* **11**: e0164165.
- Barbour, A.G., (1984) Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med* **57**: 521-525.
- Blevins, J.S., H. Xu, M. He, M.V. Norgard, L. Reitzer & X.F. Yang, (2009) Rrp2, a sigma54-dependent transcriptional activator of *Borrelia burgdorferi*, activates rpoS in an enhancer-independent manner. *J Bacteriol* **191**: 2902-2905.
- Bontemps-Gallo, S., K. Lawrence & F.C. Gherardini, (2016) Two Different Virulence-Related Regulatory Pathways in *Borrelia burgdorferi* Are Directly Affected by Osmotic Fluxes in the Blood Meal of Feeding Ixodes Ticks. *PLoS Pathog* **12**: e1005791.
- Bourret, T.J., K.A. Lawrence, J.A. Shaw, T. Lin, S.J. Norris & F.C. Gherardini, (2016) The Nucleotide Excision Repair Pathway Protects *Borrelia burgdorferi* from Nitrosative Stress in Ixodes scapularis Ticks. *Front Microbiol* **7**: 1397.

- Boylan, J.A., J.E. Posey & F.C. Gherardini, (2003) *Borrelia* oxidative stress response regulator, BosR: a distinctive Zn-dependent transcriptional activator. *Proc Natl Acad Sci U S A* **100**: 11684-11689.
- Bunikis, I., S. Kutschan-Bunikis, M. Blonde & S. Bergström, (2011) Multiplex PCR as a tool for validating plasmid content of *Borrelia burgdorferi*. *J. of Microbiol Methods* **86**: 243-247.
- Burtnick, M.N., J.S. Downey, P.J. Brett, J.A. Boylan, J.G. Frye, T.R. Hoover & F.C. Gherardini, (2007) Insights into the complex regulation of rpoS in *Borrelia burgdorferi*. *Mol Microbiol* **65**: 277-293.
- Caimano, M.J., D. Drecktrah, F. Kung & D.S. Samuels, (2016) Interaction of the Lyme disease spirochete with its tick vector. *Cell Microbiol* **18**: 919-927.
- Caimano, M.J., C.H. Eggers, K.R. Hazlett & J.D. Radolf, (2004) RpoS is not central to the general stress response in *Borrelia burgdorferi* but does control expression of one or more essential virulence determinants. *Infect Immun* **72**: 6433-6445.
- Caimano, M.J., R. Iyer, C.H. Eggers, C. Gonzalez, E.A. Morton, M.A. Gilbert, I. Schwartz & J.D. Radolf, (2007) Analysis of the RpoS regulon in *Borrelia burgdorferi* in response to mammalian host signals provides insight into RpoS function during the enzootic cycle. *Mol Microbiol* **65**: 1193-1217.
- Caimano, M.J., M.R. Kenedy, T. Kairu, D.C. Desrosiers, M. Harman, S. Dunham-Ems, D.R. Akins, U. Pal & J.D. Radolf, (2011) The hybrid histidine kinase Hk1 is part of a two-component system that is essential for survival of *Borrelia burgdorferi* in feeding *Ixodes scapularis* ticks. *Infect Immun* **79**: 3117-3130.
- Carroll, J.A., C.F. Garon & T.G. Schwan, (1999) Effects of environmental pH on membrane proteins in *Borrelia burgdorferi*. *Infect Immun* **67**: 3181-3187.
- Chattopadhyay, M.K., C.W. Tabor & H. Tabor, (2003) Polyamines protect *Escherichia coli* cells from the toxic effect of oxygen. *Proc Natl Acad Sci U S A* **100**: 2261-2265.
- Childs, A.C., D.J. Mehta & E.W. Gerner, (2003) Polyamine-dependent gene expression. *Cell Mol Life Sci* **60**: 1394-1406.
- Chu, C.Y., P.E. Stewart, A. Bestor, B. Hansen, T. Lin, L. Gao, S.J. Norris & P.A. Rosa, (2016) Function of the *Borrelia burgdorferi* FtsH Homolog Is Essential for Viability both In Vitro and In Vivo and Independent of HflK/C. *MBio* **7**: e00404-00416.

- Cohen, S.S., (1998) Bacterial Metabolism and Polyamines. In: A guide to the polyamines. S.S. Cohen (ed). New York, USA: Oxford University Press, pp. 94 - 121.
- Drecktrah, D., M. Lybecker, N. Popitsch, P. Rescheneder, L.S. Hall & 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.
- Dulebohn, D.P., C.L. Richards, H. Su, K.A. Lawrence & F.C. Gherardini, (2017) Weak Organic Acids Decrease *Borrelia burgdorferi* Cytoplasmic pH, Eliciting an Acid Stress Response and Impacting RpoN- and RpoS-Dependent Gene Expression. *Front Microbiol* **8**: 1734.
- Elias, A.F., J.L. Bono, J.A. Carroll, P. Stewart, K. Tilly & P. Rosa, (2000) Altered stationary-phase response in a *Borrelia burgdorferi* rpoS mutant. *J Bacteriol* **182**: 2909-2918.
- Elias, A.F., P.E. Stewart, D. Grimm, M.J. Caimano, C.H. Eggers, K. Tilly, J.L. Bono, D.R. Akins, J.D. Radolf, T.G. Schwan & P. Rosa, (2002) Clonal polymorphism of *Borrelia burgdorferi* strain B31 MI: implications for mutagenesis in an infectious strain background. *Infect Immun* **70**: 2139-2150.
- Falco, R.C., D. Fish & J. Piesman, (1996) Duration of tick bites in a Lyme disease-endemic area. *Am J Epidemiol* **143**: 187-192.
- Fisher, M.A., D. Grimm, A.K. Henion, A.F. Elias, P.E. Stewart, P.A. Rosa & F.C. Gherardini, (2005) *Borrelia burgdorferi* sigma54 is required for mammalian infection and vector transmission but not for tick colonization. *Proc Natl Acad Sci U S A* **102**: 5162-5167.
- 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 & J.C. Venter, (1997) Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* **390**: 580-586.
- Gherardini, F.C., J.A. Boylan, K. Lawrence & J. Skare, (2010) Metabolism and Physiology of *Borrelia*. In: *Borrelia: Molecular Biology, Host Interaction and Pathogenesis*. D.S. Samuels & J. Radolf (eds). Norfolk, UK: Caister Academic Press, pp. 103 - 138.

- Gilbert, M.A., E.A. Morton, S.F. Bundle & D.S. Samuels, (2007) Artificial regulation of ospC expression in *Borrelia burgdorferi*. *Mol Microbiol* **63**: 1259-1273.
- He, M., Z. Ouyang, B. Troxell, H. Xu, A. Moh, J. Piesman, M.V. Norgard, M. Gomelsky & X.F. Yang, (2011) Cyclic di-GMP is essential for the survival of the lyme disease spirochete in ticks. *PLoS Pathog* **7**: e1002133.
- Hubner, A., X. Yang, D.M. Nolen, T.G. Popova, F.C. Cabello & M.V. Norgard, (2001) Expression of *Borrelia burgdorferi* OspC and DbpA is controlled by a RpoN-RpoS regulatory pathway. *Proc Natl Acad Sci U S A* **98**: 12724-12729.
- Hyde, J.A., D.K. Shaw, R. Smith Iii, J.P. Trzeciakowski & J.T. Skare, (2009) The BosR regulatory protein of *Borrelia burgdorferi* interfaces with the RpoS regulatory pathway and modulates both the oxidative stress response and pathogenic properties of the Lyme disease spirochete. *Mol Microbiol* **74**: 1344-1355.
- Igarashi, K. & K. Kashiwagi, (1999) Polyamine transport in bacteria and yeast. *Biochem J* **344 Pt 3**: 633-642.
- Igarashi, K. & K. Kashiwagi, (2010) Modulation of cellular function by polyamines. *Int J Biochem Cell Biol* **42**: 39-51.
- Indest, K.J., R. Ramamoorthy, M. Sole, R.D. Gilmore, B.J. Johnson & M.T. Philipp, (1997) Cell-density-dependent expression of *Borrelia burgdorferi* lipoproteins in vitro. *Infect Immun* **65**: 1165-1171.
- Kashiwagi, K., S. Miyamoto, E. Nukui, H. Kobayashi & K. Igarashi, (1993) Functions of potA and potD proteins in spermidine-preferential uptake system in *Escherichia coli*. *J Biol Chem* **268**: 19358-19363.
- Katona, L.I., (2015) The Fur homologue BosR requires Arg39 to activate rpoS transcription in *Borrelia burgdorferi* and thereby direct spirochaete infection in mice. *Microbiology* **161**: 2243-2255.
- Kostick, J.L., L.T. Szkotnicki, E.A. Rogers, P. Bocci, N. Raffaelli & R.T. Marconi, (2011) The diguanylate cyclase, Rrp1, regulates critical steps in the enzootic cycle of the Lyme disease spirochetes. *Mol Microbiol* **81**: 219-231.
- Lin, T., L. Gao, C. Zhang, E. Odeh, M.B. Jacobs, L. Coutte, G. Chaconas, M.T. Philipp & S.J. Norris, (2012) Analysis of an ordered, comprehensive STM mutant library in infectious *Borrelia burgdorferi*: insights into the genes required for mouse infectivity. *PLoS One* **7**: e47532.
- Lin, Y.H., J.A. Romo, T.C. Smith, 2nd, A.N. Reyes, S.L. Karna, C.L. Miller, T.A. Van Laar, R. Yendapally, J.P. Chambers & J. Seshu, (2017) Spermine and spermidine

596 alter gene expression and antigenic profile of *Borrelia burgdorferi*. *Infect Immun*
597 **85**: e00684-16.
598

599 Miller-Fleming, L., V. Olin-Sandoval, K. Campbell & M. Ralser, (2015) Remaining
600 Mysteries of Molecular Biology: The Role of Polyamines in the Cell. *J Mol Biol*
601 **427**: 3389-3406.
602

603 Morris, D.R. & C.M. Jorstad, (1970) Isolation of conditionally putrescine-deficient
604 mutants of *Escherichia coli*. *J Bacteriol* **101**: 731-737.
605

606 Munro, G.F., K. Hercules, J. Morgan & W. Sauerbier, (1972) Dependence of the
607 putrescine content of *Escherichia coli* on the osmotic strength of the medium. *J*
608 *Biol Chem* **247**: 1272-1280.
609

610 Narasimhan, S., T.J. Schuijt, N.M. Abraham, N. Rajeevan, J. Coumou, M. Graham, A.
611 Robson, M.J. Wu, S. Daffre, J.W. Hovius & E. Fikrig, (2017) Modulation of the
612 tick gut milieu by a secreted tick protein favors *Borrelia burgdorferi* colonization.
613 *Nat Commun* **8**: 184.
614

615 Norris, S.J., (2014) vls Antigenic Variation Systems of Lyme Disease *Borrelia*: Eluding
616 Host Immunity through both Random, Segmental Gene Conversion and
617 Framework Heterogeneity. *Microbiol Spectr* **2**: MDNA3-0038-2014.
618

619 Ouyang, Z., R.K. Deka & M.V. Norgard, (2011) BosR (BB0647) controls the RpoN-
620 RpoS regulatory pathway and virulence expression in *Borrelia burgdorferi* by a
621 novel DNA-binding mechanism. *PLoS Pathog* **7**: e1001272.
622

623 Petzke, M. & I. Schwartz, (2015) *Borrelia burgdorferi* Pathogenesis and the Immune
624 Response. *Clin Lab Med* **35**: 745-764.
625

626 Pfaffl, M.W., (2001) A new mathematical model for relative quantification in real-time
627 RT-PCR. *Nucleic Acids Res* **29**:e45.
628

629 Pritt, B.S., P.S. Mead, D.K. Johnson, D.F. Neitzel, L.B. Respicio-Kingry, J.P. Davis, E.
630 Schiffman, L.M. Sloan, M.E. Schriefer, A.J. Replogle, S.M. Paskewitz, J.A. Ray,
631 J. Bjork, C.R. Steward, A. Deedon, X. Lee, L.C. Kingry, T.K. Miller, M.A. Feist,
632 E.S. Theel, R. Patel, C.L. Irish & J.M. Petersen, (2016) Identification of a novel
633 pathogenic *Borrelia* species causing Lyme borreliosis with unusually high
634 spirochaetaemia: a descriptive study. *Lancet Infect Dis* **16**: 556-564.
635

636 Radolf, J.D., M.J. Caimano, B. Stevenson & L.T. Hu, (2012) Of ticks, mice and men:
637 understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev*
638 *Microbiol* **10**: 87-99.
639

640 Ramamoorthy, R. & M.T. Philipp, (1998) Differential expression of *Borrelia burgdorferi*
641 proteins during growth in vitro. *Infect Immun* **66**: 5119-5124.

- Samuels, D.S., (1995) Electrotransformation of the spirochete *Borrelia burgdorferi*. *Methods Mol Biol* **47**: 253-259.
- Samuels, D.S., (2011) Gene regulation in *Borrelia burgdorferi*. *Annu Rev Microbiol* **65**: 479-499.
- Schwan, T.G., J. Piesman, W.T. Golde, M.C. Dolan & P.A. Rosa, (1995) Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc Natl Acad Sci U S A* **92**: 2909-2913.
- Shah, P. & E. Swiatlo, (2008) A multifaceted role for polyamines in bacterial pathogens. *Mol Microbiol* **68**: 4-16.
- Skare, J.T., J.A. Carroll, X. Yang, D.S. Samuels & D.R. Akins, (2010) Gene regulation, Transcriptomics and Proteomics. In: *Borrelia: Molecular Biology, Host Interaction and Pathogenesis*. D.S. Samuels & J.D. Radolf (eds). Norfolk, UK: Caister Academic Press, pp. 67 - 101.
- Sonenshine, D.E. & J.M. Anderson, (2014) Mouthparts and digestive system. In: *Biology of Ticks*. D.E. Sonenshine & R.M. Roe (eds). New-York (USA): Oxford University Press, pp. 122-162.
- Stevenson, B., T.G. Schwan & P.A. Rosa, (1995) Temperature-related differential expression of antigens in the Lyme disease spirochete, *Borrelia burgdorferi*. *Infect Immun* **63**: 4535-4539.
- Stewart, P.E. & P.A. Rosa, (2017) Physiologic and Genetic Factors Influencing the Zoonotic Cycle of *Borrelia burgdorferi*. In: *Curr Top Microbiol Immunol*. Springer, Berlin, Heidelberg, pp. 1-20.
- The staff of the Jackson Laboratory, (1966) *Biology of the Laboratory Mouse*. Dover Publications, Inc., New York.
- Thomas, T., M.A. Gallo, C.M. Klinge & T.J. Thomas, (1995) Polyamine-mediated conformational perturbations in DNA alter the binding of estrogen receptor to poly(dG-m5dC).poly(dG-m5dC) and a plasmid containing the estrogen response element. *J Steroid Biochem Mol Biol* **54**: 89-99.
- Tilly, K., D. Grimm, D.M. Bueschel, J.G. Krum & P. Rosa, (2004) Infectious cycle analysis of a *Borrelia burgdorferi* mutant defective in transport of chitobiose, a tick cuticle component. *Vector Borne Zoonotic Dis* **4**: 159-168.
- Waymouth, C., (1970) Osmolality of mammalian blood and of media for culture of mammalian cells. *In Vitro* **6**: 109-127.

- Wishart, D.S., T. Jewison, A.C. Guo, M. Wilson, C. Knox, Y. Liu, Y. Djoumbou, R. Mandal, F. Aziat, E. Dong, S. Bouatra, I. Sinelnikov, D. Arndt, J. Xia, P. Liu, F. Yallou, T. Bjorn Dahl, R. Perez-Pineiro, R. Eisner, F. Allen, V. Neveu, R. Greiner & A. Scalbert, (2013) HMDB 3.0--The Human Metabolome Database in 2013. *Nucleic Acids Res* **41**: D801-807.
- Wishart, D.S., C. Knox, A.C. Guo, R. Eisner, N. Young, B. Gautam, D.D. Hau, N. Psychogios, E. Dong, S. Bouatra, R. Mandal, I. Sinelnikov, J. Xia, L. Jia, J.A. Cruz, E. Lim, C.A. Sobsey, S. Shrivastava, P. Huang, P. Liu, L. Fang, J. Peng, R. Fradette, D. Cheng, D. Tzur, M. Clements, A. Lewis, A. De Souza, A. Zuniga, M. Dawe, Y. Xiong, D. Clive, R. Greiner, A. Nazzyrova, R. Shaykhtudinov, L. Li, H.J. Vogel & I. Forsythe, (2009) HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res* **37**: D603-610.
- Wishart, D.S., D. Tzur, C. Knox, R. Eisner, A.C. Guo, N. Young, D. Cheng, K. Jewell, D. Arndt, S. Sawhney, C. Fung, L. Nikolai, M. Lewis, M.A. Coutouly, I. Forsythe, P. Tang, S. Shrivastava, K. Jeroncic, P. Stothard, G. Amegbey, D. Block, D.D. Hau, J. Wagner, J. Miniaci, M. Clements, M. Gebremedhin, N. Guo, Y. Zhang, G.E. Duggan, G.D. Macinnis, A.M. Weljie, R. Dowlatbadi, F. Bamforth, D. Clive, R. Greiner, L. Li, T. Marrie, B.D. Sykes, H.J. Vogel & L. Querengesser, (2007) HMDB: the Human Metabolome Database. *Nucleic Acids Res* **35**: D521-526.
- Wyss, C. & P. Ermer, (1996) *Borrelia burgdorferi* is an Adenine and Spermidine Auxotroph. *Microbial Ecology in Health and Disease* **9**: 181-185.
- Yang, X., M.S. Goldberg, T.G. Popova, G.B. Schoeler, S.K. Wikel, K.E. Hagman & M.V. Norgard, (2000) Interdependence of environmental factors influencing reciprocal patterns of gene expression in virulent *Borrelia burgdorferi*. *Mol Microbiol* **37**: 1470-1479.
- Yao, X. & C.D. Lu, (2014) Functional characterization of the potRABCD operon for spermine and spermidine uptake and regulation in *Staphylococcus aureus*. *Curr Microbiol* **69**: 75-81.

731

732

733

734

735

736

737

738

739 Table 1. Bacterial Strains and Plasmids used in this study

Strains	Source
<i>B. burgdorferi</i>	
B31-A3	(Elias <i>et al.</i> , 2002)
B31-A3 Δ <i>rpoN</i>	(Fisher <i>et al.</i> , 2005)
B31-A3 Δ <i>rpoS</i>	(Burtnick <i>et al.</i> , 2007)
B31-A3 Δ <i>bosR</i>	(Katona, 2015)
B31-68-LS	(Chu <i>et al.</i> , 2016)
B31-68-LS – BBi- <i>potABCD</i>	This study
<i>Escherichia coli</i>	
Top10F': <i>mcrA</i> , Δ (<i>mrr-hsdRMS-mcrBC</i>), Φ 80 <i>lacZ</i> Δ M15, <i>lacX74</i> , <i>recA1</i> , <i>araD139</i> , Δ (<i>ara-leu</i>)7697, <i>galU</i> , <i>galK</i> , <i>rpsL</i> , <i>endA1</i> , <i>nupG</i>	Invitrogen
Plasmids	
pUC57	Genscript
pBBi- <i>potABCD</i>	This Study

740

741 Table 2. PCR primers used in this study.

PCR Primers	Sequence	Source
potAF	GCCATATGGATAATTGTATTATCCTAGAGATTAA	This study
potAR	CGGATCCTTATTCCTTATGCATAACATGAAT	This study
GTPF- <i>EcoRI</i>	ACTGCTGAATTCTCCACCTATTTTGGCTGCCA	This study
GTPR- <i>aatII</i>	ACTGCTGACGTCACATCCGGATGGGCCTAGTA	This study
QrpoSF	CTGGACAAAGAAATAGAGGGATCTG	This study
QrpoSR	CAAGGGTAATTTTCAGGGTTAAAAGAA	This study
QospCF	TGGTACTAAAACATAAAGGTGCTGAAGAA	This study
QospCR	GCATCTCTTTAGCTGCTTTTGACA	This study
QpotAF	GCTGGTTGTAAGTTTGCTTGG	This study
QpotAR	ATCTTCTGGGCGTATTACAAGG	This study
QpotBF	AGATCTTGGAGCAAGAATGTG	This study
QpotBR	GTTTAGAGCCTCCTAGCAAATC	This study
QpotCF	CGGAAATCGCAGGAAGCATAG	This study
QpotCR	ATCCCTGTCCAGTGGTGAAA	This study
QpotDF	GCACAAAGCGCTATGCTAAA	This study
QpotDR	TGGAGCATCAATAGGAATTACA	This study
QnapAF	CCCTCAATGGAAAGCATTGTTTG	This study
QnapAR	GCATCCATAAATGTTTCTCAAGATCAC	This study
QcoADRF	AGCTGGGAATCATAACAGCATTTA	This study
QcoADRR	GTCCTGTTCTTGCAGCTTCT	This study
QenoSF	GTGCACACTCTGACAACTCT	This study
QenoSR	ACCTCTGCTGCCATTCTTATT	This study

742

743 *Figure Legends*

744 **Fig. 1.** The PotABCD uptake system is essential for *B. burgdorferi*.

745 Growth and survival curve of wild-type B31-68-LS and BBi-*potABCD* strains with or
746 without 500 μ M IPTG. Cells were enumerated by plating in BSK-II plating. Data
747 represent means \pm standard deviation of three independent experiments.

748

749 **Fig. 2.** Spermidine is the preferred polyamine transported by *B. burgdorferi*.

750 Curve of uptake of radioactive spermidine and putrescine by *B. burgdorferi* B31-A3
751 strain was measured over a 10-min time course. Cells were grown to mid-log phase,
752 washed and resuspended to a cell density of 6×10^8 cells/ml in HN buffer supplemented
753 with 6 mM glucose. Tritium labelled polyamine (5 μ Ci) was added to the cell

suspensions and incubated at 34°C for the duration of the experiment. Polyamine amounts (pmol) in samples were determined against a calibration performed with authentic labeled substrate. The protein concentration was determined using a BCA Protein Assay with bovine serum albumin as a standard. Dotted lines represent the linear regression. Data represent means \pm standard deviation of two independent experiments.

Fig. 3. Relative expression of *potABCD* operon genes during nymph feeding.

Graph of the expression of *rpoS* and *ospC* (as markers for the induction of the Rrp2-RpoN-RpoS pathway), and the four genes of the *potABCD* operon were analyzed by RT-qPCR. RNA was extracted from 3 pools of 5 ticks and cDNA was generated as described in the methods section. Scutal index was used to standardize feeding progress (Bontemps-Gallo *et al.*, 2016, Falco *et al.*, 1996). The scutal index is indicated under each bar. Relative gene expression was calculated using levels expression of *enoS* as reference. Data represent means \pm standard deviation of three independent pools for each condition.

Fig. 4. Expression of *potABCD* operon under osmotic or oxidative stress.

(A) Graph of the expression analyses of the *potABCD* operon of *B. burgdorferi* grown in BSK-II at 250, 450 and 650 mOsM. (B) Graph of the expression analyses of the *potABCD* operon of *B. burgdorferi* grown in BSK-II after a treatment with 5 mM *tert*-butyl hydroperoxide compared to an untreated culture. The expression of *coADR*, *napA* (as marker of the induction of the oxidative stress response), and the four genes of *potABCD* operon were analyzed by RT-qPCR. Relative gene expression was calculated using levels expression of *enoS* as reference. Data represent means \pm standard deviation of three independent experiments.

Fig. 5. Expression of *potABCD* operon in wild-type, $\Delta rpoN$, $\Delta rpoS$ and $\Delta bosR$ mutants.

Graph of the expression analyses of the *potABCD* operon of *B. burgdorferi* grown in BSK-II at 250 and 450 mOsM. Relative gene expression was calculated using levels expression of *enoS* as reference. Data represent means \pm standard deviation of three independent experiments.

785

786 *Supplemental Material*

787 **Fig. S1.** Spermidine is not an osmoprotective or oxidative protective molecule.

788 (A) Growth of wild-type B31-A3 strain at 150, 250, 450, 650, 850 mOsM with or without
789 5 mM spermidine (SPD). An aliquot of each culture was examined daily by dark-field
790 microscopy and plated on solid BSK-II. (B) Early stationary phase (5×10^7 cells/ml)
791 cultures were incubated for 3 h with 0, 1, 5 or 10 mM of *tert*-butyl hydroperoxide (*t*-
792 butyl) and plated on BSK-II. Plates were incubated at 34°C under microaerobic
793 conditions for 7-14 d to allow enumeration of CFU. Untreated samples were standardized
794 to 100%. Data represent means \pm standard deviation of three independent experiments.