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

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Running Head: PotABCD is essential for *Borrelia burgdorferi*

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1 **Summary**

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

20

21 **Introduction**

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

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

52 and osmolarity (Blevins *et al.*, 2009, Bontemps-Gallo *et al.*, 2016, Caimano *et al.*, 2004,
53 Caimano *et al.*, 2007, Elias *et al.*, 2000, Fisher *et al.*, 2005, Hubner *et al.*, 2001). Growth
54 and changes in environmental conditions activate the Rrp2/RpoN/RpoS regulatory
55 cascade which is required for successful transmission from a tick to a mammal (Caimano
56 *et al.*, 2016). Over the last two decades, several signals responsible for activating this
57 pathway have been identified including a temperature shift (23 → 34°C) (Schwan *et al.*,
58 1995, Stevenson *et al.*, 1995), cell density (3×10^8 cells ml⁻¹) (Indest *et al.*, 1997,
59 Ramamoorthy & Philipp, 1998, Yang *et al.*, 2000), low osmolarity (250 mOsM)
60 (Bontemps-Gallo *et al.*, 2016) and acid stress (Carroll *et al.*, 1999, Yang *et al.*, 2000,
61 Dulebohn *et al.*, 2017).

62 In other bacteria, polyamines (*e.g.* putrescine, spermidine, spermine) are involved in
63 both the oxidative and osmotic stress responses (Altendorf *et al.*, 2009, Chattopadhyay *et*
64 *al.*, 2003, Cohen, 1998, Munro *et al.*, 1972, Shah & Swiatlo, 2008), both of which are
65 encountered by *B. burgdorferi* during tick feeding. In general, the polyamine content in
66 bacterial cells is regulated by both biosynthesis and transport (Igarashi & Kashiwagi,
67 1999). In *E. coli*, the most commonly used bacterial model for polyamine research, the
68 intracellular concentration of putrescine (22.1 nmol/10⁹ cells) is higher than spermidine
69 (3.8 nmol/10⁹ cells) (Morris & Jorstad, 1970). Uptake experiments in *E. coli* demonstrate
70 that spermidine/putrescine transport (via PotABCD) is a spermidine-preferential uptake
71 system (Kashiwagi *et al.*, 1993) that also transports putrescine but with lower affinity.
72 Both molecules can bind nucleic acids and proteins to stabilize their interactions (Childs
73 *et al.*, 2003), modulate the rate of transcription (Thomas *et al.*, 1995) and stimulate
74 growth (Igarashi & Kashiwagi, 2010). Despite extensive research efforts, the role of
75 putrescine and spermidine in bacterial cells remains unclear (Miller-Fleming *et al.*, 2015).

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

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

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

96

97 **Results**

98

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

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

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

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

126

127 *Spermidine is specifically transported by B. burgdorferi*

128

129 Previous studies of *potABCD* systems in other bacteria have demonstrated that these
130 transporters are capable of a broad range of uptake selectivity for polyamines (Kashiwagi
131 *et al.*, 1993, Yao & Lu, 2014). Some transport spermine, spermidine and/or putrescine
132 with equal efficiency while others demonstrate a selective preference. To determine if
133 *potABCD* in *B. burgdorferi* demonstrated preferential uptake of polyamines, we
134 performed kinetic uptake assays with tritium (^3H) labelled polyamines. *B. burgdorferi*
135 cells were grown to a density of $\sim 5 \times 10^7$ cells/ml, harvested, washed thoroughly and
136 resuspended to a cell density of 6×10^8 cells/ml. Cells were incubated with either ^3H -
137 spermidine or ^3H -putrescine and cell-associated radioactivity was analyzed at 1, 2, 6 and
138 10 min (Fig. 2). Uptake of ^3H -spermidine increased linearly 2.4-fold between 1 and 10
139 min, while no ^3H -putrescine uptake was observed during the same time interval. Because
140 the inducible strain was unable to survive without IPTG, we were unable to assay the
141 uptake selected polyamines in BBi-*potABCD*. These data indicated that, unlike the
142 characterized polyamine transport systems in other bacteria (Kashiwagi *et al.*, 1993, Yao
143 & Lu, 2014), *potABCD* in *B. burgdorferi* seemed to be specific for the uptake of

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

150

151 *potABCD* is up-regulated during nymph tick feeding

152

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

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

174

175 *potABCD* is regulated by low osmolarity

176

177 During the feeding process of the tick vector, *B. burgdorferi* encounters and must survive
178 two significant stresses in the midgut: oxidative and osmotic (Bontemps-Gallo *et al.*,
179 2016, Sonenshine & Anderson, 2014). Furthermore, putrescine and spermidine have been
180 shown to be involved in protecting bacteria from both stresses (Chattopadhyay *et al.*,
181 2003, Cohen, 1998, Munro *et al.*, 1972, Shah & Swiatlo, 2008). We hypothesized that
182 one or both stresses was responsible for the activation of the *potABCD* operon during tick
183 feeding. To test this hypothesis, we followed the expression of each gene by RT-qPCR at
184 250, 450, and 650 mOsM and after exposure to *tert*-butyl hydroperoxide (Fig. 4).
185 Expression of the *potA*, *potB* and *potC* genes encoding the putative ATP-binding protein
186 and the two membrane transport proteins increased 10-fold at low osmolarity compared
187 to medium or high osmolality (Fig. 4A). Surprisingly, expression of the *potD* gene,
188 encoding the periplasmic binding protein, increased 19-fold (Fig. 4A). The higher
189 expression of *potD* compared to the three other genes suggests an internal promoter for
190 *potD*. Recently, Arnold *et al.* demonstrated by RNA-seq that the *potABCD* operon had
191 only one promoter regardless of the phase of growth (Arnold *et al.*, 2016). However,
192 Adams *et al.* showed by using an *in vivo* expression technology-based approach that the
193 *potABCD* operon had one primary promoter and multiple secondary promoters (Adams *et*
194 *al.*, 2017). These data together with our data from feeding ticks (Fig. 3), strongly
195 suggested that the *potABCD* operon could be expressed as one polycistronic mRNA from
196 a single promoter or as multiple transcripts from secondary promoters to control the ratio
197 of the mRNA of each gene and the stoichiometry of the different proteins. These data
198 may also suggest that increased expression of the putative periplasmic binding protein
199 (PotD) was required to increase the uptake of polyamines.

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

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

210

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

212

213 Because *potABCD* expression followed a similar pattern to genes regulated by the
214 Rrp2/RpoN/RpoS regulon in a feeding tick under low osmolarity, we hypothesized that
215 this regulatory pathway controlled *potABCD* expression. To test this hypothesis, we
216 assayed the expression levels of *potABCD* in mutant strains deficient in the key
217 regulatory factors ($\Delta rpoN$, $\Delta rpoS$, and $\Delta bosR$) known to directly or indirectly modulate
218 the Rrp2/RpoN/RpoS cascade (Caimano *et al.*, 2016, Samuels, 2011). Wild-type *B.*
219 *burgdorferi*, $\Delta rpoN$, $\Delta rpoS$, and $\Delta bosR$ mutant strains were grown in BSK-II at 250 or
220 450 mOsM to approximately $\sim 5 \times 10^7$ cells/ml and RNA was harvested for RT-qPCR
221 analyses. As observed previously, the *potABCD* operon was induced in wild-type cells at
222 low osmolarity (Fig. 4 and 5). *potABCD* induction in the $\Delta rpoN$ mutant was similar to
223 levels observed in WT cells, while the $\Delta rpoS$ mutant exhibited significantly reduced
224 transcript levels under low osmolarity (Fig 5). These data were surprising since the
225 current understanding is that that inactivation of either *rpoN* or *rpoS* results in the same
226 phenotype (Caimano *et al.*, 2016, Samuels, 2011). Fisher *et al.* previously demonstrated
227 using microarray analyses that a small set of genes in the Rrp2/RpoN/RpoS regulon were
228 RpoS-dependent, RpoN-independent (Fisher *et al.*, 2005). In their study, they found that
229 *potD* expression was decreased in an *rpoS* mutant but not in an *rpoN* mutant. Those
230 previous observations and the data presented here demonstrate that *potABCD* is regulated
231 in an RpoS-dependent, RpoN-independent manner.

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

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

241

242 **Discussion**

243

244 Polyamines such as putrescine, spermidine, spermine and cadaverine are found in both
245 prokaryotes and eukaryotes and are essential for normal cell growth, modulating the rate
246 of transcription (Childs *et al.*, 2003), promoting efficient translation, altering and
247 stabilizing the structure of RNA (Thomas *et al.*, 1995) and protecting the cell from
248 deleterious effects of acid, oxidative and nitrosative stresses (Miller-Fleming *et al.*,
249 2015). Typically, bacterial cells transport, synthesize and/or modify polyamines to
250 maintain an intracellular concentration that is optimal to promote maximum function and
251 these processes are coordinately regulated (Miller-Fleming *et al.*, 2015). Most bacteria
252 maintain higher intercellular levels of spermidine than putrescine. *E. coli* is a notable
253 exception in that higher levels of putrescine (22.1 nmol/10⁹ cells) are detected compared
254 to spermidine (3.8 nmol/10⁹ cells) (Morris & Jorstad, 1970). The synthesis of putrescine
255 and spermidine is accomplished by the decarboxylation of precursor amino acids such as
256 ornithine (via ornithine decarboxylase, encoded by *speC*) or arginine (arginine
257 decarboxylase, encoded by *speA*), while the synthesis of spermine or cadaverine are rare
258 (Miller-Fleming *et al.*, 2015).

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

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

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

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

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

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

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

316

317 **Experimental Procedures**

318

319 *Bacterial strains, media and growth conditions*

320 The bacterial strains used in this study are described in the Table 1. *B. burgdorferi* strains
321 were grown in BSK-II medium (Barbour, 1984) at 34°C under microaerobic conditions
322 (3-5% O₂, 5% CO₂, 90% N₂). Cell densities were determined by dark-field microscopy.
323 The osmolality of BSK-II medium is 450 mOsM. High and low-osmolality media, were
324 generated as previously described (Bontemps-Gallo *et al.*, 2016). Every 24 h, an aliquot
325 from each culture was examined by dark-field microscopy and plated on BSK-II. Plates
326 were incubated at 34°C under a microaerobic atmosphere for 7-14 d to determine CFU.
327 Spermidine and putrescine (Sigma-Aldrich, St. Louis, MO, USA) were added to BSK-II
328 as indicated. Osmolarity of the growth media (mOsM) was measured with a vapor
329 pressure osmometer (Model 3320, Advanced Instruments, Inc., Norwood, MA, USA).

330

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

332 Plasmids and primers designed for PCR are listed in Tables 1 and 2, respectively. A
333 fragment containing *bb0643-flaBp-Kan-flacp-potA* was synthesized by Genscript USA
334 and cloned into pUC57 and was named pBBi- *potABCD*. The plasmid was transformed
335 into *B. burgdorferi* strain B31-68-LS (Chu *et al.*, 2016) as described previously (Samuels,
336 1995) and kanamycin-resistant colonies were analyzed by PCR and sequenced to confirm
337 the mutation (Table 2). The mutant strain was designated as BBi-*potABCD*.

338 Plasmid profile of the wild-type and the BBi-*potABCD* were performed by using the
339 multiplex PCR system from Bergström laboratory (Bunikis *et al.*, 2011). Wild-type B31-
340 68-LS was missing cp9 and lp5. BBi-*potABCD* was missing cp9, lp28-1 and lp5.

341

342 *Uptake of ³H-Spermidine and ³H-Putrescine*

343 Polyamine transport was determined using a previously described assay by Tilly *et al.*
344 (Tilly *et al.*, 2004). Briefly, cells were grown in BSK-II to mid log phase (~5 x 10⁷
345 cells/ml), harvested by centrifugation, washed three times with an equal volume of HN
346 buffer (50 mM Hepes, 20 mM NaCl, pH 7.6) supplemented with 6 mM glucose and
347 resuspended to a cell density of 6x10⁸ cells/ml in HN buffer supplemented with 6 mM
348 glucose. A 50 µl aliquot was reserved for protein quantification. Tritium labeled
349 polyamine (5 µCi) was added to 1.0 ml cell suspensions and incubated at 34°C for the
350 duration of the experiment. 100 µl aliquots were removed at each time point and applied
351 to a 0.22 µm AcetatePlus filter (Osmonics, Inc., Minnetonka, MN) using a vacuum
352 manifold and washed three times with 5 ml HN buffer before measuring scintillation
353 counts. Spermidine [terminal methylene-³H] trihydrochloride (ART1749) and Putrescine
354 [2,3-³H] dihydrochloride (ART0279) were purchased from American Radiolabeled
355 Chemicals, Inc, (St. Louis, MO). Polyamine amounts (pmol) in samples were determined
356 against a calibration performed with authentic labeled substrate. The protein
357 concentrations were determined using the PierceTM BCA Protein Assay with bovine
358 serum albumin as a standard (ThermoFisher Scientific, Rockford, IL, USA).

359

360 *Tick rearing and feeding*

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

371

372 *RNA purification and RT-qPCR*

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

388

389 *ROS susceptibility and protection assays.*

390 Strains were grown in pyruvate-free BSK-II medium under microaerobic conditions at
391 34°C to a cell density of 2×10^7 cells/ml and cells were exposed to the different

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

397

398 **Ethics statement**

399 Mouse infection studies were carried out in accordance with the Animal Welfare Act
400 (AWA1990) and followed the guidelines of the National Institutes of Health, Public
401 Health Service Policy on Humane Care (PHS 2002) and Use of Laboratory Animals and
402 the United States Institute of Laboratory Animal Resources, National Research Council,
403 Guide for the Care and Use of Laboratory Animals. All animal work was done according
404 to protocols approved by the Rocky Mountain Laboratories, NIAID, NIH Animal Care
405 and Use Committee. The Rocky Mountain Laboratories are accredited by the
406 International Association for Assessment and Accreditation of Laboratory Animal Care
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408

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423

424 **Author Contributions**

425 S.B-G. designed and performed experiments, interpreted data and wrote manuscript.

426 K. L. and C. L. R. performed experiments and edited manuscript.

427 F. C. G. contributed to experimental design, data analysis, and wrote manuscript.

428

429 **Conflict of Interest**

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

431

432 **References**

433 Adams, P.P., C. Flores Avile, N. Popitsch, I. Bilusic, R. Schroeder, M. Lybecker & M.W.
434 Jewett, (2017) In vivo expression technology and 5' end mapping of the *Borrelia*
435 *burgdorferi* transcriptome identify novel RNAs expressed during mammalian
436 infection. *Nucleic Acids Res* **45**: 775-792.

437

438 Altendorf, K., I.R. Booth, J. Gralla, J.C. Greie, A.Z. Rosenthal & J.M. Wood, (2009)
439 Osmotic Stress. *EcoSal Plus* **3**.

440

441 Arnold, W.K., C.R. Savage, C.A. Brissette, J. Seshu, J. Livny & B. Stevenson, (2016)
442 RNA-Seq of *Borrelia burgdorferi* in Multiple Phases of Growth Reveals Insights
443 into the Dynamics of Gene Expression, Transcriptome Architecture, and
444 Noncoding RNAs. *PLoS One* **11**: e0164165.

445

446 Barbour, A.G., (1984) Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol*
447 *Med* **57**: 521-525.

448

449 Blevins, J.S., H. Xu, M. He, M.V. Norgard, L. Reitzer & X.F. Yang, (2009) Rrp2, a
450 sigma54-dependent transcriptional activator of *Borrelia burgdorferi*, activates
451 rpoS in an enhancer-independent manner. *J Bacteriol* **191**: 2902-2905.

452

453 Bontemps-Gallo, S., K. Lawrence & F.C. Gherardini, (2016) Two Different Virulence-
454 Related Regulatory Pathways in *Borrelia burgdorferi* Are Directly Affected by
455 Osmotic Fluxes in the Blood Meal of Feeding Ixodes Ticks. *PLoS Pathog* **12**:
456 e1005791.

457

458 Bourret, T.J., K.A. Lawrence, J.A. Shaw, T. Lin, S.J. Norris & F.C. Gherardini, (2016)
459 The Nucleotide Excision Repair Pathway Protects *Borrelia burgdorferi* from
460 Nitrosative Stress in Ixodes scapularis Ticks. *Front Microbiol* **7**: 1397.

461

462 Boylan, J.A., J.E. Posey & F.C. Gherardini, (2003) *Borrelia* oxidative stress response
463 regulator, BosR: a distinctive Zn-dependent transcriptional activator. *Proc Natl*
464 *Acad Sci U S A* **100**: 11684-11689.
465

466 Bunikis, I., S. Kutschan-Bunikis, M. Blonde & S. Bergström, (2011) Multiplex PCR as a
467 tool for validating plasmid content of *Borrelia burgdorferi*. *J. of Microbiol*
468 *Methods* **86**: 243-247.
469

470 Burtnick, M.N., J.S. Downey, P.J. Brett, J.A. Boylan, J.G. Frye, T.R. Hoover & F.C.
471 Gherardini, (2007) Insights into the complex regulation of rpoS in *Borrelia*
472 *burgdorferi*. *Mol Microbiol* **65**: 277-293.
473

474 Caimano, M.J., D. Drecktrah, F. Kung & D.S. Samuels, (2016) Interaction of the Lyme
475 disease spirochete with its tick vector. *Cell Microbiol* **18**: 919-927.
476

477 Caimano, M.J., C.H. Eggers, K.R. Hazlett & J.D. Radolf, (2004) RpoS is not central to
478 the general stress response in *Borrelia burgdorferi* but does control expression of
479 one or more essential virulence determinants. *Infect Immun* **72**: 6433-6445.
480

481 Caimano, M.J., R. Iyer, C.H. Eggers, C. Gonzalez, E.A. Morton, M.A. Gilbert, I.
482 Schwartz & J.D. Radolf, (2007) Analysis of the RpoS regulon in *Borrelia*
483 *burgdorferi* in response to mammalian host signals provides insight into RpoS
484 function during the enzootic cycle. *Mol Microbiol* **65**: 1193-1217.
485

486 Caimano, M.J., M.R. Kenedy, T. Kairu, D.C. Desrosiers, M. Harman, S. Dunham-Ems,
487 D.R. Akins, U. Pal & J.D. Radolf, (2011) The hybrid histidine kinase Hk1 is part
488 of a two-component system that is essential for survival of *Borrelia burgdorferi* in
489 feeding *Ixodes scapularis* ticks. *Infect Immun* **79**: 3117-3130.
490

491 Carroll, J.A., C.F. Garon & T.G. Schwan, (1999) Effects of environmental pH on
492 membrane proteins in *Borrelia burgdorferi*. *Infect Immun* **67**: 3181-3187.
493

494 Chattopadhyay, M.K., C.W. Tabor & H. Tabor, (2003) Polyamines protect *Escherichia*
495 *coli* cells from the toxic effect of oxygen. *Proc Natl Acad Sci U S A* **100**: 2261-
496 2265.
497

498 Childs, A.C., D.J. Mehta & E.W. Gerner, (2003) Polyamine-dependent gene expression.
499 *Cell Mol Life Sci* **60**: 1394-1406.
500

501 Chu, C.Y., P.E. Stewart, A. Bestor, B. Hansen, T. Lin, L. Gao, S.J. Norris & P.A. Rosa,
502 (2016) Function of the *Borrelia burgdorferi* FtsH Homolog Is Essential for
503 Viability both In Vitro and In Vivo and Independent of HflK/C. *MBio* **7**: e00404-
504 00416.
505

506 Cohen, S.S., (1998) Bacterial Metabolism and Polyamines. In: A guide to the
507 polyamines. S.S. Cohen (ed). New York, USA: Oxford University Press, pp. 94 -
508 121.
509

510 Drecktrah, D., M. Lybecker, N. Popitsch, P. Rescheneder, L.S. Hall & D.S. Samuels,
511 (2015) The *Borrelia burgdorferi* RelA/SpoT Homolog and Stringent Response
512 Regulate Survival in the Tick Vector and Global Gene Expression during
513 Starvation. *PLoS Pathog* **11**: e1005160.
514

515 Dulebohn, D.P., C.L. Richards, H. Su, K.A. Lawrence & F.C. Gherardini, (2017) Weak
516 Organic Acids Decrease *Borrelia burgdorferi* Cytoplasmic pH, Eliciting an Acid
517 Stress Response and Impacting RpoN- and RpoS-Dependent Gene Expression.
518 *Front Microbiol* **8**: 1734.
519

520 Elias, A.F., J.L. Bono, J.A. Carroll, P. Stewart, K. Tilly & P. Rosa, (2000) Altered
521 stationary-phase response in a *Borrelia burgdorferi* rpoS mutant. *J Bacteriol* **182**:
522 2909-2918.
523

524 Elias, A.F., P.E. Stewart, D. Grimm, M.J. Caimano, C.H. Eggers, K. Tilly, J.L. Bono,
525 D.R. Akins, J.D. Radolf, T.G. Schwan & P. Rosa, (2002) Clonal polymorphism of
526 *Borrelia burgdorferi* strain B31 MI: implications for mutagenesis in an infectious
527 strain background. *Infect Immun* **70**: 2139-2150.
528

529 Falco, R.C., D. Fish & J. Piesman, (1996) Duration of tick bites in a Lyme disease-
530 endemic area. *Am J Epidemiol* **143**: 187-192.
531

532 Fisher, M.A., D. Grimm, A.K. Henion, A.F. Elias, P.E. Stewart, P.A. Rosa & F.C.
533 Gherardini, (2005) *Borrelia burgdorferi* sigma54 is required for mammalian
534 infection and vector transmission but not for tick colonization. *Proc Natl Acad Sci*
535 *U S A* **102**: 5162-5167.
536

537 Fraser, C.M., S. Casjens, W.M. Huang, G.G. Sutton, R. Clayton, R. Lathigra, O. White,
538 K.A. Ketchum, R. Dodson, E.K. Hickey, M. Gwinn, B. Dougherty, J.F. Tomb,
539 R.D. Fleischmann, D. Richardson, J. Peterson, A.R. Kerlavage, J. Quackenbush,
540 S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M.D. Adams, J. Gocayne, J.
541 Weidman, T. Utterback, L. Watthey, L. McDonald, P. Artiach, C. Bowman, S.
542 Garland, C. Fuji, M.D. Cotton, K. Horst, K. Roberts, B. Hatch, H.O. Smith & J.C.
543 Venter, (1997) Genomic sequence of a Lyme disease spirochaete, *Borrelia*
544 *burgdorferi*. *Nature* **390**: 580-586.
545

546 Gherardini, F.C., J.A. Boylan, K. Lawrence & J. Skare, (2010) Metabolism and
547 Physiology of *Borrelia*. In: *Borrelia: Molecular Biology, Host Interaction and*
548 *Pathogenesis*. D.S. Samuels & J. Radolf (eds). Norfolk, UK: Caister Academic
549 Press, pp. 103 - 138.
550

551 Gilbert, M.A., E.A. Morton, S.F. Bundle & D.S. Samuels, (2007) Artificial regulation of
552 ospC expression in *Borrelia burgdorferi*. *Mol Microbiol* **63**: 1259-1273.
553

554 He, M., Z. Ouyang, B. Troxell, H. Xu, A. Moh, J. Piesman, M.V. Norgard, M. Gomelsky
555 & X.F. Yang, (2011) Cyclic di-GMP is essential for the survival of the Lyme
556 disease spirochete in ticks. *PLoS Pathog* **7**: e1002133.
557

558 Hubner, A., X. Yang, D.M. Nolen, T.G. Popova, F.C. Cabello & M.V. Norgard, (2001)
559 Expression of *Borrelia burgdorferi* OspC and DbpA is controlled by a RpoN-
560 RpoS regulatory pathway. *Proc Natl Acad Sci U S A* **98**: 12724-12729.
561

562 Hyde, J.A., D.K. Shaw, R. Smith Iii, J.P. Trzeciakowski & J.T. Skare, (2009) The BosR
563 regulatory protein of *Borrelia burgdorferi* interfaces with the RpoS regulatory
564 pathway and modulates both the oxidative stress response and pathogenic
565 properties of the Lyme disease spirochete. *Mol Microbiol* **74**: 1344-1355.
566

567 Igarashi, K. & K. Kashiwagi, (1999) Polyamine transport in bacteria and yeast. *Biochem*
568 *J* **344 Pt 3**: 633-642.
569

570 Igarashi, K. & K. Kashiwagi, (2010) Modulation of cellular function by polyamines. *Int J*
571 *Biochem Cell Biol* **42**: 39-51.
572

573 Indest, K.J., R. Ramamoorthy, M. Sole, R.D. Gilmore, B.J. Johnson & M.T. Philipp,
574 (1997) Cell-density-dependent expression of *Borrelia burgdorferi* lipoproteins in
575 vitro. *Infect Immun* **65**: 1165-1171.
576

577 Kashiwagi, K., S. Miyamoto, E. Nukui, H. Kobayashi & K. Igarashi, (1993) Functions of
578 potA and potD proteins in spermidine-preferential uptake system in *Escherichia*
579 *coli*. *J Biol Chem* **268**: 19358-19363.
580

581 Katona, L.I., (2015) The Fur homologue BosR requires Arg39 to activate rpoS
582 transcription in *Borrelia burgdorferi* and thereby direct spirochaete infection in
583 mice. *Microbiology* **161**: 2243-2255.
584

585 Kostick, J.L., L.T. Szkotnicki, E.A. Rogers, P. Bocci, N. Raffaelli & R.T. Marconi,
586 (2011) The diguanylate cyclase, Rrp1, regulates critical steps in the enzootic cycle
587 of the Lyme disease spirochetes. *Mol Microbiol* **81**: 219-231.
588

589 Lin, T., L. Gao, C. Zhang, E. Odeh, M.B. Jacobs, L. Coutte, G. Chaconas, M.T. Philipp
590 & S.J. Norris, (2012) Analysis of an ordered, comprehensive STM mutant library
591 in infectious *Borrelia burgdorferi*: insights into the genes required for mouse
592 infectivity. *PLoS One* **7**: e47532.
593

594 Lin, Y.H., J.A. Romo, T.C. Smith, 2nd, A.N. Reyes, S.L. Karna, C.L. Miller, T.A. Van
595 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.

642
643 Samuels, D.S., (1995) Electrotransformation of the spirochete *Borrelia burgdorferi*.
644 *Methods Mol Biol* **47**: 253-259.
645
646 Samuels, D.S., (2011) Gene regulation in *Borrelia burgdorferi*. *Annu Rev Microbiol* **65**:
647 479-499.
648
649 Schwan, T.G., J. Piesman, W.T. Golde, M.C. Dolan & P.A. Rosa, (1995) Induction of an
650 outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc Natl Acad*
651 *Sci U S A* **92**: 2909-2913.
652
653 Shah, P. & E. Swiatlo, (2008) A multifaceted role for polyamines in bacterial pathogens.
654 *Mol Microbiol* **68**: 4-16.
655
656 Skare, J.T., J.A. Carroll, X. Yang, D.S. Samuels & D.R. Akins, (2010) Gene regulation,
657 Transcriptomics and Proteomics. In: *Borrelia: Molecular Biology, Host*
658 *Interaction and Pathogenesis*. D.S. Samuels & J.D. Radolf (eds). Norfolk, UK:
659 Caister Academic Press, pp. 67 - 101.
660
661 Sonenshine, D.E. & J.M. Anderson, (2014) Mouthparts and digestive system. In: *Biology*
662 *of Ticks*. D.E. Sonenshine & R.M. Roe (eds). New-York (USA): Oxford
663 University Press, pp. 122-162.
664
665 Stevenson, B., T.G. Schwan & P.A. Rosa, (1995) Temperature-related differential
666 expression of antigens in the Lyme disease spirochete, *Borrelia burgdorferi*.
667 *Infect Immun* **63**: 4535-4539.
668
669 Stewart, P.E. & P.A. Rosa, (2017) Physiologic and Genetic Factors Influencing the
670 Zoonotic Cycle of *Borrelia burgdorferi*. In: *Curr Top Microbiol Immunol*.
671 Springer, Berlin, Heidelberg, pp. 1-20.
672
673 The staff of the Jackson Laboratory, (1966) *Biology of the Laboratory Mouse*. Dover
674 Publications, Inc., New York.
675
676 Thomas, T., M.A. Gallo, C.M. Klinge & T.J. Thomas, (1995) Polyamine-mediated
677 conformational perturbations in DNA alter the binding of estrogen receptor to
678 poly(dG-m5dC).poly(dG-m5dC) and a plasmid containing the estrogen response
679 element. *J Steroid Biochem Mol Biol* **54**: 89-99.
680
681 Tilly, K., D. Grimm, D.M. Bueschel, J.G. Krum & P. Rosa, (2004) Infectious cycle
682 analysis of a *Borrelia burgdorferi* mutant defective in transport of chitobiose, a
683 tick cuticle component. *Vector Borne Zoonotic Dis* **4**: 159-168.
684
685 Waymouth, C., (1970) Osmolality of mammalian blood and of media for culture of
686 mammalian cells. *In Vitro* **6**: 109-127.
687

688 Wishart, D.S., T. Jewison, A.C. Guo, M. Wilson, C. Knox, Y. Liu, Y. Djoumbou, R.
689 Mandal, F. Aziat, E. Dong, S. Bouatra, I. Sinelnikov, D. Arndt, J. Xia, P. Liu, F.
690 Yallou, T. Bjorn Dahl, R. Perez-Pineiro, R. Eisner, F. Allen, V. Neveu, R. Greiner
691 & A. Scalbert, (2013) HMDB 3.0--The Human Metabolome Database in 2013.
692 *Nucleic Acids Res* **41**: D801-807.
693
694 Wishart, D.S., C. Knox, A.C. Guo, R. Eisner, N. Young, B. Gautam, D.D. Hau, N.
695 Psychogios, E. Dong, S. Bouatra, R. Mandal, I. Sinelnikov, J. Xia, L. Jia, J.A.
696 Cruz, E. Lim, C.A. Sobsey, S. Shrivastava, P. Huang, P. Liu, L. Fang, J. Peng, R.
697 Fradette, D. Cheng, D. Tzur, M. Clements, A. Lewis, A. De Souza, A. Zuniga, M.
698 Dawe, Y. Xiong, D. Clive, R. Greiner, A. Nazyrova, R. Shaykhtudinov, L. Li,
699 H.J. Vogel & I. Forsythe, (2009) HMDB: a knowledgebase for the human
700 metabolome. *Nucleic Acids Res* **37**: D603-610.
701
702 Wishart, D.S., D. Tzur, C. Knox, R. Eisner, A.C. Guo, N. Young, D. Cheng, K. Jewell,
703 D. Arndt, S. Sawhney, C. Fung, L. Nikolai, M. Lewis, M.A. Coutouly, I.
704 Forsythe, P. Tang, S. Shrivastava, K. Jeroncic, P. Stothard, G. Amegbey, D.
705 Block, D.D. Hau, J. Wagner, J. Miniaci, M. Clements, M. Gebremedhin, N. Guo,
706 Y. Zhang, G.E. Duggan, G.D. Macinnis, A.M. Weljie, R. Dowlatabadi, F.
707 Bamforth, D. Clive, R. Greiner, L. Li, T. Marrie, B.D. Sykes, H.J. Vogel & L.
708 Querengesser, (2007) HMDB: the Human Metabolome Database. *Nucleic Acids*
709 *Res* **35**: D521-526.
710
711
712 Wyss, C. & P. Ermert, (1996) *Borrelia burgdorferi* is an Adenine and Spermidine
713 Auxotroph. *Microbial Ecology in Health and Disease* **9**: 181-185.
714
715 Yang, X., M.S. Goldberg, T.G. Popova, G.B. Schoeler, S.K. Wikel, K.E. Hagman &
716 M.V. Norgard, (2000) Interdependence of environmental factors influencing
717 reciprocal patterns of gene expression in virulent *Borrelia burgdorferi*. *Mol*
718 *Microbiol* **37**: 1470-1479.
719
720 Yao, X. & C.D. Lu, (2014) Functional characterization of the potRABCD operon for
721 spermine and spermidine uptake and regulation in *Staphylococcus aureus*. *Curr*
722 *Microbiol* **69**: 75-81.
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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, Δ <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	TGGTACTAAAATAAAGGTGCTGAAGAA	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	GTGCACACTCTGACAACCTCT	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

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

759

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

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

769

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

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

779

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

781 Graph of the expression analyses of the *potABCD* operon of *B. burgdorferi* grown in
782 BSK-II at 250 and 450 mOsM. Relative gene expression was calculated using levels
783 expression of *enoS* as reference. Data represent means ± standard deviation of three
784 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.