EXPRESSION ANALYSIS OF BB0174, A MEMBRANE PROTEIN OF *Borrelia burgdorferi*, THE CAUSATIVE AGENT OF LYME DISEASE

A Senior Scholars Thesis

by

JOSEPH JAMES MODARELLI II

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

May 2012

Major: Veterinary Medicine and Biomedical Science

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Approved by:

Research Advisor: Maria Esteve-Gassent
Associate Director, Honors and Undergraduate Research: Duncan MacKenzie

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ABSTRACT

Expression Analysis of BB0174, A Membrane Protein of *Borrelia burgdorferi,* the Causative Agent of Lyme Disease. (May 2012)

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Borrelia burgdorferi (*Bb*), the causative agent of Lyme disease, is a spirochetal pathogen that colonizes mammalian hosts causing numerous neurological and physiological symptoms. This thesis focuses on the gene expression of BB0174, a membrane protein of *Bb.* Gene expression in *Bb* is dependent on many environmental signals following transmission from tick to host. The analysis of BB0174 shows high *in silico* similarities with the typical membrane associated histidine kinases of a Two-component system (TCS) for signal transduction. Thus this study will determine whether changes in temperature and pH conditions will induce changes in the levels of mRNA and protein of BB0174 in *Bb*. RNA will be purified from *Bb* growing at differing pH and temperature conditions similar to those found in the unfed tick and again from the fed tick. cDNA will then be generated and PCR analysis using specific primers for *bb0174* and neighboring regions will be conducted. The BB0174 protein levels will also be evaluated after growing *Bb* at room temperature and shifted to 37ºC. This project will allow us to determine whether or not BB0174 is in fact one of the members of a novel

TCS, and thus further our understanding of the mechanisms orchestrating gene expression in *Bb*. Taken together, out studies showed that *bb0174-bb0175* are not part of a new Two component system and that both are expressed under growth conditions that mimic the fed tick conditions (37ºC/pH6.8).

DEDICATION

This thesis is dedicated to Dr. Maria Esteve-Gassent, whose support and sacrifice has allowed me to become apart of such a great opportunity. She has forced me to learn in an environment I was completely new to, and for that I am deeply thankful. The opportunities she has presented me with through out my studies has allowed me to succeed in and out of the classroom during my undergraduate education.

ACKNOWLEDGMENTS

Dr. Maria Esteve-Gassent has been the ideal thesis supervisor. Her patience and wisdom has truly been the driving force through out this process. I would also like to thank my team, Erin McGregor and Elaine Wood, who I've spent many long hours with trudging through our research in the lab. With out their support I would have been lost, and this thesis would have never have been completed. I also thank the American Heart Association for funding Dr. Esteve-Gassent with the project "Study of the von Willebrand factor A containing proteins in *Borrelia burgdorferi"* under the scientist developing grant (11SDG4990006). Lastly I thank the Undergraduate Fellowship program of Texas A&M for their financial support and guidance.

NOMENCLATURE

LD	Lyme Disease
Bb	*Borrelia burgdorferi*
TCS	Two-Component System
HK	Histidine Kinase
RR	Response Regulator
PCR	Polymerase Chain Reaction
RT	Room Temperature (23ºC-25ºC)
Rxn	Reaction
Amp_{100}	Ampicillin 100µg/ml
Kan_{50}	Kanamycin 50µg/ml
Can_{20}	Chloramphenicol 20µg/ml
IPTG	Isopropyl β-D-1-thiogalactopyranoside
PASN	Post Absorption Supernatants
2x FSB	2X Final Sample Buffer
BSA	Bovine Serum Albumine
TBS	Tris Buffer Saline
RTSB	Reverse Transcriptase Buffer
Tm	Melting Temperature

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CHAPTER I

INTRODUCTION

Lyme disease (LD) is the most prevalent tick borne disease in the US with a total of 22,572 confirmed human cases reported to CDC in 2010. LD in the US is caused by the infection of a mammalian host with the bacterium *Borrelia burgdorferi (Bb) sensu stricto* acquired through the bite of an infected *Ixodes* ticks (2, 14, 26). *B. burgdorferi sensu stricto* is part of the *B. burgdorferi sensu lato* complex. Within this complex *B. afzelii* and *B. garnii* are the causative agents of LD in Europe and Asia together with 12 more genospecies (13, 18, 22). In addition, different *Ixodes* ticks will transmit the different Borrelia species. In this case, *B. burgdorferi sensu stricto* will be transmitted by *I. scapularis* in East US, *I. pacificus* in West US. *I. ricinus* and *I. persulcatus* will be the competent vector for the transmission of LD in Euroasia. *Ixodes scapularis,* or deer ticks, is more active during their nymphal stage, where infection of a host is more prevalent. Figure 1· describes the transmission of *Bb*, and the life cycle of *Ixodes scapularis*. Rodents, along with other various animals, play an important role as reservoir hosts, transferring *Bb* to the tick during its blood meal (7).

A significant increase in the number of reported cases has been observed in the past few years, classifying Lyme disease as a re-emerging infection. Lyme borreliosis is, therefore, an important public health issue particularly in endemic areas. According to

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· This thesis follows the style of Journal of Infection and Immunity.

the CDC, LD has surpassed AIDS as one of the fastest growing infectious epidemics in the US, with a cost to society measured at approximately $1 billion annually[A].

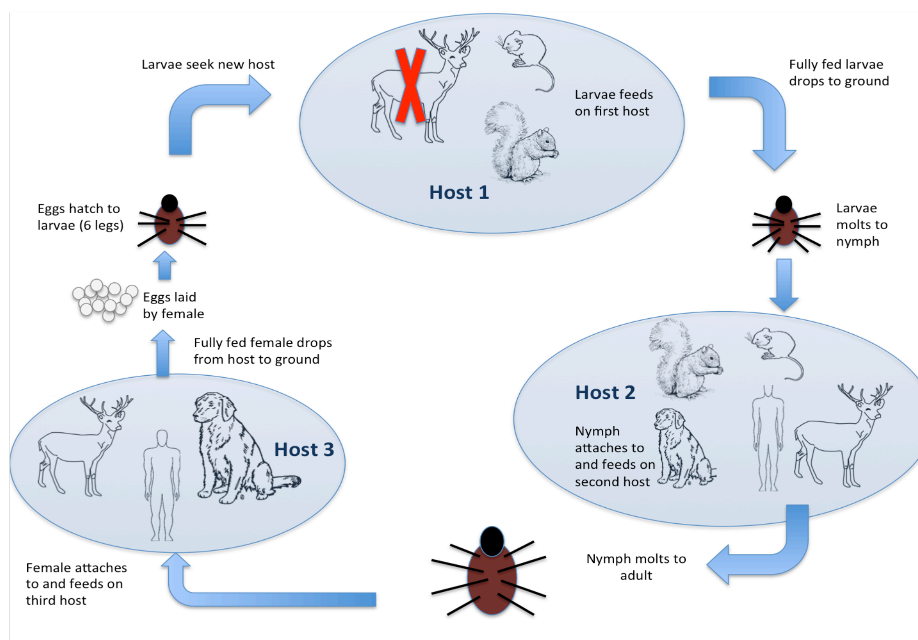

FIG 1**:** Lyme Disease Transmission Cycle. Eggs laid by an adult female deer tick in the spring hatch into larvae within a couple months. The larva will then locate a blood meal, Host 1, where if already affected with LD, will transmit the bacterium *Bb* to the larva. After feeding, the larvae will drop off Host 1, and proceed to molt into a nymph in fall months, and then remain inactive throughout the winter until early May, where nymphal activity begins. The nymph will now attach unto Host 2 (note human presence), and if previously infected with the LD spirochete, will transfer the bacterium to Host 2. Once engorged from its blood meal, the nymph will remove itself from Host 2 and molt into an adult, remaining active throughout the fall, awaiting the presence of Host 3. Mating may take place on or off the host, resulting in a gravid female, who around spring months, will detach from Host 3 and lay eggs underneath leaf litter and die. The eggs hatch later in the summer, beginning the two-year cycle.
Figure based on design by Erin McGregor.

[A] http://teenswithlyme.webs.com/statistics.htm

Bb is a spirochete bacterium that invades the blood and tissues of various mammals, mostly humans, dogs and horses. LD occurs as a multi-systemic disorder leading to carditis (10% of untreated adults), arthritis (60% of the cases) and other neurological and physiological symptoms. Erythema migrans, a "bullseye" shaped rash, is the only manifestation of LD that is sufficiently distinctive to allow clinical diagnosis in the absence of laboratory confirmation. However, the presence of an erythema migrans is only found in a small number of LD associated cases, making clinical diagnosis rare in patients affected by LD (1, 18, 23). Furthermore, there is very little information available on the tissue-specific, host pathogen interactions that lead to the pathological manifestations of infection with *Bb*.

The ability of this spirochetal pathogen to colonize mammals is dependent on its ability to rapidly alter gene expression in response to highly disparate environmental signals following transmission from infected ticks. This type of response is very much dependent on the presence of a robust signal transduction system that can induce change in gene expression (9, 12). In bacteria, the signal transduction relays on Two-component systems (TCS) (9). TCS typically consist of a membrane associated histidine kinase (HK) and a response regulator (RR).

Phosphotransfer-mediated signaling pathways allow cells to sense and respond to environmental stimuli (24). Typically the signal transduction starts by the autophosphorilation of a histidine residue in the HK upon sensing an environmental

signal. The phosphoryl group will be transferred to the RR, which will bind to DNA, RNA or a protein to regulate gene expression. *Bb* has only 2 TCS described so far (Hpk1-Rrp1 and Hph2-Rrp2) (17) while other bacterial species such as *E. coli* have an average 52 TCS (12).

Our hypothesis is that *Bb* might have other TCS that will be regulating gene expression upon recognition of different stimuli when this bacterium travels from the tick to the mammalian host. One of the candidates to be part of a TCS is BB0174. This protein has a signal peptide in the N-terminus, a transmembrane domain in the center of the protein and a phosphorylation site (H_{180}) in the C-terminus of the protein (Figure 2) and it is a protein of unknown function. Furthermore, upstream of BB0174 it is encoded BB0175 that has a phosphorylation site in amino acids 176-179 (KKRS) and it is also a protein of unknown function. Thus these two proteins shall be considered for functioning as an operon in *Bb*, where their expression may be as a single transcript or not at all.

Consequently, the main objective of this proposal is to determine the conditions at which BB0174, a membrane protein of *Borrelia burgdorferi* is expressed. We will be experimenting with two types of DNA extracted from *B. burgdorferi* B31 clonal derivative MSK5. Moreover, the presence of an operon comprising BB0174-BB0175 will also be evaluated by means of PCR analysis. This will help understanding whether BB0174 and BB0175 could be part of a novel TCS in *B. burgdorferi*, setting the basis

for further research identifying the mechanism by which this system regulates the gene expression in the causative agent of Lyme disease.

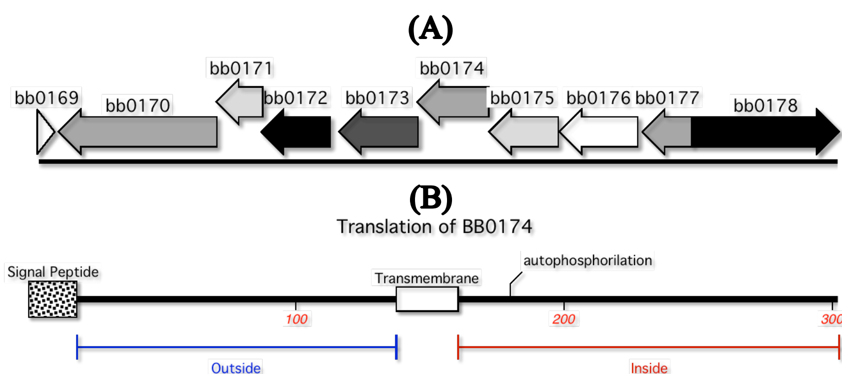

FIG 2: bb0174 Chromosomal Region. **(A)** Chromosome region where bb0174 and bb0175 are encoded. **(B)** Structural components of BB0174: the signal peptide comprises amino acids 1-18, with cleavage site in between amino acid 18-19 (LYS-YE), transmembrane domain extends from amino acid 138 to 160, the hypothetical histidine for autophosphorilation is in position 180. Consequently the amino acids 19-137 are exposed to the outside, while amino acids 161 through 302 are inside the cell.

CHAPTER II

METHODS

Cloning *bb0174* into pCR®2.1 TOPO®

In order to work with the gene of interest we cloned it in the commercially available cloning vector pCR®2.1 TOPO® (Invitrogen™). To do so we followed the steps described bellow.

TABLE 1: PCR Reaction (Ex Taq). Illustrates "recipe" for PCR reaction (Takara).

	1 rxn
10x buffer	2.500 µl
10x dNTP	2.500 µl
Primer Mix	2.000 µl
Ex Taq polymerase	0.125 µl
DNA	2.000 µl
ddH_20	15.875 µl

PCR amplification and cloning steps

The *bb0174* gene without the first 78 nucleotides (encoding for the signal peptide) was amplified by PCR using *B. burgdorferi* B31 template genomic DNA and primers with engineered restriction enzyme sites for NdeI (forward) and XhoI (reverse). The PCR reaction protocol was: 94°C for one minute, followed by 30 cycles of 94ºC for one minute, annealing at 45°C for one minute, and amplification at 72°C for three minutes and a final 4°C (TABLE 1). Amplicons were confirmed by running a 0.8% agarose gel

at 100 volts for 45 minutes. Positive PCR products were cloned into pCR®2.1 TOPO® vector following manufactures recommendations. Briefly, 4µl of the PCR product were incubated with 1µl of the vector at room temperature (RT) over night. Final reaction was transformed into TOP10 Component cells (Invitrogen™) by electroporation (Electroporator 2510 Eppendorf) at, 25µF capacitor, 2.5kV, and the Pulse Controller Unit to 200Ω). Cells were recovered in SOC media for one hour at 37ºC. Positive clones were selected overnight at 37ºC on LB agar plates containing 100µg/ml of Ampicillin (Amp_{100}) and 50µg/ml of Kanamycin (Kan_{50}), 40µg X-Gal and 1mM IPTG. Twelve positive colonies were inoculated in LB broth with Amp_{100} and Kan_{50} and incubated overnight at 37ºC for further analysis.

TABLE 2: EPB Recipe. This recipe makes 50ml of Easy Prep Buffer (EPB). One-milliliter aliquots were stored at -20ºC.

1M Tris pH8	500 µl
0.5M EDTA pH 8	100 µl
Sucrose	6.500 g
Lysozyme	0.100 g
Pancreatic RNase	0.005 g
BSA	0.005 g

TABLE 3: EcoRI Reaction.

	1 rxn
10 x buffer	2.0 µl
EcoR1	0.5 µl
DNA	10.0 µl
ddH_20	7.5 µl

Confirmation of positive clones

Cells from overnight cultures were harvested by centrifugation at 10,000 rpm for four minutes. Pellets were re-suspended in easy prep buffer (EPB) (TABLE 2) followed by boiling for one minute and centrifuging at 12000rpm for 15 minutes. The supernatants were used in the digestion reaction with EcoRI enzyme at 37ºC for 90 minutes (TABLE 3). Positive clones (PCR 2.1 *bb0174*) were saved at -80ºC for further use.

TABLE 4: Recipe for Nde1 and Xho1.

* = 10 x buffer #4

	1 rxn
10 x buffer*	4.0 µl
Nde1	3.0 µl
Xho1	3.0 µl
BSA	0.4 µl
DNA	25 µl
ddH2O	3.6 µl

Expression of rBB0174

Cloning of bb0172 in the expression vector pET23a.

In order to express recombinant BB0174*, bb0174* cloned in pCR®2.1 and the pET23a expression vector (Novagen ®) were digested with NdeI and XhoI (New England Biolabs) restriction enzymes respectively at 37ºC for two hours (TABLE 4). Digestion products were separated in a 0.8% agarose gels. Bands corresponding with digested *bb0174* and pET23a were excised and DNA extracted using the Gel and PCR Clean up

kit by Promega following manufacturer's recommendations. DNA was eluted in 30µl of ultraclean water and used in the ligation reaction specified in TABLE 5 overnight at 16ºC. After ligation was completed DNA was cleaned by ethanol precipitation, air-dried and re-suspended in 20µl of ultrapure water. Two µl of the clean ligation reaction was used to transform TOP10 cells using the same electroporation and recovery protocols described above. Positive clones were selected on LB agar plates containing Amp_{100} following the same screening process described in the previous section. In this case, positive clones were confirmed by digestion with NdeI and XhoI as well as by sequencing reactions.

TABLE 5: Ligation Reaction. Recipe for Ligation reaction using Promega T_4 DNA Ligase.

	1 rxn
10 x buffer*	2 µl
Insert (*bb0174*)	10 µl
Vector (pET23a)	5 µl
T_4 DNA ligase	1 µl
H_2O	2 µl

Sequencing reaction

Positive clones for pET23a(*bb0174*) by restriction digestion were grown overnight and plasmidic DNA extracted using the PureYield™ Plasmid Miniprep System (Promega). Ten µl of each clone were submitted for sequencing (Eton Biosciencies) utilizing specific primers to the T7 Promoter and T7 Terminator under which *bb0174* was cloned

in pET23a. Results were analyzed using the MacVector computer program (MacVector, Inc.) to determine whether *bb0174* was in frame with the promoter, the histidine tag and the terminator sequence.

Transformation into Rosetta® component cells

DNA from the positive pET23a(*bb0174*) was electroporated into the expression *E. coli* strain Rosetta® using the conditions described above. After recuperating the transformed cells in SOC for one hour at 37ºC, they were plated on LB agar containing Amp_{100}, and chloramphenicol 20µg/ml **(**Can_{20}) at 37ºC overnight. Two colonies were picked, incubated at 37ºC overnight, and used for a small-scale induction to check conditions at which the protein was expressed. Positive clones were stored at -80ºC for further use.

Expression and purification of rBB0174

The first step to purify the rBB0174 was to induce their expression in *E. coli*. To this end, a culture of the *E. coli* strain Rosetta^{TM} expressing rBB0174 was started in LB broth media containing Amp_{100} and Can_{20} at 37ºC, shaking overnight. A 1:100 dilution of each of the cultures was used to start one liter of LB Broth containing Amp_{100} and Can_{20}. Large cultures were shaken for 4-5 hours at 37ºC or until OD_{600nm}=0.5 to 0.8. When cultures reached the right bacterial density, BB0174 expression was induced by adding 1ml of Isopropyl β-D-1-thiogalactopyranoside (IPTG) and incubated for 2 hours at 37°C with continuous shaking. After the two hour induction, cells were harvested by centrifugation for 20 minutes at 4000rpm and 4ºC. Pellets were stored at -80°C until use.

Protein purification

After thawing on ice the pellets of Rosetta™ cells expressing the rBB0174, 25ml of Lysis Buffer (50mM sodium phosphate, 8M urea, 300mM NaCl, 20mM imidazole pH 7.4) were added to re-suspend the cells. Cells were disrupted by French press (Thermo scientific) three times to ensure complete lysis. Lysates were centrifuged for 20 minutes at 4000rpm and 4ºC to eliminate cell debris. The pellet was saved at -80°C until purification of the protein was confirmed. Supernatants were mixed with 5ml of Nickel beads (His60 Superflow™ resin, Chlontech) previously equilibrated with Wash Buffer (50mM sodium phosphate, 8M urea, 300mM NaCl, 40mM imidazole pH 7.4) and incubated overnight at 4°C with continuous gentle shacking. After the overnight binding, beads were clean by centrifugation for five minutes at 500 rpm and 4ºC to remove any unspecific protein binding. Post absorption supernatants (PASN) and washing fraction were saved at -80ºC until purification of the recombinant protein was confirmed. Beads with the recombinant His tag containing proteins were allowed to pack at room temperature in a chromatography column (BioRad). While the beads settle, two sets of 25 elution collection tubes were prepared. BB0174 was eluted by adding 10ml of the elution buffer (50mM sodium phosphate, 8M urea, 300mM NaCl, 300mM imidazole pH 7.4) to the column. Approximately 20ml of elution buffer is needed to ensure the adequate purification of the recombinant proteins. Fifty µl of each elution fraction was combined with 50µl of 2X Final Sample Buffer (2x FSB) to check for the faction in whom the recombinant protein gets purified. Elution fractions were saved at -80°C until use to immunize chickens to generate specific antibodies (IgY).

SDS-PAGE gel

In order to determine in which elution fractions the rBB0174 was mostly present, an aliquot of the induction pellet, PASN, wash fractions and each of the elution fractions were separated in a 12% SDS-PAGE gel. Gels were stained in Coomassiee Blue (0.25% coomassiee brilliant blue dye R250, 45% methanol, 10% acetic). After destaining (45% methanol, 10% acetic acid), the more concentrated elution fractions were selected and prepared for purification. The gels were stored (10% ethanol, 5% glycerol) and dried for further reference.

Protein clean up and concentration

The fractions containing BB0174 were cleaned and concentrated by using the Amicon filtration system (Millipore) with cut off pores of 3kDa or 5kDa to ensure the retention of the protein of interest (30kDa). The concentrated protein was further cleaned by dialysis to ensure the elimination of the denaturing agent Urea and the Imidazol used to elute the protein during the purification steps. To this end, the fractions being cleaned are placed in a dialysis cassette (Slide-A-Lyser® Cassette, Thermos Scientific) and in 500ml of dialysis buffer (50mM sodium phosphate, 300mM NaCl) for two hours at RT and continuous stirring with buffer changed every hour, followed by an overnight dialysis with fresh buffer at 4ºC. After dialysis, the concentrated fractions were recovered and stored at -80°C. Aliquots were used to determine protein concentration.

Protein quantification

Protein concentration was determined by using the Pierce BSA Assay Kit (Thermo

Scientific) so as to adjust the protein needed to immunize laying hens. Briefly, BSA (Bovine Serum Albumin) protein standards were made with concentrations ranging from 0 to 250µg/ml. The test protein was diluted 1:5 and 1:10 and 25µL of each protein dilutions and standards were placed in triplicates in a 96 well plate (Corning). After adding 200µl of the Working reagent (following manufacturer's recommendations) plates were incubated for 30 minutes at 37ºC and in the dark. After incubation, protein concentration was measured in a plate reader (BGI LABTECH OMEGA).

Generation of specific BB0174 antibodies

In order to determine the conditions at which *bb0174* expresses in *Bb*, we will analyze not only its RNA levels but also those of the BB0174 protein by immunoblot assay after growing *Bb* under different conditions. To this end we need specific antibodies that were generated in-house utilizing the laying hens model currently in use in our laboratory to generate specific antibodies towards borrelial proteins. Briefly, we immunized two laying hens with 50µg of rBB0174 per hen at days 0, 14 and 21. TiterMax^{TM} (Sigma-Aldrich) was used as adjuvant during the whole immunization protocol (v:v). After day 28 none-fertilized eggs were collected and the specific IgY was purified.

Purification of specific BB0174 IgG (IgY)

To extract the IgG (IgY) from none-fertilized eggs, we collected them after day 28 of the immunization protocol described above. In this case, we collected eggs from days 30, 34 and 38. Eggs were stored at -20ºC to help during the separation of the egg yolk (where the IgY is located) from the albumin present in the egg white. Eggs were thawed and the

albumin was separated from the egg yolk. The egg yolks were pooled and re-suspended in 300ml of sterile ultrapure water. To dilapidate the yolk pH was adjusted to 7 and stored overnight at -20ºC. After thawing the yolk emulsification, lipids were separated by centrifugation at 4000rpm for 20 minutes ant 4ºC. Supernatants were collected and the pH was re-adjusted to 7.6. Finally the IgY was desalted out with $(NH_4)_2SO_4$ stirring for one hour at RT, re-suspended in Tris buffer saline (TBS) aliquoted and stored at -20ºC until use.

Expression of *bb0174* and *bb0175* in *B. burgdorferi B31*

In order to determine under which conditions *bb0174* and *bb0175* were expressed so as to determine the presence of an operon, we followed the methodologies described below.

B. burgdorferi B31 bacterial cultures

B. burgdorferi B31 strain MSK5 was grown in BSK-II medium complemented with 6% inactivated rabbit serum at RT at pH 7.6 in order to mimic the unfed tick conditions. After bacterial cultures reached a cell density of $5*10^7$cells/ml a subculture was started at $5*10^5$cells/ml density at 37ºC pH 6.8 so as to mimic the tick feeding conditions. When bacterial cultures reached a cell density of $5*10^7$cells/ml (either at RT or at 37ºC), spirochetal cells were harvested by centrifugation for 20 minutes at 4000rpm and 4ºC and subsequently washed in HBSS. Cells pellets were used for RNA extractions.

RNA extraction

To isolate the RNA from the *Bb* bacterium the RiboPure™-Bacteria Kit protocol (Applied Biosystems) was utilized following manufacture's instructions with slight modifications to optimize yields. Briefly, *Bb* cultures growing at RT or 37ºC were harvested as described above and pellets resuspended in 200µl of HBSS. To lyse the cells 400µl of RNA*wiz* were added and the mix was vigorously vortexed. After ensure complete mixing of the cells with the phenol reagent, 250µl of chloroform was added and incubated at RT for 10 minutes with mixing every 2 minutes. To separate the aqueous from the organic phase, lysates were centrifuged for five minute at 10,000rpm and 4ºC. The aqueous phase was collected and mixed with 0.5 volumes of 100% ethanol. At this point the RNA was purified by using manufacturer's recommendations. RNA was eluted in a total of 60µl of elution buffer. RNA extraction was treated with DNase for 30minutes at 37ºC as recommended by the manufacturer in order to eliminate any DNA contamination in the purification sample. DNase reaction was stopped by incubating for 2 minutes with DNase inhibitor reagent provided in the kit. RNA concentration and quality was measured by using a Nanodrop and stored at -80ºC until use.

cDNA generation

In order to synthesis cDNA from our RNA extracts, we used the High Capacity RNA-to-cDNA Kit (Applied Biosystems) following manufacturer's protocol. Briefly, 20µl of the extracted RNA was combined with 20µl Reverse transcriptase buffer (RTSB), and 2µl of the RTSB Enzyme Mix. Reactions were incubated at 37ºC for 60 minutes followed by 5

minutes incubation at 95ºC in a thermocycler. cDNA samples were stored at -20ºC until use in PCR reactions.

TABLE 6: PCR Reaction Using GoTaq® (Promega).

	1 rxn
5 x buffer	5.000 µl
dNTP	1.000 µl
Primer mix	2.000 µl
Go Taq	0.125 µl
cDNA	2.000 µl
ddH_2O	14.875 µl

TABLE 7: Primers. Primer utilized in this study. Underlined portions represent restriction enzymes.

Primer Name	**Sequence (5' → 3')**	**Tm (ºC)**
BB0170 Forward	GTTAAACCGATTCCTGGAGAG	58
BB0170 Reverse	CAGCCAAAACTTGATGCTC	62
BB0171 Forward	CGAGGTGGCTTTCAAAGAAG	45
BB0171 Reverse	TCTCAACCATACAACCCTCTCA	49
BB0172 Forward	GCTTGATTTTTTTAATTTTATCC	45.3
BB0172 Reverse	CGGGATTACTCCCGCCAATCCCAA	63.1
BB0173 Forward	GAAGATGATACATCTTAGTGCTGG	53
BB0173 Reverse	CTTCCCTGATAAAATTTTCCAGAT	51.2
BB0174-NdeI-F	ACGCCATATGATGCCGACTCGTTATTAT	69
BB0174-XhoI-R	ACGCCTCGAGAATGTTAACATTAGCACC	69
BB0174 Forward	CCTGGAACAAGCACCTATTG	50
BB0174 Reverse	CACTTTGATCCCCACCATCT	47
BB0175 Forward	TTTCATGAGTTTAGGCCG	55
BB0175 Reverse	TGTTGACTTGCTAAACCC	52
BB0176 Forward	TTACTTGAAGGGGTTCCG	56
BB0176 Reverse	ATCCCTTTCACGAGATGC	56

Polymerase Chain Reaction (PCR)

PCR reactions were conducted following manufactures recommendations, briefly

illustrated by TABLE 6. Different primer mixes (TABLE 7) comprising the priming Forward and Reverse of each of the genes tested were used. The program used in this part of the study was as follows: 94°C for one minute, and 30 cycles of 94ºC for 1 minute, annealing temperature 55°C for one minute, and polymerization temperature of 72°C for three minutes. Samples were held at 4ºC until loaded in to a 0.8% agarose gel.

Quantitative Real Time PCR reaction

In order to quantify the levels of expression of the different genes studied we carried out Real time PCR utilizing the cDNA generated from the RNA extraction obtained after growing *B. burgdorferi* B31 strain under different conditions. To perform the gene expression analysis we used customized plates for *B. burgdorferi* from Lonza StellArray™ Gene expression Arrays. Plates were divided in 3 sets of 32 wells each. Each set contains 31 different primer sets (one set per well) to detect the levels of expression of the genes outlined in TABLE 8. Briefly, cDNA (400ng/well) was mixed with 2xSYBR® Green Master Mix (Aplied Biosystem) and water in order to have a total of 20µl/well. One plate was used per cDNA sample (RT vs. 37ºC in duplicate) in order to acquire enough data points to compare expression profiles. After loading the plate with the PCR master Mix containing the cDNA, plates were centrifuged for 2 minutes at 500rpms and loaded on an ABI 7500 Real Time PCR thermocycler using the following cycler:

1) One cycle of 50°C for 2 minutes (helps to dissolve primers)

2) One cycle of 94ºC for 10 minutes

3) Forty cycles of: 95°C for 15 seconds 60°C for 1 minute

4) Dissociation curve (Melt curve)

Ct values were recorded and utilized in the Global Pattern Recognition™ Data analysis tool offered by Lonza StellArray™ to determine the expression profile of the genes studied.

TABLE 8: List of Genes. List of genes in the customized StellArray™ (Lonza) plates

Gene	**Locus**	**Location**	**Function**
SodA	*bb0153*	Chromosome	Superoxide dismutase A (Mn-dependent)
BosR	*bb0647*	Chromosome	Borrelia oxidative stress regulator
NapA	*bb0690*	Chromosome	neutrophil activating protein A
CsrA	*bb0184*	Chromosome	Carbon storage regulator A
GAPDH	*bb0057*	Chromosome	Glycolytic pathway
RpoN	*bb0450*	Chromosome	RNA polymerase Sigma factor (σ-54)
RpoS	*bb0771*	Chromosome	RNA polymerase Sigma factor
Rrp1	*bb0419*	Chromosome	Response regulator protein
Hpk1	*bb0420*	Chromosome	Sensory transduction histidine kinase
OspA	*bba15*	Lp54	Outer surface protein
OspC	*bbb19*	Cp26	Outer surface protein
OspF	*bbr42*	Cp32-4	Outer surface protein (renamed as ErpY)
DbpA/B	*bba24/bba25*	Lp54	Decorin binding proteins A and B
FlaB	*bb0147*	Chromosome	Flagellin subunit (p41)
BBK32	*bbk32*	Lp54	Fibronectin binding protein
BBA34	*bba34*	Lp54	Bacterial extracellular solute binding protein
BBA64	*bba64*	Lp54	Hypothetical protein (antigen P35)
BBA65	*bba65*	Lp54	Hypothetical protein
BBA66	*bba66*	Lp54	Outer surface protein
BB0172	*bb0172*	Chromosome	Hypothetical protein (von Willebrand factor type A domain protein)
BB0174	*bb0174*	Chromosome	Hypothetical protein
BB0173	*bb0173*	Chromosome	Hypothetical protein (von Willebrand factor type A domain protein)
BB0175	*bb0175*	Chromosome	Hypothetical protein
P66	*bb0603*	Chromosome	Integral outer membrane protein P66
BB0831	*bb0831*	Chromosome	Xylose operon regulatory protein
BB0171	*bb0171*	Chromosome	Tetratricopeptide repeat domain protein
MvaD	*bb0686*	Chromosome	Diphosphomevalonate decarboxylase
BB0687	*bb0687*	Chromosome	Phosphomevalonate kinase, putative
Mvk	*bb0688*	Chromosome	Mevalonate kinase
XylR1	*bb0693*	Chromosome	Xylose operon regulatory protein
RecA	*bbb0131*	Chromosome	Recombinase A

Immunoblot assay

In order to determine the levels of BB0174 in *Borrelia burgdorferi* after growing under different pH and temperature conditions we ran immunoblot assays. To this end, after growing and harvesting cells following the protocol described above, cells were re-suspended in 50µl of HBSS buffer and lyzed by adding 50µl of the 2xFSB. After boiling for 5 minutes, 5µl of the cell lysate were separated on a 12%SDS-PAGE gel. One gel was used for coomassie blue staining an another gel was transferred to nitrocellulose membranes and blocked overnight at 4ºC in Tris Buffer saline (TBS) containing 0.2% Tween 20 and 10% skim milk. After blocking membranes were incubated for 1hr at room temperature with 1:1500 dilution of the Chicken anti-BB0174 antibody. After the primary antibody incubation, membranes were washed 4 times 5 minutes each with TBS containing 0.2% Tween 20 (TBS-T) followed by 1hr incubation at RT in 1:3000 dilution of the anti-chicken –HRP conjugated antibody when detecting the rBB0174, and 1:25,000 dilution of the anti-chicken-HRP conjugated antibody when detecting BB0174 in *Borrelia burgdorferi*. After washing 6 times for 5 minutes in TBS-T membranes were incubated with ECL detection reagent (GE Healthcare) when detecting rBB0174 and with ECL prime detection reagent (GE Healthcare) when detecting BB0174 on *B. burgdorferi.* Membranes were exposed for 30 seconds to 5 minutes on autoradiography films and developed in a dark room.

CHAPTER III

RESULTS

Cloning *bb0174* into pCR®2.1 TOPO®

After *bb0172* was amplified by PCR we confirmed the correct size of the amplicon by running a 0.8% agarose gel (figure 3, lane 2). This 900 base pairs amplicon was cloned into the cloning vector pCR® TOPO® (Invitrogen) to purify enough DNA to continue with the cloning of *bb0174* into the expression host. The positive clones were also stored at -80ºC for further use. A representative cartoon of this cloning step is outlined in figure 4A.

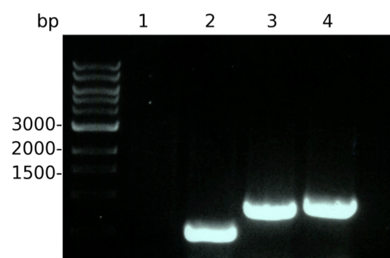

FIG 3: PCR Amplification of *bb0174.* Lane 3 shows *bb0174* at roughly 900 base pairs.

Expression of rBB0174

Cloning of bb0172 in the expression vector pET23a

After cloning *bb0174* into pCR2.1® we were ready to start the cloning of this gene into the expressing vector pET23a (Novagen). A cartoon representing the final clone is depicted in figure 4B. To this end, we digested both the pET23a vector and the pCR2.1

(*bb0174*) construct with, NdeI and XhoI restriction enzymes (New England Biolabs). Digestion products were separated in a 0.8% agarose gel as shown in figure 4C. The correct bands for digested pET23a and bb0174 were excised from the gel and purified using the Promega PCR and gel Clean-up kit and ligated as described in the material and methods section. After transforming Top 10 *E. coli* cells with the ligation products and after selecting on Ampicillin plates, positive clones were screened by PCR using primers specific for *bb0174* and also primers aligning with the T7 promoter and terminators present in pET23a clone to verify both, that we have the right insert cloned and that the vector is pET23a. This step was necessary since pCR2.1® which also contains ampicillin resistance that can contaminate the final ligation product that was used in the transformation. After picking the right clones, we stored them at -80ºC for further use.

Sequencing reaction

Positive clones for pET23a (*bb0174*) by restriction enzyme digestion and PCR were grown overnight and plasmidic DNA was extracted from each of the clone and were submitted for sequencing utilizing specific primers to the T7 Promoter and T7 Terminator under which *bb0174* was cloned in pET23a. With the sequencing reaction we found that *bb0174* was cloned in frame with the T7 promoter, the six histidines were present in frame in the C-terminator end of the protein followed by the T7 terminator. With these results we confirmed that *bb0174* has been cloned correctly (figure 4). We used clone number 10 for expression and purification of recombinant BB0174 protein. Sequencing reaction for BB0174 clone #10 using T7-promoter, and T-7 terminator can be depicted in figure 5.

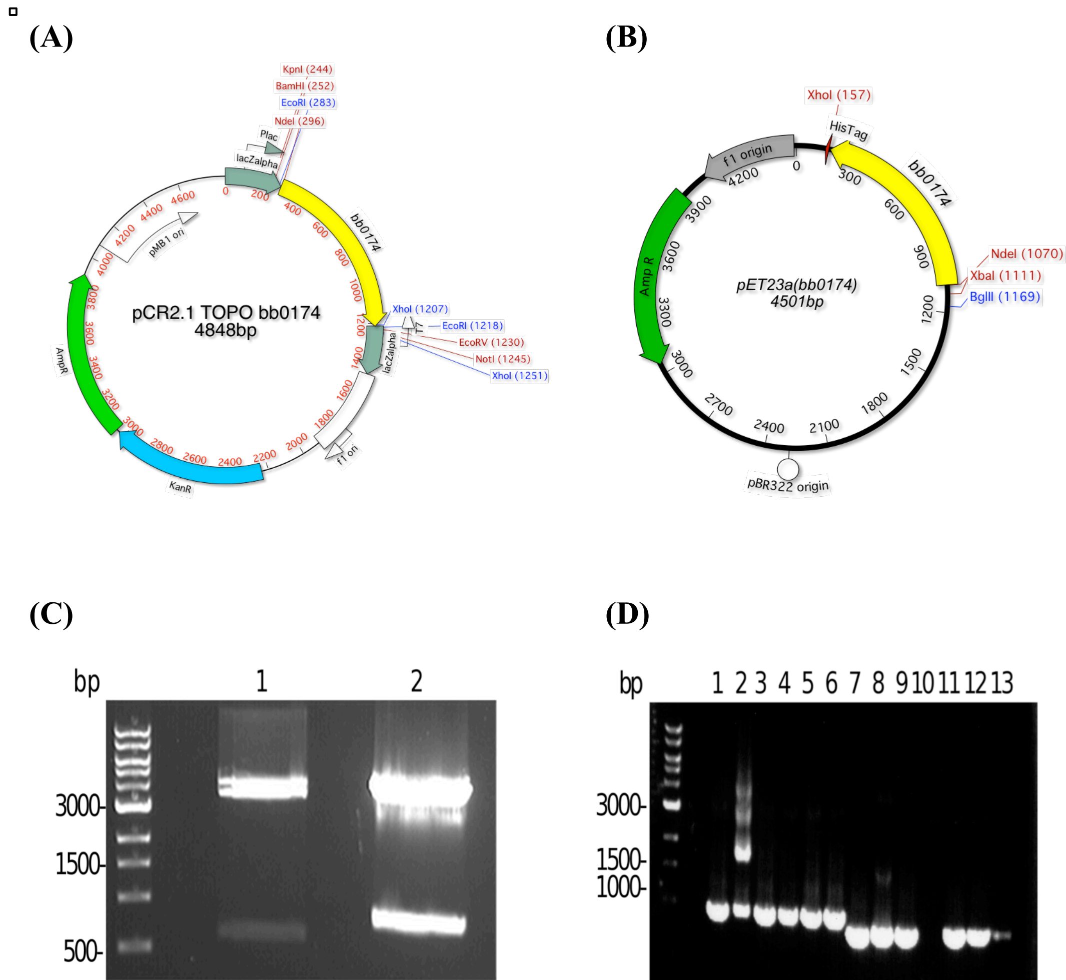

FIG 4: pCR2.1 TOPO®. Representative cartoons of the cloning of ***bb0174*** in pCR2.1 TOPO ® (A) and the expression vector pET23a (B). Cloning of *bb0174* in expression vector pET23a (C). Lane 1 represents digested pET23a with NdeI and XhoI enzymes (NEB), lane 2 represents *bb0174* cloned in pCR®2.1 and digested with the same enzymes (D). PCR amplification reaction to screen for

(A)

```
Sequencing: BB174 clone #10 using T7-promoter:
                10        20        30        40        50
          NCAAGAGATTTCCTCTAGAATAATTTTGTTTAACTTTAAGAAGGAGATAT
          NGTTCTCTAAAGGAGATCTTATTAAAACAAATTGAAATTCTTCCTCTATA
           X  R  D  F  L  *  N  N  F  V  *  L  *  E  G  D  I>
          ___TRANSLATION OF AC-BB174-10-T7-PROMOTE_D02.S____>

                60        70        80        90       100
          ACATATGATGCCGACTCGTTATTATGTAGGTGATTCTGTTGATTTTAAAG
          TGTATACTACGGCTGAGCAATAATACATCCACTAAGACAACTAAAATTTC
            H  M  M  P  T  R  Y  Y  V  G  D  S  V  D  F  K>
          ___TRANSLATION OF AC-BB174-10-T7-PROMOTE_D02.S____>

               110       120       130       140       150
          TTTCATTAATTTTAAATGATGGTGAAGAGTTTTCCCCTGTAGATTTTAAA
          AAAGTAATTAAAATTTACTACCACTTCTCAAAAGGGGACATCTAAAATTT
          V  S  L  I  L  N  D  G  E  E  F  S  P  V  D  F  K>
          ___TRANSLATION OF AC-BB174-10-T7-PROMOTE_D02.S____>

               160       170       180       190       200
          GATATTAATATTAAAGATGAATTTGTTGAAGTAAATTCGATTAGTTTTAA
          CTATAATTATAATTTCTACTTAAACAACTTCATTTAAGCTAATCAAAATT
           D  I  N  I  K  D  E  F  V  E  V  N  S  I  S  F  N>
          ___TRANSLATION OF AC-BB174-10-T7-PROMOTE_D02.S____>
```

(B)

```
Sequencing reaction: BB174 clone#10 using T7-terminator:
                10        20        30        40        50
          NNGGGTAGACAACTCAGCTTCTTTCGGGCTTTGTTAGCAGCCGGATCTCA
          NNCCCATCTGTTGAGTCGAAGAAAGCCCGAAACAATCGTCGGCCTAGAGT
            <P  L  C  S  L  K  K  R  A  K  N  A  A  P  D  *
           <___TRANSLATION OF AC-BB174-10-T7-TERMINA_E02____

                60        70        80        90       100
          GTGGTGGTGGTGGTGGTGCTCGAGAATGTTAACATTAGCACCTCTATTAA
          CACCACCACCACCACCACGAGCTCTTACAATTGTAATCGTGGAGATAATT
          <H  H  H  H  H  H  E  L  I  N  V  N  A  G  R  N  F
         <___TRANSLATION OF AC-BB174-10-T7-TERMINA_E02.S____

               110       120       130       140       150
          ATTCTTCAAAATTCGATGCTGCCGTTCTTAAATCCTCTAAAACAAACGAC
          TAAGAAGTTTTAAGCTACGACGGCAAGAATTTAGGAGATTTTGTTTGCTG
           <E  E  F  N  S  A  A  T  R  L  D  E  L  V  F  S
         <___TRANSLATION OF AC-BB174-10-T7-TERMINA_E02.S____

               160       170       180       190       200
          AAGCTATCAAATACCAAATCAACACCACTAAATTTACTAAAATCAGAAAG
          TTCGATAGTTTATGGTTTAGTTGTGGTGATTTAAATGATTTTAGTCTTTC
         <L  S  D  F  V  L  D  V  G  S  F  K  S  F  D  S  L
         <___TRANSLATION OF AC-BB174-10-T7-TERMINA_E02.S____
```

FIG 5: Sequencing Reaction for pET23a(*bb0174*). Utilizing the T7 promoter (A) and T7 terminator (B) primers. The first 200 nucleotide sequenced are represented. Highlighted in yellow, is the His*tag, in green, are the NdeI and XhoI digestion recognition sites and in red the starting Met.

Expression and purification of rBB0174

After confirming that the pET23a(bb0174) construct had all the required components in frame for optimal expression of BB0174, Rosetta^{TM} expression host cells were transformed with this construct and small scale inductions were done in order to determine how long the induction reaction needs to take so as to get rBB0174 expressed in optimal amounts. As shown in figure 6A, two to three hours are enough to induce the expression of rBB0174. Larger cultures of pET23a(*bb0174*) construct in Rosetta were started and induced for 2 hours. Cells were harvested and lysated using a French press to prevent degradation of the recombinant protein expressed. After the proteins bind to the Nickle bead column as described in material and methods, each one of the fractions collected was separated in 12% SDS-PAGE gels. As shown in figure 6B, BB0174 was present in elution fraction 4 through 10. These fractions were pooled and dialyzed to remove the Urea and Imidazol that were present during the purification process. After dialysis, the purified protein was concentrated by using the Amicon centrifugation system. Proteins were quantified by using the BCA assay giving a concentration ranging around 200µg/ml. The clean protein was used to immunized chicken and obtain rBB0174 specific IgY that was used in the evaluation of BB0174 levels in *B. burgdorferi* growing at different conditions.

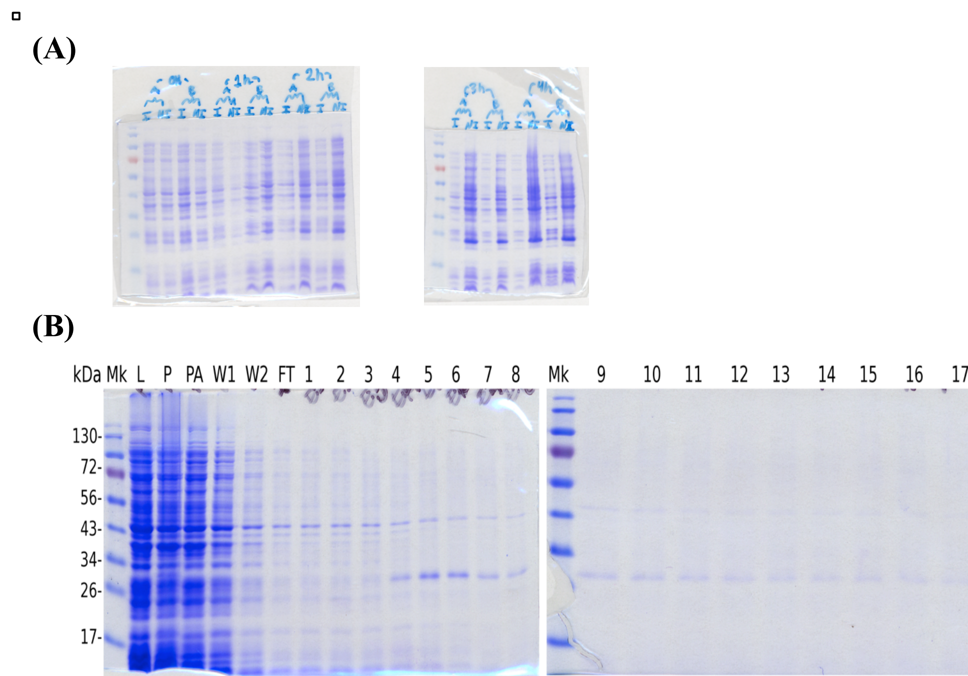

FIG 6: SDS-Page gel. Mentioned in order of lanes: Marker, lysate, pellet, PASN, Wash 1, Wash 2, Elutions 1-17.

Expression of B. burgdorferi B31 genes

In order to determine under which conditions *bb0174* and *bb0175* were expressed *B. burgdorferi B31* strain MSK5 was grown at RT/pH7.6 and 37ºC/pH6.8. As observed in FIG 7A PCR reaction using genomic Borrelial DNA and each of the primer sets designed for *bb0170, bb0171, bb0172, bb0173, bb0174, bb0175,* and *bb0176* showed an amplicon. This confirms that they can be used in the study of the gene expression using PCR reaction and cDNA form Borrelia growing under different temperature and pH conditions. When analyzing the gene expression in the borrelial sample growing under RT conditions, only *bb0173, and bb0176* showed any amplification (figure 7B)**.** On the

other hand, when analyzing the cultures shifted to 37ºC simulating the tick feeding conditions, all genes were expressed except *bb0171*. This lack of expression could be explained by the fact that *bb0171* expressing conditions will differ from those used in this experiment. Due to the low amplification levels when using the primers for *bb0171*, and *bb0174*, we decided to design new ones and check whether they were giving better amplification yields in the PCR reactions from cDNA obtained from cultures growing at 37ºC culture. Figure 7D shows that with the new primer sets generated better amplification. Another gene that gave some discrepancies in the *in vitro* gene expression study was *bb0175*, which was amplified in some of the preparations but not in all of them (Figure 7D). Figure 7D also shows the absent of amplification when using the *bb0174-bb0175* primer mix. This primer mix was checking for the presence of an operon encoding for BB0174 and BB0175. This result suggest the absence of an operon in this region, even though both genes are expressed under the same conditions and are regulated by Rrp1 (17).

Furthermore, real time PCR analysis shows that the genes studied in this thesis, are not as tightly regulated as expected. In Figure 8 we can see that *bb0171*, *bb0173* and *bb0175* are down regulated when shifted to 37ºC while *bb0172* and *bb0174* are up regulated under the same conditions. In order to validate the data, *ospC* and *flaB* genes are used as controls, since *ospC* should be up-regulated at 37ºC and the *flaB* should not show any change based on previous studies. These results suggest that those genes will have to be studied *in vivo*, using the tick model for the transmission of Lyme disease, as has been shown for the regulator Rrp1 and Hpk1 (8, 17), which regulates some of those genes.

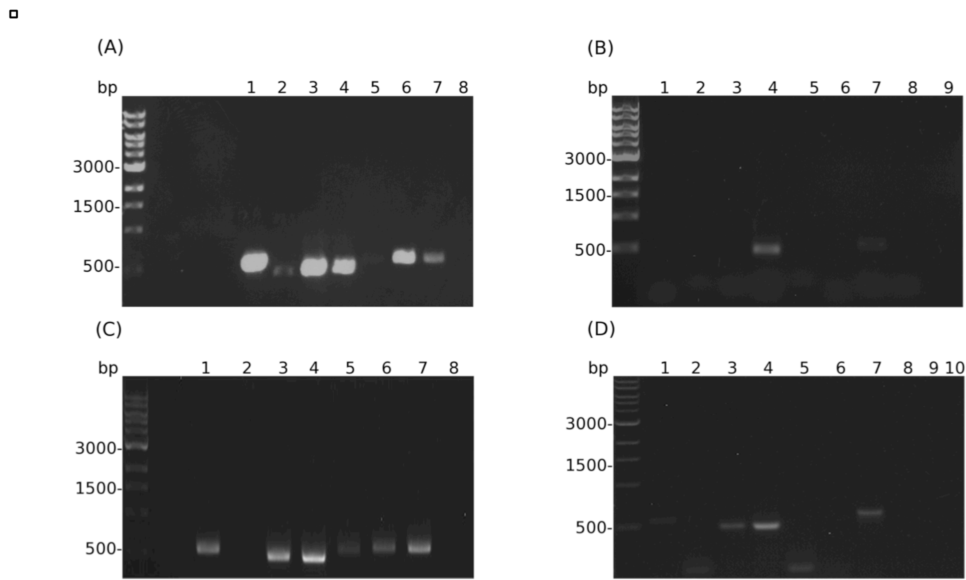

FIG 7: Gene Expression. (**A**). Positive Primers: Lanes labeled accordingly (lanes 1-8): *bb0170, bb0171, bb0172, bb0173, bb0174, bb0175, bb0176,* negative control. (**B**). RT cDNA: Lanes labeled accordingly (1-9*): bb0170, bb0171, bb0172, bb0173, bb0174, bb0175, bb0176,* negative control, water control. (**C**). 37ºC cDNA (new): Lanes labeled accordingly*; bb0170, bb0171, bb0172, bb0173, bb0174, bb0175, bb0176,* negative control. (**D**). 37ºC cDNA: Lanes labeled accordingly (1-10): *bb0170, bb0171, bb0172, bb0173, bb0174, bb0175, bb0176, bb0172-bb0173* primer mix, bb0174-bb0175 primer mix, negative control.

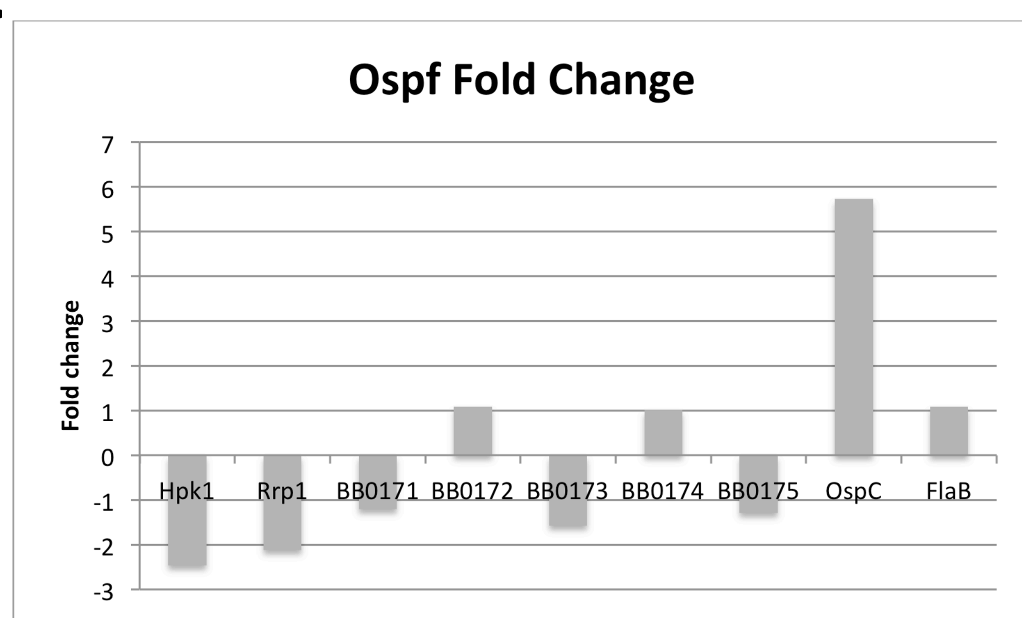

FIG 8: Real-Time PCR. Gobal pater recognition analysis using Lonza StellArray^{TM} online tools. Bars represent the fold change of gene expression when samples are shifted from RT to 37ºC.

In order to confirm that BB0174 was expressed in Borrelia we also evaluated the presence of the protein by immunoblot assay. Figure 9 shows that the IgY generated recognized the recombinant BB0174 (FIG.9A) but also, it recognized BB0174 expressed in Borrelia burgdorferi only after shifting from RT conditions to 37ºC and pH6.8 which mimics the tick feeding conditions (FIG.9B).

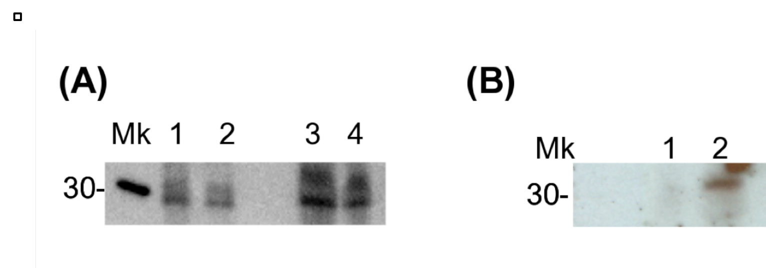

FIG 9: Immunoblot Assay of BB0174. (A) E. coli expressing in noninduced 0-hour in lane 1, induced 0-hour in lane 2. Lane 3 depicts induced at 3-hour, and noninduced at 3-hour. (B) Detection of BB0174 in *Bb* growing in different conditions, Lane 1 is at RT pH7.6, and lane 2 shifted to 37ºC at pH6.8.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The ability of *Borrelia burgdorferi* to colonize the mammalian host is dependent on its ability to rapidly alter gene expression in response to highly disparate environmental signals following transmission from infected ticks. This type of response is very much dependent on the presence of a robust signal transduction system that can induce change in gene expression (9, 12). In bacteria, the signal transduction relays on Two-component systems (TCS) (9). TCS typically consist of a membrane associated histidine kinase (HK) and a response regulator (RR) (19). Our understanding of the role of TCS play in the biology of *B. burgdorferi* is limited, but have begun to slowly identify some of the key components as well as the effector molecules (6, 8, 10, 11, 17, 19, 20). *B.burgdorferi* harbours two TCS comprised by the histidine kinate 1 (HK1, *bb0420*) and response regulartor 1 Rrp1 (*bb0419*) (6, 17) and the Hk2 (*bb0764*) and the Rrp2 (*bb0763*) (3, 4, 15, 21, 25). We know understand that the Rrp2 sigma factor pathway in which Rrp2 will activate the expression of RpoN and this one will regulate the expression of RpoS wich will directly regulate the expression of a number of proteins in *B. burgdorferi* that will allow the survival of the bacteria in the mammalian host (3, 4, 15, 16, 21, 25). On the other hand, the Hk1-Rrp1 TCS has been recently studied and research shows that this pathway uses the cyclic di-GMP as the signal transduction molecule in Borrelia (6, 10, 11, 17, 19). These two component systems have been shown to regulate a significant number of genes, and it has been proben that the Hk1-Rrp1

system is essential to orchestrate the genes responsible for the survival of *B. burgdorferi* in the tick vector (6, 11) while the Hk2-Rrp2 is essential for the survival of the bacteria in the mammalian host (4, 5, 21, 25). Eventhough some advances have been made in this field, many more TCS have been described in other bacterial pathogens (9). Therefore, further studies are needed in this regard. After analyzing the genes in *B. burgdorferi* that are regulated by Rrp1, we observed that one of the, *bb0174* protein had all the domains required to be a membrane sensor with a signal peptide, a transmembrane domain in the center of the protein with an intracellular C-terminus domain with a potential autophosphorilation site (InterPro online analysis). Since there are not many sensors described to date in this spirochaetal species, we hypothesized that due to the in silico domain prediction., BB0174 could be a sensor candidate and could be the HK component of the TCS. When looking at the neighboring genes we realized that *bb0175* was encoding for a protein that by in silico prediction was intracellular and had a number phosphorilation sites. Therefore, we hypothetise that this two proteins could be part of another sensoring pathway that *B. burgdorferi* could use to feel change in environmental conditions. Both of those proteins are annotated as hypothetical proteins so our study iniciated by the cloning and production of recombinant BB0174 to use in the generation of specific antibodies that will allow us to unveil where and when this protein is expressed and whether or not it will be part of a novel TCS or will be a member of those already described in this zoonotic pathogen.

In order to determine the expression of *bb0174* in in vitro conditions, total RNA was purified from Borrelia growing at different conditions (RT vs 37ºC). After RNA was isolated cDNA was generated and PCR was performed using the primer sets described in TABLE 7. Our PCR analysis show that *bb0174* was expressed when shifting the bacteria from unfed (RT/pH7.6) to fed (37ºC/pH6.8) tick conditions. Moreover this results are in accordance with the immunolobot analysis performed in this thesis, in which BB0174 levels are undetectable when the bacterium is growing under unfed tick conditions, and increases to detectable levels when the bacterium is shifted to the fed tick conditions. In addition to these qualitative analysis we also performed real time PCR analysis of the levels of mRNA of *bb0174* and other genes in the same chromosomal area that are regulated by the same HK1-Rrp1 TCS (17). Conidering the fact that Rrp1 is a repressor of *bb0170*, *bb0173*, *bb0174* and *bb0175*, when its levels are reduced, those of the repressed genes should inrease (17, 6). Consequently when doing the RT-PCR analysis, we observed that the Rrp1 gene was downregulated upon transition from the unfed tick to the fed tick-mammalian host conditions. At the same time *bb0174* was upregulated but not the *bb0175* gene. These results suggest that, *in vitro* conditions are not optimal for the study of the expression of these genes, and as observed by Rodgers et al (17, 6) they have to be studied in the tick model of Lyme disease. Moreover this observation is showing that there is no operon comprising *bb0174-bb0175* as suggested by the PCR results using cDNA and in contrast with the in silico analysis.

In silico analysis of the *bb0174* sequence suggest that is could be an aerotolarance receptor.This type of protein will be of great advantage for *B. burgdorferi* to sense the levels of oxygen in the environment surronding this bacteriu,, and could be of importance for itssurvival in a microaerobic millieu. In order to test this hypothesis more studies will be done in the tick model as well as in vitro under anaerobic and microaerofilic conditions.

Taken together, out studies showed that *bb0174-bb0175* are not part of a new Two component system and that both are expressed under growth conditions that mimic the fed tick conditions (37ºC/pH6.8). In addition we have been able to express and purify recombinatn BB0174 that have been used in the generation of specific antibodies in laying hens. This protocol is very advantageous since we can generate a large amount of antibodies in the egg yolk and we avoide the euthanization of animals for research porposes.

REFERENCES

1. **Aguero-Rosenfeld, M. E., G. Wang, I. Schwartz, and G. P. Wormser.** 2005. Diagnosis of lyme borreliosis. Clin Microbiol Rev **18:**484-509.

2. **Barbour, A. G., and D. Fish.** 1993. The biological and social phenomenon of Lyme disease. Science **260:**1610-1616.

3. **Blevins, J. S., H. Xu, M. He, M. V. Norgard, L. Reitzer, and 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.

4. **Boardman, B. K., M. He, Z. Ouyang, H. Xu, X. Pang, and X. F. Yang.** 2008. Essential role of the response regulator Rrp2 in the infectious cycle of *Borrelia burgdorferi*. Infect Immun **76:**3844-3853.

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

6. **Caimano, M. J., M. R. Kenedy, T. Kairu, D. C. Desrosiers, M. Harman, S. Dunham-Ems, D. R. Akins, U. Pal, and 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.

7. **De Silva, A. M., and E. Fikrig.** 1995. Growth and migration of *Borrelia burgdorferi* in *Ixodes* ticks during blood feeding. Am J Trop Med Hyg **53:**397-404.

8. **Freedman, J. C., E. A. Rogers, J. L. Kostick, H. Zhang, R. Iyer, I. Schwartz, and R. T. Marconi.** 2010. Identification and molecular characterization of a cyclic-di-GMP effector protein, PlzA (BB0733): additional evidence for the existence of a functional cyclic-di-GMP regulatory network in the Lyme disease spirochete, *Borrelia burgdorferi*. FEMS Immunol Med Microbiol **58:**285-294.

9. **Gao, R., and A. M. Stock.** 2009. Biological insights from structures of two-component proteins. Annual review of microbiology **63:**133-154.

10. **He, M., Z. Ouyang, B. Troxell, H. Xu, A. Moh, J. Piesman, M. V. Norgard, M. Gomelsky, and X. F. Yang.** 2011. Cyclic di-GMP is essential for the survival of the lyme disease spirochete in ticks. PLoS Pathog **7:**e1002133.

11. **Kostick, J. L., L. T. Szkotnicki, E. A. Rogers, P. Bocci, N. Raffaelli, and 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.

12. **Krell, T., J. Lacal, A. Busch, H. Silva-Jimenez, M. E. Guazzaroni, and J. L. Ramos.** 2010. Bacterial sensor kinases: diversity in the recognition of environmental signals. Annual review of microbiology **64:**539-559.

13. **Marques, A. R.** 2010. Lyme disease: a review. Curr Allergy Asthma Rep **10:**13-20.

14. **Milch, L. J., and A. G. Barbour.** 1989. Analysis of North American and European isolates of *Borrelia burgdorferi* with antiserum to a recombinant antigen. J Infect Dis **160:**351-353.

15. **Ouyang, Z., J. S. Blevins, and M. V. Norgard.** 2008. Transcriptional interplay among the regulators Rrp2, RpoN and RpoS in *Borrelia burgdorferi.* Microbiology **154:**2641-2658.

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

17. **Rogers, E. A., D. Terekhova, H. M. Zhang, K. M. Hovis, I. Schwartz, and R. T. Marconi.** 2009. Rrp1, a cyclic-di-GMP-producing response regulator, is an important regulator of *Borrelia burgdorferi* core cellular functions. Mol Microbiol **71:**1551-1573.

18. **Rudenko, N., M. Golovchenko, L. Grubhoffer, and J. H. Oliver, Jr.** 2011. Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. Ticks Tick Borne Dis **2:**123-128.

19. **Ryjenkov, D. A., M. Tarutina, O. V. Moskvin, and M. Gomelsky.** 2005. Cyclic diguanylate is a ubiquitous signaling molecule in bacteria: insights into biochemistry of the GGDEF protein domain. J Bacteriol **187:**1792-1798.

20. **Salman-Dilgimen, A., P. O. Hardy, A. R. Dresser, and G. Chaconas.** 2011. HrpA, a DEAH-box RNA helicase, is involved in global gene regulation in the Lyme disease spirochete. PLoS One **6:**e22168.

21. **Samuels, D. S.** 2011. Gene regulation in *Borrelia burgdorferi*. Annual review of microbiology **65:**479-499.

22. **Stanek, G., V. Fingerle, K. P. Hunfeld, B. Jaulhac, R. Kaiser, A. Krause, W. Kristoferitsch, S. O'Connell, K. Ornstein, F. Strle, and J. Gray.** 2010. Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect.

23. **Taylor, R. S., and I. N. Simpson.** 2005. Review of treatment options for lyme borreliosis. J Chemother **17 Suppl 2:**3-16.

24. **West, A. H., and A. M. Stock.** 2001. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci **26:**369-376.

25. **Yang, X. F., S. M. Alani, and M. V. Norgard.** 2003. The response regulator Rrp2 is essential for the expression of major membrane lipoproteins in *Borrelia burgdorferi.* Proc Natl Acad Sci U S A **100:**11001-11006.

26. **Zeidner, N. S., M. S. Nuncio, B. S. Schneider, L. Gern, J. Piesman, O. Brandao, and A. R. Filipe.** 2001. A portuguese isolate of *Borrelia lusitaniae* induces disease in C3H/HeN mice. J Med Microbiol **50:**1055-1060.

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