

ADAPTATION FACTORS OF *BORRELIA* FOR HOST AND VECTOR

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Abstract: The life transmission cycle of *B. burgdorferi* requires migration of spirochetes from tick's gut to its salivary glands during vertebrate's blood sucking, penetrating to the vertebrate's tissues and their colonization. A special feature of these bacteria, despite its relatively small genome, is the ability to adapt in different host environments. These unusual properties of borreliae are associated with large number of plasmids, which show a high variability as a result of recombination with each other. Changes in the synthesis of outer proteins are the first strategy of borreliae in avoiding the destructive effect of the host's immune system. Then, by colonizing tissues, they initiate production of Erp and CRASP proteins, which bind regulators and components of complement and repress the cytolytic effect of the host's serum. Some evidences indicate that the spirochetes use quorum sensing as a mechanism to control protein expression. *B. burgdorferi* probably utilizes a LuxS/autoinducer-2-dependent quorum sensing mechanism. However, it is not yet known how *B. burgdorferi* detect AI-2. Analysis of the results of expression studies of the luxS gene shows that the molecular mechanisms of this phenomenon in *B. burgdorferi* are only fragmentarily known. Continuation of quorum sensing studies may be essential in improving the construction of vaccines as well as therapy of Lyme disease.

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INTRODUCTION

Every year, infection with spirochetes belonging to the collective species, *Borrelia burgdorferi* sensu lato causes thousands cases of illnesses of people all over the world [24, 34]. There are three main species: *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* that are thought to be the etiological factors of the disease entity called borreliosis or Lyme disease [19, 24, 44]. In order to live, they obligatory require binding with the organism of the host. In their life cycle, they are transmitted to the vertebrates – hosts by the invertebrates – ticks (different species of *Ixodes*). In unfavourable conditions, the spirochetes can transform into survival forms. Studies show that they exist in three different growth stages, typically as spherical and non-motile cysts, including tightly wind spirochetes, which are resistant to almost every antibiotic [1]. The others are L forms spheroplasts devoid of cell wall, insensible to many used in

borreliosis antibiotics (having effect on the cell wall). There are also blebs forms (vesicle forms) which are excreted outside the maternal cell, and gemmas covered with membrane (both forms include DNA and outer antigens) [1]. A study of cytomorphic variations of *B. burgdorferi* isolates from patients, with or without antibiotic treatment, showed that penicillin can induce membrane-derived vesicles (cysts or spheroblast L-forms) *in vivo* [42]. The phenomenon that *B. burgdorferi* has the ability to convert (and reconvert) to cystic forms, both *in vivo* and *in vitro*, may be regarded as an explanation why the infection may be persistent and reactivate [13]. It is probable that all germinative forms of the bacterium (and not only the motile form) should be destroyed so that Lyme borreliosis can be treated effectively. There are evidences that the spirochete can survive inside such cells as macrophages, lymphocytes and endothelium cells, and in this way it can also avoid antibiotics.



The life transmission cycle of *B. burgdorferi* requires the migration of spirochetes from the tick's gut to its salivary glands during vertebrate's blood sucking, penetrating to the vertebrate's tissues and their colonization, achievement of the chronic infection state, and finally, the colonization of uninfected feeding ticks. This process is undoubtedly connected with bacteria recognizing the environmental signals, which modulate the expression of fundamental genes that ensure success in adaptation. The unusual feature of these bacteria is their ability to adapt and develop in different host environments. Perhaps more unusual feature of *B. burgdorferi* is that in spite of its relatively small genome, it is able to carry out the course of events required during transmission [26].

The special construction of the outer membrane, that is the outer layer of the *B. burgdorferi* s.l. cell and is loosely connected with lower lying structures, also expresses the adaptation for carrying out such complicated cycle. Non-typical of this membrane is fact that the genes encoding its proteins (*ospA* and *ospB*) are in the extra-chromosomal plasmid, and may be conducive for antigenic changes in the structure of these proteins. What is more, this membrane shows an ability to translocate from one end of the cell to another. This is called capping or patching, and may have significance in the phenomenon of adherence of the microorganism to the host's cells [6].

In the organism of a host-mammal, the bacteria from *Borrelia* genus may use the host's enzymes; such as plasminogen and its activator, which can attach to the surface of the bacteria's cell [59]. The bacterium uses plasmin, being the active form of plasminogen, for digesting the extracellular glycoproteins with a large molecular mass. Probably, the ability to digest the host's tissue connections enables the bacterium to translocate. Straubinger *et al.* [59] suggest that *B. burgdorferi* actively migrates through the tissues and finds a niche where it is able to survive, i.e. period of antibiotic therapy, and it does not need mediation of blood vessels for colonization of tissues. Therefore, *Borrelia* avoids penetration of blood vessels because of antibodies and the danger of phagocytosis by neutrophils and macrophages. Regarding this situation, the spirochetes stay in the connective, poorly vascularized tissue. But such a hypothesis of migration in tissues has not been confirmed by some reports in the literature about the detection of *B. burgdorferi* in the humoral fluids in people, especially patients with skin rash after exposure to ticks [53, 64].

Genome of *Borrelia burgdorferi* s.l.

Three pathogenic *Borrelia* species can permanently infect humans and other mammals, despite the active immune response of host. The ability to infect different animal species probably results from the specific structure of these bacteria's genome. It is thought that this feature has a connection mainly with factors encoded in enormous amounts of plasmids found in borreliae's genome [29], which show

a high variability as a result of recombination with each other. In this bacterium, the chromosome is in the linear form of the DNA molecule and is described as the first of this type in the world of bacteria. Its size is about 0.96 Mbp. There are two types of *Borrelia*'s plasmids: circular (9) and linear (12), and they consist of about 613,000 bp. The first linear plasmids were detected 20 years ago, in the first place in yeast, then in bacteria including *Borrelia*. Such a large number of plasmids have not been met in any other bacterium [24]. What is more interesting, the unique plasmids having less than 50% similarity to any other plasmids, occur in all three pathogenic species *Borrelia* genus. This phenomenon may be the cause of the adaptation of these bacteria to different environments, as well as their pathogenicity [29].

It has been found that the range of hosts species of vertebrates and invertebrates is indirectly associated with factors encoded mainly in huge number of plasmids found in *Borrelia*. Another characteristic feature of these plasmids is the abundance of parallel genes in them. During the passage, the plasmids may be lost because of lack of the maintenance pressure. The incapability of *Borrelia* to survive in the organism of the host may accompany such a loss [29]. To a certain degree, the chromosome is also engaged in processes of fusion. It has been shown that the right end of the chromosome in *B. burgdorferi* is variable because of the ability to "catch" (attach) the plasmid material.

The presence of 853 genes encoding proteins that have a meaning in the processes of replication, transcription, translation, energetic metabolism and membrane transport, were found in the chromosome of *Borrelia* spirochetes. On plasmids, however there are mainly genes encoding outer surface proteins, (Osp).

For example, the genome of *B. burgdorferi* s.s. species consists of a linear chromosome including 910,725 bp, in which G + C pairs constitute 28.6% and 21 plasmids (9 circular and 12 linear) consisting of about 613,000 bp. Today, the genome of *B. burgdorferi* s.s. (B31) is completely known, but in the case of *B. garinii* only the main chromosome and some of the plasmids have been sequenced. The comparative analysis of genomes have shown that three genetic elements: chromosome and plasmids cp26 (circular) and lp54 (linear) are common for both of these species and are even strikingly collinear [29]. However, some plasmids are limited to *B. burgdorferi* s.s. because neither has been found in *B. garinii*, nor their traces in the form of protein products.

Adaptation of metabolism of *B. burgdorferi* to environmental conditions

Vector-borne bacterial pathogens, such as *B. burgdorferi*, encounter different conditions as they are transmitted to various hosts. Sensing changes in these environments and accordingly, modulation of the metabolic level, is important for adaptation and survival of *B. burgdorferi* within



various hosts. The physiological flexibility of spirochetes also indicates the fact that they reproduce in many organs and tissues of the infected mammals and birds. The occurrence of these bacteria in different organs and tissues of a warm blood host requires from them the synthesizing of specific proteins appropriate for every kind of environment. Among the proteins produced by *B. burgdorferi* during infection, there are those which facilitate the interactions between bacteria and selected cells of a host or extracellular components. For the purpose of diverse synthesis of proteins in the life cycle, the bacteria must have a receptor system for detecting changes in the environment, and a regulating one to regulate the expression of appropriate genes and proteins. Such regulating mechanisms must exist in order to control the expression of genes in the individual bacterium, as well as in whole population.

Results of works on the genome of *B. burgdorferi* indicate that these spirochetes have only rudimentary mechanisms of own metabolism, and are almost completely dependent on the host in the metabolism of fats, carbohydrates, proteins, aminoacids and iron [27].

One of the factors controlling the ability of pathogenic bacteria that colonize the host's organism is the availability of alimentary constituents, including ions of iron, which take part in many essential biochemical processes. The availability of these ions in structural fluids and mucosal membranes is limited and their concentration is too small to maintain the vital functions of bacteria. Analysis of sequenced genomes of pathogenic bacteria confirmed their great possibilities in range to receive ions of iron associated with the activity of many copies of the same gene. However, the strategy of *B. burgdorferi* differs from other bacteria, namely, in the process of evolution, it eliminated the genes encoding most of the enzymes dependent on the iron [43].

Many studies have shown that environmental factors influence the synthesis of proteins associated with the infection in mammals. They are produced in small amounts at the temperature of 22°C, but when the amount is much bigger – in temperatures of 22–34°C [54]. The spirochetes are exposed to big changes of temperature while they are living in the ticks. Ph is also an important parameter influencing the synthesis of surface proteins in spirochetes.

Selective expression of membrane lipoproteins enables survival in the host

Surface lipoproteins Osp (outer surface proteins) belong to the most important protein antigens of *B. burgdorferi* s.l., playing an essential role in the pathogenicity of Lyme borreliosis, OspA, OspB, OspC, OspD, OspE and OspF anchored in the outer membrane due to the occurrence of the lipid group at the amino end [25]. The survival of *B. burgdorferi* in tick and in the organism of mammals is simplified, at least, partly by the selective expression of these lipoproteins. The first strategy of borreliae, in order to avoid the destructive activity of host's immune system,

are changes in the synthesis of surface proteins (Osp) and adaptation to different host's microenvironments – in mammal and in tick [11]. Many studies have demonstrated that *B. burgdorferi* selectively shows an expression of individual osp genes in the individual stages of its life cycle. In this way, the expression of *ospA* and *ospB* is immediately activated when the spirochetes penetrate into the vector, arthropod. During the transmission from the vector to the host-vertebrate, the expression of *ospA* and *ospB* is immediately reduced and synthesis of OspC, DbpA and BBK32 is increased [21, 28].

Selective and temporal expression of *ospA* and *ospB* in ticks suggests that these two proteins may be engaged in the early colonization of spirochete and in the survival in tick, vector. Studies by Pal *et al.* [47], showing that OspA mediates in the adherence of spirochete to the tick's gut through binding TROSPA proteins, confirm this theory and indicate that the expression of the gene supports the maintenance of the natural cycle of the spirochete. Furthermore, the osp genes encoding proteins that have an antigenic character occur in many allelic forms within each species of *B. burgdorferi* s.l., which undoubtedly has a connection with cheating the immune system of the host.

In the USA, the genes *ospA* and *ospB* are highly conservative among isolates of *B. burgdorferi* [7]. They are encoded by the DNA of linear plasmid p154, activated by the common promotor [10] and have a similar sequence and structure [9]. Before the Lyme disease agent was detected, OspA proteins had been an object of intensive studies, whereas the role of OspB in the life cycle of borreliae is not well known [41]. Earlier studies identified a mutant without *ospB* in the cloned population of *B. burgdorferi* with one changed base in the sequence, and with deletion of one nucleotide in the reading frame in the operon of *ospB* gene [52]. These changes in the *ospB* gene cause reduction of the expression and cutting of this protein in such way that the ability of penetration and infection of spirochete towards epithelium cells are reduced [52]. Other studies suggest that OspB are present on the surface of *B. burgdorferi* only in unfed ticks, and antigens against OspB repress the colonization of borreliae in gut of *I. scapularis* [25, 46]. Damage of the template in the operon *ospAB* repress the colonization of *B. burgdorferi* and maintenance in the tick's gut [65]. Despite these studies clarifying the important role of OspA in the *in vivo* spirochete-tick interaction, the independent role of OspB in the life cycle of the spirochete remains unclear.

The role of OspB protein in *B. burgdorferi* was investigated by Neelakanta *et al.* [43] who obtained cells devoid of this protein. They have shown that such borreliae are able to infect and remain alive in mouse, and also migrate to the feeding tick, adhere to and survive in its gut. But this adherence is not tight, and the addition of one copy of *ospB* gene to the wild strain of *B. burgdorferi* without OspB strengthen it a great deal. The authors suggest that there is proof that OspB plays an important role in *I. scapularis*,



and effective maintenance in this species of tick is dependent on the expression of many genes of borreliae.

Erp and CRASP proteins help to avoid the immune response of the host

It has not yet been found that these microorganisms, responsible for the symptoms of the disease, produce toxins. Through the colonization of tissues they initiate the occurrence of the inflammatory state, and at the same time, very effectively avoid the destructive effects of the host's immunological mechanisms. One such defence mechanism is the production of Erp and CRASP proteins, which through binding the complement regulators – factor H and FHL-1, as well as C3 C3b, C3c and C3d components, repress the cytolytic activity of the host's serum [20].

All spirochete strains causing Lyme disease have many varieties of DNA molecules, which replicate as circular plasmids. These plasmids are called cp32 because of their circular structure (*cp-circular plasmid*) and size of about 32 kbp [17, 56]. They are very similar to each other, with the exception of three loci: one associated with the replication of plasmid and segregation, and two connected with encoding the Erp lipoprotein exposed on the surface [23, 49, 56]. A surprising fact is that these plasmids are the genome of lysogenic bacteriophages which evidently infect all spirochetes causing Lyme disease [16, 56, 67].

Each element of cp32 includes mono or bicystronic erp locus, which may differentiate in sequences in individual plasmids [5, 56]. Erp lipoproteins are from the group of specific proteins synthesized by *B. burgdorferi* during the infection of mammal, and in this time their function is the same as in case of other proteins from this group. It consists in binding the protein factor H from the host's serum during the alternative way of complement activation [2, 3, 30, 35, 57]. Factor H is usually bound by the receptors on the surface of host's cells, where it protects these cells by repressing disintegration of C3 component and protects it against C3b degradation. It has been suggested that binding factor H by the Erp and other surface proteins of *B. burgdorferi* is a way to protect the pathogen against the destructive activity of the complement [36].

Members of the cp32 family and erp loci of four isolates of *B. burgdorferi* have been described so far [5]. Currently, it is known that, e.g. strain B31 of *B. burgdorferi*, includes 10 different cp32 and 17 erp genes. Furthermore, if one bicystronic locus is present in three identical copies and another locus, *erpH* is a natural defect, such bacterium from B32 strain may synthesize 13 different Erp lipoproteins at the same time [16, 17]. Babb *et al.* [3] have shown that each locus is preceded by highly conservative sequences of DNA in which a transcription promotor and two different, separated from each other places occur, which in specific way binds the two different cytoplasmatic proteins of the bacteria. The same authors have shown that a region binding the proteins being close to erp promotor, called

operator 2, is indispensable for appropriate regulation of the transcription of erp gene. Continuing their studies, Babb *et al.* [5] have detected an EbfC protein encoded by a chromosome, which binds a specific sequences of DNA in the 5' end of all erp loci. The authors conclude that localization of *ebfc* gene on the chromosome of *B. burgdorferi* suggests that cp32 prophages take part in utilizing proteins of the host's cells for their own use, and that EbfC protein probably plays an additional role in the bacterial cell.

The discussed studies concerned the expression of Erp proteins in *B. burgdorferi* during mammal's infection, whereas Miller *et al.* [40] have analyzed the expression of Erp during the cycle: penetration of tick and infection of mammals, which showed that bacterium in *in vivo* conditions regulates the synthesis of Erp. The bacteria show low expression of Erp protein in the unfed tick, but when the infected tick feeds on mouse, *B. burgdorferi* intensifies the production of Erp basically in all cells that penetrated into mammal. The infected mouse produces antibodies of the IgM class against all investigated Erp proteins, then there is a strong response in the form of IgG immunoglobulins. The latter grow the most intensively in the 11th month of infection, which suggests the continuation of the exposition on Erp proteins of the host's immune system during even the chronic stage of infection. If uninfected larvae obtained *B. burgdorferi* while feeding on mouse, basically all bacteria transferred into it did not produce Erp proteins, which according to the authors suggests that the production is also continued during the infection in mouse. Some time after translocation of bacteria into larvae, the synthesis of Erp in their cells is drastically reduced. The expression of Erp proteins in *B. burgdorferi* during the infection in mammal is stable with the hypothetic function of binding factor H in order to protect the bacteria against the host's immune response.

Another protein, called surface protein, binding the regulator of complement, CRASP-1 (*complement regulator-acquiring surface protein*) also binds the protein factors H and FHL-1 from human serum, and is implicated in processes associated with the survival of *B. burgdorferi* species causing Lyme disease [51]. Cytoplasmatic proteins control the activation of an alternative way of complement on the level of C3b component by factor B that binds it. Additionally, FH and FHL-1 accelerate the disintegration of C3 convertase, C3bBb and function as a co-factor for factor I causing the degradation of C3b [68]. Currently, five CRASPs, exposed on the surface, isolated from the complement, proteins binding FH and FHL-1 in it (CRASP-1 and CRASP-2), or only factor H (CRASP-3, -4, and -5 and Erp proteins) have been identified [2]. Among CRASP proteins, the protein CRASP-1 in *B. burgdorferi* binds the main factors FH and FHL-1 giving immunity to the complement in the *in vitro* culture [35]. The latest studies have shown that inactivation of gene encoding CRASP-1 in *B. burgdorferi* gives results in the serum-sensitive phenotype and addition of mutated strain with CRASP-1, restores the resistance



for lysis initiated by the complement [12]. These data suggests that CRASP-1 causes avoiding and/or survival of spirochetes in the human organism [51], but the expression of CRASP-1 during the infection in humans is still under discussion [61]; e.g. studies by McDowell *et al.* [39] have shown that the serum of patients with Lyme disease did not include antigens specific for denaturated recombinants of CRASP-1, tested with the Western blot method. However, the latest studies of Rossmann *et al.* [51] have shown that the serum of patients with Lyme disease was immunoreactive for undenaturated CRASP-1, investigated with the ELISA method and immunoblot test, but reactive for CRASP-1 denaturated in the Western blot. Therefore, occurring antigens are restrictive for undenaturated structurally determinants of functionally active proteins, and the occurred antigens do not interrupt in binding FH by CRASP-1. The authors conclude that the results, by showing the expression of CRASP-1 in the immunogenic forms of spirochetes during the infection of humans, suggest the engagement of CRASP-1 strategy for avoiding its immune response by binding FH. In the work by Kraiczky *et al.* [35], serum samples from human Lyme disease patients in the USA and Germany who had a range of disease symptoms, were examined for the presence of BbCRASP-2-directed antibodies. Sequences of *cspZ* genes encoded this protein were determined for a variety of *Borrelial* strains of different genospecies. The results indicate that *cspZ* sequences are very conservative among borreliae of Lyme disease, independently of their geographic distribution, and that antibodies recognizing BbCRASP-2 are frequently produced by humans with Lyme disease. *In vitro* studies of *cspZ* and other BbCRASP-encoding genes were also performed to help elucidate the mechanisms by which BbCRASP levels are controlled, and seems to be a unique regulatory mechanism for each class of BbCRASP that result in distinct *in vivo* expression profiles [15].

The *vls* gene is very essential for the survival of borreliae in mammals

For the survival of borreliae, the *vls* gene is most essential, which encodes surface proteins of 34 kDa. They consist of two stable components and one variable. The maintenance of pathogenic species of borreliae, in spite of active immune response of the host, is partly facilitated by the specific structure of the *vls* gene. This gene is a complex consisting of an expressive part, *vlsE* and contiguous kit including 11–15 silent *vls* cassettes [14]. Segments of the cassettes not subject to expression, recombine with the *vlsE* region during host infections of mammals, as a result they obtain surface protein VlsE in antigenic variance. VlsE is the surface-exposed protein, on the outer, protein-lipid membrane of *B. burgdorferi* spirochetes causing Lyme disease. During the infection of mammals, but not during the colonization of tick or in the laboratory culture, the segments of silent *vlsE* cassettes randomly recombine

with the expressive *vlsE* locus, continually creating new versions of *vlsE* gene [32, 45, 66].

The effect of conversions of sequences is that variants of surface VlsE protein change their antigenic properties, which evidently allow bacteria to avoid rearrangements – modifications of antibodies by the host. It seems to be an essence of the nature of this gene, because it has been observed that mutants of these bacteria, devoid of plasmids with *vls*, are not able to infect mammals in the long-term [37, 38, 48, 66], and that *vls* loci are present in all investigated spirochetes causing Lyme disease [33, 63]. The analogical systems of antigenic variants are also associated with the existing infections with spirochetes of recurrent typhus [55], in the protozoonosis such as *Trypanosoma* spp., and other important pathogens [22].

Borreliae loses genetic material and pathogenicity during passage

Species from *Borrelia* genus may lose their pathogenicity during passaging several times without a selective pressure for this property [29]. This loss is accompanied by the loss of genetic material. It is not possible to assess the range of this loss based only on data from PFGE or PCR, especially if the whole genome sequence is not known [29]. Therefore, Glöckner *et al.* [29] have sequenced and analysed plasmids from *B. garinii* strains coming from low and high passage, and also *B. afzelii* strains, in order to describe all sequence differences and to detect the cause of pathogenicity loss. It has occurred that not the whole plasmid but only a part was lost during the passage in PBI strain of *B. garinii*. The rest of the plasmid was saved by accident, or because it was important. The lost part consisted of cassettes of *vls* gene, whose frequent exchanges are engaged in the escape of *B. burgdorferi* from the host's response. Perhaps this selective loss may be caused by the repair mistakes in the locus after recombination. The authors have found that the lack of selective pressure allows this clone with cut plasmid to prosper in culture. They conclude that in order for the entire culture to lose the locus, cassette switching errors must be frequent or the mutated clone has a substantial growth advantage.

In the PKo strain of *B. afzelii* from the high passage, the whole plasmid was absent. This plasmid also included the *vls* genes, which may be deduced from the presence of a few *vls* cassette derived readouts. The loss of the whole plasmid will happen if plasmids are not correctly divided during the division between daughter cells. So far, we have discovered that two sequences, in the collection of the fragments, come from DNA of a high passage strain, which indicates that this was a two-stage process of losing the plasmid. According to this scenario, the group of the *vls* genes was lost, like as the first one, as in *B. garinii* PBI, and then the remainder of the plasmid.

The interesting fact is that in a tick-vector, the locus of the *vls* gene seems to be muffled, therefore it is stable in



this host. This explains why this accidental loss does not occur in nature.

The authors hypothesize that this additional loss happened only by coincidence, caused by the unessential nature of the plasmidial remains. This makes the *vls* locus a visible but sensitive factor of the successful prospering of *Borrelia* species in the individual hosts – vertebrates. Further studies on the additional strains from low and high passages should reveal if the loss of the genome cassettes of the *vls* is a leading or single event causing the loss of pathogenicity.

Quorum sensing

The list of known species of bacteria that use quorum sensing mechanism to regulate genes' expression, which enables a simultaneous response of the whole population to the environmental changes, has grown recently.

For the first time, the quorum sensing phenomenon was described in the marine bacteria *Vibrio fischer* which have an ability of bioluminescence. They occur in sea water and also in the luminous organ of cuttlefish. Their concentration in water is low and they do not emit light. But when their concentration in the luminous organ of cuttlefish grows up to 10^9 cells/ml, all bacteria start to emit light energy [20]. The mechanism of this phenomenon lies in the fact that bacteria excrete a specific factor called autoinducer AI-2. The excreted outside the cells autoinducer penetrates the cells once more and when its concentration in the cell increases to the proper level, it raises the expression of some genes being inactive. It has been shown that other species of marine bacterium, *V. harveyi* use AI-2 in the quorum sensing mechanism to regulate bioluminescence, and AI-2 induces bacteria to produce light independent of the autoinducer source [8].

Two general types of autoinducers have been identified in bacteria. The first type is specific for the species producing it, for example, homoserine lactones or certain polypeptides, and the second type, autoinductor-2 (AI-2), which well conserved across species. AI-2 is produced from methionine and ATP through a five-step process catalyzed by *S*-adenosylmethionine synthetase (MetK), a methyltransferase, *S*-adenosylhomocysteine/5-methylthioribose nucleosidase (Pfs), and LuxS [18]. The final step involves an apparently spontaneous cyclization of the LuxS product (4,5-dihydroxy-2,3-pentanedione) with borate to produce AI-2. The first three enzymes in this course appear to be important for bacterial survival [60]. Since AI-2-mediated *quorum sensing* is implicated in the regulation of virulence properties in a wide variety of pathogenic bacteria, LuxS has also been an essential enzyme of many bacteria in nature [58].

In order to understand the pathogenic properties of infectious agents such as *B. burgdorferi*, a valuable step is the defining of the metabolic capabilities and regulatory mechanisms controlling gene expression [4]. Stevenson and Babb [58] have shown that *B. burgdorferi* encodes

a functional LuxS enzyme enabling it to synthesize AI-2. What is more, addition of this autoinducer to the culture of *B. burgdorferi* has profound effects on the expression levels of many bacterial proteins. This indicates the importance of this quorum sensing system in the regulation of *Borrelial* protein expression.

In the studies of Hübner *et al.* [31], the *luxS* gene was expressed by *Borrelia burgdorferi* strain 297 cultured *in vitro*, or in dialysis membrane chambers implanted in rat peritoneal cavities. Although the *Borrelial luxS* gene functionally complemented a LuxS deficiency in *Escherichia coli* DH5 α , AI-2-like activity was not detected within *B. burgdorferi* culture supernatants or concentrated cell lysates. Finally, a *luxS*-deficient mutant of *B. burgdorferi* was infectious at wild-type levels when inoculated into mice, indicating that the expression of *luxS* is probably not required for infectivity but, at the very least, is not essential for mammalian host adaptation. These findings may also challenge the notion that a LuxS/AI-2 *quorum sensing* system is operative in *B. burgdorferi*. However, the studies of von Lackum *et al.* [62] demonstrated that *B. burgdorferi* encodes functional Pfs and LuxS enzymes for the breakdown of toxic products of methylation reactions. According to these observations, *B. burgdorferi* was shown to synthesize the final product, 4,5-dihydroxy-2,3-pentanedione (DPD) during laboratory cultivation. DPD undergoes spontaneous rearrangements to produce a class of pheromones collectively named autoinducer 2 (AI-2). The addition of *in vitro*-synthesized DPD to the culture of *B. burgdorferi* manifested in differential expression of a distinct subset of proteins, including the outer surface lipoprotein VlsE. Although many bacteria for regeneration of methionine can utilize the other LuxS product, homocysteine, *B. burgdorferi* did not show such an ability. It is hypothesized that *B. burgdorferi* produces LuxS for the express purpose of synthesizing DPD, and utilizes a form of that molecule as an AI-2 pheromone to control gene expression [4]. Whereas the studies of Riley *et al.* [50] have demonstrated that single operon encoding four enzymes occurs in *B. burgdorferi*, two of them are associated with the synthesis of DPD, one was found only in borreliae causing Lyme disease and is an activated-methyl donor, and the fourth is the gene encoding phosphohydrolyase. All four genes have shown that coexpression and high metabolic activity was accompanied by the growth of the cell level of methyl donor, increased the detoxication of methylation products, and growth of the DPD synthesis. Therefore, the authors conclude that the production of DPD is directly correlated with the level of cell metabolism, and perhaps functions as an extracellular signal and/or intercellular for bacteria.

SUMMARY

This review focuses some strategies to adopt to the vertebrate hosts and arthropod vector that *B. burgdorferi* have developed despite its relatively small genome.



Special properties of *B. burgdorferi* are associated with large number of plasmids, rare in other bacteria, which show a high variability as a result of recombination with each other. Changes in the synthesis of outer proteins, Osp are the strategy of borreliae in order to avoid the destructive effect of the immune system of the host. Then, by colonizing tissues, they initiate production of Erp and CRASP proteins, which bind the regulators of complement and repress a cytolytic effect of the host's serum. Some evidence indicates that Lyme disease spirochetes use quorum sensing as a mechanism to control the protein expression. The quorum sensing phenomenon is associated with autoinducer AI-2 and i.a. the LuxS protein is committed to its synthesis. The review of results of the expression studies of *luxS* gene shows that molecular mechanisms of this phenomenon in *B. burgdorferi* are still only fragmentarily known. Continuation of the quorum sensing studies, as well as other mechanisms of controlling the expression of genes in *B. burgdorferi*, will help to understand the pathogenic properties of this bacterium, and to improve the construction of vaccines and the therapy of Lyme disease.

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