

A look into the characteristics of *Borrelia garinii*

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-Abstract-

This case study explores the different properties and aspects of an unknown sequence fragment of DNA. This DNA fragment turned out to be part of the genome of *Borrelia garinii*. Using online tools like NCBI BLAST, ORF finder and KBase certain genes were determined. A literary study provides information about *B.garinii* and its properties and significance, using BLASTp different proteins were analyzed. To research the diagnostics of infection by *B.garinii* an overexpression vector is designed using Benchling.

-Introduction-

This case study analyzed an unknown DNA fragment that was identified as originating from the pathogen *Borrelia garinii* to gain insight into the different properties of the fragment, using online tools such as NCBI BLAST, ORF finder, KBASE and PDB or AlphaFold. Additionally, Benchling was used to design an overexpression vector for diagnostic purposes.

Borrelia garinii is a spiral shaped bacterium (spirochete) with a flagellum between the inner and outer membrane. It contains a small genome and belongs to the *Borrelia* genus. *Borrelia garinii* is **transmitted by ticks** and has an obligate enzootic life cycle [1, 3]. Early stages of infection cause erythema migrans: a red rash which can be accompanied with fever and fatigue. Late stage infection can cause chronic joint inflammation and neuroborreliosis. After a tick bite the *Borrelia* bacteria moves through the skin and into the tissue. Here the bacteria faces the immune system. It turns off the complement system by regulatory protein on its surface, allowing the bacteria to survive. [2] **Chemotaxis** causes *Borrelia* to spread to other parts of the body after the initial bite spot of the tick. The adaptive immune system is helpless because the *Borrelia* bacteria expresses VlsE; a surface protein which repeatedly changes its structure by recombination. Therefore antibodies will not provide protection after or during infection.

-Materials and Methods-

BLAST, ORF Finder and KBase

A BLASTn was performed with the Core nucleotide database in the selected fragment in order to know the bacteria who corresponded to it. In addition ORF Finder was used to analyze the fragment and find proteins with the following settings: minimal ORF length of 300 nucleotides, genetic code 11, “ATG” and alternative initiation codons including nested ORFs. As well, KBase was applied for gene/protein annotation to obtain CDS and tRNA.

Literary review and phylogeny

With the aim of understanding *B. garinii* a research was conducted focusing on the bacteria itself, its pathogenicity and virulence factors, Lyme disease, its genetic information and diversity and its significance in (global) health. For the phylogeny SILVA was used to obtain S16 rRNA, Clustal Omega for the alignment and iTOL for the visualization.

Protein and comparative analysis

Three proteins from fragment 6, CheA (fragment 1), CheW (fragment 2) and UvrC (fragment 3), were selected for annotation and analysis. CheA was identified with NCBI smartBLAST and its 3D structure retrieved from PDB; CheW was identified with BLASTp, functionally characterized via NCBI Conserved Domain Database and its structure found in PDB. UvrC was profiled with BLASTp, InterPro and UniProt, also its 3D model obtained from AlphaFold. BLASTp-derived sequences were then aligned in Clustal Omega to get a phylogeny, which was visualized in iTOL and formatted in Sublime Text.

Overexpression Vector Design

Protein annotations were sourced from UniProt, and structures were retrieved from the PDB and AlphaFold. The plasmid carrying the ospC gene was obtained from NCBI, and the expression vector sequence from Thermo Fisher Scientific. Primer design and the cloning strategy were prepared in Benchling to insert ospC into the vector for overexpression of OspC.

-Results-

Scientific name and strain	Total score	Query cover	E value	Accession number
<i>Borrelia garinii</i> SZ	36934	100%	0	CP007564.1
<i>Borrelia garinii</i> NMJW1	36934	100%	0	CP003866.1
<i>Borreliella bavariensis</i> strain JAASAAF1029 chromosome	36928	100%	0	CP075245.1
<i>Borreliella bavariensis</i> strain JANAGAM1369 chromosome	36923	100%	0	CP075235.1
<i>Borreliella bavariensis</i> strain NT24 chromosome	36923	100%	0	CP059009.1

Table 1: BLASTn results of fragment 6

Table 2: Gene and protein annotation results

Showing tRNA, identified protein products not hypothetical (CDS), the products, genomic start positions, strand orientation and sequence lengths obtained from KBase (Prokka) analysis.

Fragment	Product	Start	Strand (+/-)	Length (bp)
1&2	Chemotaxis protein CheW	10,785	-	543
1&2	Protein-glutamate methyltransferase/protein-glutamine glutaminase cheB	7,707	-	1,158
3	UDP-N-acetylglucosamine 1-carboxyvinyltransferase murA	1,645	-	1,284
3	Photosystem I assembly protein Ycf3	12634	-	132
1&2	6-phosphogluconate dehydrogenase, NADP(+)-dependent, decarboxylating gndA	14,286	-	1,395
1&2	CTP synthase pyrG	1,788	-	1,602
1&2	Phosphoenolpyruvate-protein phosphotransferase ptsI	1,858	-	1,722
3	UvrABC system protein C uvrC	16077	+	1,812
1&2	Chaperone protein HtpG	16,249	+	1,851
1&2	Chemotaxis protein CheA	9,904	-	2,142

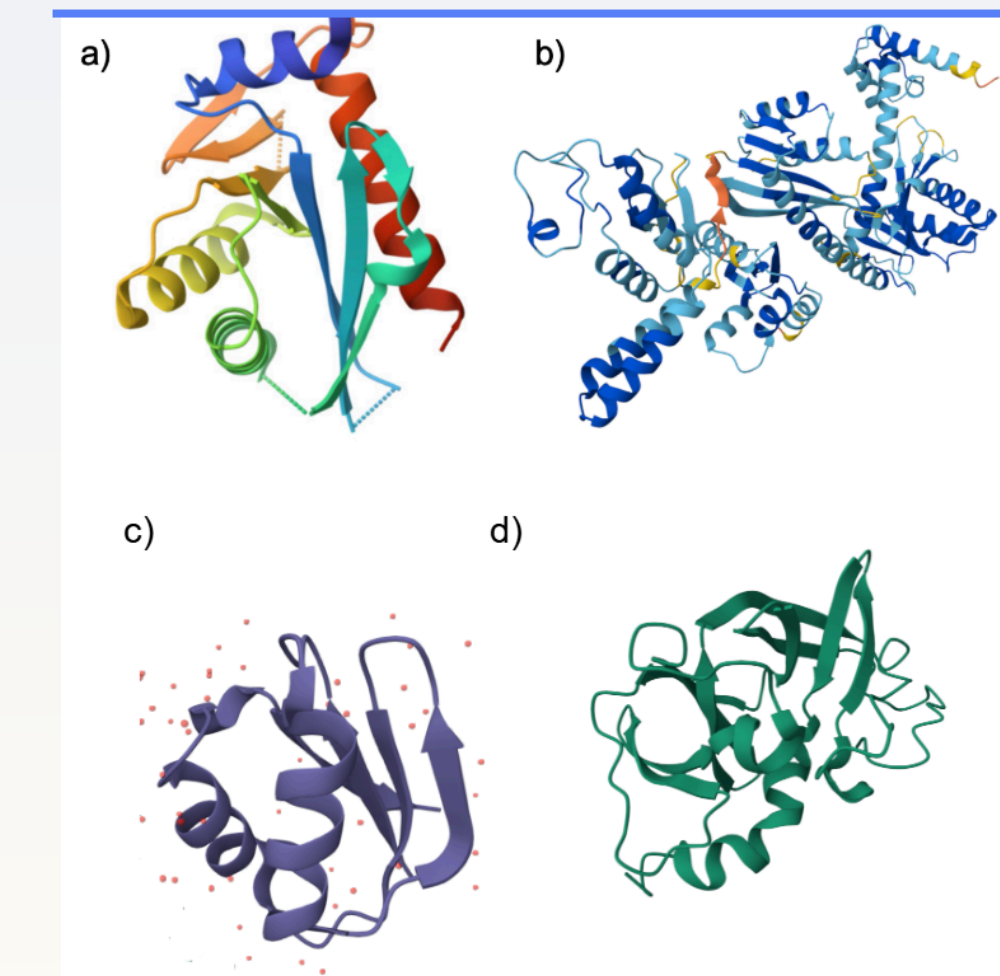


Figure 3 - 3D structures of the proteins
3D structure of Thermatoga maritima UvrC (a) and predicted 3D structure of UvrC (b)
3D structures of CheA and CheW from E. coli, X-Ray diffraction protein structure with a resolution of 2.10 Å (c)
Solution Nuclear Magnetic Resonance structure (d)

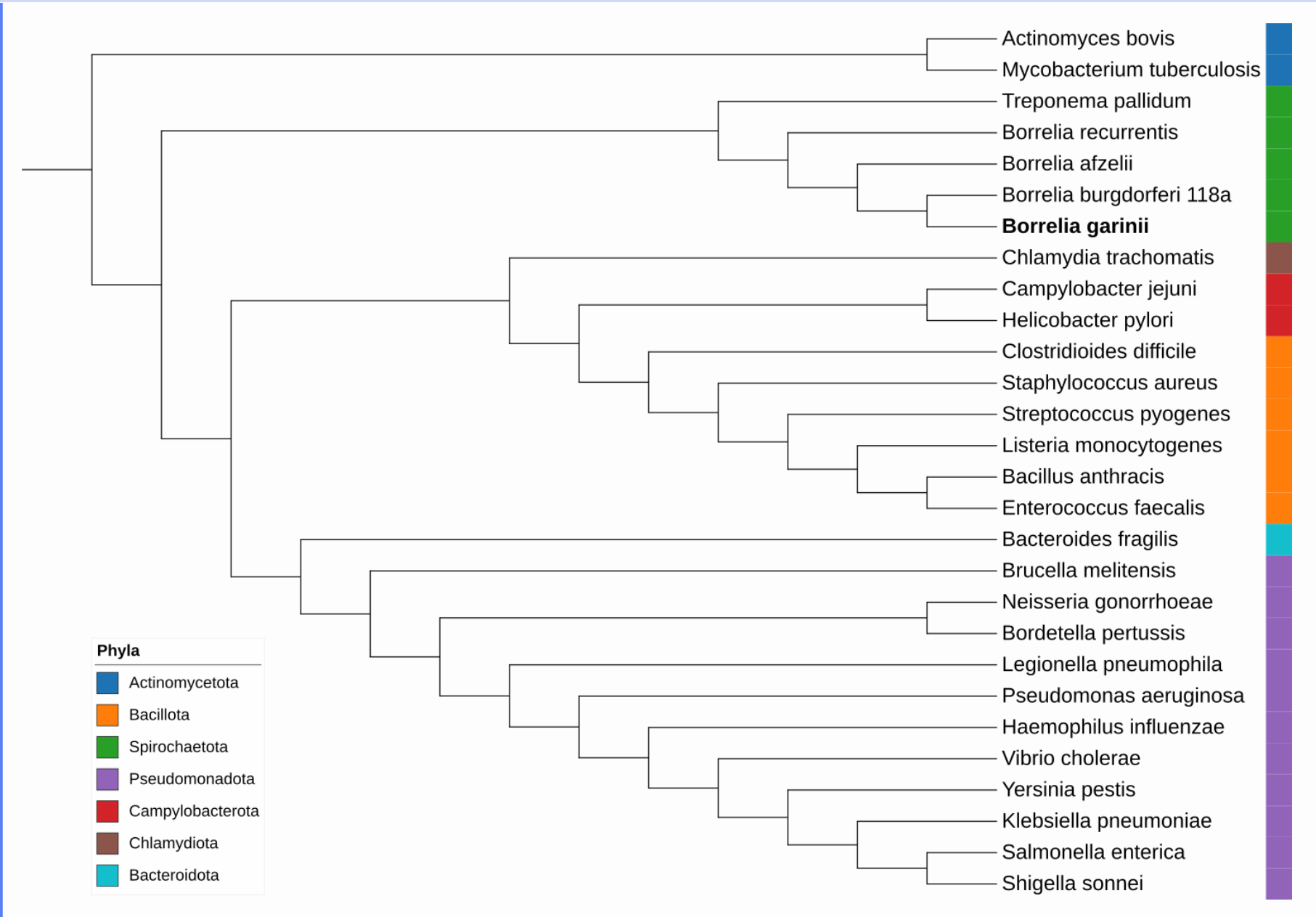
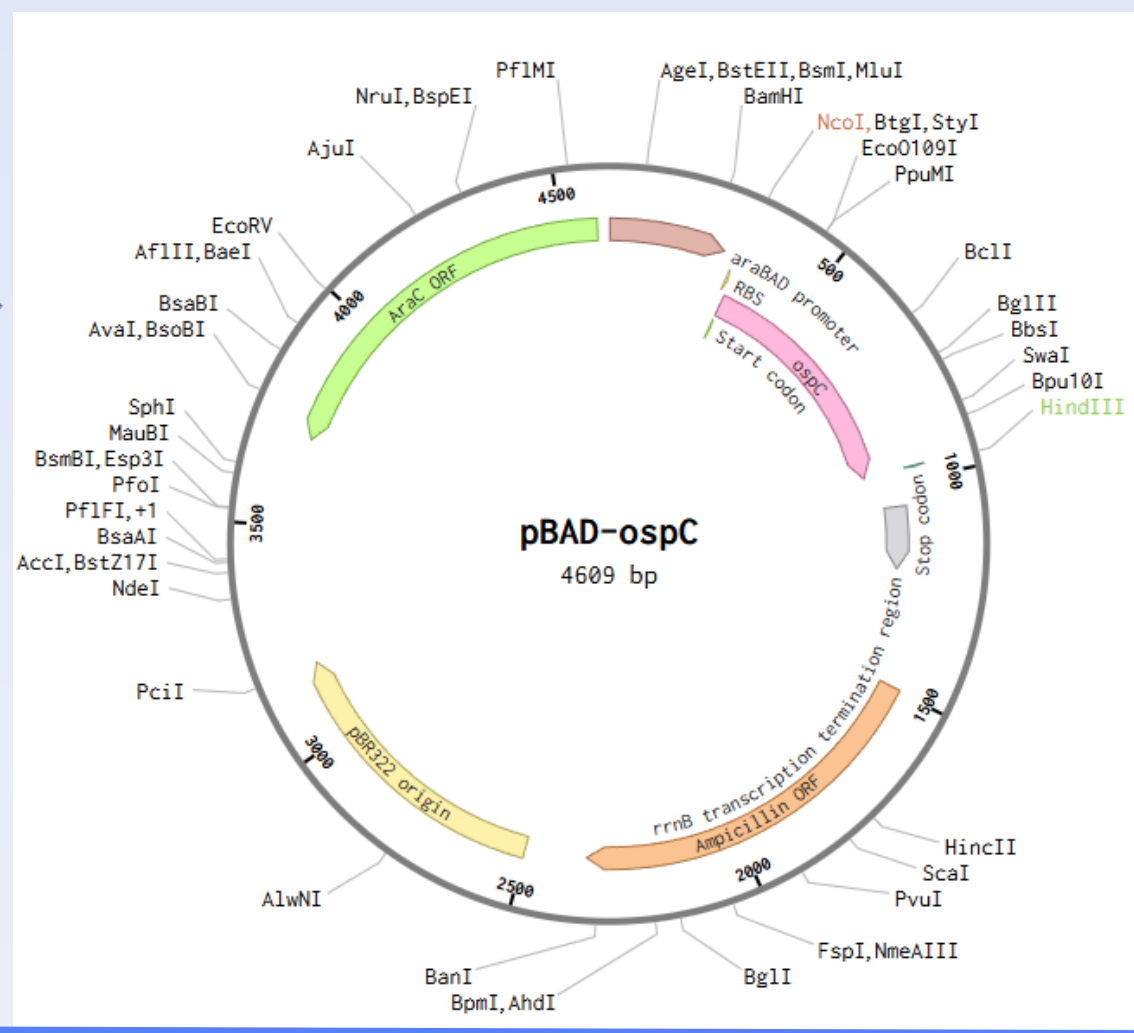


Figure 1: Bacterial phylogeny based on 16S rRNA.
Rectangular phylogenetic tree of representative bacteria inferred from 16S rRNA sequences, leaves are labeled with species names and branches are colored by phylum.

Figure 2- In silico overexpression vector of outer surface protein C (ospC) in pBAD
The RBS is on the vector, and the start codon is located 7–10 nucleotides away from it, on the ospC gene.
The primers used were a forward primer with 31.03% GC and a reverse primer with 38.46% GC content.



-Conclusion-

The DNA fragment was identified as *Borreliella garinii* by BLASTn. Its ORFs, genes and proteins were then recognized using NCBI ORFfinder and KBase Prokka.

Literature research examined *B. garinii* transmission by Eurasian Ixodes ticks, its plasmid rich genome with surface and complement evasion factors enabling infection and immune escape. The review also described Lyme disease presentation, its placement within Lyme borreliae, and how diagnostics (VlsE, OspC) and vaccines (OspA, OspC) are being developed.

Three KBase proteins were analyzed: CheA, a histidine kinase that initiates chemotaxis signaling; CheW, an adaptor that links CheA to chemoreceptors; and UvrC, a nuclease that performs the dual cuts in nucleotide excision repair around damaged DNA.

An in silico pBAD vector was made to overexpress OspC from *B. garinii* in *E. coli* TOP10, but because OspC is nontoxic and needed in high amounts, a higher expression system such as pRSET and improved higher GC primers would be more suitable. It requires an experimental test.



-References-

