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Communication

Identification of evolutionary conserved DNA sequence and corresponding S21 ribosomal protein region for diagnostic purposes of all *Borrelia* spirochetes

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It is still under investigation, whether all *Borrelia* sp. causing Lyme borreliosis and other diseases are already identified and properly classified as human pathogens. For this reason, it is of great importance to develop a diagnostic ELISA test that detects all *Borrelia* sp. The aim of this study was to identify conserved DNA and protein regions present in all currently known *Borrelia* sp. In experimental studies 31 available *Borrelia* sp. genomes were aligned and screened for the presence of evolutionary conserved regions. As a result of bioinformatics analysis, one evolutionally conserved DNA region encoding a core fragment of the S21 ribosomal protein was identified. Both a couple of genus-specific PCR primers and the S21 protein B-cell epitope were designed for prospective diagnostic purposes.

Keywords: *Borrelia* sp., Lyme disease, diagnostic

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Abbreviations: ELISA, Enzyme-Linked Immunosorbent Assay; IgG, Immunoglobulin G; PCR, Polymerase Chain Reaction; PDB, Protein Data Bank

INTRODUCTION

It is still under investigation if all the *Borrelia* species causing Lyme borreliosis and other diseases (Barstad *et al.*, 2018; Cutler *et al.*, 2016; Jahfari *et al.*, 2017; Stanek & Reiter, 2011), mainly around the N hemisphere, are fully identified and properly classified as human pathogens (Schneider *et al.*, 2008; Rudenko *et al.*, 2008). In the summer, people visiting the woodlands are exposed to the attack of ticks that can be vectors of virulent *Borrelia* sp. From a diagnostic point of view, the high genetic diversity of *Borrelia* sp. makes it difficult to detect specifically those species that are responsible for the development of the disease. Therefore, it is not easy to make progress in the field of accurate medical treatment of infected people and epidemiological studies. In the present study, conserved DNA sequences and the corresponding S21 ribosomal protein region were identified and found to be common to all *Borrelia* sp. deposited at the Gene Banks in NCBI (National Center for Biotechnology Information) and DDBJ (DNA Databank of Japan). Identification of all *Borrelia* sp. at a time can help in the detection of a rare pathogenic strain that cannot be identified using currently available tests specific to *Borrelia* sp. sensu lato and *Borrelia* sp. sensu stricto (Jahfari *et al.*, 2017). In the *16SrDNA* gene it is possible to identify conserved regions shared by all *Borrelia* sp. (Parola *et al.*, 2011; Ni *et al.*, 2014). However, greater variability within the *flaB* gene requires optimization of DNA amplification conditions if genus-specific primers are used (Wodecka *et al.* 2010). The conserved region of S21 gene discovered in our study can therefore be used as an alternative target in the diagnostics of *Borrelia* sp. by PCR, but mainly, as an additional test to exclude false positive results obtained for the *16SrDNA* or *flaB* genes, in the case of contamination of the samples by previous amplicons. However, we recommend the use of PCR-based tests to detect *Borrelia* sp. directly from ticks, rather than from clinical samples.

The immunological assays available commercially and assays developed in many laboratories worldwide have a widely divergent sensitivity and specificity (Kodym *et al.*, 2018). They are based on native whole-cell antigens, purified antigens (flagellar components), or whole-cell antigens combined with recombinant antigens. Furthermore, the vast majority of borrelial proteins are cross-reactive (Reed, 2002). The complexity of the antigenic composition among *Borrelia* genospecies and differential expression of proteins in the host and vector (temporal and spatial antigenic variability) has posed challenges for the serodiagnosis of borreliosis. This variability impedes the development of a single immunodiagnostic assay for all major *Borrelia* genospecies. The optimal combination of the most specific epitopes in the form of recombinant chimeric proteins could increase the discrimination abilities of serodiagnostic tests. In the present study we also designed a chimeric antigen for the specific detection of *Borrelia* sp.

MATERIALS AND METHODS

In this study, 31 complete *Borrelia* sp. genomes were aligned using MAFFT v. 7.271 software (Katoh *et al.*, 2002), and a DNA fragment encoding the most conserved protein within the entire *Borrelia* sp. proteome, S21, was identified. The conserved amino-acid sequence of the S21 protein was then compared with all protein sequences deposited at the Proteins Data Bank of Japan. Next, we used RaptorX Structure Prediction server (http://raptorx.uchicago.edu/) to predict the 3D structure of the whole S21 ribosomal protein and its conserved fragment (Figs. 3A and 3B, respectively). The 5MMJ PDB protein structure from the small subunit of the chloroplast *Spinacia oleracea* ribosome was selected by the RaptorX online software as a template (Bieri *et al.*, 2017) after comparison with all available 3D protein structures from Protein Data Bank (PDB) (Källberg*et al.*, 2012). B-cell epitopes present within the whole S21

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                           *       20         *       40         *       60         *       80         *
Primers     : GAAAAAGCATTRAAACGTTTYAAAAGAATGATTGAAAAAGAAGCAAT       GAATGGAARAGAAGAGAATAYTATGAAAARCCATCCAC
CP015629.1  : GAAAAAGCACTAAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCCAC
CP000049.1  : GAAAAAGCACTAAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCCAC
CP005851.1  : GAAAAAGCATTAAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCAAC
CP007022.1  : GAAAAAGCATTAAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCAAC
CP005745.1  : GAAAAAGCATTGAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCTAC
CP005829.1  : GAAAAAGCATTGAAACGCTTCAAAAGAATGATTGAAAAAGAAGCAATAATCAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCTAC
CP010308.1  : GAAAAAGCATTGAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCCAC
CP006647.2  : GAAAAAGCATTGAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCCAC
CP004217.1  : GAAAAAGCATTGAAACGTTTCAAAAGAATGATTGAAAAAGAAGCAATAATTAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCCAC
CP004267.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATCATCAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCTAC
CP003426.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATCATCAGAGAATGGAAAAGAAGAGAATATTATGAAAAACCATCTAC
CP000976.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATCATCAGAGAATGGAAAAGAAGAGAATATTATGAAAAGCCATCTAC
CP000993.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATCATCAGAGAATGGAAAAGAAGAGAATATTATGAAAAGCCATCTAC
CP009058.1  : GAAAAAGCATTAAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAGAGAAGAGAATACTATGAAAAGCCATCCAC
CP002933.1  : GAAAAAGCATTAAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAGAGAAGAGAATACTATGAAAAGCCATCCAC
CP000395.1  : GAAAAAGCATTAAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAGAGAAGAGAATACTATGAAAAGCCATCCAC
CP009212.1  : GAAAAAGCATTAAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAGAGAAGAGAATACTATGAAAAGCCATCCAC
CP003882.1  : GAAAAAGCATTAAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAGAGAAGAGAATACTATGAAAAGCCATCCAC
CP007564.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGTGAATGGAAAAGAAGAGAATACTATGAGAAACCATCCAC
CP003866.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGTGAATGGAAAAGAAGAGAATACTATGAGAAACCATCCAC
CP003151.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGTGAATGGAAAAGAAGAGAATACTATGAGAAACCATCCAC
CP000013.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGTGAATGGAAAAGAAGAGAATATTATGAGAAACCATCCAC
CP009117.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
CP009656.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
AE000783.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
CP005925.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
CP001205.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
CP002312.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
CP002228.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
CP002746.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGCGAATGGAAAAGAAGAGAATACTATGAAAAACCATCCAC
CP009910.1  : GAAAAAGCATTGAAACGTTTTAAAAGAATGATTGAAAAAGAAGCAATTATTCGTGAATGGAAAAGAAGAGAGTATTATGAAAAACCATCCAC
S21 protein   E  K  A  L  K  R  F  K  R  M  I  E  K  E  A  I  I  R  E  W  K  R  R  E  Y  Y  E  K  P  S  T
fragment
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Bor_spF 5′  GAAAAAGCATTRAAACGTTTYAAAAGAATGATTGAAAAAGAAGCAAT
Bor_spR 5′  GTGGATGGYTTTTCATARTATTCTCTTCTYTTCCATTC
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Figure 1. Multiple sequence alignment of the S21 ribosomal protein gene fragment for 31 genomic sequences deposited at the National Centre for Biotechnology Information (NCBI).
Bor_spF and Bor_spR are the degenerated primer sequences selected for PCR detection of all *Borrelia* sp.

>A0A0E1U9C3 *Borrelia burgdorferi* 64b, S21 ribosomal protein
MVTVTVDKNENLEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVKKEKAFKRKQAKKVRKLKQKTNR
The most conserved protein region in *Borrelia* sp.

UniProt Accession number	Species name	Amino-acid sequence of conserved region (Predicted B-cell epitope)
A0A0A7UV08	*Borreliella chilensis*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
G0AL63	*Borreliella bissettii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
D6RX98	*Borreliella valaisiana*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A1L6VPK9	*Borrelia mayonii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
C0APY9	*Borreliella spielmanii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A0E1U9C3	*Borrelia burgdorferi*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
C0APG6	*Borreliella finlandensis*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
B7XSS9	*Borrelia garinii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A1L8ZCR4	*Borreliella bissettii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
B7J1I4	*Borrelia burgdorferi*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
O51271	*Borrelia burgdorferi*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
Q662A9	*Borreliella bavariensis*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
Q0SNQ5	*Borrelia afzelii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A1G4PL64	*Borreliella japonica*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
I0FC33	*Borrelia crocidurae*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A142JBW0	*Borrelia hermsii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
W5SZF6	*Borrelia coriaceae*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
W5SHF8	*Borrelia crocidurae*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
W5SMU4	*Borrelia anserina*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
B5RR73	*Borrelia recurrentis*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
B2RZW7	*Borrelia hermsii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
B5RL82	*Borrelia duttonii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
W5SDG3	*Borrelia miyamotoi*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A1D8TDL4	*Borrelia miyamotoi*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A172XAR9	*Borrelia turicatae*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A0A0E1C180	*Borrelia hermsii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
A1QZ52	*Borrelia turicatae*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
W6TIG2	*Borrelia parkeri*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
W5SR45	*Borrelia duttonii*	LEKALKRFKRMIEKEAIIREWKRREYYEKPSTIRVK
		************************************

Figure 2. Multiple sequence alignment of the S21 protein fragment of 29 *Borrelia/Borreliella* sp. amino acid sequences deposited in the Protein Bank of Japan.
Highlighted in grey is the conserved region characteristic for ribosomal S21 protein of *Borrelia/Borreliella* sp. The predicted B-cell epitope is distinguished on a black background.

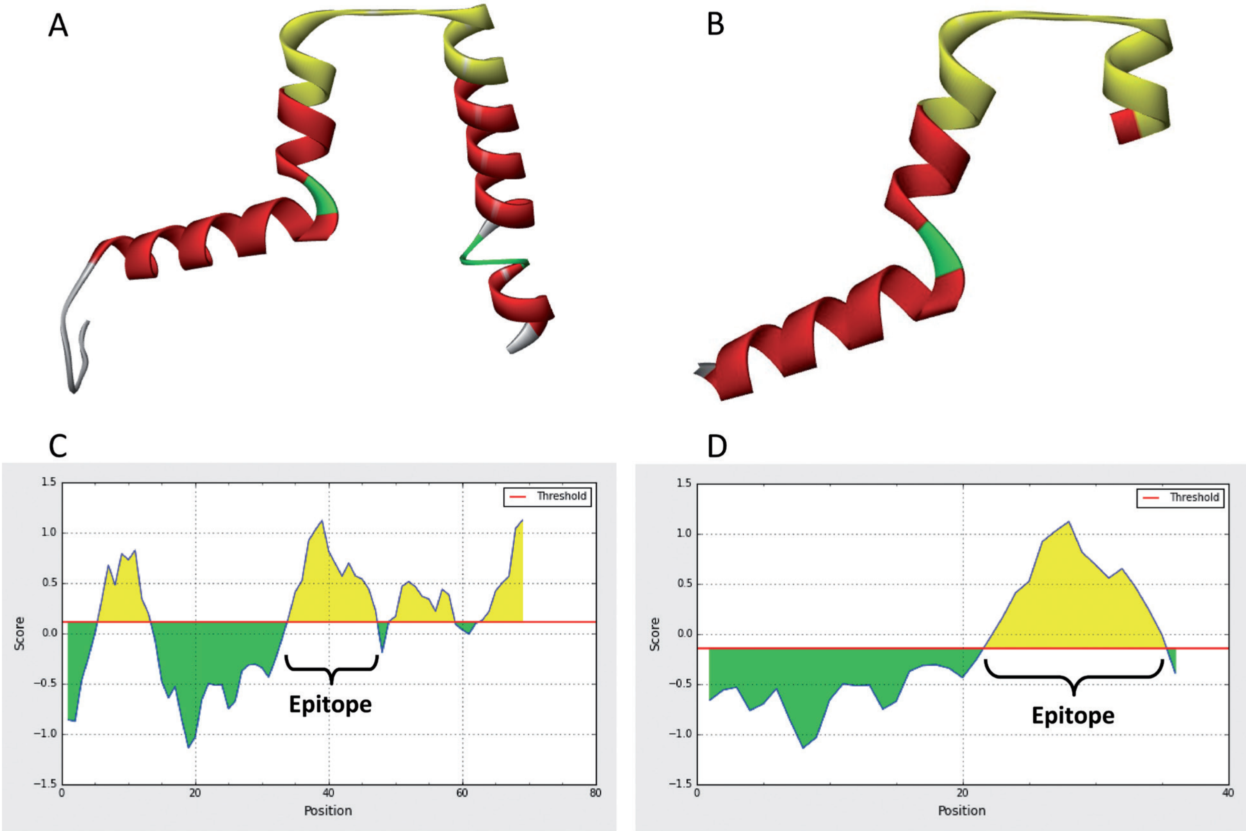

Figure 3. The structure of ribosomal S21 protein of *Borrelia burgdorferi* 64b obtained using homology modelling.
(**A**) – the entire amino-acid sequence of the S21 protein; (**B**) – the conserved region within S21 proteins from *Borrelia/Borreliella* sp.; (**C**) – predicted B-cell epitope within the whole S21 ribosomal protein; (**D**) – predicted B-cell epitope within the conserved region of the S21 ribosomal protein of *Borrelia/Borreliella* sp.

ribosomal protein and the S21 conserved protein motif were predicted using B Cell Epitope Prediction Tools (http://tools.immuneepitope.org/main/bcell/), (Larsen *et al.*, 2006).

RESULTS AND DISCUSSION

Multiple alignments of *Borrelia* sp. genomes allowed for the selection of the most conserved DNA protein coding region (Fig. 1). The identified DNA region was used to design slightly degenerate PCR primers: Bor_spF and Bor_spR specific to all *Borrelia* sp. Translation of the selected DNA region revealed, irrespective of point mutations in the DNA sequences from different genomes, an identical amino-acid chain among all of the analyzed *Borrelia* sp, suggesting that this core part of the ribosomal S21 protein is evolutionary conserved. All amino-acid sequences with 100% similarity identified using BLAST server at DDBJ belonged to the genus *Borrelia/Borreliella* (Fig. 2). The conserved part of S21 ribosomal protein may potentially serve as an intracellular antigen, indicating active human infection caused by all of the *Borrelia* spirochetes on the basis of ribosomal metabolism. The structure prediction analysis confirmed a similar 3D-conformation for both the entire S21 protein and for the conserved region only (Figs. 3A and 3B, respectively). Therefore, based on bioinformatics analysis, we can speculate that it will be possible to immunize animals for the production of specific antibodies using the S21 protein conserved region. Because the selected S21 protein fragment has a molecular weight below 5kDa, it may act as a hapten that is capable of inducing an immune response and recognizing specific antibodies only when attached to a large carrier such as a protein or assembled into one chimeric protein with other fragments of various antigens. This is why we suggest combining two genus-specific protein fragments, the S21 conserved peptide (Fig. 2) and, for example, a part of the FlaB protein: QIRGLSQASRNTSKAINFIQTTEGNL. Successful application of chimeric proteins, assembled using molecular biology techniques, in immunological assays for the diagnosis of viral, bacterial and parasitic diseases has already been demonstrated (Alcaro *et al.*, 2003; Holec-Gąsior *et al.*, 2012a, Holec-Gąsior *et al.*, 2012b; Drapała *et al.*, 2014; Ferra *et al.*, 2015). To date, only a few studies have shown the reactivity of this kind of proteins with specific IgG antibodies from human sera of individuals with Lyme borreliosis (Gomes-Solecki *et al.*, 2000; Schreterova *et al.*, 2017). However, chimeric proteins are a new generation of recombinant products which have the potential to replace the native antigen (e.i. crude fractions of sonicated cells of microorganisms). Furthermore, the construction of recombinant chimeras containing genes from several genospecies can allow generating one protein that confers antigenicity to multiple strains. In addition, the use of pure chimeric proteins as diagnostic antigens provides greater flexibility in adapting the test to different assay formats. Thus, the S21-FlaB fusion peptide proposed in this study appears to be a very promising antigen. The core region of the S21 protein contains the B-cell epitope (Figs. 3C and 3D), whereas FlaB, the major endoflagellar filament protein (Motaleb *et al.,* 2000), acts as an antigen targeting the initial antibody response of the host (Aguero-Rosenfeld *et al.,* 1993). The advantage of the chimeric antigen designed in this study is 100% specificity to all *Borrelia* sp. and the presence of two different antigens.

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