# AN ATTEMPT TO DISCRIMINATE *BORRELIA BURGDORFERI* SENSU LATO COMPLEX MICROBES ACCORDING TO THE PROTEIN PROFILE

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## 1 Introduction

Lyme borreliosis (LB), the most common vector-borne disease in Europe, North America and parts of Asia, is caused by the tick-borne spirochete *Borrelia* species. In 1982, the bacterium that causes LB was first isolated by Willy Burgdorfer and colleagues from the hard tick *Ixodes dammini* collected on Long Island, N. Y. [1]. The isolate was subsequently identified as a new species of the genus *Borrelia* and was named *Borrelia burgdorferi* in 1984 [2]. In the past years classification of Lyme disease borreliae has been investigated intensively. More than 20 species have been identified within the genus *Borrelia* so far [3, 4]. These species are usually categorized into two major groups, the relapsing-fever borreliae and the Lyme borreliosis borreliae, that differ their ecological and genetic characteristics [3]. Relapsing fever has been associated with *B. hermsii, B.turicatae, B. parkeri, B. crocidurae, B. hispanica* and *B. duttonii*. Category of Lyme borreliosis borreliae is named *B. burgdorferi* sensu lato complex. This complex is composed of the following species: *B. turdii* (the name has been corrected from *B. turdae*), *B. tanukii, B. bissettii, B. valaisiana, B. lusitaniae, B. burgdorferi* sensu stricto, *B. andersonii, B. japonica, B. garinii,* and *B. afzelii. B. burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii* are the dominant species associated with infection in humans [5, 6, 7, 8, 9].

*Borrelia* microbes are fastidious, microaerophilic bacteria that grow at 33°C in a complex Barbour-Stoenner-Kelly liquid medium [1]. Like other *Borrelia* species, *B. burgdorferi* sensu lato is a spiral-shaped, gram-negative bacterium containing from 7 to 11 periplasmic flagella. It varies from 10 to 30μm in length and 0.2 to 0.5μm in width [3].

LB may attack a lot of organs or tissues, resulting in the skin, cardiac, neurological and musculoskeletal disorders. Erythema migrans occurs at more than 90% of patiens. Lyme carditis, arthritis, neuroborreliosis and acrodermatitis chronica atrophicans are others clinical manifestations [9].

## 2 Material and methods

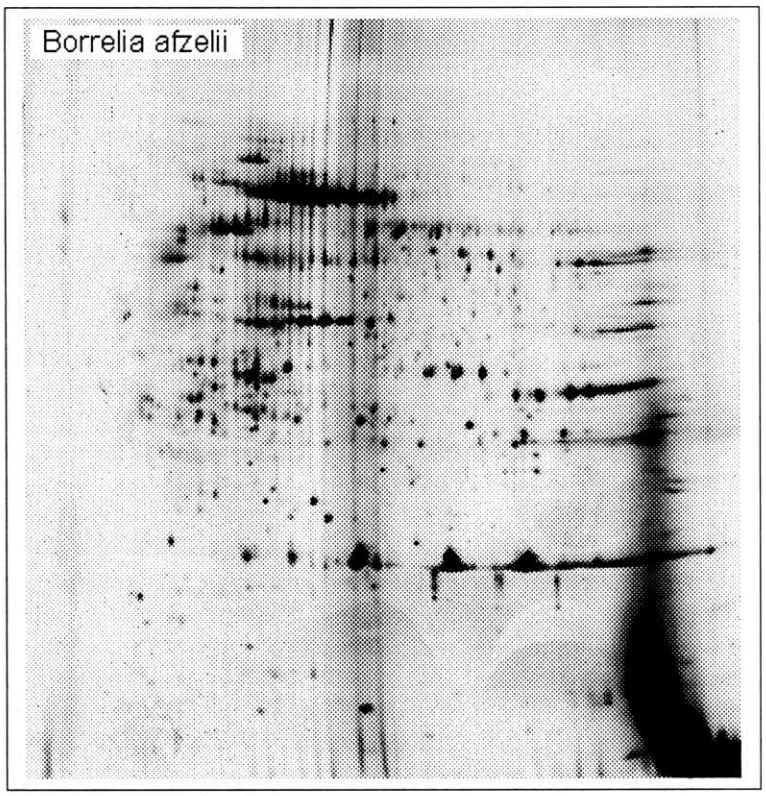
### 2.1 Bacterial cultures

In our study three *Borrelia* subspecies of *B. burgdorferi* sensu lato complex were used: the B-31 strain of *B. burgdorferi* sensu stricto (the ninth passage), the CB-61 strain of *B. garinii* (the twelfth passage) and CB-43 strain of *B. afzelii* (the twelfth passage). These microbes were grown in Barbour-Stoenner-Kelly-H (BSK-H) medium (Sigma, St. Louis, MO, USA) supplemented with 6% rabbit serum and ATB (antibiotic mixture for *Borrelia*) at 34 °C. The number of spirochaetes was calculated by dark-field microscopy according to *Mangusson* et al. [11].

The suspension of microbes for 2-D electrophoresis was prepared in cold PBS ($5x10^8$ and $5x10^9$), centrifuged, and the pellets were immediately homogenised in lysis buffer (137mM NaCl, 10% glycerol, 1% p-octyl-â-D-glucopyranoside, 50mM NaF, 1mM $Na_3VO_4$ and proteinase inhibitors).

### 2.2 2-D electrophoresis (2-DE)

Bacterial proteins were precipitated overnight in 20% TCA in acetone (-18°C) containing 0.2% DTT and then solubilized in IEF buffer (9M urea, 4% CHAPS, 70mM DTT and 2% carrier ampholytes pH 9–11). Commercial strips with nonlinear immobilized pH 3–10 gradient were used. The strips were swollen in rehydration buffer containing 2M thiourea, 7M urea, 4% CHAPS, 0.5% Triton X-100, 10mM DTT and 0.4% Pharmalytes pH 3–10 overnight. Protein concentration in IEF buffer was determined by modified BCA assay. 75μg of protein was loaded for 2-DE of whole cell bacterial lysates. IEF was performed with Multiphor II, with continually increasing voltage from 300 to 5000 V overnight. In the second dimension gradient 9–16% SDS-PAGE was used. Electrophoresis was carried out using a Protean II xi Multi Cell, at a constant current of 40mA/gel for 3.5h. Proteins were visualised by silver staining. The gels were scanned


Borrelia afzelii

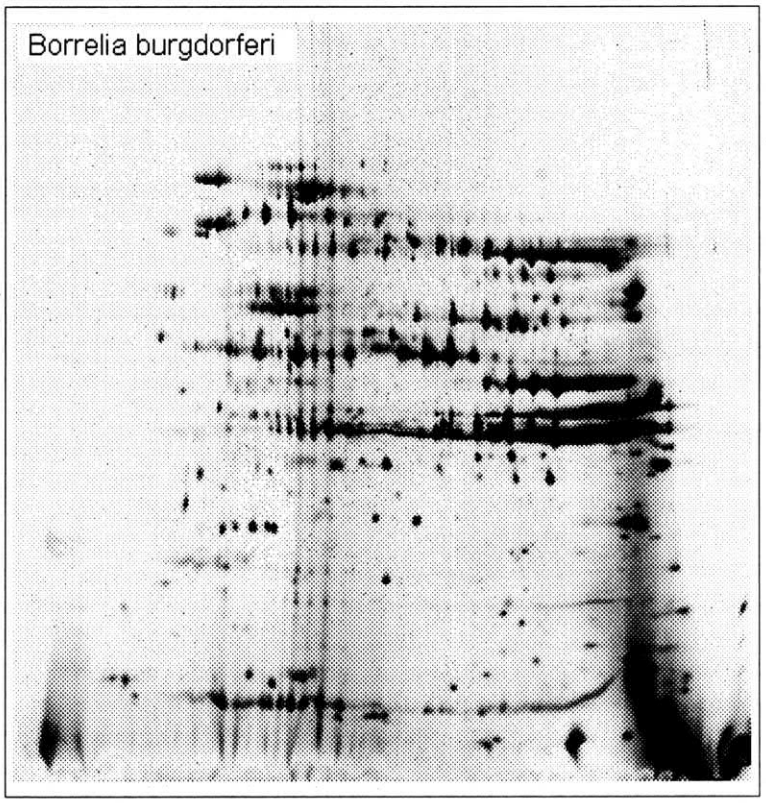

Borrelia burgdorferi


*Fig. 1*

using a laser densitometer Personal Densitometer linked to Sun Enterprise 250 workstation. The 2--DE image analysis was carried out using Melanie III package (BioRad).

### 2.3 Mass spectrometric study

The spectra were recorded by using a MALDI-TOF mass spectrometer (Voyager-DE$^{TM}$ STR, Perseptive Biosystems, Framingham, MA, USA). After cultivation, suspension of microbes ($1x10^6$ and $1x10^7$ microbes/tube) was washed in cold PBS, centrifuged, and resuspended in acetonitrile-0.1% trifluoracetic acid (70:30, vol/vol). The sample was mixed with 3,5-dimethoxy-4-hydroxycinnamic acid matrix solution (sample:matrix 10:1) and 1.5µl was deposited in a target of 100-target sample plate. Each spectrum was obtained in the linear mode by counting 100 laser shots at an accelerating voltage of 20kV by using a 0.3-µs delay time and mass range 1000-25000.

## 3 Results

The proteins of *B. afzelii, B. burgdorferi* and *B. garinii* (Fig. 1), were separated by 2-DE. After comparing of the patterns it was found that some spots were placed at the same position, but a lot of spots differ in spot position and several spots are typical for certain subspecies. *B. afzelii* appears as a very different. Those results are the same as published *Jungblut* et al. [12]. They identified 19 protein spots by peptide mass fingerprinting using MALDI MS. The identified spots P83/100, P41, Hsp70, GAPDH were at the same position, whereas OspA, OspB and OspC varied in spot position among borrelia species.

Some differences among subspecies are evident from the first obtained MS spectra of ACN fraction of *Borrelia* microbes. There are just three peaks on spectra which were common for all three tested *Borrelia*: 6700, 6950 and 8010. One peak (13655) was found in spectra of *B. burgdorferi* and *B. garinii*, but not in spectrum of *B. afzelii*. Any results about similary study were published in literature.

## 4 Concluding remarks

That are the first results of study of protein composition of the *Borrelia burgdorferi* sensu lato complex microbes. We will be identify protein spots by using MALDI-TOF mass spectrometry in a further research. It is necessary to improve method MALDI-TOF mass spectrometry measurement of ACN fraction of bacteria, too.

## 5 References


1. BURGDORFER, W., et al. Lyme disease – a tick-borne spirochetosis? *Science*, 1982, 216, p. 1317–1319.
2. JOHNSON, RC. *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease. *Int. J. Syst. Bacteriol.*, 1984, 34, p. 496–497.
3. BARBOUR, AG. – HAYES, SF. Biology of *Borrelia* species. *Microbiol. Rev.*, 1986, 50, p. 381–400.
4. SCHWAN, TG. – BURGDORFER, W. – ROSA, PA. *Borrelia*. In MURRAY, PR., et al. (ed.). Manual of clinical microbiology. 6th ed. American Society for Microbiology, Washington, 1995, p. 626–635.
5. ROBERTS, DM., et al. The *bdr* Gene Families of the Lyme Disease and Relapsing Fever Spirochetes: Potential Influence on Biology, Pathogenesis, and Evolution. *Emerging Infectious Diseases*, 2000, 6, p. 110–122.
6. ZÜCKERT, WR. – MEYER, J. – BARBOUR, AG. Comparative Analysis and Immunological Characterization of the *Borrelia* Bdr Protein Family. *Infection and Immunity*, 1999, 67, p. 3257–3266.
7. ANDA, P., et al. A new *Borrelia* species isolated from patiens with relapsing fever in Spain. *Lancet*, 1996, 348, p. 162–165.
8. GERN, L. – HUMAIR, PF. Natural history of *Borrelia burgdorferi* sensu lato. Review Article. *Wiener Klinische Wochenschrift*, 1998, vol. 110, no. 24, p. 856–858.
9. WANG, G., et al. Molecular Typing of *Borrelia burgdorferi* Sensu Lato: Taxonomic, Epidemiological, and Clinical Implica-tions. *Clinical Microbiology Reviews*, 1999, 12, p. 633–653.
10. BARBOUR, AG. Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.*, 1984, 57, p. 521–525.
11. MANGUSSON, HJ. – HILL, C. – FLEISCHMAN, R. The minimal infectious inoculum of *Spirochaeta pallida* (Nichols strain) and consideration of its rate of multiplication *in vitro*. *Amer. J. Syph. Gonorrh. Ven. Dis.*, 1948, 32, p. 1–18.
12. JUNGBLUT, P. – GRABHER, G. – STÖFFLER, G. Comprehensive detection of Immunorelevant *Borrelia garnii* antigens by two-dimensional electrophoresis. *Electrophoresis*, 1999, 20, p. 3611–3622.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