The Outer Membrane of Borrelia; The Interface Between Them and Us

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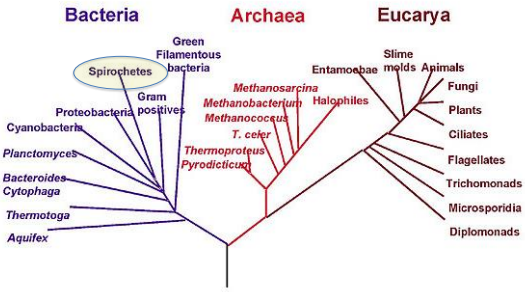
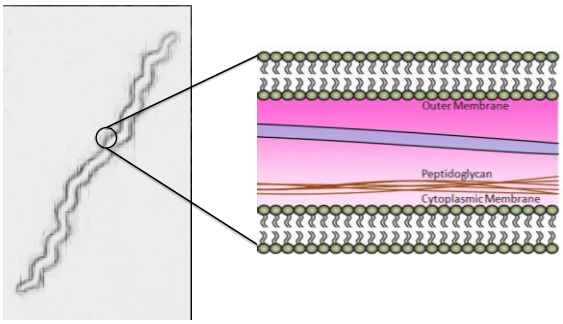
Lecture Outline

- I will give an overview of the outer membrane of Borrelia
- I will present evidence from various sources showing how Borrelia can:
  - Adhere to extracellular matrix
  - Invade tissues
- I will briefly discuss the biofilm formation
- I will then talk about the research projects at Huddersfield
  - Evasion of the complement immune response
  - SodA
  - Enolase

Phylogenetic Tree of Life



The outer membrane of *Borrelia*

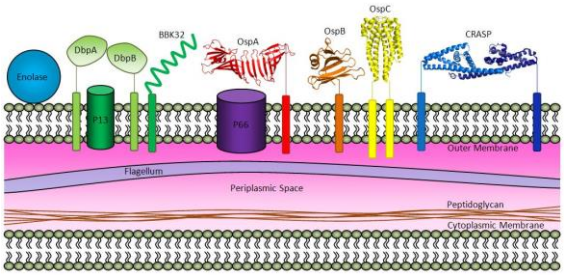


Borrelia outer membrane morphology

- The basic structure consists of a cytoplasmic membrane surrounded by peptidoglycan and a loosely associated outer membrane (OM).

  Unique characteristics:
  - Abundance of lipoproteins
  - Glycolipids (no lipopolysaccharide)
  - The presence of cholesterol

(Ben-Menachem et al., 2003; Schroder et al., 2003; Stubs et al., 2009)



Adhesins

- **Adhesins are bacterial cell-surface components that facilitate adhesion or adherence to cells or extracellular matrix**

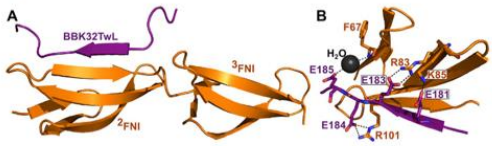
The extracellular matrix

- During persistent infection *Borrelia* is localized to the extracellular matrix
  - Imai *et al* (2013) Dynamics of connective-tissue localization during chronic Borrelia burgdorferi infection, Lab Invest. Aug;93(8):900-10
- This provides a protective niche.
- Numerous proteins on the surface of Borrelia are involved, binding to various proteins of the extracellular matrix
  - DbpA binds to decorin
  - BBK32 binds to fibronectin

Recent Research on BBK32

- **Borrelia burgdorferi protein BBK32 binds to soluble fibronectin via the N-terminal 70 kDa region, causing fibronectin to undergo conformational extension** Gemma Harris, W. Ma, LM, Maurer, JR. Potts and DF.Mosher, *J. Biol. Chem. published online June 24, 2014*

Figure 4

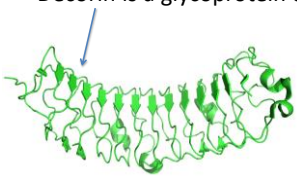


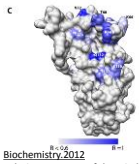
Fibronectin Binding

- BBK32 is a surface lipoprotein
- Binds fibronectin
- Binds to glycosaminoglycans (GAGs)
- bbk32 was also shown to be expressed during tick engorgement as well as in the mammalian host.
- The mechanism of binding is very similar to FnBPA from *S. aureus*

Decorin Binding Proteins DbpA and DbpB

- There is a direct association between *B. burgdorferi* and the proteoglycan **decorin**,
- Decorin is a glycoprotein and binds to collagen





Biochemistry 2012
Solution structure of decorin-binding protein A from Borrelia burgdorferi.
Wang X.

The question of Biofilm

- We need to be careful about how we define biofilm.

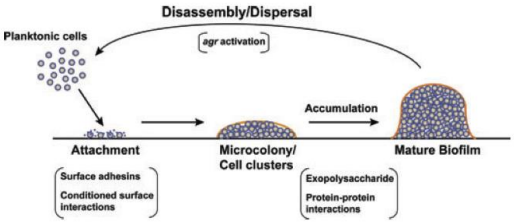
What is Biofilm?

- **Biofilms can be defined as sessile communities of surface-attached cells encased in a self-produced extracellular matrix.**
  - Polysaccharides, proteins, and extracellular DNA are the primary matrix components in other bacteria.
- **Treatment is challenging due to the resistance to both antimicrobials and host defenses.**

Borrelia has Biofilm-like properties

- **Borrelia burgdorferi binds to, invades, and colonizes native type I collagen lattices**
  - Zambrano *et al.* INFECTION AND IMMUNITY, 2004, p. 3138–3146
- Borrelia burgdorferi binds strongly to the extracellular matrix and cells of the connective tissues.
- Bb grew and formed microcolonies

Staphylococcal biofilm life cycle



Kiedrowski and Horswill Ann. N.Y. Acad. Sci. 1241 (2011) 104–121

? extracellular matrix ?

- There is a lack of evidence to show that Borrelia secretes significant amounts of any polysaccharide, DNA or proteins to form an extracellular matrix.
  - (one paper suggests the polysaccharide alginate, but this has not been repeated and there is a strong possibility of contamination)
- There are **many** unanswered questions- What genes are involved? Where does the eDNA come from?
- More research is required

- **Biofilms can be defined as sessile communities of surface-attached cells encased in a self-produced extracellular matrix.**

Yes- There is lots of evidence for this.
The Borrelia proteins involved have been identified and we have a reasonable understanding of how this occurs.

So far, no strong evidence for this (in my opinion).

Research at Hudderfield

- Lipoproteins can be readily identified by their characteristic signal sequence
- Genome data allows us to identify all potential lipoproteins
- MKKVKSKYLA LGLLFGFISC

Positive charge

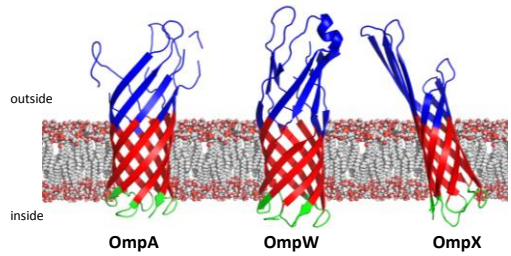
Non-polar

Cysteine amino acid

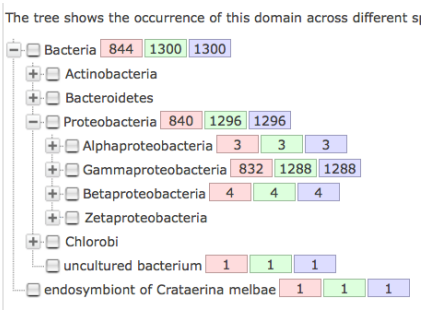
Research at Huddersfield

- Very few beta-barrels are known in Borrelia
  - P66, BamA (BB0795), P13?
- Our aim is to identify and characterise novel membrane spanning beta-barrel proteins in Borrelia
- The major target is the highly conserved **OmpA-type membrane-spanning domain**

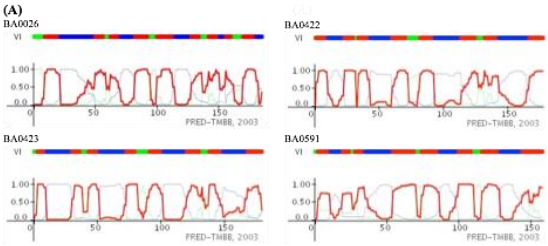
Some *E. coli* Beta-barrels



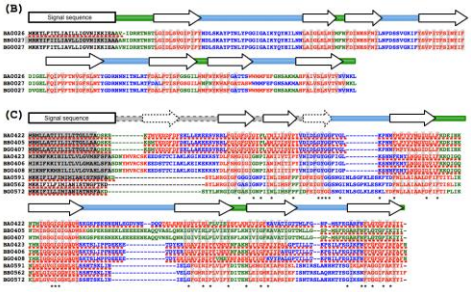
Pfam family PF01389

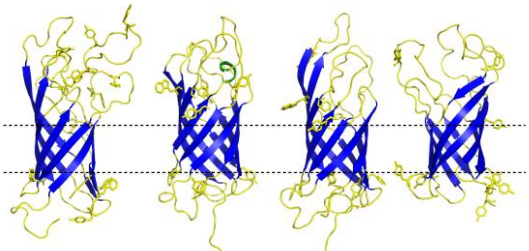


Topology Prediction



Amino acid sequence alignment



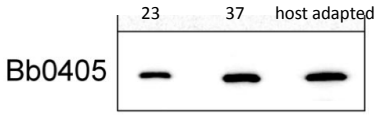


Literature Search

- BB0405 is expressed in human and tick- see next slide (Brooks et al, 2006)
- BB0405 is surface exposed (Yang et al, 2011)
- BG0407 may bind Factor-H (Bhide et al, 2009)

- BB0405 and BG0407 are homologues of BAPKO_0422
- 87% sequence identity

Bb0405 is expressed in both tick and host



Brooks et al, INFECTION AND IMMUNITY, Jan. 2006, p. 296–304

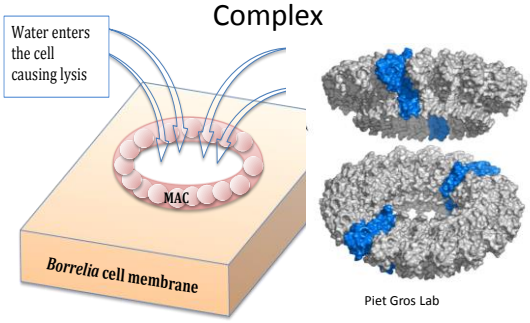
The Complement System

- Part of the innate immune response
- A large number of plasma proteins activated by three initiation pathways.
  - The Classical Pathway
  - The Alternative pathway
  - The Lectin Pathway

- The alternative pathway is continuously activated at a low level
- non-selectively attacks all surfaces in contact with host plasma.
  - Such as the outer membrane of an invading bacterial cell.

- This system is regulated by various proteins- (e.g.. Factor H)

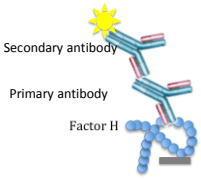
A cascade of events leads to the formation of the Membrane Attack Complex

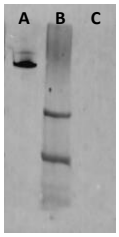


Piet Gros Lab

BAPKO_0422 binds to human Factor H

- Affinity Ligand Binding Immunoblot - ALBI



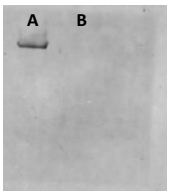


Lane A = Human Factor H Positive control
Lane B = BAPKO_0422
Lane C = Negative control (another protein from Borrelia -SodA)

General overview of the methods

- Blocking – milk powder 5% made up in TBS – 2 hours
- Factor H wash 73µg/ml (14hr)
- Primary antibody was 1:1000 (1hr)
- Secondary 1:5000 (1hr)
- Washes between each step – 3 washes at 5min each with TBS-Tween

Negative controls (done the same day, with identical solutions but **no Factor-H wash**



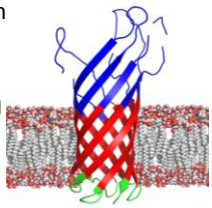
Lane A = Human Factor H Positive control
Lane B = BAPKO_0422

The absence of a band in lane B shows that the primary and secondary antibodies do not bind to BAPKO_0422

Loading BSA, SodA or LDAO detergent alone are also blank

Conclusion

- We have identified four outer membrane proteins in Borrelia
- Cloned, produced pure recombinant protein
- Basic structural characterisation
  - Circular dichroism
  - Molecular envelope by SAXS
  - Demonstrated fH binding by ALBI



Acknowledgments

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Biochemical Society
Advancing Molecular Bioscience