

Genome reduction in blattabacteria, the endosymbionts of cockroaches

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Blattabacteria, a unique group of insect endosymbionts

- Obligately intracellular presence in bacteriocytes in the abdominal fat body
- Exclusively known as endosymbionts of cockroaches
- Function yet unknown, possibly linked to N recycling
- Blattabacteria are pertaining to the class **Flavobacteria** and therefore belong to the phylum **Bacteroidetes** (formerly: CFB)
Low GC content: 28 mol%
- Goal: Revealing the role of the blattabacteria in the symbiosis
How have they adapted to their specific way of life?
Is the pattern of genome evolution the same like in other insect endosymbionts ?

Other insect endosymbionts

- 6 gamma-proteobacteria are yet completely sequenced:
3 *Buchnera aphidicola* strains from aphids,
2 *Blochmannia* spp. from carpenter ants and
Wigglesworthia glossinidia from tsetse fly
- All functionally related to host nutrition:
Supplementation of limited diets
- Common features of their genomes are:
significantly reduced sizes (620 - 800 kb)
Loss of not-essential information (e.g. metabolic genes)
often via transiently pseudogenes
- However, not known if this kind of endosymbiont-typical
evolution is general or specific for (gamma-) proteobacteria

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The hosts: 3 cockroach species



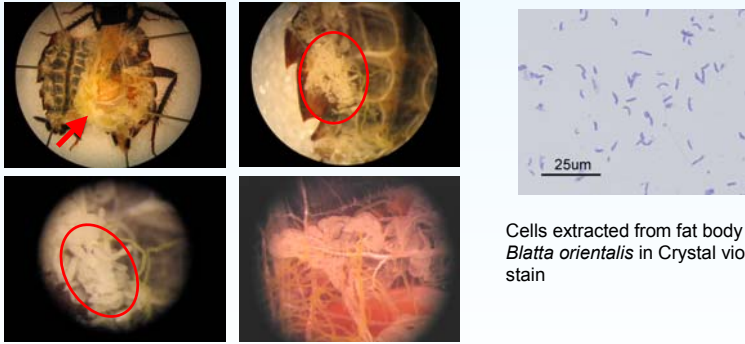
Comparative table

	<i>Blattella germanica</i>	<i>Blatta orientalis</i>	<i>Periplaneta americana</i>
Color	Pale brown	Dark brown or black	Reddish brown
Size	10 - 15 mm	22 - 27 mm	28 - 50 mm
Wings	Both sexes with wings	Male has fully-developed wings and female rudimentary wings	Wings well developed
Capacity to fly	Rarely fly	Both the male and female are unable to fly	With capacity to fly
Adult life span	300 days	From 34 to 180 days	Up to one year
Habitat / Localization	cracks and crevices of walls, sewer systems	Sewer systems, floor drains, basements	Boilers, baths, drainages, sewer system, air vent, larger building such as restaurants, grocery stores

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M+M: Extraction of blattabacteria

- Isolation of blattabacteria:
Dissection of animals and collection of fat body,
Extraction of endosymbionts



Cells extracted from fat body of *Blatta orientalis* in Crystal violet stain

Blatta orientalis fat body: Overview (top left) and details.

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M+M: PFGE, 16S rDNA analysis, FISH, genome sequencing

- PFGE = Pulsed field gel electrophoresis:
Inclusion of extracted cells in agarose plugs
Analysis in two different size ranges: 5 - 200 kb and 50 - 850 kb
- 16S rDNA analysis:
Isolation of genomic DNA,
Amplification and sequencing with standard primers,
Analysis with ClustalW, MEGA3 and PhyML (v2.4.1).
- FISH = Fluorescence in situ hybridization:
Fixation of extracted endosymbionts,
Hybridisation with two newly designed genus-specific probes (BLB) and
comparison with general probes (EUB)
- Genome sequencing:
Endosymbiont of *Blattella germanica*
Shot gun libraries with pGEM-T vector,
Sequencing with BigDye,
Sequence editing and assembly with Staden package,
BLASTX analysis

Blattabacteria are the only bacteria in the fat body of cockroaches.

- Sequencing of 16S rDNA:

⇒ for each cockroach only one sequence type found, affiliation with known blattabacterial sequence types, no indication of other bacteria

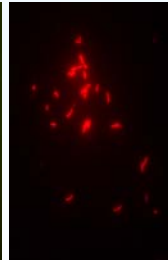
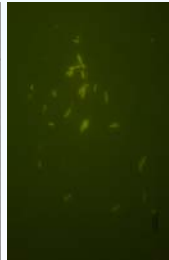
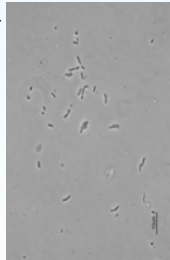
- FISH: Test of cells extracted from fat body tissue

Identity check for blattabacteria:

Phase contrast

EUB

BLB1 (genus probe *Blattabacterium*)



all cells show signals with genus-specific probes



again no indication of presence of other bacteria in the fat bodies

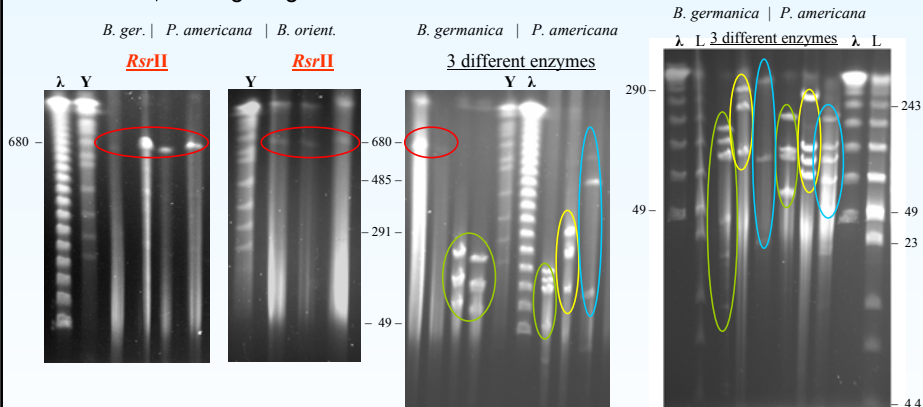


Blattabacteria are the only endosymbionts in cockroach fat body.

Results: PFGE

⇒ Long range: 50 – 850 kb

⇒ Short range: 5 – 200 kb



The blattabacteria of the 3 cockroach species have identical genome sizes of 650 kb. Despite identical sizes: restriction patterns are different and species-specific.

Results: PFGE

Size estimation of genomic DNA fragments of *Blattabacterium* spp..

Endosymbiont from	<i>B. germanica</i>	<i>P. americana</i>	<i>B. orientalis</i>	Species	Genome size in Mb
Restriction enzyme					
<i>RsaI</i>	# of fragments size (range, n) Σ	1 650 (630 – 680, 7) 650	1 650 (640 – 650, 7) 650	1 650 (640 – 650, 6) 650	
<i>ApaI</i>	# of fragments range (kb) Σ	9 4.5 – 175 653	5 75 – 220 650	n.d.	
<i>KspI</i>	# of fragments range (kb) Σ	3 20 – 500 650	5 20 – 215 645	5 45 – 215 630	
<i>SmaI</i>	# of fragments range (kb) Σ	3 133 – 300 658	4 100 – 280 645	n.d.	

Free-living

<i>Bacteroides thetaiotaomicron</i>	6.29
<i>B. fragilis</i>	5.31
<i>Porphyromonas gingivalis</i>	2.34
<i>Cytophaga hutchinsonii</i>	4.42

Endosymbiotic

<i>Blattabacterium</i> sp.	0.65
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➡ The 4 restriction enzymes give congruent results of approx. 650 kb. Comparison with other CFB = Bacteroidetes shows significant reduction of the genome size like typical for insect endosymbionts.

Results: Shot gun sequencing of the *Blattella germanica* endosymbiont genome

- Background:
Scarce genome data for the Bacteroidetes in databases:
only 4 completed genomes, 1 more can be BLASTed (but none of Flavobacteria), approx. 20 projects, 5 for Flavobacteria
- Up to now:
250 kb in 219 contigs (as single or double readings)
Contig lengths: 300 b – 2.5 kb
- BLASTX: # of matches

<i>B. thetaiotaomicron</i>	13	<i>Cytophaga</i>	47
<i>B. fragilis</i> (2 strains)	23	<i>Porphyromonas</i>	14
		<i>Chlorobium</i>	6

➡ The homologies of the partial genome information shows affiliation with the Bacteroidetes phylum. However, due to low amount of high homology matches, the *Blattabacterium* genome seems to be clearly distinct from the ones of other Bacteroidetes.

Summary

- 16S rDNA amplification and FISH show that blattabacteria are the only bacterial symbionts in the fat body of cockroaches.
- Genome size determination for the 3 blattabacteria from cockroach species yields identical sizes of approximately 650 kb.
- Despite same sizes: restriction patterns are distinct and species-specific.

Conclusions

- Although blattabacteria diversified during co-evolution with their hosts, the size of their genomes seems to be a stable parameter.
- Like other insect endosymbionts, blattabacteria have reduced genomes of small size.
- Preliminary results of the genome sequencing of the *B. germanica* endosymbiont confirm the relatively isolated position of the blattabacteria within the Bacteroidetes.

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