# A search for Reb-like protein encoding endosymbionts in local ciliate communities

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### Abstract

**The endosymbiotic bacterium *Caedibacter* may be found in the cytoplasm of *Paramecium*, conveying the "killer" trait to these paramecia. We have been using PCR with primers for one of the proteins (RebB) produced by *C. taeniospiralis* to search for similar endosymbionts in local freshwater ciliates. The Reb proteins constitute the R bodies which are secreted by killer paramecia and kill other paramecia not harboring this endosymbiont. We have also been using degenerate RebB primers to search for similar genes. Our goal is to discover how extensive similar endosymbionts are in local ciliates.**

A particle called Kappa was discovered in 1943 (Sonneborn, 1943) as a cytoplasmic factor (plasmagene) conveying the "killer" phenotype to paramecia. It is now known to be the endosymbiotic bacterium *Caedibacter*, with different species present in *Paramecium aurelia*, *P. caudatum*, *P. biaurelia*, and *P. tetraurelia* (Schmidt *et al.*, 1987). This endosymbiont releases the R-body protein complex which is secreted from the ciliate causing death in paramecia without Kappa. While the coding sequences of the four R-body-encoding Reb genes have been determined in a few laboratory strains, little is known about the occurrence of this symbiont in wild ciliate communities and the evolutionary history of the Reb gene complex remains unclear. In addition to being found in ciliate endosymbionts, R-bodies are also found in some free-living bacteria (Quackenbush, 1978). Recent evidence indicates that cryptomonads' trichocyst ribbon genes are related to Kappa Reb genes, supporting the hypothesis that horizontal transfer from bacteria has occurred in the evolutionary history of this gene (Yamagishi *et al.*, 2011).

We have begun a project to discover if this or similar endosymbionts are present in local freshwater ciliate communities.

### Materials and Methods

Live locally-collected or kappa-containing paramecia were concentrated either by 1) heat-killing ciliates followed by centrifugation, or by 2) filtration. DNA was isolated using the MoBio PowerSoil DNA Isolation Kit. Locally-isolated paramecia were *P. multimicronucleatum*, three strains of *P. tetraurelia*, and *Paramecium sp.* The Kappa-containing strain was *P. tetraurelia* 116K which contains *Caedibacter taeniospiralis*. PCR amplification was done using the ciliate SSU rDNA primers 384-F and 1147-R (Dopheide *et al.*, 2008), and several RebB primer pairs. Primers RebB-F (5' ATG AGT AAT GTA AAT TCA C 3') and RebB-R (5' TTA ACC ATT TTT AGC GGC 3') recognize the *C. taeniospiralis* RebB coding segment (Heruth, personal communication). We also used the following RebB degenerate primer pairs: RebB1-F (5' GTN AAY DVN CAR ATH CAN GAY 3') and RebB1-R (5' NAD NAD RTT NSW YTG YTG YTG 3'); RebB1-F and RebB2-R (5' NNW NTK YTG YTG RTT 3'); and RebB3-F (5' CAR ATH CAN GAY DSN GTN WSN CAR 3') and RebB3-R (5' NAR NGT RTT YTG YTG YTG YTG 3'). These degenerate primers were designed by Heruth (personal communication) to recognize nucleotide sequences in conserved regions of Type 51 and Type 7 Kappa RebB CDSs as well as related RebB sequences from non-endosymbiotic bacteria that produce RebB protein. They were designed to amplify approximately 150 bp segments of the RebB CDS. Gel electrophoresis was performed using either standard 2-3% agarose gels or 2% agarose in the E-Gel system (Life Technologies). For sequencing, amplicons were purified on a CloneWell 0.8% agarose gel (Life Technologies) and cloned using the TOPO-TA cloning kit (Invitrogen) followed by colony sequencing (GeneWiz). Geneious software (Drummond *et al.*, 2011) was used for sequence analysis.

### Results

The RebB-F/RebB-R primer pair successfully amplified the RebB CDS from the DNA extracted from *P. tetraurelia* 116K (Figures 1 & 2). The 281 nt sequence of this fragment was identical to published *C. taeniospiralis* RebB CDS sequences (accession numbers AY422720, U04523, U04524)(Data not shown). The 384-F/1147-R SSU rDNA primer pair amplified 116K rDNA (Figure 1) which upon sequencing varied from several published *P. tetraurelia* by only a single C/T transition over 639 nt. (It also perfectly matched one published sequence for *P. primaurelia*.)(Data not shown.)

Unfortunately, our test of the designed degenerate RebB primers failed to clearly amplify the RebB CDS in *P. tetraurelia* 116K and in several locally-collected paramecia when annealing temperature was 5$^{o}$C below the melting temperature (Tm) of the primers (Figures 2) as well as when it was 42$^{o}$C (Figure 3).

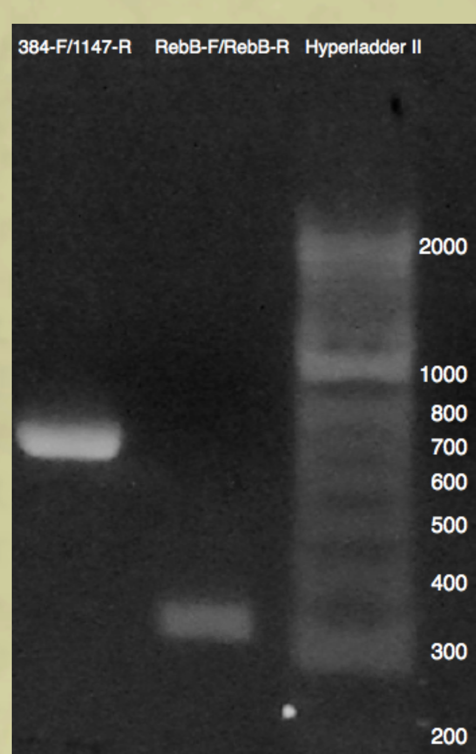


***Figure 1. PCR-amplified P. tetraurelia 116K RebB DNA and SSU rDNA.*** DNA isolated from Kappa-containing *P. tetraurelia* 116K was PCR amplified using the SSU rDNA primer pairs 384F/1147R and RebB-F/RebB-R (lanes 1 and 2). The marker was Hyperladder II (Bioline USA). Fragments of the predicted size were observed and sequenced.

### Discussion

The designed degenerate primers have apparently proven to be ineffective tools for the discovery of new RebB-related sequences in ciliates. However, further investigation is needed to identify the segment amplified by the RebB1-F/RebB1-R primer pair in 116K as well as the segment amplified by the RebB-F/RebB-R primer pair in our *P. multimicronucleatum* and in our *P. tetraurelia* strain #2 (Figure 3). However, it should be noted that these amplicons may not necessarily come from the paramecia, since our ciliates are grown in rye medium with the bacterium *Klebsiella pneumoniae* and other possible microbes.

### Literature Cited

Dopheide *et al.*, 2008. *Applied & Environ. Microbiology*, 74: 1740–1747.

Heruth, D. P. Children's Mercy Hospital & Clinics, School of Med., UMKC.

Drummond *et al.*, 2011. Geneious v5.5, Available from http://www.geneious.com

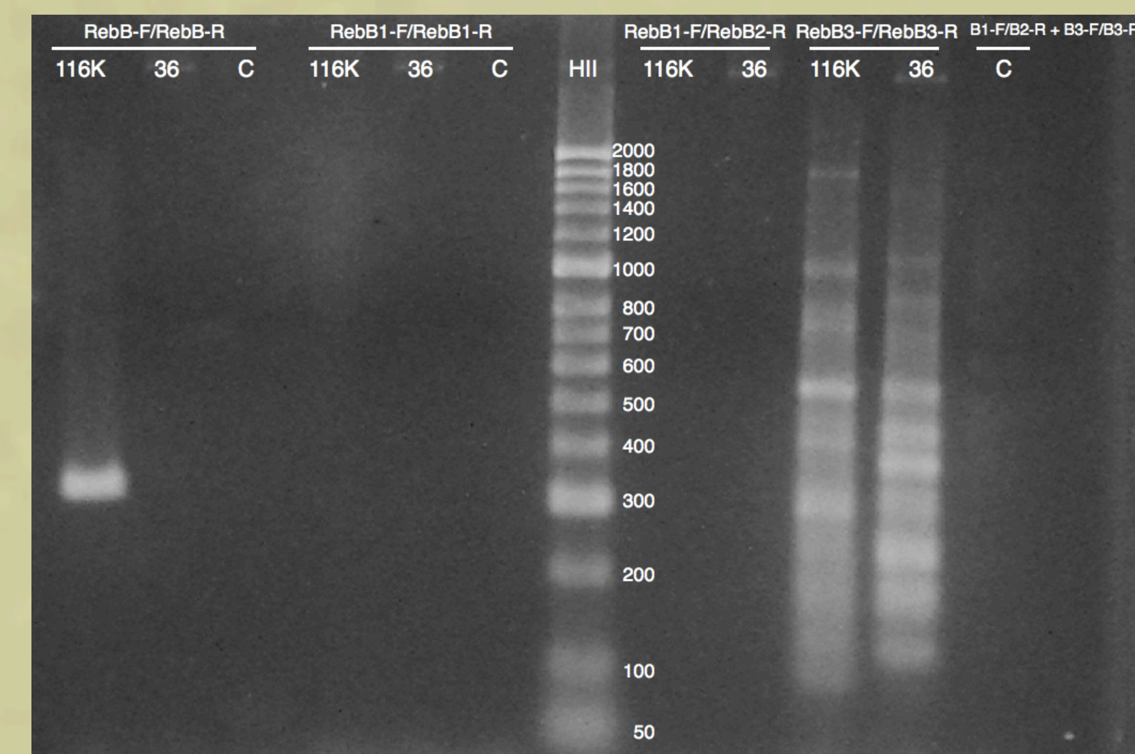


***Figure 2. PCR-amplification with RebB and degenerate RebB primers.*** PCR was performed with an annealing temperature 5$^{o}$C below the lowest Tm of the primer pair. Primers used were the RebB-F/RebB-R pair and degenerate primer pairs RebB1-F/RebB1R, RebB1-F/RebB2-R, and RebB3-F/RebB3-R. 116K is the Kappa-containing *P. tetraurelia* 116K, 36 is local *Paramecium sp.*, and C is a control without template DNA. HII is Hyperladder II marker.

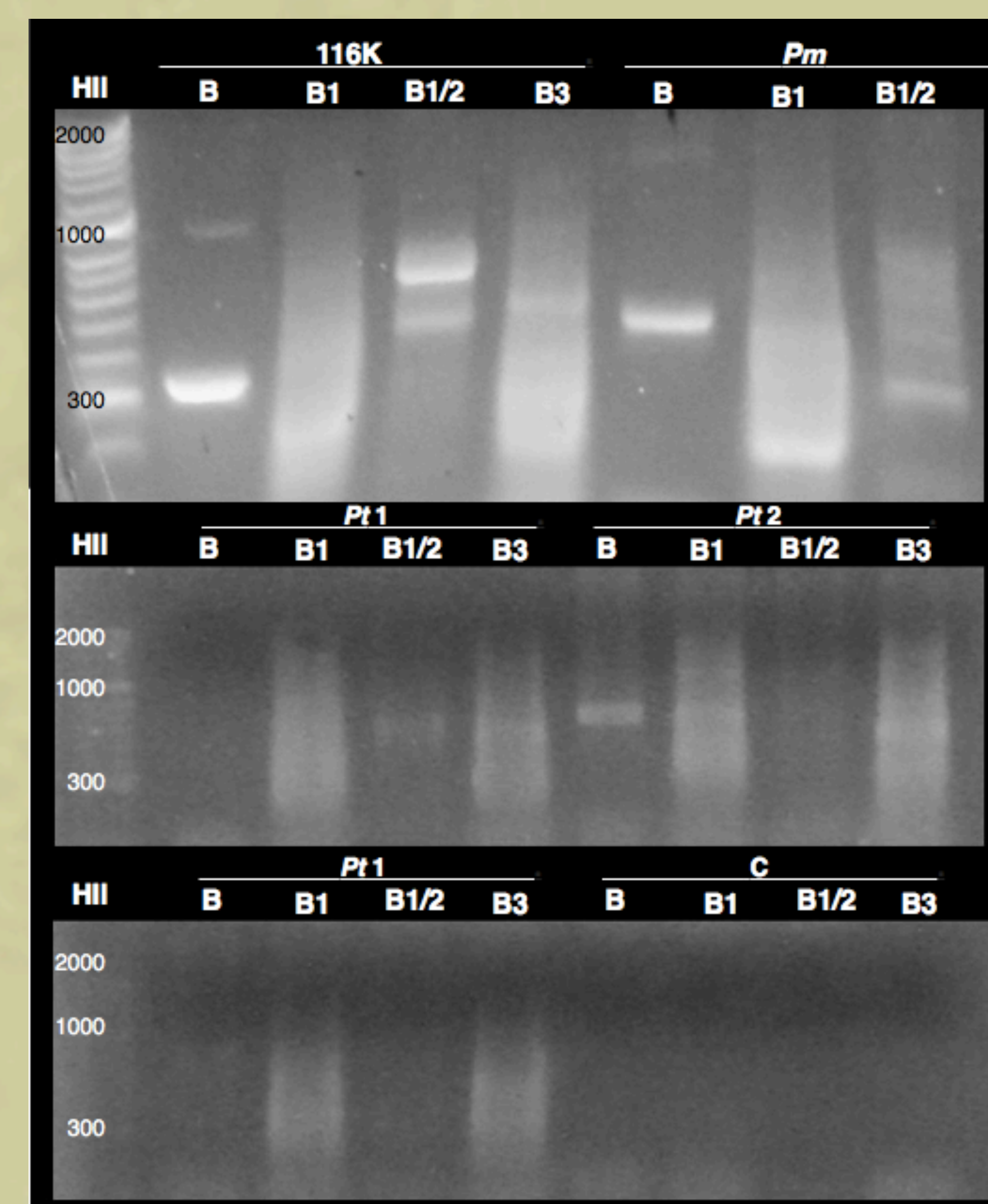


***Figure 3. PCR-amplification with RebB and degenerate RebB primers--annealing temperature 42$^{o}$C.*** PCR was performed with an annealing temperature of 42$^{o}$C. Primers were the RebB-F/RebB-R pair (B) and the degenerate primer pairs RebB1-F/RebB1R (B1), RebB1-F/RebB2-R (B1/B2), and RebB3-F/RebB3-R (B3). 116K is the Kappa-containing *P. tetraurelia* 116K, *Pm* is local *P. multimicronucleatum*, *Pt1*, *Pt2*, and *Pt3* are local strains of *P. tetraurelia*. C is a control without template DNA. HII is Hyperladder II marker.