**Caedibacter caryophila** sp. nov., a Killer Symbiont Inhabiting the Macronucleus of *Paramecium caudatum*

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*Caedibacter caryophila* sp. nov. lives in the macronucleus of certain strains of *Paramecium caudatum*. The type strain is 221, carried in *P. caudatum* C221. *C. caryophila* is distinguished from other caedibacteria on the basis of host specificity, R body morphology, behavior of R bodies, and guanine-plus-cytosine content of its deoxyribonucleic acid.

Bacteria, fungi, algae, and even other protozoa have been observed as endosymbionts of protozoa (1). Unique morphological or behavioral phenotypes can characterize such symbiotic relationships (6). Endosymbionts of the genus *Caedibacter* cause their paramecium hosts to kill susceptible paramecia (cells without these symbionts), whereas killer paramecia themselves are resistant. This is a classic example of a unique behavioral phenotype associated with endosymbionts. Furthermore, caedibacteria can produce unusual inclusion bodies (R bodies) which make them morphologically unique.

All members of the genus *Caedibacter* are obligate endosymbionts of *Paramecium* spp. that confer killer traits upon their ciliate hosts (7). *Caedibacter* spp. are distinguished from other bacterial endosymbionts of paramecia that also confer killer traits upon their hosts by their ability to produce R bodies (7). R bodies are long (up to 20 μm), proteinaceous ribbons (approximately 0.5 μm wide and 3 μm thick) that are rolled up inside the bacterial cell, forming a hollow cylindrical structure. All *Caedibacter* spp. have been described as cytoplasmic endosymbionts occurring in *Paramecium biaurelia* or *Paramecium tetraurelia* (6, 7). We report here an obligate bacterial endosymbiont of *Paramecium caudatum* that confers a killer trait upon its host and produces R bodies. Furthermore, this bacterium resides in the macronucleus rather than the cytoplasm (Fig. 1). The purpose of this paper is to name this organism in accordance with the rules of the Bacteriological Code. We propose the name *Caedibacter caryophila* sp. nov. to indicate that the new species is a bacterium which kills and lives in a nucleus.

The genus *Caedibacter* contains bacteria which occur in *P. biaurelia* and *P. tetraurelia*. All *Caedibacter* spp. are toxic to certain susceptible strains of paramecia. They are gram negative and nonmotile, and up to 30% (usually less than 10%) of the cells in any given population contain single (rarely double) refractile inclusion bodies (R bodies). Cells with R bodies are called bright forms (as determined by phase-contrast microscopy) (5), whereas R-body-free cells are referred to as nonbright forms. Nonbright forms are considered as the reproductive forms, and some of them change into bright forms by producing R bodies (5). The R body is a proteinaceous ribbon which is tightly rolled up within the cell. Cells containing R bodies are usually thicker (Fig. 2) than cells that do not contain R bodies and have certain spherical bacteriophage-like structures or covalently closed circular deoxyribonucleic acid (DNA) plasmids (2).

The type species is *Caedibacter taeniospiralis* (4). The detailed description of *C. caryophila* sp. nov. (see below) shows that this species complies with all diagnostic characteristics that qualify it for membership in the genus *Caedibacter*.

Gram-negative, R-body-producing bacteria similar in appearance to those described by Estévé (3) were originally discovered in strains C220 and C221 of *P. caudatum*. A few months after their cultivation in the laboratory, the endosymbionts in strain C220 lost their ability to produce R bodies. However, this strain still is a host to nonbright forms. Nonbright forms of both strains are generally uniform in size, with a diameter of 0.4 μm. Their average length is 1.0 to 1.5 μm, but occasionally much larger rods can be found which resemble a row of nonbright forms sticking together end to end. Bright forms are bigger, about 0.7 μm wide and 1.5 to 2.5 μm long (Fig. 3). They are always present in strain C221 with low numbers in rapidly growing cultures. The numbers of bright forms per nucleus, however, increased considerably when such cultures were starved. Bright forms contain R bodies and bacteriophages (Fig. 3).

*C. caryophila* is well adapted to its life in a nucleus. The symbionts were usually seen in clusters which are distributed with the dividing macronucleus. Symbiont-free cells were never found in cultures of strain C221 or C220. The high infection rate of 100% may be due to the killing activity; that is, symbiont-free cells may be killed immediately. However, no killing activity was detected for strain C220. The infection rate of 100% in this stock therefore must be due to very effective (accurate) mechanisms for the balanced distribution of symbionts at the division of the host nucleus. It was impossible to free paramecia from their symbionts by rapid growth. Attempts to infect symbiont-free *P. caudatum* strains with isolated nonbright forms or homogenates from C220 were unsuccessful. Paramecia of strain C220 are obviously not harmed by their nonbright symbionts. On the other hand, strain C221 paramecia suffer and eventually die, but only in starving cultures. Their death is probably caused by their nonbright forms. Strain C221 is a killer with prelethal symptoms of the paralysis type. Cells from strain C220 do not kill, but they are still resistant against the toxin. It is not known whether they were killers prior to loosing their bright forms.

*C. caryophila* has a guanine-plus-cytosine (G+C) content of 34.6 mol% (calculated from thermal denaturation profiles [Tm] [8]). When isolated DNA from strain C220 symbionts and the DNA from C221 nonbright forms was digested with EcoRI restriction enzymes and electrophoresed on a gel, their banding patterns were very similar (Fig. 4). In addition,
FIG. 1. Electron micrograph of *P. caudatum* C221. In the macronucleus (Ma), a cluster of bacterial endosymbionts is surrounded by large areas filled with small chromatin bodies and nucleoli (arrows). The cytoplasm (Cy) is free of bacteria. T, Trichocysts. ×10,000. Bar, 1 μm.


These results together with the observations that strain C220 endosymbionts could produce bright forms when first cultured in the laboratory and that this strain is still resistant against killing strongly support the conclusion that both strains are of the same species, and that an unknown event destroyed the ability for R body production in C220 endosymbionts.

Both nonbright and bright forms (and their R bodies) can be isolated from paramecium homogenates by ion-exchange chromatography (Ecteola; Serva, Heidelberg, Federal Republic of Germany) or Percoll (Pharmacia Fine Chemicals) density gradient centrifugation. *C. caryophila* DNA, extracted from such preparations, does not hybridize to R body DNA.

FIG. 2. *C. caryophila* floating in culture medium after the host cell was crushed with the cover glass. Arrows indicate bright forms. Unfixed, unstained, phase-contrast, microflash. ×1,600. Bar, 10 μm.
C. caryophila R bodies are a useful diagnostic characteristic for the species. They are approximately 0.8 µm in width and diameter and are larger than any other R bodies from caedibacteria; they are also visible with the light microscope. They are associated with bacteriophages, which are always seen on the inner terminus of unrolled R bodies (Fig. 5). These inner ends form an acute angle, whereas the outer one is blunt (Fig. 5A and B). Unrolling takes place in a telescoping fashion from the inside (Fig. 5C). The R body proteins exhibit some antigenic cross-reactivity against antisera prepared for type 51 and type 7 R bodies (H. J. Schmidt, F. R. Pond, and H. D. Görtz, J. Cell Sci., in press).

**Description of Caedibacter caryophila sp. nov.**

Caedibacter caryophila (ca.ry.o'phi.la; Gr. noun caryum, nucleus; Gr. adj. philus, loving; M. L. adj. caryophila, nucleus loving). Obligate macronuclear endosymbionts of *P. caudatum*. Gram-negative rods with rounded termini, probably best described as elongated elipsoids. Within any population of *C. caryophila*, a small percentage (usually <10%) of the individuals contain R bodies. R-body-containing cells are larger (1.5 to 2.5 µm long by 0.7 µm wide) than cells without R bodies (1.0 to 1.5 µm by 0.4 µm). *C. caryophila* R bodies are about twice as large (approximately 0.8 µm long by 0.5

**TABLE 1. Diagnostic characteristics of *C. caryophila* sp. nov.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Host species</td>
<td><em>P. caudatum</em></td>
</tr>
<tr>
<td>Site of endosymbiosis</td>
<td>Macronucleus</td>
</tr>
<tr>
<td>Size of nonbright forms (width by length)</td>
<td>0.4-0.5 µm by 1.0-1.5 µm</td>
</tr>
<tr>
<td>Size of bright forms (width by length)</td>
<td>0.7 µm by 1.5-2.5 µm</td>
</tr>
<tr>
<td>Killing</td>
<td>Paralysis type</td>
</tr>
<tr>
<td>DNA G+C content</td>
<td>34.6 mol%</td>
</tr>
<tr>
<td>R body width (after isolation)</td>
<td>0.8 µm</td>
</tr>
<tr>
<td>Mode of unrolling</td>
<td>From inside</td>
</tr>
<tr>
<td>Outer terminus</td>
<td>Blunt</td>
</tr>
<tr>
<td>Inner terminus</td>
<td>Acute</td>
</tr>
<tr>
<td>Extrachromosomal elements</td>
<td>Phage</td>
</tr>
</tbody>
</table>
\[\mu m\] in diameter in the rolled up form inside the bacterial cell) as those reported to occur in the other species of Caedibacter. R-body-containing cells are toxic to susceptible strains of paramecia, with paralysis being the only observable prelethal effect. Appearance of R bodies in C. caryophila is accompanied by the production of bacteriophage particles. DNA base composition is approximately 35 mol% G+C as determined by the thermal denaturation method (8). The type strain is 221 carried in P. caudatum C221 (ATCC 50168).

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