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 13 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 50% (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).

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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

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**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.

**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.
coding sequences from pKAP47 plasmids (Schmidt et al., in press).

*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). A summary of diagnostic characteristics is given in Table 1.

**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>
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FIG. 3. Electron micrograph of *C. caryophila* 221 in the macronucleus of *P. caudatum*. B, Bright forms; N, nonbright forms. Arrows indicate phages associated with R bodies (lamellar structures). ×39,000. Bar, 1 μm.

FIG. 4. Banding patterns of DNAs (digested with EcoRI) from *C. caryophila* 221 (lane a) and 220 (lane b) nonbright forms. The DNAs were separated according to size by electrophoresis on a 0.8% agarose gel and stained with ethidium bromide. Lane M gives fragment sizes (in kilobases) of the λ DNA marker digested with HindIII. Arrows indicate the similar banding patterns of some dominant bands.
FIG. 5. Electron micrographs of isolated R bodies from C. caryophila. Negative stain, phosphotungstic acid. (A) Acute inner terminus, showing bacteriophages (arrows), of an unrolled R body (×24,000; bar, 1 μm). (B) Blunt outer terminus of an unrolled R body (×30,000; bar, 1 μm). (C) R body starting to unroll from inside in a telescoping fashion (×24,000; bar, 1 μm).

μm 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).

We thank C. Kulessa and P. Nikolaus for excellent technical assistance.

This research was supported by grants from the Deutsche Forschungsgemeinschaft (Schm 544, 2-2, 3; SFB 310, C2), awarded to H.J.S. and H.-D.G., and by grants GM36293 from the National Institutes of Health and IN8420591 from the National Science Foundation, awarded to R.L.Q.

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