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Arthromitus (*Bacillus cereus*) Symbionts in the Cockroach *Blaberus giganteus*: Dietary Influences on Bacterial Development and Population Density

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Abstract

The filamentous spore-forming bacterium *Arthromitus*, discovered in termites, millipedes, sow bugs and other soil-dwelling arthropods by Leidy (1850), is the intestinal stage of *Bacillus cereus*. We extend the range of *Arthromitus* habitats to include the hindgut of *Blaberus giganteus*, the large tropical American cockroach. The occurrence and morphology of the intestinal form of the bacillus were compared in individual cockroaches (n=24) placed on four different diet regimes: diurnally maintained insects fed (1) dog food, (2) soy protein only, (3) purified cellulose only, and (4) a dog food-fed group maintained in continuous darkness. Food quality exerted strong influence on population densities and developmental stages of the filamentous bacterium and on fecal pellet composition. The most dramatic rise in *Arthromitus* populations, defined as the spore-forming filament intestinal stage, occurred in adult cockroaches kept in the dark on a dog food diet. Limited intake of cellulose or protein alone reduced both the frequency of *Arthromitus* filaments and the rate of weight gain of the insects. Spores isolated from termites, sow bugs, cockroaches and moths, grown on various hard surfaces display a branching mobility and resistance to antibiotics characteristic to group I Bacilli whose members include *B. cereus*, *B. circulans*, *B. alvei* and *B. macerans*. DNA isolated from

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pure cultures of these bacilli taken from the guts of *Blaberus giganteus* (cockroach), *Junonia coenia* (moth), *Porcellio scaber* (sow bug) and *Cryptotermes brevis* (termite) and subjected to Southern hybridization with a 23S-5S *B. subtilis* ribosomal sequence probe verified that they are indistinguishable from laboratory strains of *Bacillus cereus*.

Keywords: *Bacillus cereus*, intestinal symbionts, segmented filamentous bacteria, symbiotic bacilli, drug resistance, spore-forming bacteria, cellulose diet, motility pattern formation

1. Introduction

The genus *Arthromitus*, given several species names (e.g. *cristatus, chaseii, intestinalis*) were described as minute plants "rooted" to the intestine of *Julus* (millipede) and to intestinal fungi (trichomycete symbionts). *Arthromitus* was beautifully depicted in a large teaching chart of Joseph Leidy (circa 1850) now in the Academy of Natural Sciences, Philadelphia (Warren, 1998). Morphologically distinctive spore-forming filaments attached to the intestinal wall later were documented in termites, cockroaches, isopod crustaceans and other soil-dwelling animals by Leidy (1881). Similar forms were subsequently described in ducks and named *Coleomitus* (Grassé, 1925).

The strain of *Arthromitus* in the isopod crustacean *Porcellio scaber* (sow bugs) was revealed to be *Bacillus cereus* by morphological, physiological, 16s rRNA, and all other measured criteria (Margulis et al., 1990,1998; Jorgensen et al., 1997). Darkness enhanced filament length in sowbug symbionts (Jorgensen el al., 1997). Additional genetic and physiological characterization showed it to belong to group I Bacilli (Piest, 1993). Members of this group are generally resistant to antibiotics and display pattern-forming ability on low-nutrient agar surfaces. Huge populations of *Arthromitus* were found not only in the sow bugs resident in one of the monkey houses (New England Science Center, Worcester, MA), but also in the intestines of the golden lion tamarin (*Leontopithecus rosalia*). Each morning at sunrise, just after the tamarins synchronously defecate, the sow bugs quickly ingest the monkey's feces. Since all *P. scaber* walk over the monkey's food, the bacilli must pass easily from soil to food to arthropod and mammal intestine (Margulis et al., 1998). Single and two-celled ("diplo") bacilli as well as spores and filaments were directly observed in both sow bug and tamarin intestines. Literature reports supplemented by our observations of 12 different arthropod species and other potential *Arthromitus* habitats were summarized in Table 1 of Margulis et al., 1998. After spore-forming filamentous bacteria were observed in conspicuous quantities in laboratory-raised adults of the large (5±0.5 cm, Fig. 1) tropical American cockroach, *Blaberus giganteus*, this study of the effect of diet on *Arthromitus* development was undertaken.

Figure 1. *Blaberus giganteus*, left to right: 1st instar (1), 4th instar (4), 5th instar (5), adult (A) used for this study. Bar = 2 cm.

2. Materials and Methods

Blaberus giganteus, originally collected in the Caribbean and raised for five years in the laboratory of Professor Rosemary Redfield (Department of Biology, UBC, Vancouver, Canada), were used in this study. Twenty adult *Blaberus giganteus* individually isolated into separate plastic chambers (10×5×5 cm), were assigned to one of four experimental groups, each consisting of five insects. Three groups were exposed to an ambient diurnal photoperiod while a fourth, except for brief exposures to light during feeding and care, was maintained in a laboratory drawer in constant darkness. The three diurnal groups of cockroaches were maintained on different diet regimes. The control, group 1 was fed a complete diet of dry pellets of dog food that contained about 20% protein, 8% fat, 4% crude fiber, 1% vitamins and minerals as well as moisture and other carbohydrates (Alpo brand). Group 2 was fed only soy bean protein. The diet of group 3 was limited to cellulose delivered as filter paper shreds. The cockroaches in group 4 were fed the same complete dog food pellet diet as group 1 but were maintained in continuous darkness. The four groups are hereafter referred to as "complete", "soybean protein", "cellulose" and "dark", respectively. The experiment ran for 40 days (March–May 1998). Each cockroach was identified and weighed on an electronic balance weekly before

sacrifice. Insects on complete diet regimes (groups 1 and 4) were provided with dry dog food. Insects in the soy protein group (group 2) were provided with 100% Naturade soybean protein powder (Naturade Inc., Paramount, CA, catalogue item No. 2702). The cellulose group members were fed only Whatman CF11-type cellulose, and for each diet type distilled water was available in troughs *ad libitum* within each plastic isolation container.

Intestinal spore-forming filamentous bacteria were studied microscopically and their population numbers were determined using a Petroff-Hauser chamber counting slide. Fecal pellets crushed or dropped on glass microscope slides (to which insect saline solution or water was added if necessary) were subject to direct microscopic observation. Study of morphology and quantification of numbers of live cells and filament lengths were made with brightfield, Nomarski differential interference contrast (DIC), and phase contrast microscopy. Observations were recorded with a 3/4" SONY U-Matic video tape deck attached to a SONY DXC-107A series camera.

Cockroach behavior from normal to moribund was noted. Normal *B. giganteus* are active, skirmish if disturbed and photophobic. Docile cockroaches will right themselves if turned over. Docility was judged by impairment or failure of the escape reaction when handled, whereas lethargic cockroaches showed these reactions but were very slow in all of their movements. Moribund cockroaches do not move at all except for an occasional antenna or leg twitch. Observations were made at least once a week on all live cockroaches. One cockroach from each of the four groups was sacrificed every 7–10 days.

For brilliant blue staining of laboratory bacilli cultures, each cockroach was surface sterilized in 70% ethanol for one minute prior to dissection. Insect hindguts were aseptically removed and boiled 4 min in insect Trager's (1934) solution. Boiled, macerated hindguts were used as inocula. Samples, transferred to culture tubes with nutrient agar broth and incubated on the laboratory bench for two weeks, by which time flocculent growth appeared, were aseptically streaked onto nutrient agar plates. These plates incubated at ambient conditions, approx. 25°C. Laboratory *Bacillus* strains summarized in Rudner et al. (1998) were used as controls. *Bacillus cereus* strain 6A1 (H.O. Halvorson) and *Bacillus macerans* strain 22A1 (J.M. Nadirova) were obtained from the Bacillus Genetic Stock Center (BGSC), Columbus, Ohio and the pattern-forming bacilli, *B. tipchirales* (T/C) and *B. vortex* (V) from the Rudner collection. These prototrophs that grow in minimal medium lacking growth factors are identical in their Southern hybridization profiles. A number of laboratory cultures of *Arthromitus* bacilli taken from insects in the Margulis laboratory were used as comparisons for the brilliant blue colony patterns. The insects included 3 termites, 1 beetle and 1 moth.

To easily visualize colony morphology and motility patterns published techniques were employed (Rudner et al., 1998; Mendelson and Salhi, 1996). In

summary: nutrient-poor hard agar (1.5–2.5%) and nutrient-rich soft agar (0.6%) plates with 160 µg/ml Xgal (5-bromo-4-chloro-3indolyl-β-D-galactoside) were prepared. These were point inoculated with either one or nine droplets (5 µl) that each contained $1–5\times10^5$ bacilli from liquid cultures. They were incubated at 37°C from 2 to 21 days. To stop growth for analysis of the pattern morphology, nutrient-poor hard agar plates were stained with 0.1% brilliant blue in 50% methanol. They were destained in 10% acetic acid. It was unnecessary to stain nutrient-rich soft agar plates because their patterns are clearly visible with the blue product of Xgal. Drug resistance was checked on LB (Luria broth) plates that contained either 10 µg/ml chloramphenicol (Cm) or 25 µg/ml lincomycin (Lm) and 1 µg/ml erythromycin (Erm), 10 µg/ml kanamycin (Km) or 100 µg/ml ampicillin (Am), 15 µg/ml tetracyclin (Tc), 125 µg/ml spectinomycin (Sp) or 1000–2000 µg/ml streptomycin (Sm).

DNA preparation for Southern hybridizations after digestion with EcoRI was carried out as previously described (Rudner et al., 1994, 1998). Since *B. cereus* strains resist lysozyme (2 mg/ml), lysis was accomplished in 2.5% SDS by repeated heating at 60°C for 10 minutes followed by freezing at –70°C. A purified 2.3 Kb insert of 23S-5S rDNA from *Bacillus subtilis* was labeled with the DECAprime II DNA labeling kit (Ambion) using [α-^{32}P]-dATP and used as a hybridization probe.

3. Results

Arthromitus and diet

In all four groups the number of filamentous bacteria counted in suspension from in vivo samples was approximately the same, $1.15–1.45\times10^4$ cells per ml at time zero. Since foregut, midgut and rectum harbored very few or no *Arthromitus* filaments, no attempt to quantify bacillus populations in any alimentary segment but the hindgut was made. Large diameter rods, four cells in length or longer, were recorded as filamentous. At time 0 the average quantity of filaments in the hindguts of the four individuals was 1.25×10^4 per ml. By the first week a slight increase in numbers of *Arthromitus* in the control group (complete diet) (range $1.25–2.24\times10^4$, average 1.59×10^4, n=4, minimal number of cells to be called a filament) was observed. By the fourteenth day all four groups were easily distinguished. Group 1 and group 4 cockroaches fed complete dog food diets showed the most significant rise in hindgut *Arthromitus* populations; those maintained in darkness consistently contained the largest populations of *Arthromitus*. The dog food control, exposed to diurnal light cycles, had the highest *Arthromitus* populations relative to the other two light-exposed regimes. Group 1 and group 4 (dark) cockroaches in all

Table 1. *Arthromitus* hindgut populations of suspended filaments ($\times 10^4$) per ml

Day	Soybean protein	Cellulose	Complete (control)	Complete (darkness)
0	1.15	1.32	1.15	1.45
7	1.25	1.63	1.25	2.24
14	1.56	1.56	2.50	5.94
20	1.88	0.31	3.40	7.23
30	4.38	0.31	8.13	8.42

Table 2. Weight (grams) of individual cockroach hosts (C) of *Arthromitus* on different diet regimes

Day	C1	C2	C3	C4	C5
Complete (control)					
0	5.90	3.50	4.70	6.10	4.80
7		4.34	6.17	6.89	6.25
14			6.13	6.89	6.02
20				6.92	6.25
30					6.33
Soybean protein					
0	6.02	6.07	6.42	6.70	7.25
7		7.79	7.29	7.79	7.17
14			7.42	6.94	7.20
20				7.07	7.19
30					7.45
Cellulose					
0	4.21	4.32	4.07	6.41	6.08
7		4.38	4.02	6.98	6.28
14			4.02	6.94	6.35
20				7.00	6.42
30					7.13
Complete (darkness)					
0	7.40	5.20	6.10	3.89	5.00
7		5.51	7.39	4.73	5.68
14			7.31	5.29	5.63
20				5.30	5.45
30					5.25

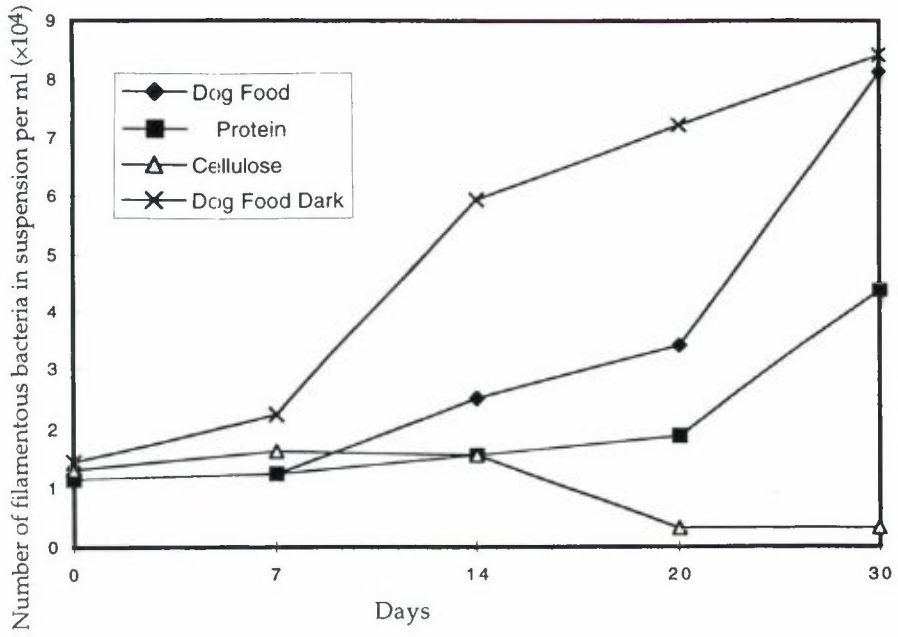

Figure 2. *Arthromitus* hindgut population changes in cockroaches fed different diet regimes.

protein-fed groups (1, 2 and 4) showed an increase in numbers of filaments during the experiment, whereas the frequency of *Arthromitus* declined in the cockroaches fed cellulose. The number of filaments, over 8000 per ml in the control, are tabulated (Table 1) and plotted in Fig. 2. Cockroach weights at intervals of approximately one week are presented in Table 2.

Morphological differences between *Arthromitus* filaments within the hindguts were easily detected. As many as five different morphotypes conform to descriptions of the *Arthromitus* genus, i.e., "diplos", chains of at least 3–5 cells, spore-containing filaments, motile filaments and spores (Figs. 3A,B). Filament lengths (>4 cells) extended from 9 to >180 μm. The average width was 1 to 2 μm. When transferred to culture medium the filaments disintegrate primarily to single cells and "diplos", probably due to dramatic environmental differences. Large rod cells that form chains tend to replace filaments. In nutrient broth and on damp plates, the chains tend to break up into single typical bacillus cells.

Fecal pellets and behavior

The effects of diet on the appearance of the insects and their fecal pellets became abundantly clear by the second week. Those cockroaches fed complete

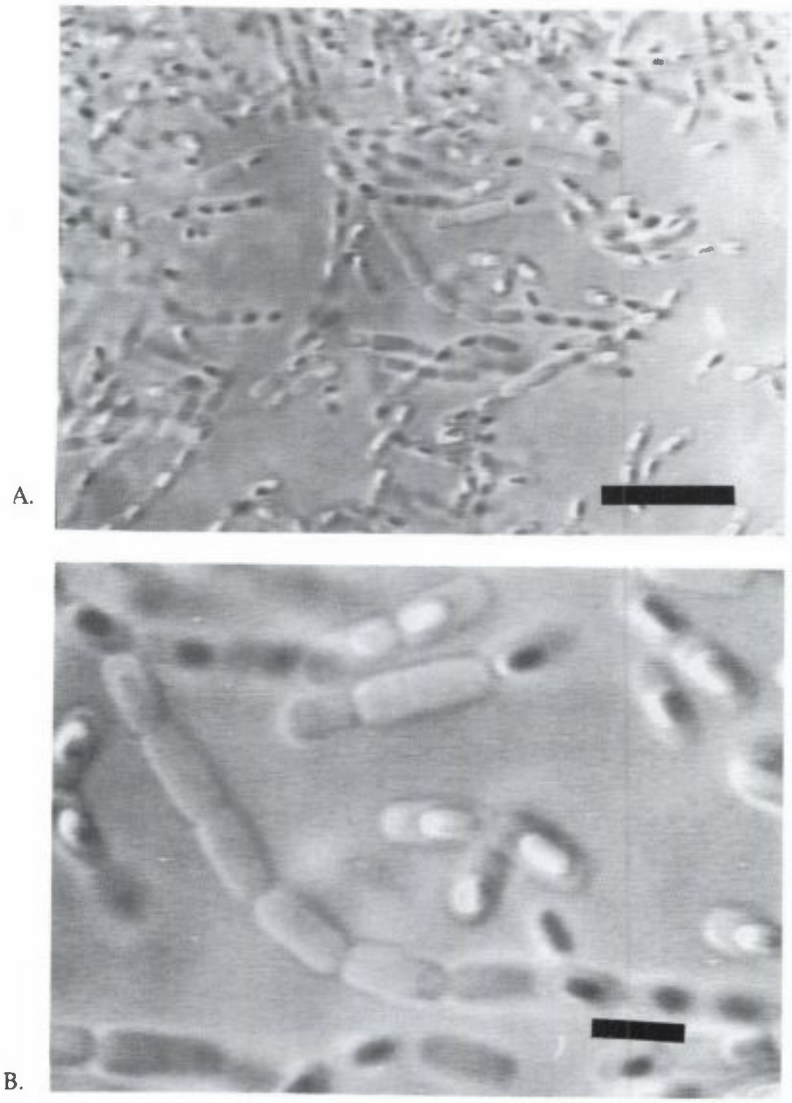

Figure 3. *Arthromitus* (*Bacillus cereus*) from *Blaberus giganteus*: light micrographs. In A. bar = 10 μm, in B. bar = 5 μm.

dog food diets produced small, red fecal pellets that appeared dehydrated. The fecal pellets of the cockroaches fed soy protein were dark brown. Unconsolidated and watery, they resembled diarrhea, staining the paper towel lining of the cockroach compartments. The fecal pellets of the cockroaches fed pure cellulose were extremely large and white. They seemed to be composed primarily of fibrous cellulose strands. The fact that individuals

fed cellulose gained very little weight (Table 2), and that the cellulose remained intact, implies virtually no digestion of the carbohydrate polymer took place when cockroaches were fed on filter paper. Both *Blaberus* intestinal tissue and *Arthromitus* lack the enzymes (i.e. cellulase) that digest pure cellulose. No filamentous bacteria were found anywhere in the intestinal fluid of the cockroaches fed cellulose. However, on four occasions, *Arthromitus* filaments were observed in the crushed fecal pellets of these cockroaches.

Behavioral differences among the cockroaches fed on different dietary regimes were noted. Those incubated in continuous darkness but fed dog food diets were very active. Those on dog food diets and diurnal light regimes were docile; they tended not to resist handling. Those fed soy bean protein tended to be lethargic and inactive. Cellulose-fed cockroaches demonstrated the greatest resistance to handling.

Pattern formation

The pattern-forming ability of *Blaberus giganteus* bacilli were examined on two nutrient substrates used previously for other members of group I Bacilli, low-nutrient high agar and high-nutrient low agar (Rudner et al., 1998). Patterns produced by two laboratory strains of *B. cereus* (6A1 and 22A1) on nutrient-poor hard agar are shown in Fig. 4A and B. The first looks like tree branches with tip-splitting growth as in the *T* morphotype (top row of Fig. 4D) whereas the second pattern is typical chiral growth where the branches have the same handedness (described for the *C* morphotype). The B*laberus giganteus* bacilli (Fig. 4D lower row, Fig. 4E) displayed feathery branching. The cockroach patterns resemble those in *Bacillus licheniformis* (see Fig. 6Bf in Rudner et al., 1998) and also those from the moth *Junonia coenia* (middle row Fig. 4D). The patterns emerge after 4–7 days of incubation at 37°C. Pattern formation occurs faster, by 24 h, on nutrient-rich soft agar with group II Bacilli (e.g., *B. subtilis*, Fig. 4C). These patterns, "the deep-branching morphotype" (Rudner et al., 1998) were absent in *Blaberus giganteus* and in *Junonia coenia*, whereas bacilli from the moth *J. coenia* exhibited considerable swarming (Fig. 4F middle row). The *B. giganteus* bacilli, that at first showed no pattern on soft agar (Fig. 4 upper row), after growth for several weeks displayed a feathery border (Fig. 4F lower row). Laboratory cultures of bacilli that had been isolated from other insects were spot inoculated for colony morphology comparisons all at once. Similar feathery branching patterns on low-nutrient hard agar were observed for *Arthromitus* isolates symbiotic in two termites (*Reticulitermes flavipes*, a subterranean rhinotermitid, and *Cryptotermes cavifrons*, a dry-wood-eating termite). *Arthromitus* isolated from the Bess bug (or patent leather bug, *Passalus cornutus*) also displayed the feathery branching pattern on soft agar.

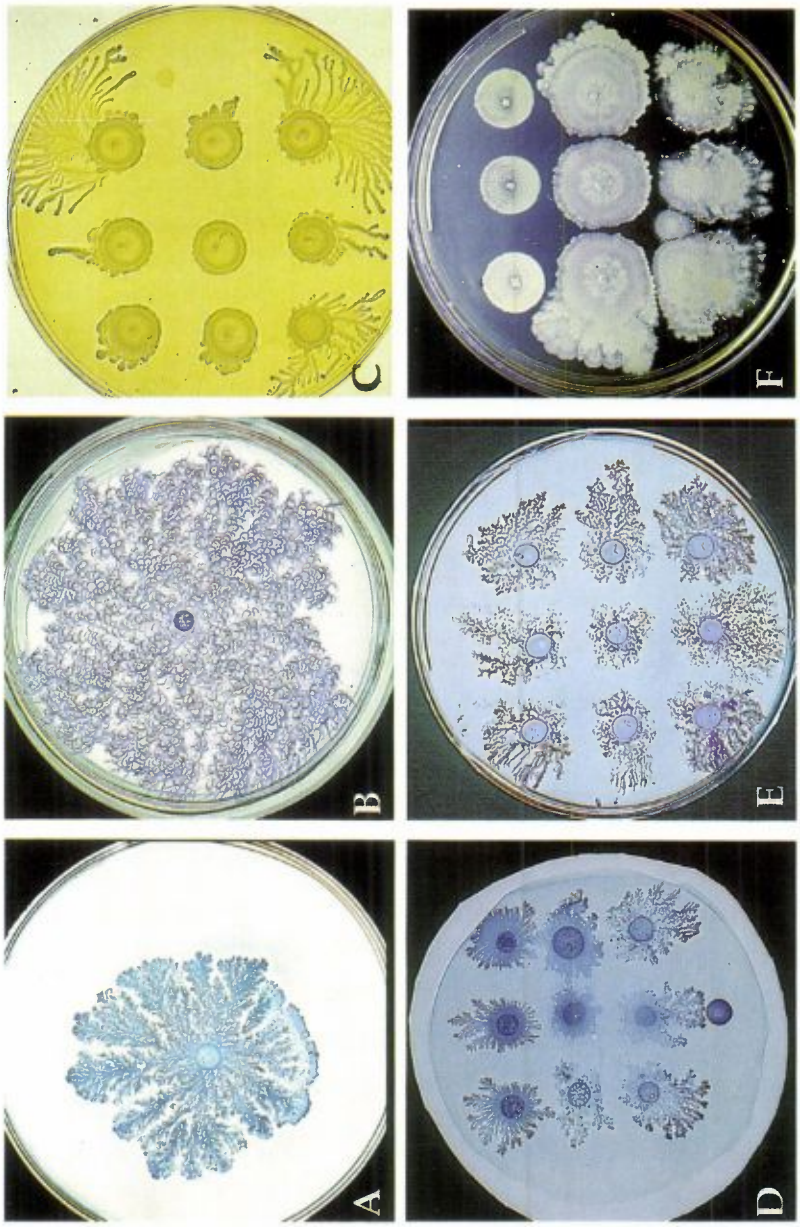

Figure 4. The emergence of patterns from a laboratory strain of *B. cereus*, *B. subtilis*, *T* morphotype and from *Blaberus giganteus* and *Junonia coenia* bacilli on nutrient-poor, hard agar or nutrient-rich soft agar-Xgal plates. **A.** A fully grown suspension of *B. cereus* strain 1A6 was inoculated as 5 µl on a 2 g/l peptone, 1.5% agar, incubated for 4–7 days at 37°C. **B.** A fully grown suspension of *B. cereus* strain 22A1 was inoculated 5 µl on a 1.25 g/l peptone, 1.25% agar, incubated for 4–7 days at 37°C.

Drug resistance and β-galactosidase activity

Bacillus cereus, a member of group I Bacilli, resist antibiotics on hard surfaces (Rudner et al., 1998). Among the antibiotics to which laboratory strains were resistant were chloramphenicol (Cm), lincomycin (Lm) and erythromycin (Erm), kanamycin (Km), ampicillin (Am), tetracyclin (Tc), spectinomycin (Sp) and the histidine analog aminotriazole (AT; Gropp et al., 1994) however, they were not resistant to rifampin (Rif). The *Blaberus giganteus* bacilli exhibited partial antibiotic resistance. They resist only Cm, Km, Am and AT. Considerable swarming was associated with antibiotic resistance, especially to Am and Km. The colonies appear like those at the bottom of Fig. 4F. The *Blaberus* bacilli did not produce a blue color on Xgal plates (the phenotype of a fl-galactosidase-like activity) as do reference strains of *B. cereus*.

Southern hybridizations and 16S sequencing

The *B. subtilis* ribosomal 23S-5S (*rrn*) probe hybridized to all group I Bacilli with similar intensity at stringency of 63°C. The EcoRI *rrn* restriction profiles of *B. giganteus* and the Bess bug, *P. cornutus* bacilli yielded the same banding pattern as did *B. cereus* strains 6A1 and 22A1 (Fig. 5). The latter strain was originally given to us by the Bacillus Genetic Stock Center as *B. macerans* BKM B-51 and when the 16S DNA was sequenced showed 100% homology to standard *B. cereus* (Rudner et al., 1998). All DNA preparations that originated from *Arthromitus*, i.e., as symbiotic bacilli from the guts of *B. giganteus* (cockroach), *J. coenia* (moth), *Porcellio scaber* (sow bug) and *Cryptotermes brevis* (termite) displayed the same band patterns as *B. cereus*-1 standard (Fig. 5). We counted 11 ribosomal homologs compared to the 10 *rrn* operons that were mapped for *B. subtilis* (Fig. 5). The other DNAs from group I Bacilli (known as *B. tipchirales* (T/C) and *B. vortex* (V)) that produce extensive patterns on nutrient-poor hard agar plates are included for comparison (Fig. 5; Rudner et al., 1998).

Figure 4. Continued. **C.** Patterns of *B. subtilis* strain BD 170 (trpC2, thrA5) inoculated as 9×5 µl onto TB-0.6% agar containing 160 µg/ml Xgal and incubated for 48 h at 37°C. **D.** Three different suspensions were inoculated on as 5 µl on a 2 g/l peptone, 1.5% agar: top row *T* morphotype of *B. tipchirales;* middle row *Junonia coenia* bacilli; lower row *Blaberus giganteus* bacilli. **E.** Patterns of *B. giganteus* bacilli on a 1.25 g/l peptone, 1.25% agar plate, incubated for 7 days at 37°C. **F.** Three different suspensions of a yellow isolate of *Blaberus giganteus* bacilli (top row); *Junonia coenia* bacilli (middle row); a white isolate of *Blaberus giganteus* bacilli (lower row) were grown and inoculated as 3×5 µl onto TB-0.6% agar containing 160 µg/ml Xgal and in-cubated for 48 h at 37°C.

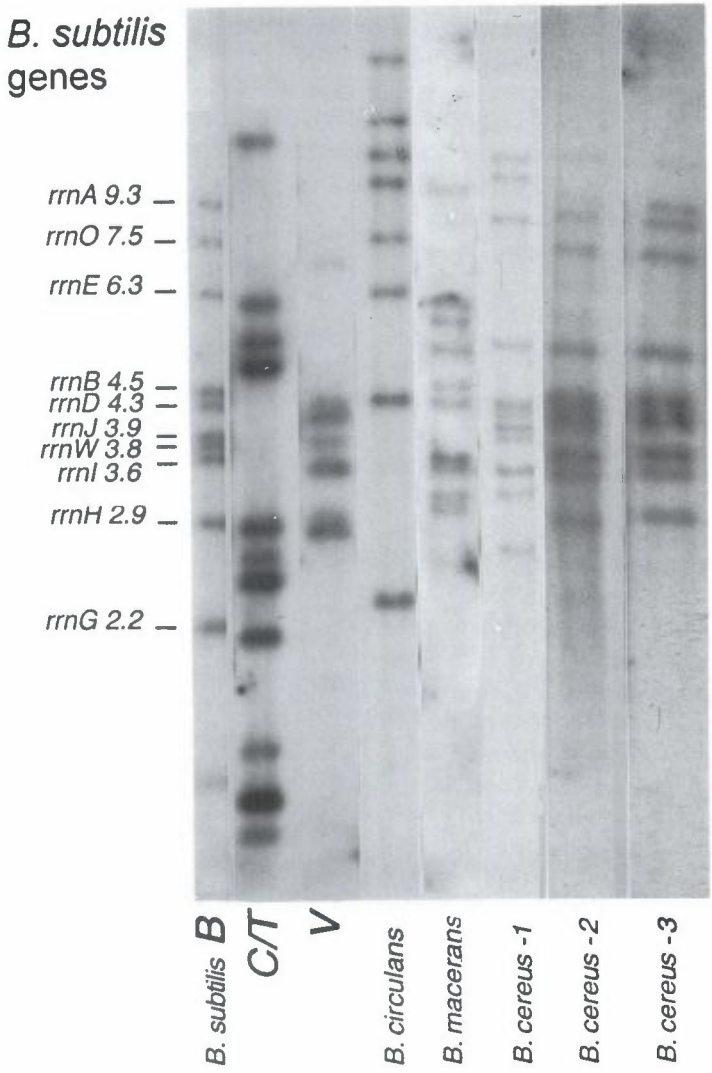

Figure 5. Southern blots of EcoRI-digested chromosomal DNAs from *B. subtilis* and group I Bacilli *T, C, V, B. circulans* strain A-4513, *B. macerans* strain B-4267, *B. cereus*-1 (6A1), *B. cereus*-2 (*Blaberus giganteus*) and *B. cereus*-3 (Bess bug, *Passalus cornutu*) hybridized with a *B. subtilis rrn* probe. The digested DNAs with EcoRI, electrophoresed on 0.8% agarose. After transfer, probed with a labeled 23S-5S fragment.

4. Discussion

Different *Arthromitus* morphotypes have been reported in wood-eating cockroaches, wood-eating termites (kalotermitids, rhinotermitids and

hodotermitids) as well as sow bugs. Such segmented and non-segmented filamentous bacteria occur most prominently in the hindgut of these arthropods (To et al., 1980; Margulis et al., 1990). At least one of each morphotype has been isolated from termites (*Pterotermes occidentis*), sow bugs (*Porcellio scaber*) and hissing cockroaches (*Gromphodorhina portentosa*) and identified as *Bacillus cereus*. We have shown here that both light conditions and diet strongly influence the population size and relative frequency of morphologically distinguishable stages, such as single stationary or flagellated bacilli, diplos, short stationary or motile filaments, segmented or unsegmented filaments, filaments replete or not with spores (Jorgensen et al., 1997; Margulis et al., 1990). The fact that diets and light-dark cycles were not carefully controlled or reported in previous studies limits direct comparisons of different arthropods. In no species of arthropod, whether millipede, termite, beetle, cockroach or here in *B. giganteus*, have all possible ambient conditions and all analyzed *Arthromitus* morphotypes been characterized. Diet, ambient light, insect developmental stage and hormonal contribution as well as genuine genetic differences in the bacteria probably all contribute to the variation.

Differences between *B. giganteus* and other insects that host *Arthromitus* were noted. Whereas the addition of gut fragments enhanced *Arthromitus* filament length in cultures of sow bugs, termites, and other arthropods (Leidy, 1881; Jorgensen et al., 1997; Ashcraft, 1994) they seemed to have no effect on the bacilli from *B. giganteus*. Intestinal material added to these bacilli cultured from *B. giganteus* intestine, induced no changes in *Arthromitus* filament length. Enhancement of filament length was noted in sowbugs (Jorgensen et al., 1997).

B. giganteus gut material was used to inoculate nutrient agar plates as well as nutrient broth. *Arthromitus*-like bacilli on more than one occasion were cultured in broth and on agar plates from *B. giganteus* but after only a few days in culture the isolated bacilli rarely resembled those observed directly in the insect hindgut. The cultures primarily consisted of spore-forming, *Bacillus*-like long rods, about 8 μm. The rods occasionally extended to as much as 4 to 5 times the original length to 32 to 40 μm long. *In vivo*, the filamentous units appeared as long chains of individually differentiated cells. The filament-forming tendency was best seen in isolated cultures 3 to 4 weeks old taken from *B. giganteus* intestines.

A dramatic change occurred in the *Arthromitus* intestinal symbiont populations over the four weeks in the 20 *Blaberus giganteus* studied. The presence and developmental stage of the potential filamentous spore-forming *B. cereus* is greatly influenced by diet and environmental light-dark conditions. Cellulose does not support growth. We infer that in the cockroaches, unlike the termites, cellulolytic enzymes (cellulases) are likely absent. Neither *B. giganteus* nor its filamentous spore-forming bacteria can survive on a cellulose diet. Protein apparently is required. Maintenance of conditions for continuous

growth of the bacteria enhances the number and length of the *Arthromitus* filaments, consistent with previous studies (Jorgensen et al., 1997; Margulis et al., 1998).

Spore-forming filamentous bacteria identical to those *in vivo* almost never appeared in nutrient broth or on agar plates. The discrepancies between in vivo and *in vitro* morphologies is most easily explained by the unique conditions inside the arthropod body. Oxygen concentration, temperature, illumination, nutrient and hormone composition and medium viscosity are just a few of the variables that differ in culture relative to the insect intestine. *Bacillus* strains are commonly reported to form filaments and chains; *Bacillus anthracis* is notorious among bacteriologists for its failure to grow as single bacilli (Gordon, 1973; Margulis et al., 1990).

The *Arthromitus* form of *Bacillus cereus* is abundant in many arthropods. We show here that they are also normal inhabitants of healthy *B. giganteus*, the large omnivorous tropical American cockroach. This conclusion that confirms Joseph Leidy's (1823–1891) original observation, was reached by Klaasen et al. (1992) for the intestinal segmented bacteria of mammals. Incomplete diets of soybean protein led not only to unhealthy cockroaches with abnormally soft fecal pellets and behavioral changes, but to a depletion in the *Arthromitus*, i.e., filamentous spore-forming stage of *B. cereus*. Nutrients, probably at least in part protein, essential for normal development of *Arthromitus* in healthy, rapidly growing *Blaberus*, must be lacking in an unbalanced diet. Two weeks in near-total darkness enhanced development of filaments in *B. giganteus*, an observation consistent with previous work that showed light sensitivity of filament development in *Porcellio scaber* (Table 4 in Jorgensen et al., 1997). The conclusion is strengthened that *B. cereus* in filamentous form, as *Arthromitus*, is a normal symbiont of *Blaberus* intestines. *Bacillus cereus* thrives on the same balanced diet and reduced light regime that support normal weight gain, growth and healthy behavior in the cockroach.

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