5′ ETS and the first 72 nt of the 16S RNA sequence sandwiched between 21 nt of vector and 5 nt of host flanking sequence at the 5′ end and 15 nt of vector sequence at the 3′ end. When required, the RNA was uniformly labeled with [α-^{32}P]CTP (cytidine triphosphate) or 3′ end-labeled with pCp with the use of RNA ligase. Processing was performed at 75°C as described (*1*) with purified activity instead of crude cell extract. For partial processing, ~1 pmol of nonradioactive substrate RNA was incubated with processing activity for 15 s to 5 min. Reactions were terminated by phenol extraction and the RNA was recovered after ethanol precipitation. Sites of in vitro cleavage were mapped by primer extension with a 5′ end-labeled oligonucleotide complementary to positions 57 to 35 of the 16S rRNA sequence. Primer (0.5 pmol) was annealed to partially processed RNA in the presence of 2.5 μg of yeast carrier RNA by incubation for 2 min at 92°C, 10 min at 47°C, and 20 min at 20°C. An enzyme mix containing deoxynucleoside triphosphates and Superscript II reverse transcriptase (100 units per reaction) (BRL) was added, and extension was performed at 47°C for 45 min. The extension products were recovered after phenol extraction and ethanol precipitation and were displayed on a denaturing polyacrylamide gel. Bulk in vivo RNA from *S. acidocaldarius* used for S1 nuclease protection and for reconstitution was isolated from growing cell cultures by boiling in SDS lysis buffer, phenol extraction, ethanol precipitation, and centrifugation through 5.8 M CsCl [P. P. Dennis and J. Chow, in *Archaea—A Laboratory Manual*, F. Robb *et al.*, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, in press)]. Bulk RNA alone showed no processing activity. Bulk RNAs isolated from *Escherichia coli*, *Bacillus subtilis*, or *Haloferax volcanii* were inactive in heterologous reconstitution.

8. The product with a 5′ end at position +1 and the intermediate with a 5′ end at −31 are identical to those observed in vivo (or in vitro) by S1 nuclease protection. The intermediate with a 5′ end at position −99 is 3 or 4 nt longer than the in vivo (or in vitro) intermediate detected by S1 nuclease protection; the difference presumably represents limited S1 digestion at the end of the protected DNA probe. For unknown technical reasons, we have been unable to generate high-quality primer extension products with bulk in vivo RNA as template.
9. The limit processing assay was performed for 1 to 64 min at 75°C with ^{32}P-labeled substrate as described (*1*) but with purified activity instead of crude cell extract. The product RNA fragment that extends from the 5′ end of the transcript to site 1 migrates anomalously fast in the electrophoresis gel, probably because of an extended hairpin containing nine consecutive G • C base pairs (Fig. 4B). The identity of this fragment was verified by increasing or decreasing the length of the flanking sequence at the 5′ end of the substrate RNA and observing the expected mobility shift of this fragment. The identity of the fragment from site 4 to the 3′ end was confirmed by RNA ligase–mediated 3′ end-labeling of the substrate RNA with pCp. Bands that are not labeled in Fig. 2 are either uncharacterized processing intermediates or are the result of thermal or contaminating nuclease–mediated degradation of the ^{32}P-labeled substrate RNA.
10. A complementary DNA (cDNA) clone of the U3-like RNA was obtained after reverse transcription and PCR amplification of RNA extracted from the purified processing activity. Two complementary oligonucleotides (JC1, 5′-CGACGGATAGAAGAATTCTGTTCGTTGGAG; and its complement, JC2) were used for first-strand synthesis. Briefly, JC1 was blocked at the 3′ end with terminal transferase and dideoxy adenosine triphosphate and phosphorylated at the 5′ end with polynucleotide kinase and adenosine triphosphate (ATP). The modified oligonucleotide was then ligated to the RNA extracted from the processing activity with RNA ligase. The complementary oligonucleotide JC2 was used to prime first-strand synthesis with avian myeloblastosis virus reverse transcriptase, and the product was tailed with terminal transferase and ATP. The tailed product was amplified by PCR with Race 37 (CGAGCTGCGTCGACAGGCT$_{17}$) and JC2 as primers. The product was cloned directly into the pCR2 vector to yield plasmid pPD1156. The cDNA insert was used to identify the chromosomal gene on a 1.2-kb Eco RV–Hind III fragment. The genomic fragment was cloned into plasmid pSP73 (Promega) to give plasmid pPD1158. The nucleotide sequence of both the cDNA and the chromosomal gene were determined (*23*).

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19. Approximately 150 pmol of the specific oligonucleotides oSP7 (5′-GTAGTTCCGCATTCAGG) or oSP2 (5′-CGACACAGTTAGACAAGCTCC), complementary to the box B and box A regions of *S. acidocaldarius* U3-like RNA (positions 85 to 69 and 32 to 12, respectively), or nonspecific oligonucleotide oSP5 (5′-ATAAGCTTAATAATGCCGCCGTAGCT) was mixed with 5 μl of processing activity (obtained after precipitation with 35% ammonium sulfate; purified ~10-fold), heated to 85°C, and cooled slowly to 37°C. Buffer [10 μl of 60 mM tris-HCl (pH 7.5), 6 mM $MgCl_2$, 1.5 mM dithiothreitol, bovine serum albumin (45 μg/ml), 4% (v/v) glycerol] was added together with 2 units of *E. coli* RNase H (Pharmacia). Incubation was continued for 10 min at 37°C and 20 min at 30°C (*13*). The treated processing activity was then heated to 75°C for 15 min, substrate was added, and incubation was continued. Portions of the mixture were removed at specified time intervals and analyzed by the primer extension assay described in Fig. 1. For the control reactions, the extract alone or the extract plus specific or nonspecific oligonucleotides was treated in a parallel manner except that no RNase H was added. In other quantitative experiments, we have estimated by densitometric analysis that the rate of consumption of substrate after RNase H digestion in the presence of the oSP7 box B oligonucleotide (Fig. 6) is ~5% of that in the control reactions.

20. Processing activity was incubated with specific oligonucleotides oSP7 or oSP2, or nonspecific oligonucleotide oSP5, and digested with RNase H as described (*19*). The RNA obtained after phenol extraction and ethanol precipitation was subjected to electrophoresis on a denaturing 6% polyacrylamide gel, electroblotted onto a Hybond N+ membrane (Amersham), and fixed by brief exposure to ultraviolet light. The membrane was probed under standard conditions (*13*) at 42°C for 16 hours with the ^{32}P-labeled product generated from the U3-like cDNA fragment from plasmid pPD1156 (*10*) with random oligonucleotide primers.
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Revival and Identification of Bacterial Spores in 25- to 40-Million-Year-Old Dominican Amber

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A bacterial spore was revived, cultured, and identified from the abdominal contents of extinct bees preserved for 25 to 40 million years in buried Dominican amber. Rigorous surface decontamination of the amber and aseptic procedures were used during the recovery of the bacterium. Several lines of evidence indicated that the isolated bacterium was of ancient origin and not an extant contaminant. The characteristic enzymatic, biochemical, and 16S ribosomal DNA profiles indicated that the ancient bacterium is most closely related to extant *Bacillus sphaericus*.

Microorganisms have been isolated from various types of ancient materials, including salt crystals, deep earth cores, and fossilized animals and plants (*1*, *2*). All such claims of ancient origin have faced the criticism of being a result of modern environmental contamination based on the sense that viable, ancient isolates are unlikely. This skepticism stems primarily from the extrapolation of survival curves of modern bacteria that suggest that accumulated damage and macromolecular decay would preclude viability (*3*).

Morphological and biochemical data about ancient bacteria are scarce, precluding detailed studies of bacterial metabolism, origins, and evolution. Sequence data derived from the 16S ribosomal RNA (rRNA) have been used to construct a phylogenetic tree for modern prokaryotes (*4*). Such data from both ancient bacterial DNA and *Bacillus* spp. from amber samples of known age can be used to analyze bacterial phylogeny and the rate of nucleotide substitution for various genes in this taxon.

Bacillus is an ancient and ubiquitous bacterial genus characteristically capable of

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forming endospores (5). The spore's contents are dehydrated within a thick, protective protein coat. The spore's resistance to heat, radiation, pressure, and chemical agents (6) is a result of the chemical environment within the spore, enzymatic dormancy, dehydration, and conformational changes of the genome (7). In the state of desiccation, some bacteria, including spore formers, may remain in a cryptobiotic state for several million years (8).

There is a symbiotic relationship between certain *Bacillus* species (for example, *B. cereus*, *B. subtilis*, *B. megaterium*, *B. pumilus*, *B. sphaericus*, and *B. circulans*) and many species of bees (9–11). This relationship dates back millions of years, as *Bacillus* DNA has been amplified from the abdominal tissue of 25- to 40-million-year-old bees that had been preserved in amber (12). The symbiosis contributes to the production, metabolic conversion, and preservation of larval bee provisions as well as to maintaining the health of the bee colony (13).

Proplebeia dominicana (family Apidae, tribe Meliponinae) is an extinct species of neotropical, stingless bee found in 25- to 40-million-year-old amber from the Dominican Republic (14). This species is relatively common in the amber, presumably because individuals became entrapped when they attempted to collect resin balls for nest construction (15). The presence of *Bacillus* spp. within these bees has been demonstrated by electron microscopy (16), by the recovery of *Bacillus* DNA from the abdomen of *P. dominicana* preserved in amber (12), and by phase-contrast microscopic observations of spore-like structures within the abdominal contents of *P. dominicana* (17).

Tissue recoveries from fossil bees in 25- to 40-million-year-old Dominican amber were performed in a decontaminated, class II laminar flow hood. The amber pieces were surface-sterilized chemically and cracked, and the tissue was harvested as described (18). We inoculated trypticase soy broth (TSB) (BBL, Cockeysville, Maryland) with the bee tissue samples to test for the presence of viable spores. An isolate of *Bacillus* sp. was obtained from the TSB inoculated with *P. dominicana* abdominal tissue from amber and coded as BCA16.

Two other ancient *Bacillus* spp. isolates have now been revived, cultured, and identified from Dominican amber, but they were not included in this report in the interest of clarity and simplicity. Additionally, more than 100 other bacterial isolates from ambers of various geological ages have been recovered (recovery rate of 0.05 isolates per gram of amber) but these have not yet been evaluated critically.

To test for contamination from the reagents, we also inoculated TSB with 100-μl samples of the solutions used in the sterilization process and with pieces from the exterior or interior of the amber. The TSB was incubated aerobically for 2 weeks at 35°C. No bacteria were isolated from any of these TSB tubes. To test for environmental contaminants, we exposed three petri plates of tryptic soy agar (TSA) in the hood throughout the entire tissue removal process (approximately 15 min). At the end of the procedure, the plate lids were replaced and the plates were incubated at 35°C for 2 weeks. No contamination

Table 1. Enzymes produced by *B. sphaericus* isolated from soil (three leftmost columns) and bees (three rightmost columns). The amount of enzyme produced was determined with the API-ZYM system. The numbers indicate the following amounts of enzyme produced: 0 = <10 nm; 1 = 10 to 19 nm; 2 = 20 to 29 nm; 3 = 30 to 39 nm; 4 = 40 to 49 nm; and 5 = ≥50 nm. ATCC, American Type Culture Collection.

Enzyme	Amount of enzyme produced by *B. sphaericus* isolated from					
	NM13	PJ23	PJ18	ATCC 13805	ATCC 17932	BCA16
Control	0	0	0	0	0	0
Alkaline phosphatase	1	1	1	0	0	0
Butyrate esterase	4	3	3	1	1	3
Caprylate esterase lipase	3	3	3	1	1	3
Myristate lipase	1	0	1	1	0	0
Leucine aminopeptidase	1	1	0	2	2	0
Valine aminopeptidase	0	1	1	1	0	0
Cystine aminopeptidase	0	0	0	1	0	0
Trypsin	0	0	0	0	0	0
Chymotrypsin	2	3	1	0	1	0
Acid phosphatase	0	1	0	0	0	0
Phosphoamidase	0	0	0	0	0	2
α-Galactosidase	0	0	0	0	0	5
β-Galactosidase	1	0	1	0	0	2
β-Glucuronidase	0	0	0	0	0	0
α-Glucosidase	0	1	0	4	0	0
β-Glucosidase	2	0	1	0	0	2
N-Acetyl-β-glucosaminidase	0	0	0	0	0	0
α-Mannosidase	0	0	0	0	0	0
α-Fucosidase	0	0	0	0	0	0

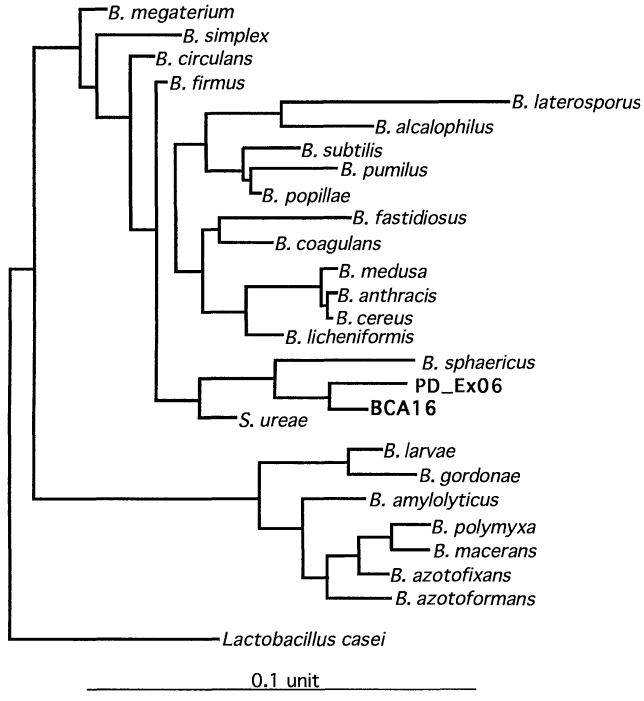

Fig. 1. Evolutionary tree for *Bacillus* spp. GenBank Accession numbers are as follows: *S. ureae*, X62174; *B. alcalophilus*, X60603; *B. amylolyticus*, X60606; *B. anthracis*, X55059; *B. azotofixans*, X60608; *B. azotoformans*, X60609; *B. cereus*, X55063; *B. circulans*, X60613; *B. coagulans*, X60614; *B. firmus*, X60616; *B. larvae*, X60619; *B. laterosporus*, X60620; *B. licheniformis*, X60623; *B. medusa*, X60628; *B. megaterium*, X60629; *B. subtilis*, X60646; *B. polymyxa*, X60632; *B. popillae*, X60633; *B. pumilus*, X60637; *B. simplex*, X60638; *B. sphaericus*, X60639; *Lactobacillus casei*, X61135; BCA16 (the putatively ancient bacterial isolate from Dominican amber), L38654; and PD_Ex06 (a PCR-amplified DNA sample of *P. dominicana* abdominal material), L38665. The tree was constructed by the maximum likelihood method with the DNAML program of PHYLIP 3.5 (11). *Lactobacillus casei* was used as the outgroup, with randomized data input (J option) and global rearrangement of the data (G option). A total of six independent runs were evaluated. All resulting trees were identical. Branch lengths were drawn to scale, with branch lengths obtained from maximum likelihood analysis and TreeTool.

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was detected on these TSA plates.

The effectiveness of the surface sterilization procedure (*18*) was further tested by inoculating 11 pieces of amber (without inclusions) with a sporulated culture of extant *B. subtilis* (about 1.6×10^7 spores per milliliter). The amber was soaked overnight in the culture before the sterilization procedure was performed. The surface-sterilized amber pieces were then transferred to tubes containing 10 ml of TSB, and the tubes were incubated at 35°C for 2 weeks. No bacterial growth was detected in any of the 11 tubes after the incubation period.

The tissue removal procedure was simulated to check for environmental contamination of the samples during the actual recovery of tissue. Two pieces of amber (without inclusions) were surface-sterilized (*18*), and the tissue recovery procedure was simulated inside the hood for approximately 45 min. Samples of the solutions used in the sterilization procedure and from both the interior and exterior of the amber were cultured in TSB and incubated at 35°C for 14 days to test for the presence of contaminants. No bacterial isolates were obtained from any of the simulations.

We identified the putatively ancient isolate BCA16 as *B. sphaericus* using morphological and biochemical identification methods (*19*). The isolate was evaluated as to its enzymatic capabilities with the API-ZYM system (API, Plainview, New York) according to the manufacturer's instructions (Table 1). The enzymatic profiles of the putatively ancient isolate was similar to the enzymatic profiles of *B. sphaericus* isolated from extant bees. The lipases most often associated with tropical bees are caprylate esterase lipase and butyrate esterase (*10*). These were also produced by the isolate (Table 1).

The presence of *Bacillus* DNA was also evaluated. DNA was extracted from samples of the abdominal contents of *P. dominicana*, the solutions used in the sterilization procedure, and small pieces from the interior and exterior of the amber. The DNA extractions were performed with Glassmilk SpinBuffer from an RPM kit (Bio 101, La Jolla, California) as described by Cano and Poinar (*20*). A 530–base pair (bp) fragment of the 16*S* ribosomal DNA (rDNA) was produced in 6 of 16 samples of DNA from the abdominal contents assayed after amplification by the polymerase chain reaction (PCR) with primers (BCA341F and BCA871R) specific for *Bacillus* spp. (*12*). The remaining 10 amplifications yielded no products. No amplified DNA was obtained from the solutions used in the sterilization procedure or from the exterior or interior of the amber. The sequence determined from PD_Ex06, which resulted from the PCR-amplified DNA sample of the same amber specimen from which BCA16 was isolated, was very similar to the 16*S* rDNA sequence determined from the BCA16 culture (Fig. 1).

Clones were sequenced with both the SP6 and T7 sequencing primers. The sequences were read manually, aligned with the aid of CLUSTAL by using the Genetic Data Environment (GDE) software program (version 2.2) (*21*), and verified visually.

The 16*S* rDNA nucleotide sequences of

```
     1          11         21         31         41       50
     |          |          |          |          |        |
  1  GAAAGCCTGA TGGAGCAACG CCGCGTGAGT GAAGAAGGAT TTCGGTTCGT   50 B.SPHAER1
  1  .......... .......... .......... .......... ..........   50 B.SPHAER2
  1  ........C. .......... ........AC ........C. ..........   50    BCA16
  1  ........C. .......... ........A. ........C. ..........   50  PD_Ex06
  1  ........C. .......... .......... .......... N.........   50 BSPH16SR

     51         61         71         81         91       100
     |          |          |          |          |        |
 51  AAAACTCTGT TGTAAGGGAA GAACAAGTAC -GTA-GTAAC TGGCTTT---   95 B.SPHAER1
 51  .......... .......... .......... .......... .........  100 B.SPHAER2
 51  ...G...... .......... .......... C.G....... ..C.G..G.C   99    BCA16
 51  ...G...... .......... .......... C.G....... ..C.G..G.C  100  PD_Ex06
 51  .......... .......... .......... A......... ....C..TAC   99 BSPH16SR

     101        111        121        131        141      150
     |          |          |          |          |        |
 96  ---GACGGTA CCTTATTAGA AAGCCACGGC TAACTACGTG CCAGCAGCCG  142 B.SPHAER1
101  .......... .......... .......... .......... ..........  150 B.SPHAER2
100  CTT....... .....CC... .......... .......... ..........  149    BCA16
101  CTT....... .....CC... .......... .......... ..........  150  PD_Ex06
100  CTT....... .......... .......... .......... ..........  149 BSPH16SR

     151        161        171        181        191      200
     |          |          |          |          |        |
143  CGGTAATACG TAGGTGGCAA GCGTTGTCCG GAATTATTG- ----TAAGCG  187 B.SPHAER1
151  .......... .......... .......... .......... ..........  200 B.SPHAER2
150  .......... .......... .....A.... .........G --GT......  199    BCA16
151  .......... .......... .....A.... .........G --GC......  200  PD_Ex06
150  .......... .......... .....A.... .........G GCGTN.....  199 BSPH16SR

     201        211        221        231        241      250
     |          |          |          |          |        |
188  CGCGCAGGTG GTTTCTTAAG TCTGATGTGA AAGCC-ACGG CTCAACCGTG  236 B.SPHAER1
201  .......... .......... .......... .......... ..........  250 B.SPHAER2
200  .T........ ..C....... .......... .....C.... ..........  249    BCA16
201  .T........ ..C....... .......... .....C.... ..........  250  PD_Ex06
200  .......... .......... .......... .....C.... ..........  249 BSPH16SR

     251        261        271        281        291      300
     |          |          |          |          |        |
237  GAGGGTCATT GGAAACTGGG AGACTTGAGT GCAGAAGAGG ATAGTGGAAT  286 B.SPHAER1
251  .......... .......... .......... .......... ..........  300 B.SPHAER2
250  .......... .......... .......... .......... .G........  297    BCA16
251  .......... .......... .......... .......... ..........  300  PD_Ex06
250  .......... .......... .......... .......... ..........  299 BSPH16SR

     301        311        321        331        341      350
     |          |          |          |          |        |
287  TCCAAGTGTA GCGGTGAAAT GCGTAGAGAT TTGGAGGAAC ACCAGTGGCG  336 B.SPHAER1
301  .......... .......... .......... .......... ..........  350 B.SPHAER2
298  ....C..... .......... .......... G......... ..........  347    BCA16
301  ....C..... .......... .......... G......... ..........  350  PD_Ex06
300  .......... .......... .......... .......... ..........  349 BSPH16SR

     351        361        371        381        391      400
     |          |          |          |          |        |
337  AAGGCGACT- -CTGGTCTGT AACTGACACT GAGGCGCGAA AGCGTGGGGA  384 B.SPHAER1
351  .......... .......... .......... .......... ..........  400 B.SPHAER2
348  .........A T......... .......G.. ....A..... ..........  397    BCA16
351  .........A T......... .......G.. ....A..... ..........  400  PD_Ex06
350  .........A T......... .......... .......... ..........  399 BSPH16SR

     401        411        421        431        441      450
     |          |          |          |          |        |
385  GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCCGTAAAC GATGAGTGCT  434 B.SPHAER1
401  .......... .......... .......... .......... ..........  450 B.SPHAER2
398  .......... .......... .......... .......... ..........  447    BCA16
401  .......... .......... .......... .......... ..........  450  PD_Ex06
400  .......... .......... .......... .......... ..........  449 BSPH16SR

     451
     |
435  AAGTGT                                                  440 B.SPHAER1
451  ......                                                  456 B.SPHAER2
448  ......                                                  453    BCA16
451  ......                                                  456  PD_Ex06
450  ......                                                  455 BSPH16SR
```

Fig. 2. Sequence alignment of the 16*S* rDNA fragment produced after amplification by PCR with the primers BCA341F and BCA871R (*12*). Sequences of BCA16, PD_Ex06, and extant *B. sphaericus* are included (*29*). Identical bases are indicated by a dot, base substitutions are indicated by the first letter of the substituting base, and gaps are indicated by a dash. B.SPHAER1 (accession number D16280) and B.SPHAER2 (accession number L14010) are mosquito isolates. BSPH16SR (accession number X60639) is strain NCDO 1767.

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BCA16 and the amplification product PD_Ex06 (Fig. 2) were compared with homologous sequences of *Bacillus* spp. listed in GenBank, with *Lactobacillus casei* as the outgroup. Phylogenetic trees were constructed with the maximum likelihood (DNAML) algorithms of PHYLIP 3.5 (*22*). The maximum likelihood tree (Fig. 1) shows that both the BCA16-derived sequence and the PD_Ex06 sequence are in a sister group with *B. sphaericus*. The DNA sequences of BCA16, PD_Ex06, and *B. sphaericus* were all further grouped by the algorithm with that of *Sporosarcina ureae*, the only other species among those analyzed that forms essentially round spores.

We calculated the rate of nucleotide substitution, *r*, by dividing the number of substitutions between the putatively ancient isolate BCA16 and extant B. *sphaericus* by the number of nucleotide sites analyzed (*23*). This number, *K*, was then divided by *T*, where *T* is the time span separating the extant and extinct *B. sphaericus* isolates. We estimated *T* at 25 to 40 million years on the basis of the age of the Dominican amber used in the extraction. The nucleotide substitution rate was calculated to be 1.8×10^{-9} to 2.4×10^{-9} substitutions per site per year.

Phylogenetic studies showed that the 530-bp fragment of the 16S rDNA from the ancient isolate was similar to the amplicons from the abdominal contents of *P. dominicana* and similar, but not identical, to the amplicon of extant *B. sphaericus* (Fig. 3). The nucleotide substitution rate we estimated was greater than those reported, which include 0.1×10^{-9} substitutions per site per year for eubacterial 16S rDNA (*24*) and 0.1×10^{-9} to 0.4×10^{-9} substitutions per site per year for aphid bacterial symbionts 16S rDNA (*25*).

There are several possible explanations for this variance. Only 33% of the 16S rDNA was analyzed in this study. This segment contains 3 of the 10 variable regions found in bacterial 16S rRNA (*26*). Sequencing of the entire 16S rRNA gene of BCA16 might have resulted in substitution rates similar to those reported. The method of arriving at these estimations may also be a source of variance. Ochman and Wilson (*24*) estimated the divergence time by tying ecological events that took place at known times in the geological past to specific branch points in the genealogical tree relating the 16S rRNAs of eubacteria, mitochondria, and chloroplasts. Moran *et al.* (*25*) reconstructed the phylogenetic trees of the endosymbiotic bacteria in aphids using the phylogenetic trees of their host. The method used in the present study for arriving at the estimation of base substitution rates was based on the age of the amber fossil (that is, 25 to 40 million years) from which the putatively ancient *B. sphaericus* was isolated (*27*).

We determined the secondary structure of the amplified segment of the 16S rRNA (Fig. 3) to ascertain the nature of the substitutions noted in the nucleotide sequence. The distribution of nucleotide changes was not random but rather was densely localized within the variable regions V3 and V4 (*28*) of the 16S rRNA molecule. Additionally, many of the substitutions were compensatory base changes within recognized helical pair regions that would preserve the structure of the molecule.

The results of the morphological, biochemical, enzymatic, and molecular characterization of isolate BCA16 support the hypothesis that a viable *B. sphaericus* spore was indeed recovered from the abdominal contents of *P. dominicana* entombed in 25- to 40-million-year-old amber. The putatively ancient isolate resembles known endosymbionts of modern bees. Furthermore, process validation studies showed that the chemically sterilized surface of the amber and the environmental controls were repeatedly negative for bacterial contamination. As the room and the hood therein were only used for the extraction of ancient materials and never modern material, the probability that the BCA16 isolate is an environmental contaminant was further diminished. On the basis of the results described here, it appears that the *B. sphaericus*–like bacterium isolated from *P. dominicana* entombed in Dominican amber is of ancient origin.

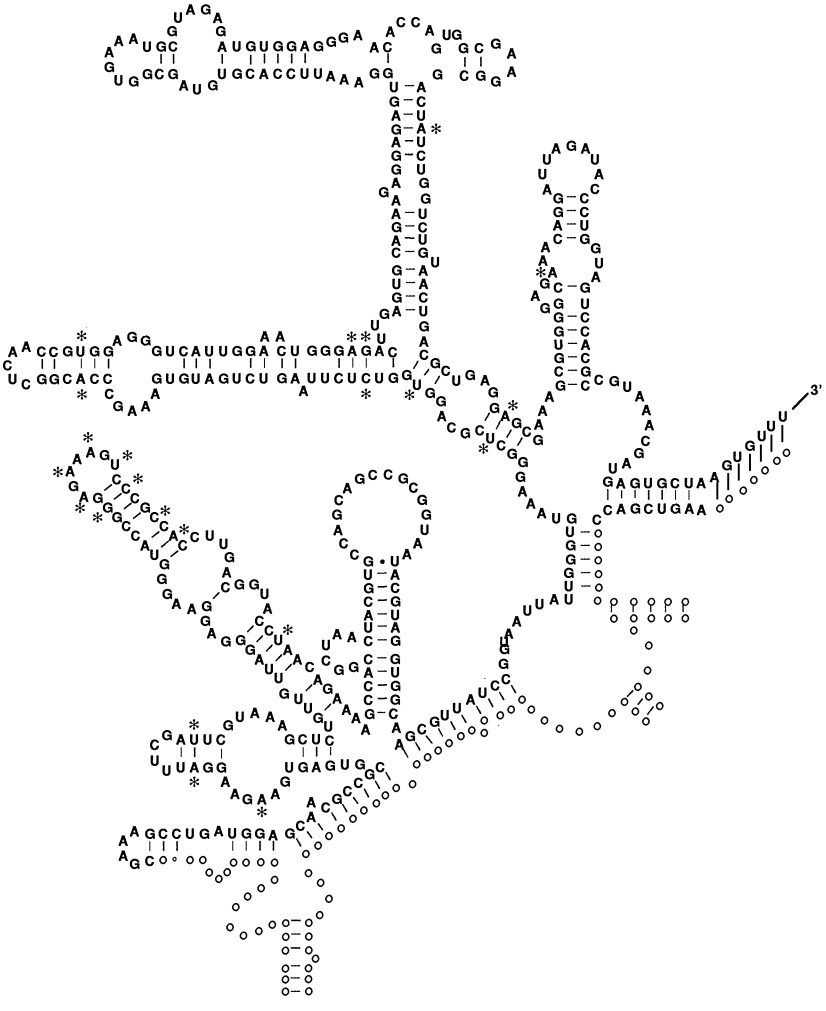

Fig. 3. Secondary structure of the BCA16 amplicon inferred from the nucleotide sequence of the PCR product in Fig. 2. The secondary structure was constructed by inference with the published secondary structures of both *Escherichia coli* and *B. subtilis* rRNA. Asterisks indicate the locations of the nucleotide substitutions noted, and open circles represent nucleotide bases not within the PCR-amplified region of the 16S rDNA gene.

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