

## Isolation of *Thermus* Strains from Hot Composts (60 to 80°C)

TRELLO BEFFA,\* MICHEL BLANC, PIERRE-FRANÇOIS LYON, GUDRUN VOGT,  
MARCELLO MARCHIANI, JOHANNA LOTT FISCHER,  
AND MICHEL ARAGNO

Laboratoire de Microbiologie, Université de Neuchâtel,  
CH-2007 Neuchâtel, Switzerland

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High numbers ( $10^7$  to  $10^{10}$  cells per g [dry weight]) of heterotrophic, gram-negative, rod-shaped, non-spore-forming, aerobic, thermophilic bacteria related to the genus *Thermus* were isolated from thermogenic composts at temperatures between 65 and 82°C. These bacteria were present in different types of wastes (garden and kitchen wastes and sewage sludge) and in all the industrial composting systems studied (open-air windrows, boxes with automated turning and aeration, and closed bioreactors with aeration). Isolates grew fast on a rich complex medium at temperatures between 40 and 80°C, with optimum growth between 65 and 75°C. Nutritional characteristics, total protein profiles, DNA-DNA hybridization (except strain JT4), and restriction fragment length polymorphism profiles of the DNAs coding for the 16S rRNAs (16S rDNAs) showed that *Thermus* strains isolated from hot composts were closely related to *Thermus thermophilus* HB8. These newly isolated *T. thermophilus* strains have probably adapted to the conditions in the hot-compost ecosystem. Heterotrophic, oval-spore-forming, thermophilic bacilli were also isolated from hot composts, but none of the isolates was able to grow at temperatures above 70°C. This is the first report of hot composts as habitats for a high number of thermophilic bacteria related to the genus *Thermus*. Our study suggests that *Thermus* strains play an important role in organic-matter degradation during the thermogenic phase (65 to 80°C) of the composting process.

Composting is a self-heating, aerobic, solid-phase, biodegradative process of organic-waste materials (7, 8). During the thermogenic phase of the composting process, the temperature usually rises to a high level (65 to 80°C) for a certain period of time. At the highest temperatures studied (65 to 69°C), only strains related to *Bacillus stearothermophilus* were identified (17, 18), but no study showed the presence of bacilli able to grow at temperatures above 70°C. Recently, moderately high numbers of obligately autotrophic (*Hydrogenobacter* spp.) and facultatively autotrophic (*Bacillus schlegelii*) thermophilic bacteria growing at temperatures above 70°C were isolated from hot composts (3), but these bacteria do not seem to be the dominant degraders at these high temperatures.

The purpose of this study was to demonstrate the presence of large numbers of highly thermophilic, heterotrophic bacteria growing at temperatures above 70°C during the thermogenic stage of the composting process.

### MATERIALS AND METHODS

**Composting facilities.** The composting facilities studied represent the main types of composting systems used in Switzerland, treating different amounts and types of organic wastes. The facilities included (i) classic, open-air windrows, for treatment of 8,000 to 10,000 t of garden and kitchen wastes per year; (ii) boxes of 80 to 90 m<sup>3</sup> in a semiclosed hall, with automated turning, and aeration of the compost controlled by the temperature measured in the heaps, for treatment of 3,000 t of garden and kitchen wastes per year; (iii) boxes of 160 m<sup>3</sup> in a closed hall, with automated turning and aeration of the compost, for treatment of 20,000 t of premethanized kitchen wastes mixed with shredded garden waste per year; (iv) a closed bioreactor of 12 to 20 m<sup>3</sup> with automated aeration and weekly turning, for treatment of kitchen waste with shredded garden waste; and (v) a closed bioreactor of 300 m<sup>3</sup> with automated aeration, for treatment of 6,000 to 8,000 t of methanized sewage sludge mixed with wood chips per year. The composting facilities were located 50 to 250 km (i, ii, iii, and iv) and 10 km (v) from the author's laboratory. None of the composting facilities studied seeded with a commercial compost starter containing thermophilic bacteria; rather,

self-seeding with previous compost, especially compost in the thermogenic phase, was done. Usually, the large pieces of wood retained by the sieving are recycled by mixing them with fresh organic material.

**Measurements of physicochemical parameters in composts.** Temperature was measured with standard temperature probes. Oxygen was measured by an electrochemical cell (O<sub>2</sub>-Ex-H<sub>2</sub>S Multiwarn P detector; Drägerwerk AG, Lübeck, Germany). For pH determination, 30 g of wet compost was suspended in 270 ml of deionized water and shaken for 30 min at 150 rpm at room temperature. In the open-air windrow composting system (site i; see above), a windrow was built (3.5 m wide by 1.4 m high by 25 m long) which consisted mainly of garden and kitchen wastes. The initial carbon/nitrogen ratio was 24. The windrow was turned daily with a specialized windrow turning machine. The temperature evolution in the compost mass at a depth of 60 cm from the top was monitored on-line each hour with a Squirrel meter-data logger 1250 series (Grant Instruments, Cambridge, United Kingdom) equipped with CS-U-V10-2V ( $\pm 0.25^\circ\text{C}$ ) temperature probes.

**Compost samples.** Thermogenic (65 to 82°C) compost samples were taken from 2- to 5-week-old organic waste at the different composting facilities. A total of about 10 kg of organic material in the thermogenic zone was sampled and mixed; 1 kg was then used for microbiological studies. Compost samples were stored at 5°C and used within 24 h. The estimations of bacterial numbers by parallel serial dilution directly from fresh (not stored) samples and from samples stored for 24 h at 5°C gave similar results (2).

**Enrichment and isolation of spore-forming bacteria.** Organic material (30 g [fresh weight], 45 to 65% moisture content) was placed in 270 ml of sterile water and shaken at 150 rpm for 30 min at room temperature. Parallel serial dilutions ( $10^{-1}$  to  $10^{-12}$ ) of the organic-material suspension were performed in an 8 g/liter solution of nutrient broth (Merck, Darmstadt, Germany). This medium was named NB. The cultures were incubated without agitation at 50, 60, 65, and 70°C for 1 to 6 days. Pure strains were isolated at 60°C on the same medium solidified with 15 g of agar-agar (Merck) per liter.

**Enrichment and isolation of non-spore-forming bacteria.** Parallel serial dilutions ( $10^{-1}$  to  $10^{-12}$ ) were performed in basal mineral medium (BMM) (3) supplemented with 8 g of NB (Merck) and 2 g of yeast extract (Merck) per liter. This supplemented medium was named MNY. Prior to the incubation, the enrichment cultures were placed in a water bath at 70°C for 5 min and then incubated without agitation at 75°C for 2 to 7 days.

In some cases, enrichment of non-spore-forming bacteria was performed under oligocarbophilic conditions in BMM supplemented with 0.1 g of yeast extract (Merck) per liter and incubated at 70°C for 3 to 10 days. This medium was named MO.

Pure colonies were isolated from the last positive dilution by successive plating on the same media solidified with 15 g of agar-agar (Merck) per liter and incubated at 70°C.

**Reference strains and media.** The reference or type strains used were as follows: *Thermus thermophilus* HB8 (14) (DSM 579), *Thermus aquaticus* YT1 (5) (DSM 625), *Thermus filiformis* Wai 33 A1T (10) (NCIMB 12588), and *Thermus*

\* Corresponding author. Mailing address: Laboratoire de Microbiologie, Université de Neuchâtel, Rue Emile-Argand 11, CH-2007 Neuchâtel, Switzerland. Phone: 41-38-23.22.61. Fax: 41-38-23.22.31.

TABLE 1. Numbers of spore-forming and non-spore-forming thermophilic bacteria at the beginning and cooling phases of the open-air windrow composting system

Time (days)	Temperature of compost sample (°C)	No. of bacteria/g (dry wt) of compost <sup>a</sup>	
		Oval-spore formers <sup>b</sup>	Non-spore formers <sup>c</sup>
0 (starting material)	25–40	10 <sup>6</sup> –10 <sup>7</sup>	10 <sup>2</sup> –10 <sup>4</sup>
4	60–65	10 <sup>9</sup> –10 <sup>10</sup>	10 <sup>4</sup> –10 <sup>6</sup>
6	65–71	10 <sup>8</sup> –10 <sup>9</sup>	10 <sup>6</sup> –10 <sup>7</sup>
10	67–78	10 <sup>8</sup> –10 <sup>9</sup>	10 <sup>8</sup> –10 <sup>9</sup>
115	20–35	10 <sup>7</sup> –10 <sup>8</sup>	10 <sup>3</sup> –10 <sup>4</sup>

<sup>a</sup> Results are the minimal and maximal values of three to five independent determinations.

<sup>b</sup> Enrichment of serial dilutions at 60°C on NB medium.

<sup>c</sup> Enrichment of serial dilutions at 75°C on MNY medium. Prior to incubation, the enrichment cultures were placed in a water bath at 70°C for 5 min.

*ruber* DSM 1279 (12). The *Escherichia coli* B (DSM 2840) reference strain was used for standard DNA percent G+C determination. *T. thermophilus* was cultivated on MNY medium (see above) at 75°C, and *E. coli* was grown on NB medium at 30°C. *T. aquaticus*, *T. filiformis*, and *T. ruber* were cultivated on the standard *Thermus* medium (20), the first two at 70°C and the last at 55°C.

**Biochemical characterization of non-spore-forming bacteria.** Sensitivity to penicillin was tested during 5 days of incubation at 65°C in liquid media containing 10 mg of benzylpenicillin (Fluka Chemie AG, Buchs, Switzerland) per liter, as described previously (3). Pigments were extracted from cell pellets with a 3:1 (vol/vol) solvent mixture of methanol and acetone, and the absorption spectra between 350 and 550 nm were determined with a spectrophotometer. Since several strains of non-spore-forming bacteria grew poorly on all the carbon sources tested (1 and 10 g of acetate, pyruvate, D-glucose, and soluble starch per liter) in BMM, yeast extract (0.2 g/liter) was added to stimulate growth. Controls with no addition of single carbon sources were also included. The oxidation of single carbon sources was assessed by respiratory-activity measurements. Growth at pH 6 to 8.5 was tested at 0.5-pH-unit intervals.

**Scanning electron microscopy.** The method used for the preparation of cell samples for scanning electron microscopy was that of Sutton et al. (19). Samples were examined with a Philips XL 20 scanning electron microscope.

**Growth experiments.** Determinations of batch growth kinetics were carried out in a KLF 2000 stirred fermentor (Bioengineering AG, Wald, Switzerland) with a working volume of 2 liters. Temperature, oxygen concentration, and agitation were automatically regulated. The pH was measured on-line and remained constant ( $\pm 0.2$  pH unit) during the growth experiments. All growth kinetics experiments were performed with MNY medium at 50% oxygen saturation.

**Oxygen uptake measurements.** The respiratory activities were measured polarographically with an oxygen electrode system (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio) between 60 and 80°C according to the procedure of Beffa et al. (3).

**DNA base composition and hybridization.** DNA purification, DNA G+C content, and DNA-DNA homology determinations were performed according to the methods of Beffa et al. (3).

**PCR analysis.** Extraction and purification of genomic DNA were performed with approximately 0.25 g (wet weight) of cells from fresh MNY cultures by the method of Pitcher et al. (15) without enzymatic treatment. DNA pellets were resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8). The integrity of the DNA was checked by horizontal gel electrophoresis in 0.8% agarose in

TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA; pH 8.3) containing ethidium bromide (0.5  $\mu$ g/ml).

The 16S rDNA was selectively amplified from purified genomic DNA by using oligonucleotide primers designed to anneal to conserved positions in the 3' and 5' regions of bacterial 16S rRNA genes. The forward primer, UNI16S-L (5'-ATTCTAGAGTTTGATCATGGCTCA), corresponded to positions 11 to 26 of *E. coli* 16S rRNA, and the reverse primer, UNI16S-R (5'-ATGGTACCGTGTGACGGGCGGTGTGTA), corresponded to the complement of positions 1411 to 1393 of *E. coli* 16S rRNA (6). The first and second underlined 5' regions represent sequences that overlap with those of the *Xba*I and *Kpn*I restriction sites, respectively. The primers were supplied by Microsynth, Balgach, Switzerland.

The reaction conditions were as follows: 12 ng of template DNA, 5  $\mu$ l of 10 $\times$  PCR buffer [750 mM Tris-HCl (pH 9.0 at 25°C), 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% (wt/vol) Tween 20], 1.5 mM MgCl<sub>2</sub>, 0.8 U of Goldstar *Taq* DNA polymerase (Eurogentec, Seraing, Belgium), 0.25  $\mu$ M forward primer, 0.25  $\mu$ M reverse primer, 170  $\mu$ M dATP, 170  $\mu$ M dCTP, 170  $\mu$ M dGTP, and 170  $\mu$ M dTTP were combined in a total volume of 50  $\mu$ l. Amplification was carried out in 200- $\mu$ l tubes in a PTC-100 thermal cycler (MJ Research Inc., Watertown, Mass.) as follows: a preliminary denaturation step was done at 95°C for 2 min, followed by 36 cycles of 1 min at 94°C (denaturation), 1 min at 56°C (except for the first six touchdown cycles, which were successively at 68, 66, 64, 62, 60, and 58°C), and 2 min at 72°C (extension).

PCR products were electrophoresed at 10 V cm<sup>-1</sup> in 1.3% agarose in TBE buffer containing ethidium bromide (0.5  $\mu$ g/ml).

For restriction enzyme digestion, 70  $\mu$ g of the PCR product was mixed with 2  $\mu$ l of 10 $\times$  restriction buffer H and 1 U of *Hinf*I (Boehringer, Mannheim, Germany) (final volume, 20  $\mu$ l). Digestion was carried out at 37°C for 2 h; then another 1 U of *Hinf*I was added and the mixture was incubated for an additional 2 h.

Digests were electrophoresed for 70 min at 10 V cm<sup>-1</sup> in 2.5% agarose in TBE buffer containing ethidium bromide (0.5  $\mu$ g/ml). Gels were photographed with a UV transilluminator on Ilford FP4Plus film (Ilford Ltd, Mobberley, United Kingdom) and printed on Ilford IS5.1M paper.

**Gel electrophoresis of proteins.** Extracts from cells growing actively on MNY medium were obtained according to the method of Beffa et al. (3). Proteins (25 to 30  $\mu$ g per lane) were resolved by denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 5% [wt/vol] stacking gel and 12.5% [wt/vol] resolving gel) at 22°C according to the procedure of Beffa et al. (3). The protein profile on the gel of the *T. aquaticus* reference strain was not satisfactory because of unknown interfering substances during the protein concentration measurements. The proteins of the *T. aquaticus* cell extract were precipitated with 14% (vol/vol) trichloroacetic acid for 30 min on ice and then centrifuged (12,000 rpm, 10 min, 4°C). The supernatant was removed, and the pellet was gently resuspended and washed with methanol by centrifugation as described above. After removal of the supernatant, the pellet was resuspended in 0.1 M Tris-HCl (pH 7.6) containing 4% (wt/vol) SDS, and the suspension was then incubated at 37°C for several hours, until the proteins were totally solubilized. Gels were stained with Coomassie brilliant blue R-250 after electrophoresis. Molecular mass (14.4- to 200-kDa) standards were purchased from Bio-Rad Laboratories (Hercules, Calif.). Controls containing precipitated and nonprecipitated cell extracts of *T. thermophilus* gave similar protein profiles on the gel (2).

**Other analytical methods.** For the dry-weight determination, 10 g of wet compost was dried for 24 h at 70°C under a vacuum. Growth was followed turbidimetrically at 436 nm with an optical path of 1 cm. The protein concentration was determined by the method of Bradford (4) with bovine serum albumin as the standard.

## RESULTS

**Compost facilities and samples.** The temperature ranged from 65 to 82°C, the oxygen concentration was found to be 2 to

TABLE 2. Non-spore-forming, thermophilic bacteria isolated from different composting systems

Composting system (main type of waste)	Temperature of compost sample (°C)	Number of bacteria/g (dry wt) of compost <sup>a</sup>	Strain isolated at last positive dilution
Open air (garden)	68–77	10 <sup>8</sup> –10 <sup>9</sup>	CT1 <sup>b</sup>
	68–77	10 <sup>4</sup> –10 <sup>5</sup>	JT-4 <sup>c</sup>
Box (garden plus kitchen)	68–80	10 <sup>8</sup> –10 <sup>9</sup>	CT2a <sup>b</sup>
Box (methanized kitchen plus garden)	70–80	10 <sup>8</sup> –10 <sup>9</sup>	CT5 <sup>b</sup>
Bioreactor, 12 m <sup>3</sup> (garden plus kitchen)	68–82	10 <sup>8</sup> –10 <sup>10</sup>	CT4 <sup>b</sup>
Bioreactor, 300 m <sup>3</sup> (sludge plus wood chips)	65–75	10 <sup>8</sup> –10 <sup>9</sup>	CT3a, <sup>b</sup> CT3b <sup>b</sup>

<sup>a</sup> Results are the minimal and maximal values of two to five independent determinations.

<sup>b</sup> Strains isolated at 75°C on MNY medium at the last positive enrichment dilution. Prior to the incubation, the enrichment cultures were placed in a water bath at 70°C for 5 min.

<sup>c</sup> Strain isolated at 70°C under oligocarbophilic conditions on MO medium at the last positive dilution.

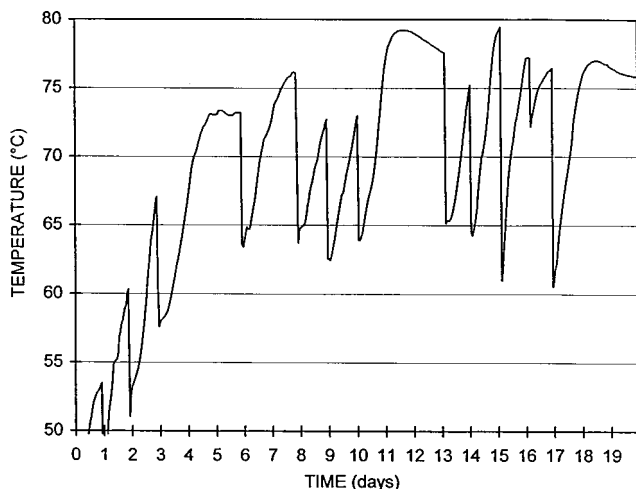


FIG. 1. Temperature recorded inside (at a depth of 60 cm from the top) a classical open-air windrow composting system intensively managed by daily turnings. The temperature was recorded automatically every hour. The windrow was turned on days 1, 2, 3, 6, 8 to 10, and 13 to 17. The experiment was started in the fall (25 October 1994).

15% (vol/vol), the pH ranged from 6 to 8.5, and the moisture content was found to be between 45 and 65%.

**Enrichment and isolation of spore-forming bacteria.** Enrichment of hot-compost samples at 50, 60, and 65°C gave essentially  $10^8$  to  $10^9$  oval-spore-forming bacteria or bacilli per g (dry weight) of compost. Enrichment at 70°C gave a mixture of spore-forming and non-spore-forming bacteria. The num-

bers of oval-spore-forming bacteria remained high ( $10^6$  to  $10^{10}$  cells per g [dry weight] of compost) during the whole composting process (Table 1). The upper limit of the temperature range for growth of all thermophilic oval-spore-forming strains isolated from hot composts was 70°C.

**Enrichment and isolation of non-spore-forming bacteria.** Enrichment of hot-compost samples (65 to 82°C) at 75°C, with preincubation of the cultures at 70°C in a water bath for 5 min, resulted in only non-spore-forming, thermophilic bacteria at  $10^7$  to  $10^{10}$  cells per g (dry weight) of compost (Tables 1 and 2). These cultures do not form pellicles at their surfaces. In the open-air composting system experiment, the fast increase of temperature from 65 to 79°C during the second week (Fig. 1) was probably related to the very high numbers of *Thermus* cells in hot compost (Table 1). Lower temperatures and lower numbers of *Thermus* cells were observed during the first days of composting and during the terminal cooling phase (Table 1).

Enrichment of compost samples at 70°C under oligocarbo-philic conditions on MO medium gave lower numbers of non-spore-forming bacteria (Table 2).

**Characterization of non-spore-forming bacteria.** The initial isolates at 75°C were usually long rods or filaments (0.5- $\mu\text{m}$  diameter). The length of the cells varied from 5 to 10  $\mu\text{m}$  to more than 30  $\mu\text{m}$ . The typical morphology of strain CT1 cultivated on MNY medium at 60°C is presented in Fig. 2. All isolates were gram negative, nonmotile, and penicillin G sensitive. Yellow-orange pigments were produced only by strains CT3a and CT3b (Table 3) and showed an absorbance peak at 452 nm and shoulders at 477 and 427 nm, similar to those obtained from the *T. aquaticus* and *T. thermophilus* reference strains.

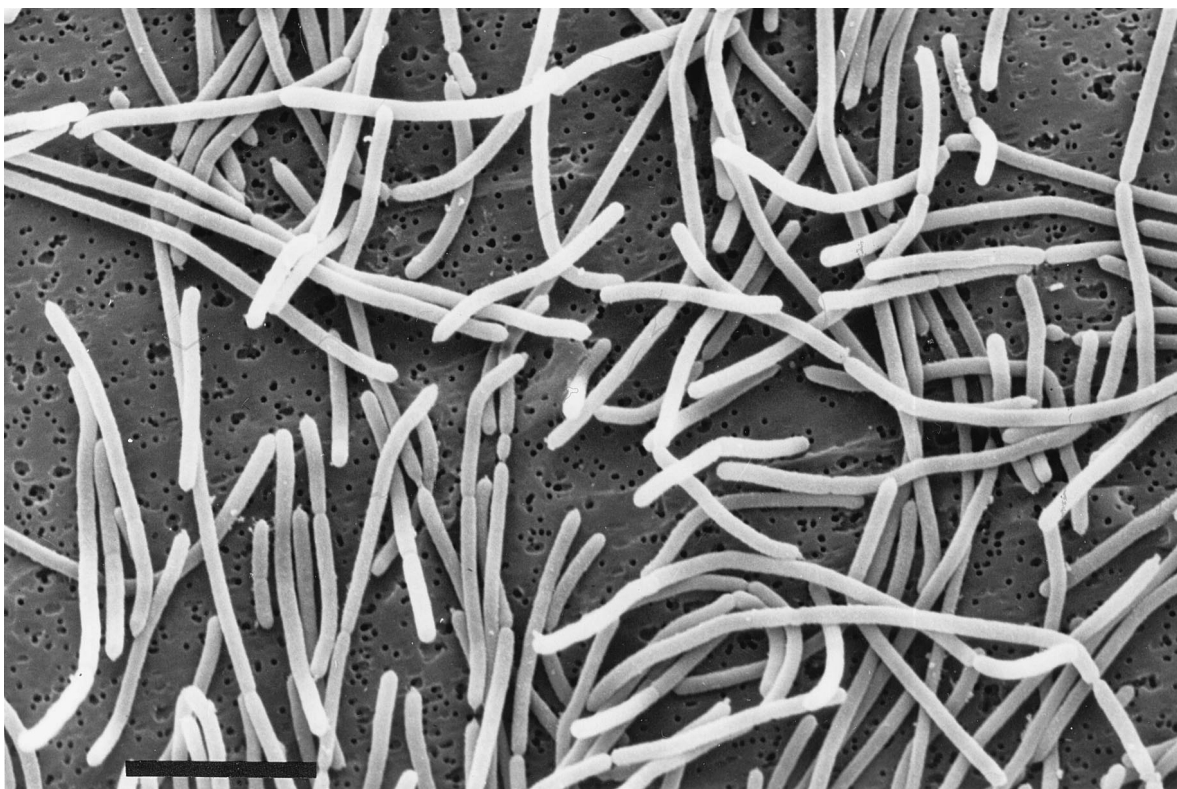


FIG. 2. Scanning electron micrograph of strain CT1 grown in batch culture with shaking in MNY medium at 60°C. Bar, 5  $\mu\text{m}$ .

TABLE 3. Main characteristics of thermophilic, heterotrophic, non-spore-forming bacteria isolated from hot composts

Strain	Pigment	G+C (mol%)	GT (°C) <sup>a</sup>	Growth on <sup>b</sup> :							
				TM <sup>c</sup>	NB	MNY plus NaCl (3%)	MNY plus NaCl (5%)	BMM plus acetate (1 or 10 g/liter)	BMM plus pyruvate (1 or 10 g/liter)	BMM plus D-glucose (1 or 10 g/liter)	BMM plus soluble starch (1 or 10 g/liter)
CT1	None	61.3	40–80	+	±	+	±	+	+	+	+
CT2a	None	63.2	40–80	+	±	+	±	+	+	+	+
CT3a	Yellow	63.4	40–80	+	±	+	±	±	+	+	±
CT3b	Yellow	62.1	40–80	+	±	+	±	+	+	+	+
CT4	None	64	40–80	+	±	+	+	+	+	+	+
CT5	None	61.7	40–80	+	±	+	±	+	+	+	+
JT4	None	60.7	40–75 <sup>c</sup>	+	±	NT <sup>d</sup>	NT	NT	NT	NT	NT

<sup>a</sup> The growth temperature (GT) was tested on standard MNY medium for all strains except JT4.

<sup>b</sup> Symbols: +, good growth; ±, poor growth. See text for details.

<sup>c</sup> TM, standard *Thermus* medium.

<sup>d</sup> NT, not tested.

**Growth characteristics.** With the exception of strain JT4, all isolates grew on standard MNY medium at temperatures between 40 and 80°C and at all the pH values tested (6 to 8.5). Growth occurred in standard MNY medium supplemented with 30 g of NaCl per liter and in a more concentrated MNY medium (BMM plus 20 to 40 g of nutrient broth and 5 to 10 g of yeast extract per liter). Strain JT4 grew quickly on *Thermus* medium and poorly on MNY medium at 70 to 75°C. Strains CT1, CT2a, CT3b, CT4, and CT5 grew at 75°C in the presence of pyruvate, acetate, D-glucose, or soluble starch (Table 3) and showed significant respiratory activities in the presence of these compounds (2).

Growth studies of strain CT-1 on MNY medium in the bioreactor (Table 4) showed fast growth and respiratory-activity rates between 65 and 75°C (specific growth rate, 0.721 to 0.862 h<sup>-1</sup>), with optimum rates at 70°C.

**DNA base composition and hybridization.** The G+C contents in the DNAs of all the strains isolated from thermogenic composts ranged from 60.5 to 63.4 mol% (Table 3) and were similar to the G+C content reported for *Thermus* spp. (10, 20). High degrees of DNA-DNA homology (73 to 83%) were observed between strain CT1 and strains CT2a, CT3a, CT3b, CT4, and CT5. Strain CT3a showed a higher degree of homology (85%) with strain CT3b. All isolates (except strain JT4) showed a higher degree of DNA-DNA homology with *T. thermophilus* (65 to 71%) than with *T. aquaticus* (50 to 62%). Only strain JT4 showed a relatively high degree of homology (72%) with *T. aquaticus*. All isolates presented poor DNA-DNA homology with *T. filiformis* (39 to 48%) and *T. ruber* (30 to 40%).

**PCR analysis.** The restriction fragment length polymorphism (RFLP) profiles of 16S rDNA of all *Thermus* strains isolated from hot composts are similar to the profiles of the reference strain *T. thermophilus* HB8 (Fig. 3). The reference

strains of *T. thermophilus*, *T. aquaticus*, *T. filiformis*, and *T. ruber* showed different RFLP profiles.

**SDS-PAGE protein profiles.** Gel electrophoresis of total proteins revealed that the compost isolates tested had relatively similar protein profiles (Fig. 4). Strain CT2a showed one to several dense bands at about 38 kDa. The profiles resembled those of *T. thermophilus* more than they did those of *T. aquaticus*.

## DISCUSSION

Thermophilic bacteria related to the genus *Thermus* were isolated from many neutral and alkaline geothermal and man-made thermal environments throughout the world (1, 10, 11, 16, 20). It is assumed that the natural habitat of *Thermus* spp. is the hot-springs ecosystem. Usually isolation of *Thermus* spp. was performed on a medium with a low concentration of organic matter (20). Isolation on a richer medium yielded mostly strains related to *T. thermophilus* (14). At this time only one study has reported the presence of two non-spore-forming strains of thermophilic bacteria in sewage sludge compost (9). Isolation was done at 60°C, and their optimum temperatures ranged from 60 to 65°C.

In the present work we report for the first time the presence of a diversity and a considerable number of bacteria related to

TABLE 4. Growth and respiratory properties of strain CT1 as functions of growth temperature

Growth temperature (°C)	Specific growth rate (h <sup>-1</sup> )	Doubling time (min)	Oxygen consumption (nmol of O <sub>2</sub> · mg [dry wt] <sup>-1</sup> · min <sup>-1</sup> )
60	0.456	91	195
65	0.794	52	380
70	0.862	48	460
75	0.721	57	370
80	0.320	129	180

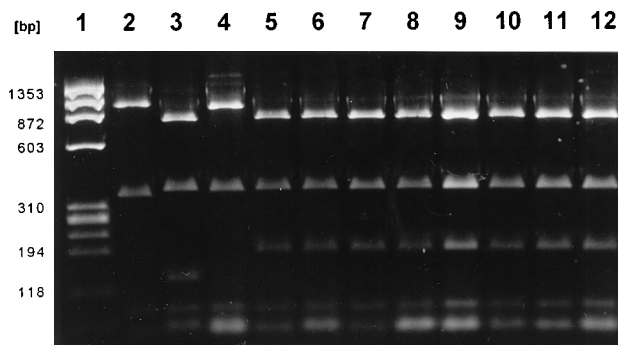


FIG. 3. RFLP profiles of 16S rDNA selectively amplified and digested with *Hinf*I of *Thermus* reference strains and seven strains isolated from hot composts. Lane 1,  $\phi$ X174RF digested with *Hae*III; lane 2, *T. ruber* (12) (DSM 1279); lane 3, *T. filiformis* Wai 33 A1T (10) (NCIMB 12588); lane 4, *T. aquaticus* YT1 (5) (DSM 625); lane 5, *T. thermophilus* HB8 (14) (DSM 579); lane 6, strain CT1; lane 7, strain CT2a; lane 8, strain CT3a; lane 9, strain CT3b; lane 10, strain CT4; lane 11, strain CT5; lane 12, strain JT4.

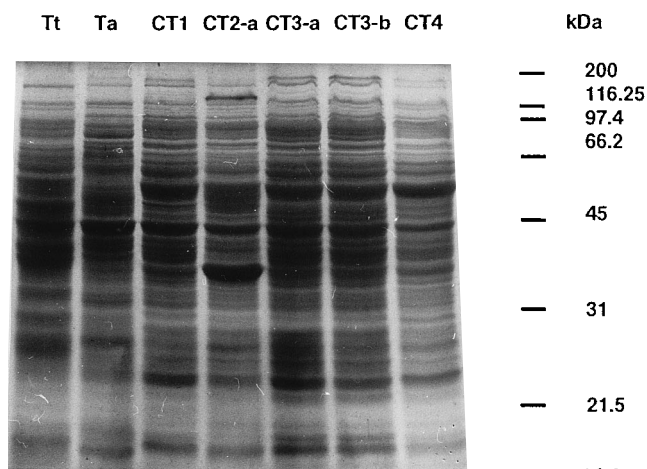


FIG. 4. Coomassie brilliant blue-stained SDS-polyacrylamide gel showing proteins of total cell extracts of thermophilic bacteria related to the genus *Hydrogenobacter* isolated from hot composts (strains CT1, CT2a, CT3a, CT3b, and CT4) and from the type strains *T. thermophilus* HB8 (Tt) and *T. aquaticus* YT1 (Ta). Molecular mass markers are on the right.

the genus *Thermus* in thermogenic (65 to 82°C) composts taken from 2- to 5-week-old organic-waste samples. The majority of the isolates are probably *Thermus* strains which have adapted to the conditions present in the hot-compost ecosystem. The nutritional characteristics, total protein profiles by gel electrophoresis, DNA-DNA hybridization (except strain JT4), and restriction fragment length polymorphism profiles of the 16S rDNA showed that the *Thermus* strains isolated from hot composts were closely related to *T. thermophilus* HB8.

Thermophilic oval-spore-forming bacilli are very active during composting when the temperatures are between 50 and 65°C (13, 17, 18) (see also above). However, in the composts studied, temperatures between 65 and 80°C were usually reached during the thermogenic phase. Phase-contrast microscopy observations of thermogenic compost samples showed the presence of high numbers of oval bacterial spores (2). In addition, no strains of thermophilic bacilli isolated from hot compost were able to grow at temperatures above 70°C. This study suggests that non-spore-forming, thermophilic bacteria related to the genus *Thermus* are the dominant active degraders in thermogenic composts at temperatures above 70°C.

The demonstrated functional bacterial diversity during the thermogenic phase seems to make it possible to compost at high temperatures (65 to 75°C) for a longer period of time. By doing that, the composting process could be performed with better destruction of potential human pathogens and allergenic molds, as well as phytopathogens and seeds.

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#### REFERENCES

1. Alfredsson, G. A., S. Baldursson, and J. K. Kristjansson. 1985. Nutritional diversity among *Thermus* spp. isolated from Icelandic hot springs. *Syst. Appl. Microbiol.* **6**:308-311.
2. Beffa, T. Unpublished data.
3. Beffa, T., M. Blanc, and M. Aragno. 1996. Obligately and facultatively autotrophic, sulfur- and hydrogen-oxidizing thermophilic bacteria isolated from hot composts. *Arch. Microbiol.* **165**:34-40.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248-254.
5. Brock, T. D., and H. Freeze. 1969. *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. *J. Bacteriol.* **98**:289-297.
6. Brosius, J., M. L. Palmer, P. J. Kennedy, and H. F. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **75**:4801-4805.
7. De Bertoldi, M., G. Vallini, and A. Pera. 1983. The biology of composting, a review. *Waste Manage. Res.* **1**:167-176.
8. Finstein, M. S., and M. L. Morris. 1975. Microbiology of municipal solid waste composting. *Adv. Appl. Microbiol.* **19**:113-151.
9. Fujio, Y., and S. J. Kume. 1991. Isolation and identification of thermophilic bacteria from sewage sludge compost. *J. Ferment. Bioeng.* **72**:334-337.
10. Hudson, J. A., H. W. Morgan, and R. M. Daniel. 1987. *Thermus filiformis* sp. nov., a filamentous caldoactive bacterium. *Int. J. Syst. Bacteriol.* **37**:431-436.
11. Hudson, J. A., H. W. Morgan, and R. M. Daniel. 1989. Numerical classification of *Thermus* isolates from globally distributed hot springs. *Syst. Appl. Microbiol.* **11**:250-256.
12. Loginova, L. G., L. A. Egorova, R. S. Golovacheva, and L. M. Seregina. 1984. *Thermus ruber* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* **34**:498-499.
13. Nakasaki, K., M. Shoda, and H. Kubota. 1985. Effect of temperature on composting of sewage sludge. *Appl. Environ. Microbiol.* **50**:1526-1530.
14. Oshima, T., and K. Imahori. 1974. Description of *Thermus thermophilus* (Yoshida and Oshima) comb. nov., a nonsporulating thermophilic bacterium from a Japanese thermal spa. *Int. J. Syst. Bacteriol.* **24**:102-112.
15. Pitcher, D. G., N. A. Saunders, R. J. Owen. 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.* **8**:151-156.
16. Ramaley, R. F., and K. Bitzinger. 1975. Types and distribution of obligate thermophilic bacteria in man-made and natural thermal gradients. *Appl. Microbiol.* **30**:152-155.
17. Strom, P. F. 1985. Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. *Appl. Environ. Microbiol.* **50**:899-905.
18. Strom, P. F. 1985. Identification of thermophilic bacteria in solid-waste composting. *Appl. Environ. Microbiol.* **50**:906-913.
19. Sutton, N. A., N. Hughes, and P. S. Handley. 1994. A comparison of conventional SEM technique, low temperature SEM and the electroscan wet scanning electron microscope to study the structure of a biofilm of *Streptococcus crista* CR3. *J. Appl. Bacteriol.* **76**:448-454.
20. Williams, R. A. D., and M. S. Da Costa. 1991. The genus *Thermus* and related microorganisms, p. 51-62. In J. K. Kristjansson (ed.), *Thermophilic bacteria*. CRC Press, Inc., Boca Raton, Fla.