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# *Hyperthermus butylicus*, a Hyperthermophilic Sulfur-Reducing Archaebacterium That Ferments Peptides

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**The hyperthermophilic peptide-fermenting sulfur archaebacterium *Hyperthermus butylicus* was isolated from the sea floor of a solfataric habitat with temperatures of up to 112°C on the coast of the island of São Miguel, Azores. The organism grows at up to 108°C, grows optimally between 95 and 106°C at 17 g of NaCl per liter and pH 7.0, utilizes peptide mixtures as carbon and energy sources, and forms $H_2S$ from elemental sulfur and molecular hydrogen as a growth-stimulating accessory energy source but not by sulfur respiration. The same fermentation products, $CO_2$, 1-butanol, acetic acid, phenylacetic acid, and a trace of hydroxyphenylacetic acid, are formed both with and without of $S^0$ and $H_2$. Its ether lipids, the absence of a mureine sacculus, the nature of the DNA-dependent RNA polymerase, and phylogenetic classification by DNA-rRNA cross-hybridization characterize *H. butylicus* as part of a novel genus of the major branch of archaebacteria comprising the orders *Thermoproteales* and *Sulfolobales*, representing a particularly long lineage bifurcating with the order *Sulfolobales* above the branching off of the genus *Thermoproteus* and distinct from the genera *Desulfurococcus* and *Pyrodictium*.**

Extremely thermophilic sulfur-reducing archaebacteria (13, 14) thrive either chemolithoautotrophically, utilizing $CO_2$ as the sole carbon source and synthesis of $H_2S$ from $S^0$ and molecular hydrogen as the energy source, or by sulfur respiration of various organic carbon and energy sources.

Some members of this group, e.g., *Desulfurococcus* spp. (26), *Pyrodictium abyssum* (K. O. Stetter, personal communication), *Thermococcus* spp. (19), and *Pyrococcus* spp. (2, 20), show a significant ability to grow anaerobically on various carbon sources without sulfur and/or hydrogen. This has been taken to indicate fermentation, although defined products have not been identified.

Although samples from hot vents with temperatures of more than 300°C have been analyzed (3, 4), the maximal growth temperatures of the most extreme hyperthermophiles (organisms growing at and above 100°C) known, *Pyrodictium occultum* (12) and *P. abyssum* (Stetter, personal communication) do not exceed 110°C.

This report describes a novel hyperthermophilic, peptide-fermenting archaebacterium, *Hyperthermus butylicus*, which was isolated from a submarine solfataric source with temperatures of up to 112°C off the coast of the island São Miguel, Azores, and grows at up to 107°C. A preliminary assignment of the phylogenetic position at the root of the order *Sulfolobales* and an analysis of the fermentation products are given.

## MATERIALS AND METHODS

**Sampling.** Temperatures in the field were measured with a thermoelement thermometer. Samples were taken by scuba diving in two ways. The first procedure consisted of suction through a 50- or 200-cm-long, 3-mm-wide aluminum needle into 50-ml plastic syringes. The needle was equipped with a bullet-shaped tip with lateral fine-mesh sieve windows for excluding solids, e.g., sand. The syringes were plugged. On the surface, the contents of two syringes amounting to about 100 ml were injected into each 100-ml serum bottle containing 0.5 g of elemental sulfur, 100 μg of resazurin as a redox indicator, and 100 kPa of $CO_2$ and 100 Pa of $H_2S$ to maintain anaerobicity. The second procedure used evacuated 100-ml serum bottles which were plugged by rubber stoppers equipped with metal rings. The bottles were fastened to a clamp on the tip of a 2-m-long rod. By a string connected to the ring, the stopper could be removed while the bottle was held to the place from which the sample was to be taken. Because of the vacuum, the bottle filled immediately. It was held sideways, retracted, and closed within 2 s. Both types of samples yielded the same results.

**Enrichment.** The samples were kept and transported at ambient temperature. The medium used for enrichment corresponded essentially to that used by Stetter et al. for *Pyrodictium* spp. (12), including the trace minerals (1), except that it contained 2.5 mg of KI per liter and 2 mg of $NiSO_4 \cdot 6H_2O$ per liter and, in addition, 0.5 g of $NH_4Cl$ per liter as the nitrogen source. Tryptone (5 to 10 [usually 6] g/liter; Difco Laboratories) served as the carbon source. The medium was made anaerobic by addition of $H_2S$-water until the redox indicator, 1 mg of resazurin per liter, became colorless. The atmosphere consisted of 800 kPa of $CO_2$ with or without addition of 200 kPa of $H_2$. In the latter case, 6 g of elemental sulfur (sonicated) per liter was added. Enrichment was performed at 106°C in 100-ml serum bottles containing 20 ml of this medium and inoculated with 1 ml each of the samples. After 2 days, irregular roundish cells resembling *Sulfolobus* sp. appeared, which under these conditions grew to about $5 \times 10^7$/ml with $S^0$ and $H_2$ and somewhat less without $S^0$ and $H_2$.

**Isolation.** Eight grams of K9A40 gellan gum (Kelco, a division of Merck & Co., Inc.) per liter was dissolved in boiling water. After addition of 1 g of $CaSO_4$ per liter, glass petri dishes were each loaded with 25 ml of the hot solution. After solidification, each plate was soaked for 2 min with 5

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ml of a saturated solution of $S^0$ in 1 M $(NH_4)_2S$ and then rinsed with water. Colloidal sulfur was precipitated in the surface by acidification with 5 to 10 ml of 1 M $H_2SO_4$ for 2 min. After extensive washing with water, the plates were equilibrated overnight in the culture medium. After decanting of the medium, the petri dishes were dried for 1 h at 37°C. They were placed in anaerobic incubation vessels in an anaerobic chamber with an atmosphere of $N_2$. The vessels were sealed, and 50 to 100 kPa of $H_2S$ was added to the atmosphere to ensure anaerobicity. Dilutions of grown cultures (0.1 ml each) were plated anaerobically and incubated at 99°C for 40 to 60 h. Small amber colonies surrounded by clear halos appeared in the sulfur layer. Single colonies were grown in liquid cultures which were plated once more. All further work was done with cultures derived from one subclone.

Large liquid cultures were grown in glass bottles containing 1.6, 12, or 50 liters of medium at 95 to 97°C or in an enameled steel fermentor (Bioengineering) in which all nonenameled parts were coated with Halar. Above 100°C, the fermentor was run at a pressure of 2,000 kPa. All of these cultures were continuously gassed with either $CO_2$ or $N_2$ and, when $S^0$ was added, with 20% (vol/vol) $H_2$ in addition. In the standard culture medium with 6 g of tryptone per liter as the carbon source and with 10 g of $S^0$ and $H_2$ per liter, the cells grew to a density of $2 \times 10^8$/ml to $3 \times 10^8$/ml within 24 to 48 h.

**Lipids.** Lyophilized cells were extracted with $CHCl_3$-methanol (2:1), the lipids were hydrolyzed, and the isoprenoid alcohols were reduced as described by Thurl and Schäfer (15).

**DNA-dependent RNA polymerase.** DNA-dependent RNA polymerase was purified to homogeneity by the polymin-P method as described for other archaebacteria (9, 23).

**Analytical techniques.** The analytical techniques used were described previously (25). The fermentation products were identified by gas chromatography-mass spectroscopy (acids) and nuclear magnetic resonance spectroscopy. Butanol was identified by distillation and determination of the boiling point, and $H_2S$ was monitored as CdS and $CO_2$ was monitored as $BaCO_3$.

**Electron microscopy.** Specimens were visualized in a Zeiss 109 electron microscope. Details are described in the figure legends. Negative staining was with 1 to 2% uranyl acetate. Rotary shadowing with platinum was done at an angle of 8°. Contrasting of sections of Epon 812-embedded cells was done with lead citrate, uranyl acetate, and lead citrate, in that order. Cryosections (courtesy of B. Humbel) were produced as described by Tokuyasu (16).

**Determination of phylogenetic position.** The phylogenetic position was determined by DNA-total rRNA cross-hybridizations as previously described (6, 20), except that instead of various concentrations of rRNA, only one concentration, 1 μg/ml, was used six times for each cross.

## RESULTS

**Isolation.** A small, moderately active submarine solfataric field was found at a depth of about 9 m around 200 m from a steep, rocky shore off São Miguel Island, Azores, in a region, where faults extend from the coast into the sea. Numerous streams of gas bubbles emerged from the sandy sea floor between rocks. One particularly active gas stream, apparently consisting mainly of steam and $CO_2$ with little $H_2S$, had created a small crater surrounded by black sulfidic deposits in the sand. About 40 cm below the sandy surface of this crater, a temperature of 112°C was measured within the sediment. Water samples were taken from this depth as described in Materials and Methods. These samples were transported at ambient temperature and enriched anaerobically at 106°C in closed serum bottles in a medium containing 10 g of tryptone per liter and 5 g of $S^0$ per liter with a gas phase consisting of $CO_2$ (800 kPa) and $H_2$ (200 kPa). Cell clones were isolated from single colonies grown for 48 h on sulfurized K9A40 gellan gum plates. All experiments were performed with an isolate obtained by repeated subcloning.

**Morphology.** The cells resembled *Sulfolobus* sp. in shape but were slightly larger (around 1.5 μm in diameter). They appeared as irregular spheres with edges between partially flattened surfaces (Fig. 1a and 2a). In exponentially growing cultures, they exhibited high phase contrast. Constricted duplex forms, probably in division (Fig. 1a and 2b), were sometimes seen but were considerably less frequent (about 1%) than with *Thermococcus* sp. and *Pyrococcus* sp. Few such duplex forms, possibly late in division, showed a thin string of cytoplasm surrounded by the envelope connecting the cells (Fig. 2c). This string appeared to break close to one of the cells and to remain attached to the other like a tail (Fig. 2d).

Many projections, resembling pili rather than flagellae, were seen all over the surface (Fig. 2a). In gassed liquid cultures, the cells appeared singly if not in division. In nonagitated cultures, however, they often formed clumps of sometimes several hundred cells (Fig. 1b).

Damaged cells and ghosts obtained by treatment with Triton X-100 (0.2%) and ultrasonication exhibited a remarkably distinct hexagonal S layer (Fig. 2e and f). This S layer was also visible in thin sections (Fig. 3a and c). It resembles S layers from *Thermoproteus* sp. and *Sulfolobus* sp. in symmetry but differs from these and from that of *Pyrodictium* sp. in both its lattice constant and its mass distribution (W. Baumeister, U. Santarius, S. Volker, R. Dürr, G. Lembke, and H. Engelhardt, Syst. Appl. Microbiol., in press).

Cells grown at high temperature often contained vacuoles within their cytoplasm, sometimes immediately below the S layer (Fig. 2f and 3a and b). Occasionally, the S layer over these vacuoles had collapsed and the membrane appeared highly irregular if not broken at these places, possibly opening the vacuole directly into the surrounding medium. The boundary between the vacuole and the cytoplasm was sharp but not bilayered like a typical membrane (Fig. 3b).

At 108°C, the highest temperature allowing continuous survival, about 25% of the cells showed long tails (data not shown).

**Requirements.** The organism utilized peptide mixtures obtained by various hydrolysis procedures, e.g., tryptone (optimal concentration, 5 to 6 g/liter), Trypticase (BBL Microbiology Systems), Merck peptone from casein, gelatin, and a chymotryptic digest of casein as carbon sources. However, it did not utilize a mixture of all 20 natural amino acids (each at 0.5 g/liter), mixtures of the amino acids from which the fermentation products could have been formed (see below), any single natural amino acid (2 g/liter), the tripeptides Gly-Ala-Gly and Leu-Gly-Gly, an octapeptide with an N-terminal Ile and a C-terminal Lys, a dodecapeptide with an N-terminal Ile and a C-terminal Val, a heptadecapeptide with an N-terminal Tyr and a C-terminal Val, a 19-mer with a C-terminal Tyr and an N-terminal Asp, glycine anhydride, or glycine methylester (each at 2 g/liter). The undigested proteins casein and bovine serum albumin (each at 10 g/liter) were also not utilized.

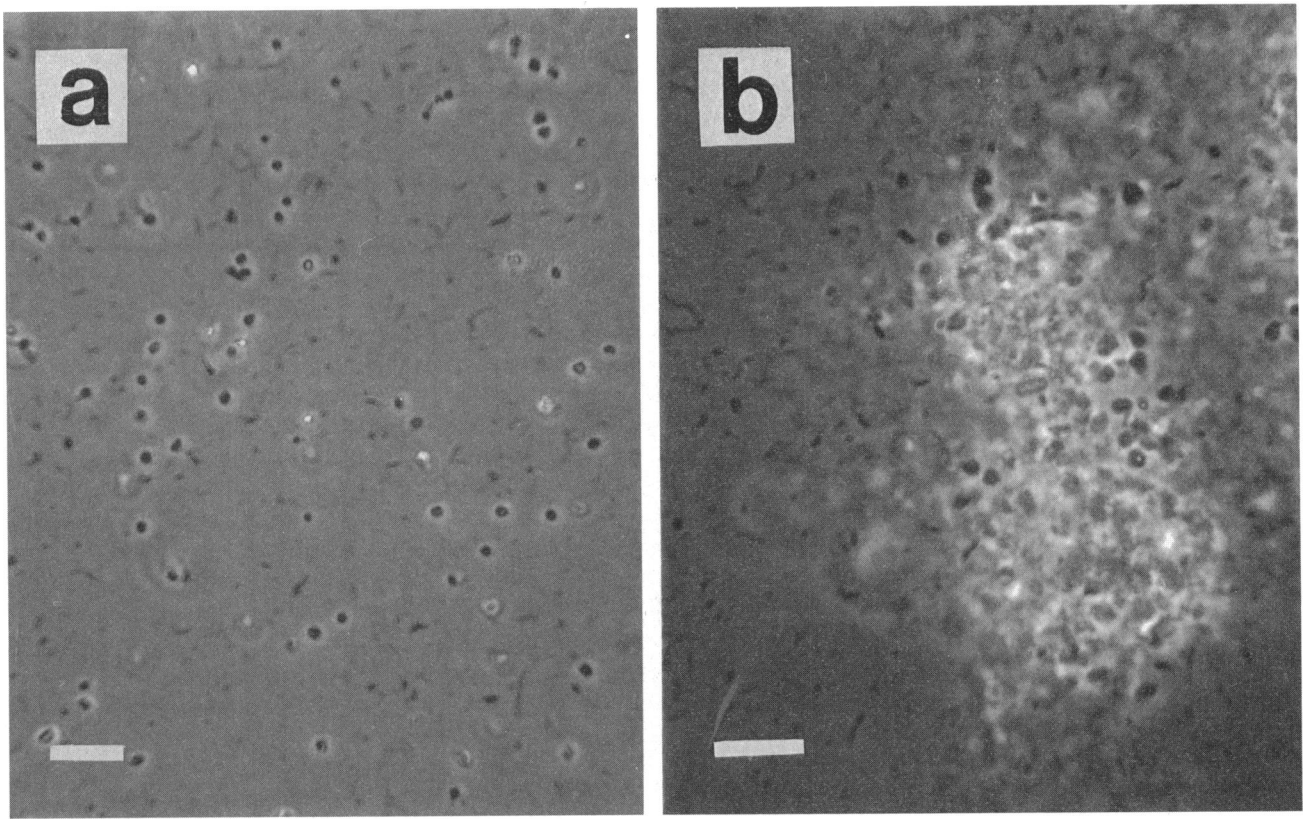


FIG. 1. Phase-contrast micrographs of *H. butylicus*. Panels: a, 10-fold concentrated from agitated liquid culture (original magnification, ×1,000); b, clump of cells from a nonagitated culture (original magnification, ×1,250); only a fraction of the cells is in focus.

Yeast extract (6 g/liter) allowed less than 5% of the growth observed with tryptone. No growth was found with 2 g each of starch, maltose, sucrose, lactose, glucose, galactose, ribose, lactic acid, lactic acid ethylester, glyoxylate, pyruvate, oxalate, fumarate, maleate, malate, malonate, and formate per liter.

Growth on tryptone (6 g/liter) was strongly stimulated by addition of $S^0$ (10 g/liter) plus $H_2$ (200 kPa in closed vessels or 20% [vol/vol] in continuously gassed cultures) concomitant with massive formation of $H_2S$ (0.31 mmol in a 25-ml culture with $10^9$ cells total). The final cell yield was increased by addition of $S^0$ and $H_2$ at 100°C from about $1 \times 10^7$/ml to $2 \times 10^8$/ml, and the generation time was decreased from about 7 to 2.5 h. No $H_2S$ formation was detected in uninoculated cultures. Without the peptidic carbon source, however, no chemolithoautotrophic growth was observed and replacement of $CO_2$ by $N_2$ did not change the stimulatory effect of $S^0$ plus $H_2$ or $H_2S$ formation. Without $S^0$ plus $H_2$, the organism required $NH_4^+$ ions as a nitrogen source. With $S^0$ and $H_2$, however, it was able to utilize the peptides in the medium as a nitrogen source.

The optimal pH was about 7, and the sharp salt optimum was at 17 g of NaCl per liter. No growth was detected after 2 days at 30 g of NaCl per liter.

No growth was found at 75°C. At 85°C, the generation time was 4.5 h. The broad optimum was between 95 and 107°C with generation times of 2 to 3 h and an unsharp minimum of about 2 h at around 107°C (Fig. 4). At 108°C, only slow growth with a generation time of 14 h was observed. At 110°C, the culture died, with a half-life of 3.5 h. Even after exposure for 24 h, the survivors grew normally at a lower temperature. At 112°C, the half-life of survival was not much lower, about 3 h, but the survivors grew with increased generation time, 6 instead of 3 h at 100°C, even after repeated transfer into fresh medium, suggesting genetic changes.

**Fermentation products.** On tryptone (0.6 g/liter) as the carbon source, the same fermentation products were produced with and without $S^0$ plus $H_2$, but their amounts were about 10 times higher with than without these compounds. These products were $CO_2$ (4.6 mol in a 100-liter fermentor culture grown to a density of $2.2 \times 10^8$ cells per ml, corresponding to about 2 g dry weight); 1-butanol, which was collected in a cooled trap from the gas outflow (67 mmol in the same 100-liter culture); nearly equal amounts of acetic, propionic, and phenylacetic acids; and about 1/10 as much hydroxyphenylacetic acid. Propylbenzene, acetophenone, and hydroxyacetophenone were qualitatively detected in the culture fluid. Since the pH did not change despite formation of carbonic acids, formation of $NH_3$ is probable under these conditions.

The characteristic smell of the cultures appeared to be composed of $H_2S$ and a trace of butyl mercaptan (which was identified as a contaminant of butanol by nuclear magnetic resonance spectroscopy) and the odors of 1-butanol and phenylacetic acid. A similar smell has been recognized in *Thermococcus* (19) and *Pyrococcus* (20) cultures.

**Lipids.** Lipids were Soxhlet extracted from lyophilized cells with chloroform-methanol (1:1) and subjected to hydrolysis (15). The resulting isopranyl alcohols were reduced, and the hydrocarbons were separated and characterized by gas chromatography-mass spectroscopy. More than 85% of

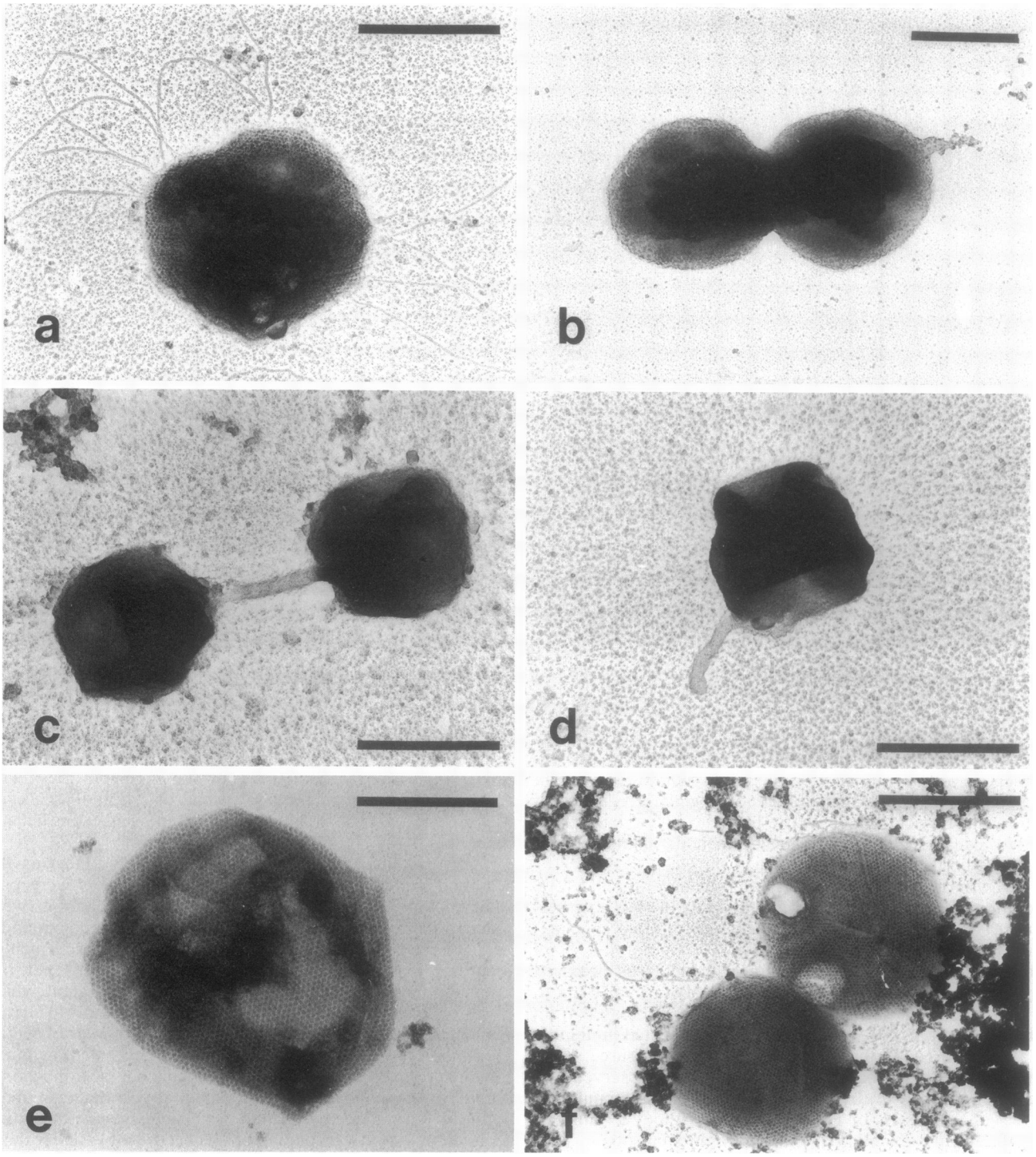


FIG. 2. Electron micrographs of *H. butylicus*. Panels: a, Single cell with pili; b, duplex form; c, cells connected by a string of cytoplasm; d, cell with a tail; e, ghost exhibiting an S layer; f, duplex form exhibiting an S layer and vacuoles. All panels but e were rotary shadowed with Pt; panel e was negatively stained with uranyl acetate. Bars, 1 μm.

the hydrocarbons were found to be derived from biphytanols with zero to two cyclopentane rings in the chains, and less than 15% were from phytanol, indicating a strong predominance of tetraether over diether lipids as characteristic for the orders *Sulfolobales* and *Thermoproteales*.

**RNA polymerase.** RNA polymerase was purified in the same manner as other polymerases from extremely thermophilic sulfur archaebacteria. Its component pattern (BAC type; 10, 21) resembles those characteristic of this group (Fig. 5). The larger components B, A, C, and E were identified by immunoblotting with antibodies against the corresponding *Sulfolobus acidocaldarius* subunits. The enzyme was insensitive to 100 μg of rifampin per ml. Optimal reaction conditions were 50 mM KCl and 4 mM $MgCl_2$.

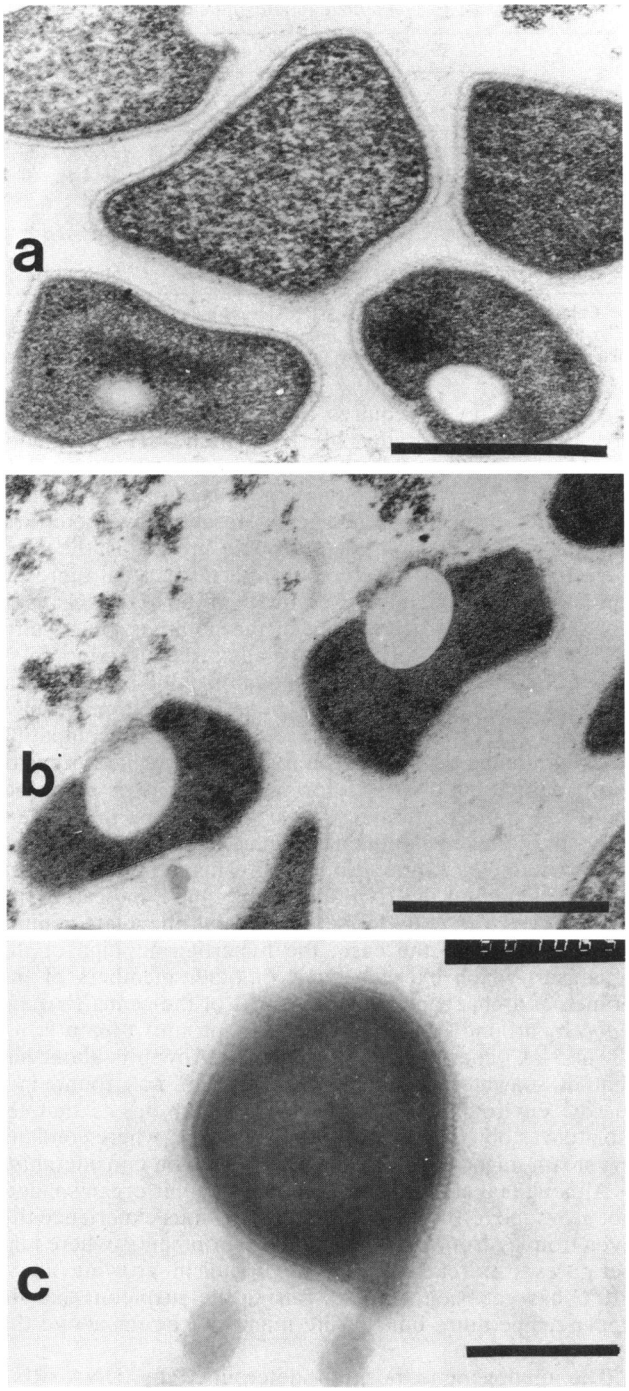


FIG. 3. (a) Thin section of *H. butylicus*. Panels: a and b, embedded in Epon 812, contrasted with lead citrate, uranyl acetate, and lead citrate, exhibiting an S layer and vacuoles; c, cryosection prepared as described by Tokuyasu (16; courtesy of B. Humbel) showing details of the S layer. Bars, 0.33 μm.

Enclosed in a capillary at low ionic strength, the enzyme decayed with a half-life of 8 h at 102°C. At 105°C, the half-life was 3.6 h, and at 108°C, it was 2.6 h.

**Phylogenetic position.** The G+C content of the DNA was 55.6%. A preliminary determination of the phylogenetic position was made by DNA-rRNA cross-hybridizations (6, 20). The resulting tree shows the genus *Hyperthermus* as a particularly long lineage, three times longer than the aerobe *Sulfolobus acidocaldarius*, branching from the *Sulfolobus* lineage above *Thermoproteus tenax*, i.e., in the vicinity of the genera *Pyrodictium* and *Desulfurococcus* (data not shown), which, however, represent particularly short branches (Fig. 6; a distance matrix is shown in Table 1).

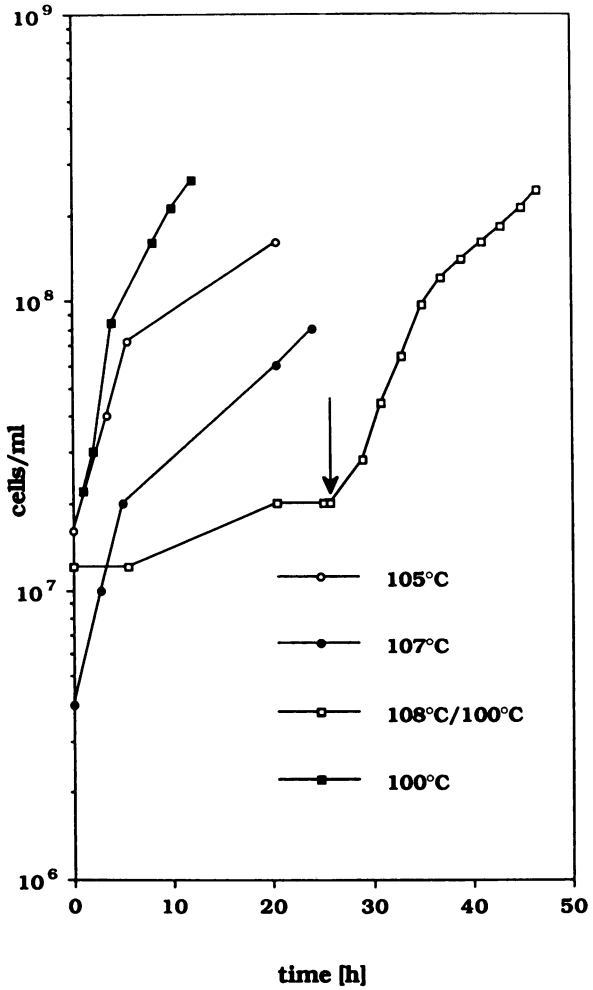


FIG. 4. Growth curves of *H. butylicus* on 0.6 g of tryptone per liter in $S^0$ and $H_2$ at various temperatures. The arrow indicates a temperature shift from 108 to 100°C.

## DISCUSSION

Although growth by fermentation has been discussed for *Desulfurococcus* (24), *Thermodiscus* (11), *Thermococcus* (19), and *Pyrococccus* (2; for a review, see reference 11) spp., *Hyperthermus* sp. is the first archaebacterium for which fermentation products have been identified. They correspond to those of *Clostridium butylicum*, which, however, utilizes glucose rather than a peptide mixture as an energy source. The nature of the products suggests their generation from glycine, alanine, phenylalanine, and tyrosine residues. The inability of the organism to utilize free amino acids could be due to a deficiency in uptake. On the other hand, proteins are also not utilized, indicating the absence of proteases.

$H_2S$ formation from elemental sulfur and molecular hydrogen, and not by sulfur respiration, appears to be an addi-

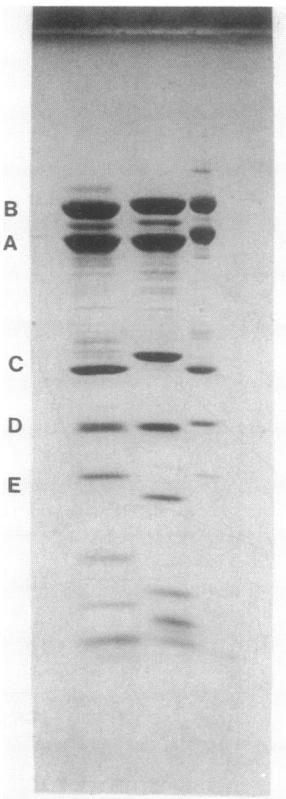


FIG. 5. Component patterns of DNA-dependent RNA polymerases of *H. butylicus* (leftmost lane), *S. acidocaldarius* (center lane) and *T. tenax* (rightmost lane) obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7; 10 to 25% gradient gel as described by Mirault and Scherrer [8]).

tional means for energy generation. $CO_2$ is not utilized as a carbon source. $H_2S$ formation results in increased growth capacity without a major change in the nature of the products, except that it allows utilization of peptide-bound nitrogen. It could thus possibly act as an accessory means to generate ATP (similar to the light-driven proton pump of *Halobacterium halobium*). A similar stimulation of heterotrophic growth by $H_2$ in the presence of sulfur has been observed for *Pyrodictium abyssum* (K. O. Stetter, personal communication). *Pyrococcus woesei*, belonging to a different order, even requires $S^0$ plus $H_2$ but not $CO_2$ for growth on carbohydrates (20). Utilization of $H_2S$ formation as an accessory energy source thus appears to be widespread among extremely thermophilic archaebacteria.

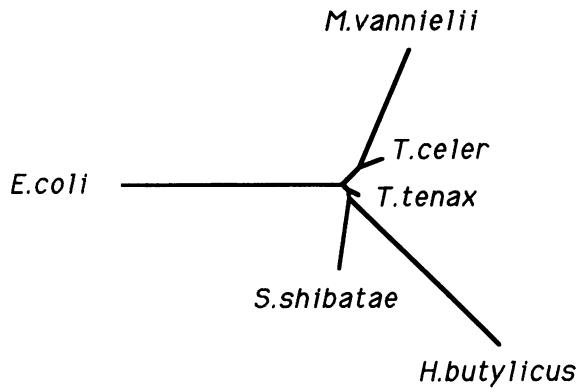


FIG. 6. Unrooted phylogenetic tree constructed from phylogenetic distance values of total rRNAs determined by quantitative cross-hybridization (6).

TABLE 1. Phylogenetic distance matrix[a]

| Species | % Sequence homology or phylogenetic distance[b] | | | | | |
|---|---|---|---|---|---|---|
| | *S. shibatae* | *H. butylicus* | *T. tenax* | *T. celer* | *M. vannielii* | *E. coli* |
| *S. shibatae* | | 34.9 | 11.6 | 17.4 | 27.9 | 34.8 |
| *H. butylicus* | 72.1 | | 26.8 | 33.8 | 46.4 | 61.8 |
| *T. tenax* | 89.2 | 77.5 | | 7.7 | 21.8 | 28.5 |
| *T. celer* | 84.5 | 72.8 | 92.7 | | 18.6 | 32.9 |
| *M. vannielii* | 76.7 | 65.4 | 81.1 | 83.5 | | 44.1 |
| *E. coli* | 72.1 | 57.9 | 76.3 | 73.4 | 66.6 | |

[a] Calculated on the basis of rRNA-DNA cross-hybridization (6).
[b] Lower left, Percent sequence homology (6); upper right, phylogenetic distance calculated as described by Jukes and Cantor (5).

It has often been asked whether fermentative heterotrophic or rather chemolithoautotrophic growth is the more ancestral type of energy metabolism. The fact that representatives of the probably most primitive group of organisms (in the sense of short distance from the putative root), sulfur archaebacteria (18, 22), which are all extreme thermophiles, use $H_2S$ formation for chemolithoautotrophic existence has been taken to indicate that this mode of growth might have preceded heterotrophic existence. This is not incompatible with the finding that this novel hyperthermophile thrives by fermentation and uses $H_2S$ formation only as an accessory device. $H_2S$ formation in *Hyperthermus* sp. might be a remnant of the chemolithoautotrophic machinery as it exists in, for example, *Thermoproteus* sp. This is in line with the extreme length of the *Hyperthermus* branch, indicating that the ability to thrive by fermentation might be a late acquisition. If this were the case, the hyperthermophilia of this organism, which exceeds those of other members of this branch of archaebacteria, except that of the genus *Pyrodictium*, by around 10°C, might not be a primitive feature.

The NaCl concentration optimal for growth is about half that of seawater, although the organism was found in a marine environment. One explanation for this is that the isolate was obtained from within sediments, where condensing steam might lower the salt concentration considerably.

Although taken from a source at 112°C, this organism does not grow above 108°C. This adds to previous experience that even from hydrothermal deep-sea environments, where temperatures can reach 420°C, no organism growing above 110°C has yet been isolated, raising the suspicion that the upper temperature limit of life might not be far above this temperature.

The phylogenetic position determined by DNA-rRNA cross-hybridization (6, 20) is in line with the high content of tetraether lipids, which characterize extremely thermophilic sulfur archaebacteria of the orders *Thermoproteales* and *Sulfolobales*. The S layer (Baumeister et al., in press), however, differs markedly from that of *Desulfurococcus* sp. (17), which has tetragonal symmetry, and from that of *Pyrodictium* sp., which has a different lattice constant and structure (Baumeister, personal communication). The G+C content, 56.5%, is about 4% lower than that of *Pyrodictium* DNA. The polymerase component pattern is characteristic of the major branch of extremely thermophilic sulfur archaebacteria that constitutes the orders *Thermoproteales* and *Sulfolobales* but is also found in the order *Thermococcales*. However, the small fraction of cells that apparently divide

by constriction ("diploforms" characteristic of the order *Thermococcales* [20]) in exponentially growing cultures and the nature of the S layer, which in *Thermococcus* spp. shows interaction of its subunits only inside the membrane (Baumeister, personal communication) support the conclusion drawn from the cross-hybridization experiment that *Hyperthermus* represents a distinct lineage of extremely thermophilic sulfur archaebacteria that branches off between the genera *Thermoproteus* and *Sulfolobus* and apart from the genera *Pyrodictium* and *Desulfurococcus*.

We have named this novel hyperthermophile *Hyperthermus butylicus* because of its ability to grow at temperatures above 100°C, forming the same products as *Clostridium butylicum*, although from a different carbon source. The organism has been deposited at the Deutsche Sammlung von Mikroorganismen (accession no., DSM 5456).

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32**:781–791.
2. **Fiala, G., and K. O. Stetter.** 1986. *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100°C. Arch. Microbiol. **145**:56–61.
3. **Fiala, G., K. O. Stetter, H. W. Jannasch, T. A. Langworthy, and J. Madon.** 1986. *Staphylothermus marinus* sp. nov. represents a novel genus of extremely thermophilic submarine heterotrophic archaebacteria growing up to 98°C. Syst. Appl. Microbiol. **8**:106–113.
4. **Jones, W. L., J. A. Leigh, F. Mayer, C. R. Woese, and R. S. Wolfe.** 1983. *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch. Microbiol. **136**:254–261.
5. **Jukes, T. H., and C. R. Cantor.** 1969. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), Mammalian protein metabolism. Academic Press, Inc., New York.
6. **Klenk, H.-P., B. Haas, V. Schwass, and W. Zillig.** 1986. Hybridization homology: a new parameter for the analysis of phylogenetic relations, demonstrated with the urkingdom of the archaebacteria. J. Mol. Evol. **24**:167–173.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
8. **Mirault, M. E., and K. Scherrer.** 1971. Isolation of preribosomes from HeLa cells and their characterization by electrophoresis on uniform and exponential-gradient-polyacrylamide gels. Eur. J. Biochem. **23**:372–386.
9. **Prangishvili, D., W. Zillig, A. Gierl, L. Biesert, and I. Holz.** 1982. DNA-dependent RNA polymerases of thermoacidophilic archaebacteria. Eur. J. Biochem. **122**:471–477.
10. **Schnabel, R., M. Thomm, R. Gerardy-Schahn, W. Zillig, K. O. Stetter, and J. Huet.** 1983. Structural homology between different archaebacterial DNA-dependent RNA polymerases analyzed by immunological comparison of their components. EMBO J. **2**:751–755.
11. **Stetter, K. O.** 1986. Diversity of extremely thermophilic archaebacteria, p. 39–74. *In* T. D. Brock (ed.), Thermophiles. John Wiley & Sons, Inc., New York.
12. **Stetter, K. O., H. König, and E. Stackebrandt.** 1983. *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulphur reducing archaebacteria growing optimally at 105°C. Syst. Appl. Microbiol. **4**:535–551.
13. **Stetter, K. O., A. Segerer, W. Zillig, G. Huber, G. Fiala, R. Huber, and H. König.** 1986. Extremely thermophilic sulfur-metabolizing archaebacteria. Syst. Appl. Microbiol. **7**:393–397.
14. **Stetter, K. O., and W. Zillig.** 1985. Thermoplasma and the thermophilic sulfur-dependent archaebacteria, p. 85–170. *In* I. C. Gunsalus, J. R. Sokatch, L. N. Ornston, C. R. Woese, and R. S. Wolfe, (ed.), The bacteria, vol. VIII. Academic Press, Inc., New York.
15. **Thurl, S., and W. Schäfer.** 1988. Lipids from the sulphur-dependent archaebacterium *Thermoproteus tenax*. Biochim. Biophys. Acta **961**:253–261.
16. **Tokuyasu, K. T.** 1973. A technique for ultracryotomy of cell suspensions and tissues. J. Cell Biol. **57**:551–565.
17. **Wildhaber, I., U. Santarius, and W. Baumeister.** 1987. Three-dimensional structure of the surface protein of *Desulfurococcus mobilis*. J. Bacteriol. **169**:5563–5568.
18. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51**: 221–271.
19. **Zillig, W., I. Holz, D. Janekovic, W. Schäfer, and W.-D. Reiter.** 1983. The archaebacterium *Thermococcus celer* represents a novel genus within the thermophilic branch of the archaebacteria. Syst. Appl. Microbiol. **4**:88–94.
20. **Zillig, W., I. Holz, H.-P. Klenk, J. Trent, S. Wunderl, D. Janekovic, E. Imsel, and B. Haas.** 1987. *Pyrococcus woesei*, sp. nov., an ultra-thermophilic marine archaebacterium, representing a novel order, *Thermococcales*. Syst. Appl. Microbiol. **9**:62–70.
21. **Zillig, W., H.-P. Klenk, P. Palm, G. Pühler, F. Gropp, R. A. Garrett, and H. Leffers.** 1989. The phylogenetic relations of DNA-dependent RNA polymerases of archaebacteria, eukaryotes, and eubacteria. Can. J. Microbiol. **35**:73–80.
22. **Zillig, W., R. Schnabel, and K. O. Stetter.** 1985. Archaebacteria and the origin of the eukaryotic cytoplasm. Curr. Top. Microbiol. Immunol. **114**:1–18.
23. **Zillig, W., K. O. Stetter, and D. Janekovic.** 1979. DNA-dependent RNA polymerase from the archaebacterium *Sulfolobus acidocaldarius*. Eur. J. Biochem. **96**:597–604.
24. **Zillig, W., K. O. Stetter, D. Prangishvilli, W. Schäfer, S. Wunderl, D. Janekovic, I. Holz, and P. Palm.** 1982. *Desulfurococcaceae*, the second family of the extremely thermophilic, anaerobic, sulfur-respiring *Thermoproteales*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. 1 Abt. Orig. Reihe C **3**:304–317.
25. **Zillig, W., K. O. Stetter, W. Schäfer, D. Janekovic, S. Wunderl, I. Holz, and P. Palm.** 1981. *Thermoproteales*: a novel type of extremely thermoacidophilic anaerobic archaebacteria isolated from Icelandic solfataras. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. 1 Abt. Orig. Reihe C **2**:205–227.
26. **Zillig, W., S. Yeats, I. Holz, A. Böck, M. Rettenberger, F. Gropp, and G. Simon.** 1986. *Desulfurolobus ambivalens* gen. nov., sp. nov., an autotrophic archaebacterium facultatively oxidizing or reducing sulfur. Syst. Appl. Microbiol. **8**:197–203.