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Hyperthermophilic Archaea and Bacteria Occurring within Indonesian Hydrothermal Areas

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Summary

From 85 samples taken during cruise 45B of the R/V SONNE within the Sunda Arc subduction zone and from solfatara fields in Java, thermophilic and hyperthermophilic archaea and bacteria were isolated. The archaea were found to belong to the genera *Methanobacterium, Methanolobus, Methanosarcina, Acidianus, Thermoproteus, Desulfurococcus, Thermoplasma, Thermococcus* and a so far unknown thermoacidophilic continental metal mobilizer. All 7 *Thermoplasma* isolates were found to represent a new genotype so far only found in Indonesia. The extremely thermophilic bacterial isolates are a new species of *Thermotoga* and a novel strict anaerobe, thriving by H_2/NO_3^- autotrophy.

Key words: Archaea – Thermoplasma – Thermophilic – Solfataras – Nitrate.

Introduction

The most extremely thermophilic organisms known up to now belong to the *Archaea* (previously: archaebacteria), the third domain of life besides the *Bacteria* and the *Eucarya* (*Woese* et al., 1978; *Woese* et al., 1990). These hyperthermophiles are the archaeal sulfur metabolizers and some methanogens, growing optimally at temperatures above 80 °C (*Stetter*, 1982; *Stetter*, 1986; *Burggraf* et al., 1990; *Stetter* et al., 1990). *Thermotoga maritima* and the closely related *Thermotoga neapolitana* are the only bacterial hyperthermophilic species known with optimal growth at 80 °C (*Huber* et al., 1986 b; *Jannasch* et al., 1988; *Windberger* et al., 1989). *Thermotoga* represents the deepest phylogenetic branch-off within the bacterial domain (*Huber* et al., 1986 b; *Woese*, 1987; *Woese* et al., 1990). Due to their exceptional position in the evolution of life and their outstanding cellular properties, hyperthermophiles are of great interest for microbiology and biotechnology.

Different hyperthermophilic archaea have been isolated from continental and submarine hydrothermal systems at the Azores, East Pacific Rise, Iceland, Italy, Japan, Polynesia, Yellowstone National Park (USA), and New Zealand (*Brock*, 1978; *Stetter* and *Zillig*, 1985; *Stetter*, 1986; *Stetter* et al., 1987; *Huber* et al., 1990). Since hydrothermal biotopes are widely dispersed and hyperthermophiles are unable to grow at normal environmental temperatures, the question concerning dispersion arises. Although very few systematic studies on the distribution of hyperthermophiles have been carried out up to now, there is evidence for the existence of an endemic group: The *Methanothermaceae* have so far only been found in the southwest of Iceland (*Lauerer* et al., 1986). During the R/V SONNE 45B cruise in Indonesia within marine areas of active volcanism along the Sunda Arc subduction zone and at solfatara fields in Bali and Java, attempts were made to address the following points: (a) the existence of novel hyperthermophilic archaea and bacteria in Indonesia, (b) the distribution of hyperthermophilic archaea within Indonesian hydrothermal systems and solfatara fields, (c) the dissemination of hyperthermophilic archaea between different hydrothermal systems through cold sea water, (d) the survival of hyperthermophiles in cold deep-sea sediments. Here we report on the first results of our screening program.

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Materials and Methods

Strains. Acidianus infernus (DSM 3191), *Pyrococcus furiosus* (DSM 3638), *Thermococcus celer* (DSM 2476), *Thermococcus litoralis* (DSM 5473), *Thermoplasma acidophilum* (DSM 1728), *Thermoplasma volcanium* (DSM 4299), *Thermotoga maritima* (DSM 3109), and *Thermotoga neapolitana* (DSM 4359) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM, Braunschweig-Stockheim, FRG).

Culture conditions. Strictly anaerobic organisms were cultured by following the anaerobic technique described by *Balch* et al. (1979). *Methanobacterium, Methanosarcina* and *Methanolobus* isolates were obtained after enrichment and plating on media described (*Balch* et al., 1979; *König* and *Stetter*, 1982). For plating, the media were solidified by 2% (w/v) agar. If not mentioned otherwise, *Thermococcus celer, Thermococcus litoralis, Pyrococcus furiosus* and *Thermococcus*-like isolates were grown on "SME" medium supplemented with 0.1% (w/v) yeast extract and 2.5% (w/v) S° (*Stetter* et al., 1983). *Thermotoga maritima, Thermotoga neapolitana* and the *Thermotoga*-like isolates were grown as described (*Huber* et al., 1986b; *Windberger* et al., 1989). *Acidianus infernus* and *Acidianus*-like isolates were grown anaerobically at pH 2 in the presence of H_2 and S° or aerobically on S° in Allen's medium (*Allen*, 1959; *Segerer* et al., 1986a). *Sulfolobus*-like isolates and the metal mobilizers were enriched and isolated aerobically at pH 2 in Allen's medium (*Allen*, 1959), supplemented with S° or ore mixture G1 (*Huber* et al., 1986a). *Thermoproteus* and *Desulfurococcus*-like isolates were enriched and grown anaerobically at pH 5.5 in Allen's medium (*Allen*, 1959) supplemented with 0.1% (w/v) yeast extract, 0.1% (w/v) peptone and 2.5% (w/v) S°. *Thermoplasma acidophilum, Thermoplasma volcanium* and the *Thermoplasma* isolates were cultivated, enriched, and cloned by plating as described by *Segerer* et al. (1988).

Light microscopy and ultrastructure. Cultures were inspected by light microscopy as described by *Burggraf* et al. (1990). Electron microscopy was performed as described previously (*König* and *Stetter*, 1982).

DNA preparation, base composition and homology. The DNA was isolated as described by *Lauerer* et al. (1986). The GC-content of the DNA and DNA-DNA homology were determined as described by *Segerer* et al. (1988).

Results and Discussion

Sampling

During cruise SO-45B, 10 anaerobic samples (each 100 ml) of shallow (depth: 30 to 82 m; T = 16 to 20 °C) and deep sea (depth: 1283 to 1743 m; T = 2.9 to 3.7 °C) sediments were taken from box grabs, piston- and box cores at different stations north, east and south of Sumbawa and in the Komodo-Rinja region. Thirteen additional sediment samples were taken by a syringe (*Stetter*, 1982) within the Satonda Crater lake (depth = 0.6 to 2 m; T = 32 °C) and from shallow submarine hot springs (depth: 0 to 2 m; T = 75 to 96 °C) at the beach of Sangeang Island, about 7 km to the north of Kelapa village. The freshly taken samples were collected in 100 ml storage bottles. Oxygen was reduced immediately by the addition of sodium dithionite and sodium sulfide until the redox indicator resazurin added before became colorless (*Stetter*, 1982). The bottles were tightly stoppered and stored at 4 °C. Eight samples of sea water (depth: 14 to 2000 m) were deoxygenated as described for the sediments. In another experiment, 3–5 l of the anaerobic water samples were passed through millipore ultrafilters (pore width: 0.4 µm) in order to concentrate the microorganisms. The filter concentrates were then stored anaerobically in tightly closed 20 ml storage tubes.

During land expeditions, anaerobic and aerobic water and mud samples were taken from the following places (Table 1): Lake Batur, Bali: Toye Bungkah Hot Springs (TB). Dieng Plateau, Java: Kawah Sikidang Crater (KS), Kawah Candradimuka Crater (KC), and Kawah Sileri Hot Lake (SL). Tangkuban Prahu, Bandung, Java: Kawah Domas Crater (KD; altitude: 1500 m above sea level), Kawah Badak Crater (KB), and Kawah Djarian Crater (KDj). Ciater, Bandung, Java: Ciater Hot Springs (C). Ganung Gede, Java: Hot Waterfall (GG). All samples were

Table 1. Samples taken from hot springs and continental solfatara fields in Bali and Java
Abbreviations: TB = Toye Bunkah; KS = Kawah Sikidang; KC = Kawah Candradimuka; SL = Kawah Sileri; KD = Kawah Domas; KB = Kawah Badak; KDj = Kawah Djarian; C = Ciater; GG = Ganung Gede.

Designation		Original pH	Original Temp (°C)
TB	1	7	42
	2	7	39
KS	1	1.5	93
	2	1.5	92
	3*	3	22
	4*	3	92
	5	1.5	50
	6*	2	24
	7*	3	27
	8	3.5	51
	9	7	92
	10*	3	50
	11	3	50
	12	2.5	92
	13	3.5	22
	14*	3.5	22
	15	5	92
	16*	3	30
	17*	2	30
KC	1	7.5	88
	2	6	90
	3	5	60
	4	7.5	80
SL	1*	6.5	55
	2	6.5	62
	3*	6	55
	4*	6.5	40
	5*	3.5	32
	6	5.8	72
	7	3.5	32
KD	1	3	91
	2	2	94
	3	5	67
	4	2	46
	5	2	86
	6*	1.5	32
	7	2	70
	8	4	94
	9	3	50
	10	3	50
	11	3	58
	12	3	93
	13	3	92
KB	1*	1	90
	2	1.5	68
	3	2	94
KDj	1*	3	92
	2	3	93
	3	5.5	92
	4	1.5	92
	5	3.5	94
	6	1.5	90
C	1	3	42
	2	3	43
GG	1	6.5	56
	2	6.5	56

* aerobic sample

carried back to the laboratory by airplane without temperature control. Growth experiments were performed at 20, 37, 60, 85, 100 and 110 °C and at pH 2, 5.5, 7 and 8.5 with various organic and anorganic substrates (e. g. *Fiala* and *Stetter*, 1986).

Archaeal isolates from sea sediments and sea water samples from the Sumbawa and Komodo-Rinja areas

In order to detect hyperthermophiles possibly existing and spreading between zones of active submarine volcanism, water and sediment samples with low ambient temperatures were incubated anaerobically at high temperatures in the presence of substrates suitable for such organisms. No hyperthermophiles could be enriched. Although only relatively few samples were taken, this result suggests that (a) these sediments did not contain significant amounts of hyperthermophiles which survived at low temperatures in the resting state and (b) the surrounding water contains less than 1 cell of hyperthermophiles per 5 liters. Probably much larger quantities of sea water within hydrothermally active areas have to be filtered (e. g. cubicmeters) in order to detect spreading cells of hyperthermophiles.

Archaeal hyperthermophiles in concentrations of up to 10^3/ml sea water have recently been discovered within the open sea plume of an erupting seamount (*Huber* et al., 1990). When samples from the Sumbawa and Komodo-Rinja areas were incubated at 30 °C, mesophilic strictly anaerobic methanogenic archaea were enriched from some sediments and were purified by plating (Table 2). The physiological properties and morphology suggest that these methanogenic isolates belong to the genera *Methanosarcina*, *Methanobacterium*, and *Methanolobus* (*Balch* et al., 1979; *König* and *Stetter*, 1982). *Methanolobus* is an obligate methylotroph which has so far been isolated only from a black marine sediment in Tindari, Sicily (*König* and *Stetter*, 1982). Our results suggest that it may be common in marine sediments. A novel highly irregular ("leach"-shaped) methanogen from Lake Satonda was up to now obtained only in syntrophic mixed culture with Clostridia.

Hyperthermophilic archaeal and bacterial isolates from the shallow submarine hot springs at the beach of Sangeang Island

From sample SG7 (original temp. = 90 °C), a new coccoid strictly anaerobic heterotrophic hyperthermophilic archaeon was isolated under anaerobic growth conditions at 90 °C in artificial sea water (SME; *Stetter* et al., 1983) supplemented with yeast extract (0.1% w/v) and elemental sulfur. The coccoid cells of the isolate measure about 0.5 to 2 μm in diameter, occur mainly in pairs (Fig. 1) and are

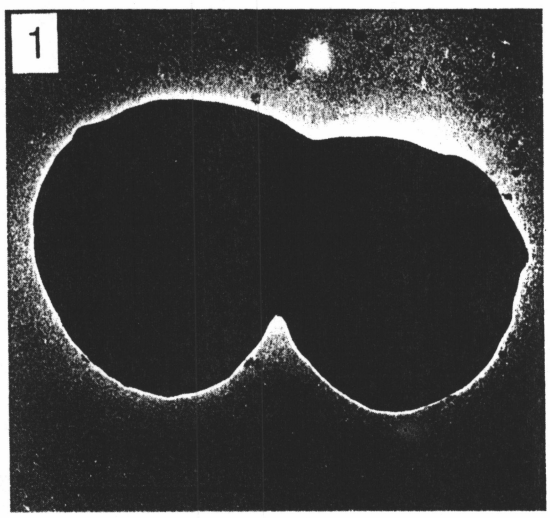

Fig. 1. Dividing cell of the hyperthermophilic archaeal isolate SG7. EM micrograph, negative staining.

Table 2. Mesophilic methanogenic archaea isolated from sea sediments with low original temperatures of the Sumbawa and Komodo Rinja areas

Sample	Station	Water depth (m)	Original temperature (°C)	Description	Isolate
FS-1	101-2	1735	2.9	greyish-green sediment	*Methanosarcina* sp.
FS-6	107-2	82	16	greyish sediment with concretions	*Methanolobus* sp.
FS-7	113-1	45	19	greyish sediment banded hematite	*Methanolobus* sp.
FS-14	Satonda Crater Lake, Stat. 16	0	27	black mud in spring	*Methanolobus* sp.
FS-15	Satonda Crater Lake, embarkation site	2	32	black sediment	*Methanolobus* sp.
FS-15a	Satonda Crater Lake bottom	60	29	black sediment, contains H_2S	*Methanosarcina* sp. enrichment culture: novel irregular leach-shaped methanogen

motile by polar monotrichous flagellation. Complex organic nutrients like yeast extract, peptone, meat extract and tryptone serve as energy source for growth. Elemental sulfur is not essential for growth. However, in the presence of S°, H_2S is formed instead of H_2 (up to 3 µmoles/ml culture medium). The new isolate grows between 46 and 96 °C with an optimum at around 88 °C. Growth occurs in the presence of 0.7 to 8% NaCl at a pH between 5.0 and 8.0. The DNA of isolate SG7 shows a GC-content of 40 mol% and is therefore similar to *Thermococcus litoralis* (38 mol% GC; *Neuner* et al., 1990) and different to *Thermococcus celer* (56 mol% GC; *Zillig* et al., 1983) and *Pyrococcus furiosus* (37 mol% GC; *Fiala* and *Stetter*, 1986). By DNA-DNA hybridization (Table 3) a specific phylogenetic relationship of SG7 with *Thermococcus litoralis* was detected, indicating that the Sangeang isolate belongs to the same species which has so far been found only in Italy (*Neuner* et al., 1990).

Table 3. DNA homology (%) between the new archaeal isolate SG7, *Thermococcus celer*, *Thermococcus litoralis*, and *Pyrococcus furiosus*

Source of filter-bound DNA	% Homology with the following sources of ^{32}P-labeled DNA			
	Tc. celer	*Tc. litoralis*	*Pc. furiosus*	Isolate SG7
Thermococcus celer	(100)	2	6	5
Thermococcus litoralis	6	(100)	2	85
Pyrococcus furiosus	3	4	(100)	7
Isolate SG7	6	88	12	(100)

From samples SG1 and SG7 (both with original temperatures of 90 °C), rod-shaped cells with a characteristic outer sheath could be enriched and isolated anaerobically at 85 °C in *Thermotoga* medium (*Huber* et al., 1986 b). Both isolates turned out to be bacterial hyperthermophiles belonging to the genus *Thermotoga*. They represent a new species as indicated by the low DNA homology with the type strain *Thermotoga maritima* DSM 3109 and with some other still undescribed *Thermotoga* isolates from elsewhere (Table 4). In contrast to the other *Thermotoga* strains, the Indonesian isolates grow in aggregates (up to 15 cells) and can therefore be easily distinguished.

Table 4. DNA homology (%) between *Thermotoga maritima*, *Thermotoga neapolitana*, and some recent *Thermotoga* isolates and isolates SG1 and SG7 from Sangeang

Source of filter-bound DNA	% Homology with the following sources of ^{32}P-labeled DNA	
	Isolate SG1	Isolate SG7
Thermotoga maritima	15	13
Thermotoga neapolitana	55	40
Isolate RQ 2 (Azores)	12	15
Isolate RQ 7 (Azores)	55	38
Sangeang isolate SG1	(100)	82

Methanogenic isolates from the Toye Bungkah hot springs, Bali

From anaerobic samples taken from the black mud of the two ponds at the hot spring of Toye Bungkah, Lake Batur, Bali (TB 1, 2; Table 1), a member of the genus *Methanobacterium* (*Balch* et al., 1979) was isolated from the pond close to the origin of the spring (TB 1). From the second pond (TB 2), a *Methanobacterium* sp. similar to TB 1 and a *Methanosarcina* sp. were isolated. The determination of their exact taxonomic position is in progress.

Hyperthermophilic and thermophilic archaea and bacteria isolated from solfatara fields at the Dieng Plateau and Tangkuban Prahu, Java

In Java, hot springs and solfatara fields at the Dieng Plateau, Tangkuban Prahu, Ciater, and Ganung Gede (Table 1) were investigated for thermophilic anaerobic and aerobic archaea and bacteria (Table 5). At the Dieng Plateau, samples were taken from three different types of solfatara fields: (a) Kawah Sikidang with strongly acidic water- and mudholes of up to boiling temperatures, (b) Kawah Candradimuka with neutral to slightly acidic almost boiling hot springs, and (c) Kawah Sileri with neutral to slightly acidic muddy blackish water with temperatures around 65 °C. Within the Tangkuban Prahu area, samples were collected at three different solfatara fields mainly with strongly acidic pH and very high temperatures: (a) Kawah Domas with many strongly gassed water- and mudholes, (b) Kawah Badak with many fumaroles and very few tiny waterholes, and (c) Kawah Djarian situated within a rain forest with trees decomposing within boiling holes of sulfur mud. In addition, samples were taken from an acidic warm spring in Ciater and from a hot waterfall with neutral pH at Ganung Gede.

No archaeal isolates were obtained from the samples of Ciater and Ganung Gede. From the acidic hot springs and mudholes at the Dieng Plateau and at Tangkuban Prahu, members of the genus *Acidianus* were isolated in Allen's medium (*Allen*, 1959; Table 5). These are chemolithoautotrophic facultative aerobes, growing by oxidation or reduction of elemental sulfur, depending on the redox potential (*Segerer* et al., 1985; *Segerer* et al., 1986 a). All isolates grow at temperatures of up to 96 °C and are by this feature similar to the type species *Acidianus infernus* (isolated in Italy). Again from the acidic hot springs of this area, many *Sulfolobus* enrichment cultures could be obtained aerobically on S° and yeast extract (data not shown), which were not further characterized up to now. From a hot acidic waterhole (KB 1; Table 5) in Kawah Badak, a novel coccoid *Sulfolobus*-shaped (*Brock*, 1978) archaeal metal mobilizer was isolated, suitable for microbial leaching of sulfidic ores at high temperatures. The organism grows chemolithoautotrophically at temperatures up to 80 °C with pyrite, chalcopyrite and sphalerite as energy sources. After 1 week at 80 °C, cell densities of 10^8/ml are obtained in the laboratory, indicating very vig-

Table 5. Hyperthermophilic and thermophilic archaeal and bacterial isolates from hot springs and mudholes in Java

Area	Name of solfatara field	Isolates from samples	Genus	Growth up to (°C)
Dieng Plateau	Kawah Sikidang	KS 1, 2, 12, 15	*Acidianus infernus* (?)	96
		KS 9, 15	*Thermoproteus* sp.	96
		KS 9, 15	*Desulfurococcus* sp.	96
		KS 5, 8, 11	*Thermoplasma* sp.	67
	Kawah Candradimuka	KC 1, 2, 4	*Thermoproteus*	96
		KC 1, 2	*Desulfurococcus*	96
		KC 4	novel unnamed H_2/NO_3^--autotrophic bacterium	80
	Kawah Sileri	SL 7	*Thermoplasma* sp.	67
		SL 3	vibrio-shaped new bacterial metal mobilizer	50
Tangkuban Prahu	Kawah Domas	KD 1, 2, 3, 5	*Acidianus infernus* (?)	96
		KD 3, 5, 7	*Thermoplasma* sp.	67
	Kawah Badak	KB 3	*Acidianus infernus* (?)	96
		KB 1	Coccoid novel archaeal metal mobilizer	80
	Kawah Djarian	KDj 3	*Acidianus infernus* (?)	96
		KDJ 3, 5	*Thermoproteus*	96

orous growth on ores compared to other thermophilic ore leachers (*Huber* et al., 1986 a). A moderately thermophilic so far unknown acidophilic vibrio-shaped ore leaching bacterium was isolated from sample SL3 (table 5). It grows optimally at 45 °C (5 hours doubling time) in the presence of sphalerite and pitch blend or, alternatively, yeast extract (*E. Drobner* and *K. O. Stetter*, unpublished).

The less acidic to neutral hot samples contained anaerobic, sulfur respiring, rod shaped organisms, growing at 96 °C in Allen's medium (*Allen*, 1959) in the presence of yeast extract, peptone and elemental sulfur. Elemental sulfur was replaceable by thiosulfate (0.1% w/v). The rods exhibit true branching and terminal spherical bodies ("golf clubs") which are typical for members of the genus *Thermoproteus* (*Zillig* et al., 1981). In the same enrichment cultures, also coccoid archaea were found, thriving organotrophically by S°-respiration at temperatures up to 96 °C (Table 5). Most likely, they belong to the genus *Desulfurococcus* (*Zillig* et al., 1982).

From locations with strongly acidic pH and moderately hot temperatures (50 °C), cell wall-less (Fig. 2) highly irregular coccoid thermoacidophilic archaea growing up to 67 °C could be enriched in Darland's medium (*Darland* et al., 1970). They were cloned by plating on medium solidified by 10% starch. After 4 days of incubation at 60 °C in the presence of a CO_2/air atmosphere (50 : 50), small (0.2 mm ∅) "friedegg"-shaped colonies became visible (Fig. 3). Very surprisingly, cell extracts of the isolates showed serological cross-reaction with antibodies prepared against the histone-like protein of *Thermoplasma acidophilum* (DSM 1728), indicating a phylogenetic relationship of the isolates to the genus *Thermoplasma* (*Stein* and *Searcy*, 1978). This genus had been described to exist only within smoldering coal refuse piles (*Darland* et al., 1970; *Brock*, 1978).

Similar to other *Thermoplasma* isolates which we had obtained recently from solfataric fields in Italy, Yellowstone National Park, the Azores, and Iceland, the Indonesian isolates are different from the type species *Thermoplasma acidophilum* and exhibit a GC-content of their DNA of 40 mol% (instead of 46 mol% for T.a.; *Segerer* et al., 1988). In DNA-DNA hybridization experiments all 7 Indonesian isolates turned out to be genetically identical (100% DNA homology; data not shown), while they exhi-

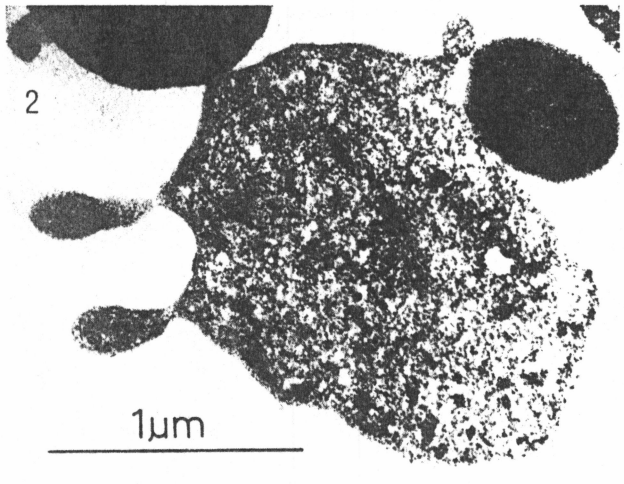

Fig. 2. Cell of the *Thermoplasma* isolate KD3. EM micrograph, ultrathin section.

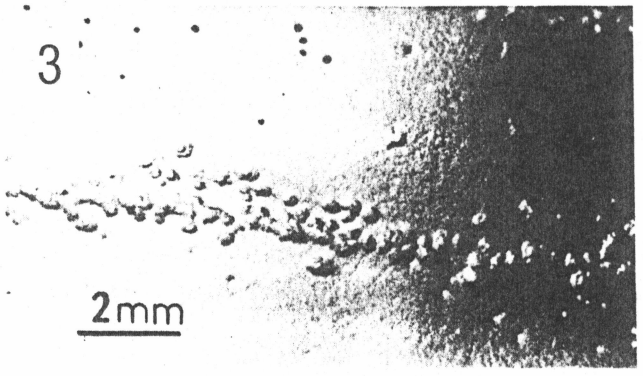

Fig. 3. Colonies of the *Thermoplasma* isolate KD3 on a starch plate.

bited no significant homology (7 to 12%; *Segerer* et al., 1988) with *Thermoplasma acidophilum* and the isolates from the other solfataric fields. Therefore, the Indonesian isolates represent a so far unknown genotype of *Thermoplasma*. Phenotypically, they can be distinguished from the type species *T. acidophilum* also by physiological properties, for example their lower minimal and higher maximal growth temperatures and their higher salt tolerance (Table 6). However, in contrast to their genetic difference, no separating features were found so far between them and some of the *Thermoplasma* isolates from other solfataric fields which had been described as *Thermoplasma volcanium* (Table 6; *Segerer* et al., 1988). Similar to both type strains, the Indonesian *Thermoplasma* isolates are organotrophic facultative anaerobes (*Segerer* et al., 1986 b). The recent isolation of members of *Thermoplasma* in Indonesia and other countries demonstrates, that solfatara fields are the (most likely primary) biotope of this group of archaea (*Segerer* et al., 1988). The ability to grow within the mesophilic temperature range and the isolation of strain SL 7 from a tropical swamp (original temperature: 32 °C) in Kawah Sileri show that tropical swamps and similar biotopes, e. g. self-heating organic waste, may be further biotopes for these acidophilic archaea.

From the hot mud sample KC 4, a novel rod-shaped flagellated Gram-negative bacterium (Fig. 4) was obtained on medium 1 (*Balch* et al., 1979) at 75 °C. The isolate (KC

Table 6. Physiological properties of *Thermoplasma acidophilum* (DSM 1728), *Thermoplasma volcanium* (DSM 4299), and the Indonesian *Thermoplasma* isolate KD3

Strain	Growth temperature (°C)			pH for growth			NaCl requirement (%)			GC content (mol%)
	min	opt	max	min	opt	max	min	opt	max	
Thermoplasma acidophilum DSM 1728	45	59	63	0.8	2	4	0.01	0.25	2	46
Thermoplasma volcanium DSM 4299*	32	59	67	0.8	2	4	0.01	0.25	2	38
Isolate KD3 DSM 4300	32	59	67	0.8	2	4	0.01	0.25	4	40

* isolated from Vulcano, Italy

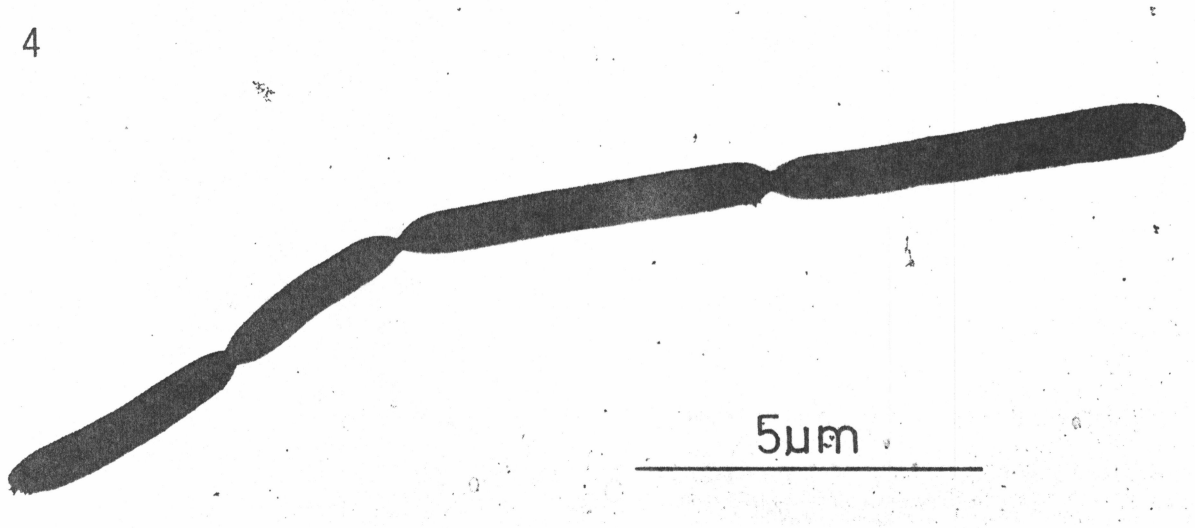

Fig. 4. Cells of isolate KC4. EM micrograph, negative staining.

4; Table 5) is a strictly chemolithoautotrophic anaerobe, growing in mineral medium in the presence of molecular hydrogen, nitrate (0.1% w/v) and CO_2 at neutral pH and temperatures of up to 80 °C. Stoichiometric amounts of ammonia are formed during growth (not shown), indicating that the new organism is gaining energy by the so far unknown H_2/NO_3^- chemolithoautotrophy. This way of nutrition is made possible by the relatively high nitrate content of up to 5 µmoles/l present in this type of hot springs (*G. Liebezeit*, pers. comm.). Batch cultures (50 l) of isolate KC 4 were grown in an enamel-protected fermentor (yield: 0.5 g wet weight/l). Phylogenetically the novel isolate trees deeply among the bacteria and does not seem to belong to any known phylum as indicated by 16S rRNA sequence comparisons (*C. R. Woese*, pers. comm.).

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