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ORIGINAL PAPER

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Cultivation and properties of *Echinamoeba thermarum* n. sp., an extremely thermophilic amoeba thriving in hot springs

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Abstract Here we describe a new, extremely thermophilic amoeba growing between 33 °C and 57 °C ($T_{opt.}$ = 50 °C). Isolates had been obtained from hot springs at Agnano Terme (Italy), Yellowstone National Park (USA), Kamchatka (Russia), and the Arenal Volcano (Costa Rica). They could be cultured monoxenically on a thermophilic alpha-proteobacterium. The morphology of the amoeba was studied using a microscope situated under a heatable polyacrylate hood. At 50 °C, the cells appeared flat with an irregular triangular or elongate shape, sometimes exhibiting fine spine-like subpseudopodia. On average, they were 22 μm long and 11 μm wide and had one nucleus with a central nucleolus. Based on morphology and on SSU rRNA comparisons, the amoeba belonged to the genus *Echinamoeba*, where it represents a new species. Referring to its extremely thermophilic lifestyle and its hydrothermal habitat, we name it *E. thermarum*.

Keywords *Echinamoeba* · Hydrothermal · Monoxenic culture · Protozoa · Thermophilic · 18S rRNA

Introduction

The genus *Echinamoeba* comprises small, naked, uninucleate amoebae, which produce typical finely pointed subpseudopodia (Page 1975). Originally, two species were isolated from non-thermal environments: a lake in Alabama (USA) and leaf litter in England (Page 1967, 1975). Later, isolates of *Echinamoeba* sp. were derived from hot water systems of hospitals (Fields et al. 1989; Rohr et al. 1998). SSU rRNA sequence comparisons show that *Echinamoeba* and the closely related *Hartmannella* belong to a monophyletic group of naked amoebae, the Leptomyxid-Saccamoeba-Hartmannellid sequence clade (Amaral Zettler et al. 2000).

Some amoebae are able to grow at rather high temperatures: several isolates of *Hartmannella* and *Saccamoeba* from the hospital hot water systems were designated to be thermotolerant (Rohr et al. 1998). They were able to multiply at temperatures up to 53 °C, but also at ambient temperature. Some amoebae of the genus *Naegleria* are described to be thermophilic since they are capable of growing at or above 40 °C (De Jonckheere 2002). Many of them are pathogens, e.g., *N. fowleri*, a causative agent for primary amoebic meningoencephalitis, which grows at temperatures up to 45 °C (De Jonckheere 2002). The distribution of possibly pathogenic amoebae in hydrothermal bathing pools in Yellowstone National Park has been studied by Ramaley et al. (2001). At 45 °C, they had isolated only apathogenic strains of *Naegleria* and *Hartmannella* and a further 16 amoebae that they could not classify. As early as 1932, Hindle had found a small amoeba in thermal springs at Dax (France) (Hindle 1932). He was able to keep this isolate in a laboratory culture for about 1 year at 54 °C. Based on the absence of any flagellate stage and its general morphology, he proposed that this amoeba might belong to the genus *Hartmannella*. Kahan (1969) reported on an amoeba isolated from the Tiberias hot springs in Israel that was able to grow at 55 °C and that he preliminary named *Vahlkampfia reichi*.

Here we report on the cultivation, properties, and worldwide distribution of a novel amoeba from hydrothermal environments. It is the first amoeba that grows fastest at temperatures of 50 °C and is unable to grow at ambient temperature. In order to distinguish it from the thermophilic and thermotolerant strains, we define its temperature requirement as "extremely thermophilic"

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(optimal growth above 45 °C, no growth at 25 °C). An isolate obtained from Yellowstone National Park was studied in detail and we refer to it in our description of a new species belonging to the genus *Echinamoeba*.

Materials and methods

Sampling

In Agnano Terme near Naples (Italy), several iron-containing springs arose ranging in temperature from 49 °C to 60 °C. From this site, several samples were taken, e.g., sample AG3 (Table 1). Further sampling was carried out in the Yellowstone National Park (USA), at various sites in Kamchatka (Russia), and at the Arenal Volcano in Costa Rica (Table 1). Sediments, microbial biofilms, and water from hot pools and brooks were collected and transferred into sterile 100-ml storage bottles. The bottles were sealed with rubber stoppers and carried to the laboratory without temperature control.

Cultivation

Amoebae and bacteria were cultivated in a medium containing per liter: $CaSO_4 \times 2H_2O$ 490 mg; $CaCl_2 \times 2H_2O$ 240 mg; $NaHCO_3$ 370 mg; $MgSO_4 \times 7H_2O$ 500 mg; KNO_3 0.16 mg; KCl 8.6 mg; and mineral solution 10 ml. The pH was adjusted to 7 with NaOH. The mineral solution contained per liter: $FeCl_3 \times 6H_2O$ 55.2 g; $ZnSO_4 \times 7H_2O$ 3.5 g; $MnCl_2 \times 4H_2O$ 250 mg; $(NH_4)_2Ni(SO_4)_2 \times 6H_2O$ 200 mg; LiCl 300 mg; $SnCl_2 \times 2H_2O$ 100 mg; and $Na_2MoO_4 \times 2H_2O$ 400 mg. For liquid cultivation, 0.02% (w/v) yeast extract and 0.1% (v/v) glycerol were added. Erlenmeyer flasks (100 ml) were filled with 10 ml medium and sealed with silicone sponge plug closures. In order to obtain plates, the medium was supplemented with 0.06% (w/v) yeast extract and 0.1% (v/v) glycerol and was solidified with 1.8% (w/v) agar. All media were sterilized by autoclaving (200 kPa, 20 min, 121 °C).

Amoebae were cultured monoxenically by feeding on one single bacteria strain (usually on the strain OSrt; see Results). The organic compounds of the medium served as substrates for the bacteria. For cultivation on agar plates, a lawn of food bacteria was grown overnight at 50 °C. The next day, the lawn was inoculated with a loopful of amoebae from liquid culture or an already existing plate. Upon further incubation at 50 °C, the amoebae grazed on the bacteria lawn in a typical manner: They moved out radially from the inoculation point and formed a ring (Fig. 1). When the amoebae had grazed almost the whole plate, it was stored at room temperature. Subculturing was done weekly. During incubation, all plates were placed into a sealable polyacrylate cylinder to prevent drying. For growth in liquid culture, a loopful of amoebae (about $1–5 \times 10^6$ cells) with concomitant OSrt bacteria was scraped off a plate and transferred into the medium, which was incubated at 50 °C in a water bath shaker (60 rpm). Bacteria were proliferating and served as food for the amoebae.

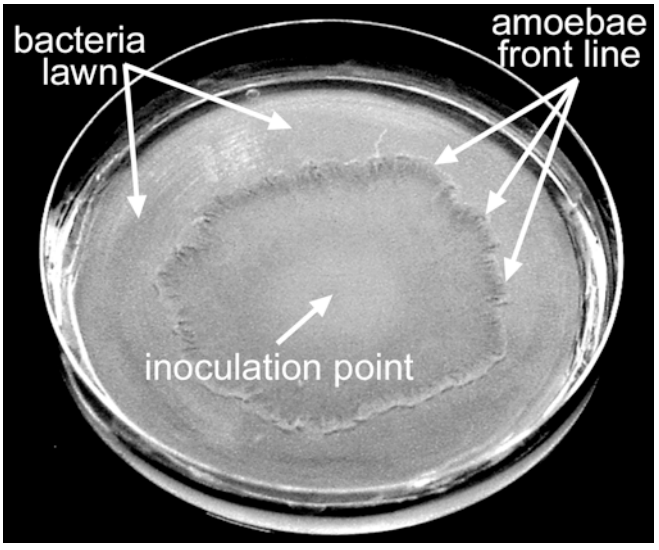

Fig. 1 *Echinamoeba thermarum* OSB1 on agar plates grazing OSrt bacteria. The plate was inoculated with about 1×10^5 amoebae and incubated at 50 °C for 1 day

Determination of growth requirements

Optimal and upper growth temperatures of the amoebae were determined in liquid culture. The media were inoculated with about 5×10^5 amoebae/ml and about 1×10^7 bacteria/ml. The high initial bacteria concentration and their fast growth ensured permanent food supply of the amoebae during their logarithmic growth phase. Growth curves were established at various temperatures by counting the amoebae in a Thoma counting chamber (Brand, Wertheim, Germany; depth 100 μm). In addition, plates with lawns of pre-grown bacteria were inoculated with 1×10^5 amoebae and incubated at different temperatures. The diameter of the grazed area was measured at regular intervals. The lowest growth temperature was assayed on plates only qualitatively.

Table 1 Description of sampling sites yielding *Echinamoeba thermarum* isolates

Sample	Origin	Max. temp.	pH	Description
AG3	Agnano Terme, Italy	60 °C	7.0	Rusty sediments and floating mats of bacteria from an artificial thermal pool
Ar1	Arenal Volcano, Costa Rica	55 °C	6.5	Sandy sediments near a hot brook; the water contains large amounts of iron
GV9	Valley of Geysers, Kamchatka, Russia	80 °C	6.5	Hot sand at the banks of a little river containing gray, fluffy organic mats
KV2	Karymsky Volcano, Kamchatka, Russia	85 °C	6.5	Iron-containing mud nearby a brook situated in the crater
MV2	Mutnovsky Volcano, Kamchatka, Russia	61 °C	6.5	Orange and green biofilms from an artificial brook running through a camp
OSB1	Yellowstone National Park, USA	60 °C	6.0	Especially hot site in a thermal brook that is formed by the outflow of Octopus Spring
P3	Puchino hot springs, Kamchatka, Russia	39 °C	8.0	Black sludge from a carbonate- and boron-containing spring

In order to study salt tolerance and the pH range of growth, agar plates were supplemented with 0.5%, 1%, and 1.5% (w/v) NaCl or buffered with 50 mM BisTris/HCl (pH 5–7), 50 mM Tris/HCl (pH 7–9), or CAPS/NaOH (pH 9 and 10), respectively. Since the plates contained no organic substrate, food bacteria were grown separately. After harvesting by centrifugation (about 4 ml of liquid culture), they were spread onto the agar surface before amoebae were inoculated.

Microscopy

The morphology of moving amoebae attached to glass surfaces was studied at 50 °C using phase contrast optics with a Zeiss Axiovert Inverse Microscope, which was situated under a heatable polyacrylate hood with temperature control (25–70 °C; ± 0.3 °C; Fig. 2; Horn et al. 1999; Baumgartner et al. 2002). To avoid drying, the amoebae were kept in a tightly sealed observation chamber (Fig. 2) in which they survived up to 2 days. Length and breadth dimensions of 100 actively moving cells and the diameter of 100 cysts were determined. For interference contrast photographs, amoebae were allowed to attach to a cover slip at 50 °C and fixed by flooding with an aqueous solution of 4% formaldehyde and 2% glutaraldehyde.

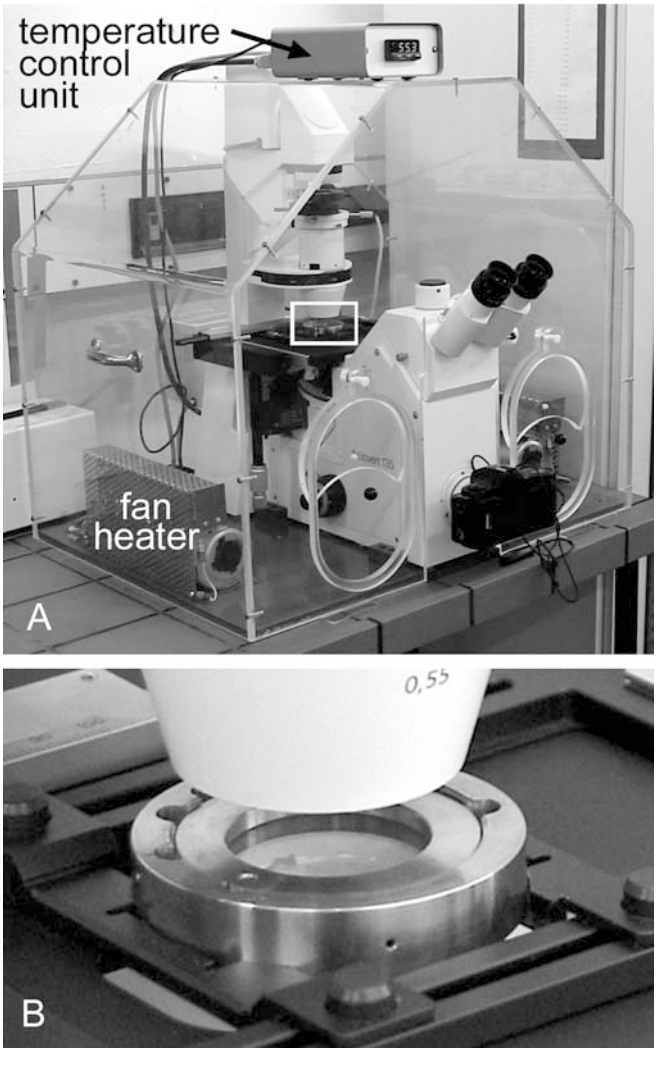

Fig. 2 **A** Microscope under a heatable polyacrylate hood. **B** Observation chamber (magnification of the white square)

DNA extraction and phylogenetic analysis

Extraction of genomic DNA and amplification of the SSU rRNA gene with eukaryotic-specific primers (Medlin et al. 1988) were carried out as described by Baumgartner et al. (2002). The PCR products covered almost the whole gene and were about 1,850 nucleotides long. For cloning the CloneAmp pAMP System cloning kit (Life Technologies, Gaithersburg, Md., USA) was used. The inserts of several clones were partly sequenced. The insert of one clone representing one sequence type was sequenced completely in both orientations. An alignment of a subset of eukaryotic SSU rRNA gene sequences that encompasses several amoebae of the Leptomyxid-Saccamoeba-Hartmannellid sequence clade was kindly provided by Linda Amaral Zettler (Marine Biological Laboratory, Woods Hole, Mass., USA; Amaral Zettler et al. 2000). The new SSU rRNA sequences were fit into this alignment. The final dataset for tree reconstruction comprised 23 taxa and 1,377 unambiguously aligned positions. Parsimony, distance matrix (minimum evolution algorithm), and maximum likelihood analyses were computed with PAUP* (Swofford 1999). As described by Amaral Zettler et al. (2000), Modeltest (Posada and Crandall 1998) was run to decide which evolutionary model best fit the data. The Tamura-Nei model was selected, with the proportion of invariant sites incorporated into the model and the gamma shape parameter for among-site variation calculated from the dataset. This model was invoked for inferring trees using the distance and the likelihood criterion. The data were bootstrap resampled 100 times.

Results

Enrichment

The first amoebic enrichment culture was obtained from sample AG3 in liquid medium without added mineral solution. After 3 days of incubation at 50 °C, amoeboid cells that were feeding on concomitantly growing bacteria were observed. However, reproducible growth was initially achieved only by addition of autoclaved ochre-colored sediment from Agnano Terme to the medium. Based on chemical analyses of this sediment (data not shown), a mineral solution was assembled that turned out to replace the natural sediment. Enrichment cultures of similar looking amoebae could be obtained from samples Ar1, GV9, KV2, MV2, OSB1, and P3 (Table 1) on the same culture medium.

Establishment of monoxenic cultures and single-cell cloning

In order to find suitable food bacteria, dilutions of enrichment cultures AG3 and OSB1 were plated on solidified medium. After incubation at 50 °C for 4 days, various types of colonies became visible. In food bacteria assays, these colonies served as starting cultures for bacteria lawns on agar plates. Fastest growth of the amoebae (indicated by most rapid grazing) was obtained on bacterial isolate OSrt that had originated from the OSB1 enrichment culture. This bacterial isolate formed pink colonies on plates. The cells were short rods about 1.5 μm long and 0.8 μm in width and were often found in pairs. Due to SSU rRNA comparison (EMBL accession no. AJ489269), the food bacterium OSrt

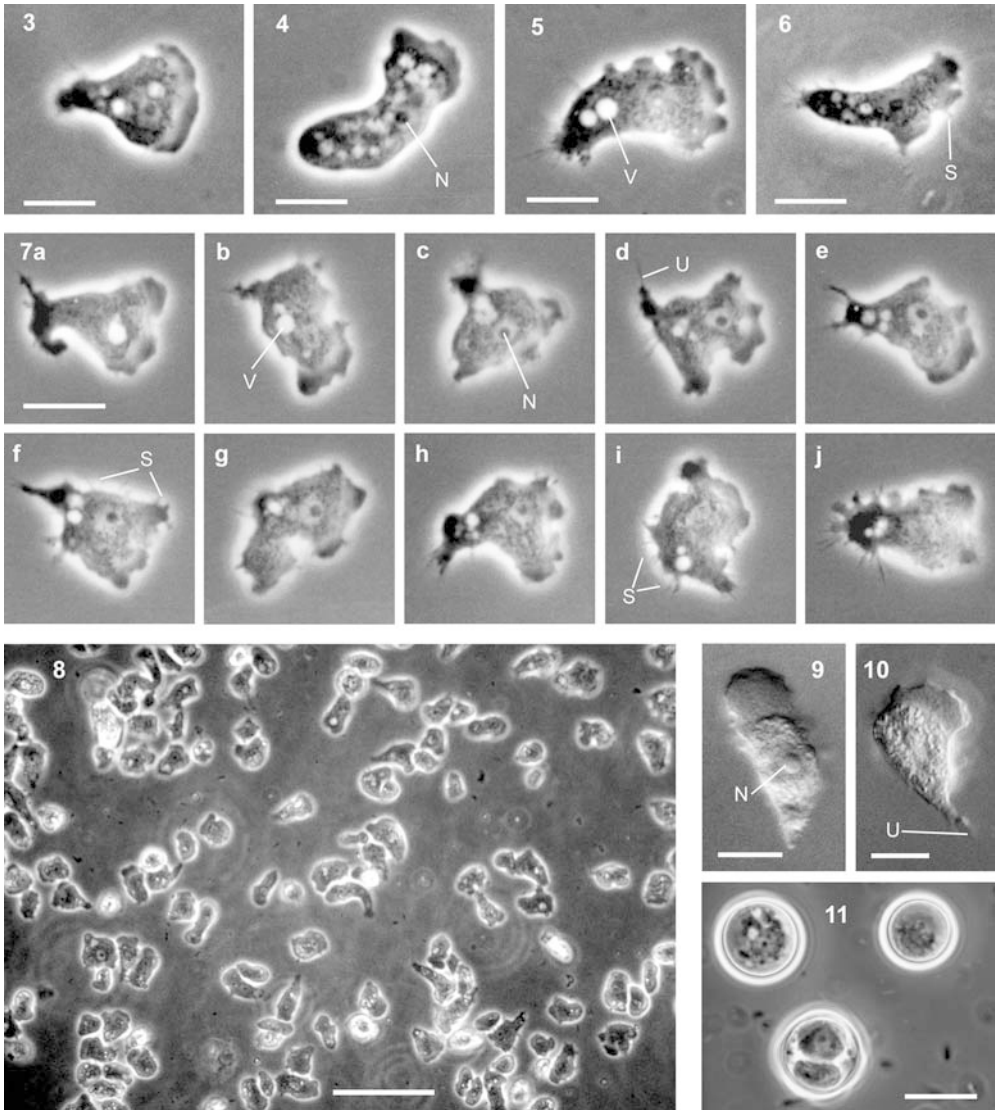

Fig. 3 Triangular *E. thermarum* cell (50 °C, phase contrast optics). Scale bar: 10 μm
Fig. 4 Elongated *E. thermarum* cell (50 °C, phase contrast optics). Scale bar: 10 μm. *N* nucleus
Fig. 5 *E. thermarum* cell with irregular hyaloplasm (50 °C, phase contrast optics). Scale bar: 10 μm. *V* vacuole
Fig. 6 *E. thermarum* cell with subpseudopodia (50 °C, phase contrast optics). Scale bar: 10 μm. *S* subpseudopodia
Fig. 7 *E. thermarum* cell in movement (50 °C, phase contrast optics). Time elapsed between two pictures about 5 s. Scale bar: 10 μm. *N* nucleus, *S* subpseudopodia, *U* uroidal filaments, *V* vacuole
Fig. 8 Active *E. thermarum* cells in overview (50 °C, phase contrast optics). Scale bar: 50 μm
Fig. 9 Elongated *E. thermarum* cell with extensive hyaline zone (cell fixed at 50 °C during locomotion, interference contrast optics). Scale bar: 10 μm. *N* nucleus
Fig. 10 *E. thermarum* cell trailing uroidal filaments (cell fixed at 50 °C during locomotion, interference contrast optics). Scale bar: 10 μm. *U* uroidal filaments
Fig. 11 Cysts of *E. thermarum* (phase contrast optics). Scale bar: 10 μm

belonged to the *Rhodobacter* group of the alpha-proteobacteria, where it represents a new species (data not shown). It was strictly aerobic and multiplied in a temperature range of 25–55 °C. The new strain tolerated salt concentrations up to 3% NaCl and grew from pH 6 to 8.

Amoebae did not feed on bacteria (strain OSrt) killed by heat (5 min at 100 °C) or by exposure to ethanol (70% v/v) for 1 h.

To obtain clonal amoebic cultures, singe cells of enrichment cultures OSB1 and AG3 were separated using optical tweezers (Ashkin et al. 1987; Huber et al. 1995). All other strains were cloned by serial dilutions. These clonal amoeba strains were all cultured monoxenically on food bacterium OSrt and were used for all further studies.

Morphology

At ambient temperatures, the amoeba cells (isolate OSB1) were rounded (about 9 μm in diameter) and free floating. At 50 °C using the heatable microscope (Fig. 2), the amoebae attached to surfaces and were actively moving. Active cells of isolate OSB1 were monopodial and flattened (Figs. 3, 4, 5, 6, 7, 8, 9, 10). They were variable in shape (Figs. 7, 8) but often displayed a triangular or elongated outline (Figs. 3, 4, 5, 6, 7a,j). The length of the amoebae ranged from 15 to 35 μm (mean 21.5 μm), and their breadth ranged from 8 to 16 μm (mean 11.2 μm). The length:breadth ratio varied from 1 to 4. At the anterior end, a hyaline zone was present with an irregular, often asymmetrical outline (Figs. 3, 4, 5, 6, 9, 10). During slow locomotion, finely pointed subpseudopodia were produced from the hyaline zone (Figs. 6, 7f,i). They were carried back along the sides of the amoebae (Fig. 7d,g). Sometimes these pseudopodia became uroidal filaments that were trailed by the posterior end (Figs. 7d, 10). Subpseudopodia could not be observed during fast locomotion. The amoebae had one vesicular nucleus with a central nucleolus (Fig. 4, 7c, 9). Several vacuoles could be observed that appeared to contain no particulate matter, while food vacuoles were inconspicuous (Figs. 5, 7b).

The amoebic isolates from Italy, Costa Rica, and Russia looked very similar. Their morphology was not studied in detail, however. Shortly after isolation, all amoeba strains lost their ability to encyst. Cysts were observed in high numbers in the enrichment culture of the isolate P3. The cysts were almost perfect spheres with a smooth surface (Fig. 11). The cyst diameter ranged from 5.8 μm to 12 μm (mean 8 μm).

Physiological characterization

Growth at different temperatures

In liquid culture, fastest growth of isolate OSB1 was observed at 50 °C, with a doubling time of about 10 h (Fig. 12). In accordance, bacteria lawns on agar plates were grazed the fastest at 50 °C, namely, within 2 days (Table 2). At 30 °C, no growth occurred, indicating that the new organism is a strict thermophile. At 33 °C, after 2 days of incubation, a grazed area of 14 mm in diameter was visible that was slowly increasing. At that temperature amoebae were transferred successfully several times. On agar plates and in liquid culture, isolate OSB1 grew at 55 °C but not at 60 °C.

The other isolates showed a similar temperature optimum concerning their grazing speed on bacteria lawns (Table 3). However, isolates Ar1 and KV2 exhibited an upper temperature limit of 57 °C. None of the isolates was able to proliferate at 60 °C or at room temperature.

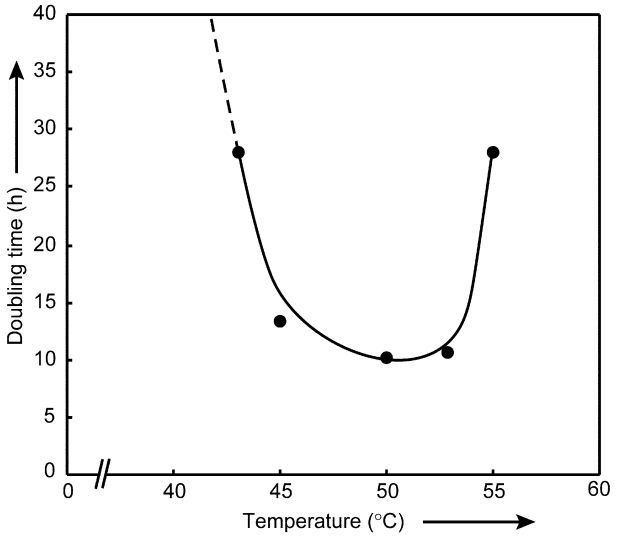

Fig. 12 Effect of temperature on doubling time of *E. thermarum* OSB1 in liquid culture

Table 2 Grazing velocity as a measure of growth of *E. thermarum* OSB1 on bacteria lawns at various temperatures: diameter (in mm) of the grazed area after different times of incubation. *X* The lawn (86 mm) was completely grazed

Days	33 °C	37 °C	45 °C	50 °C	55 °C
1	0	0	32	40	20
2	14	21	74	X	42
3	20	31	X		47
5	24	48			70
7	24	70			X

Behavior above the upper growth temperature

Amoebae of strain OSB1 that had been cultivated at 55 °C started to move more sluggishly when heated up to 60 °C in the heatable microscope. After 15 min, the first cells detached from the glass surface and acquired a spherical shape. After 60 min, all cells had lost their typical flattened locomotive form but became spherical with a granulated gray appearance when observed by phase contrast microscopy. These amoebae could not be transferred successfully.

Storage

Amoebae (trophozoites of isolate OSB1) grown on agar plates could be transferred successfully after storage at 4 °C for at least 2 weeks and at 10 °C and 20 °C for at least 3 weeks. Cysts of isolate P3 stored at room temperature remained viable at least for 3 months. Amoebae frozen in medium containing 10% DMSO and stored below −140 °C could be successfully revived after 4 years.

Salt tolerance

Amoebae (strain OSB1) proliferated on plates additionally containing up to 1% NaCl. For growth at 1% NaCl, inoculum-serving pre-cultures had to be grown at least at 0.5% NaCl. No growth occurred on plates with 1.5% NaCl, even after pre-adaptation at 1% NaCl.

Influence of pH

Amoebae (strain OSB1) propagated fast in the pH-range of pH 7–9. At pH 6 the amoebae grazed the bacteria

Table 3 Grazing velocity as a measure of growth of *E. thermarum* isolates on bacteria lawns: diameter (in mm) of grazed area after 1 and 2 days of incubation at 40 °C and 50 °C. *X* The lawn (86 mm) was completely grazed

Sample	1 day		2 days	
	40 °C	50 °C	40 °C	50 °C
P3	22	40	51	X
AG3	8	38	34	X
GV9	12	29	19	52
MV2	17	54	28	X
KV2	22	40	32	75
Ar1	23	59	43	X

lawn only slowly and not at all at pH 5 and 10. Amoebae grown at pH 7 lysed within 30 min when suspended in medium with pH 5. In contrast, amoebae survived suspension in a medium with pH 10.

SSU rRNA Phylogeny

In amoeba OSB1 three copies of the SSU rRNA gene could be detected (EMBL accession no. AJ489261–AJ489263) that differed in up to 2.6% of the nucleotides (Table 4). More than 80% of the variations were located in the eukaryotic-specific variable areas, mainly in area V4 (see Wuyst et al. 2000 for numbering system). Partial sequences obtained from further isolates also indicated the presence of multiple copies of the SSU rRNA gene. So far, however, only one copy of the SSU rRNA gene per isolate was completely sequenced (EMBL accession no. AJ489264–AJ489268). A comparison of the sequences revealed a relationship of these amoeba strains at the species level (Table 4). In view of the presence of multiple SSU rRNA genes in the same isolate, attempts to obtain higher resolution by this method do not appear to be meaningful at present.

The phylogenetic position of amoeba OSB1 within the eukaryotic domain was inferred using the consensus sequence of the three different copies of the SSU rRNA gene. Amoeba OSB1 was specifically related to *Echinamoeba exundans* (Fig. 13) but exhibited a distinct evolutionary distance (Table 4). These two sequences and the one of *Hartmannella vermiformis* formed a consistent group within the Leptomyxid-Saccamoeba-Hartmannellid clade in all analyses performed (Fig. 13).

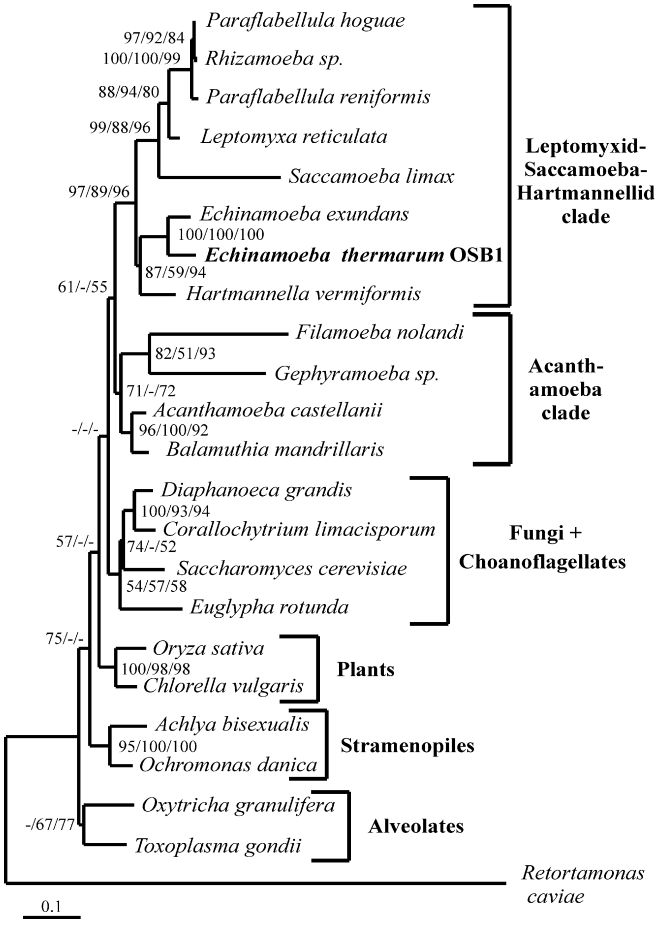

Fig. 13 Maximum likelihood tree based on small subunit ribosomal RNA sequences indicating the phylogenetic position of *E. thermarum* OSB1. In this analysis 1,377 unambiguously aligned positions were used. The distance bootstrap percentage is followed by the parsimony and likelihood bootstrap values. *Dashes* indicate bootstrap values below 50%. The scale bar corresponds to expected substitutions per 100 nucleotides

Discussion

The amoeba OSB1 possesses general features of the Gymnamoebia (Page 1988; Page and Siemensma 1991) such as the absence of a flagellate stage and tubular mitochondrial cristae (C. Fredriksson, M. Baumgartner and R. Rachel, unpublished data). It exhibits all morphological features that define the members of the genus *Echinamoeba* (Page 1975): Cells are flattened, often triangular or elongate. They have a single nucleus with a central nucleolus and produce the typical fine subpseudopodia. Therefore, we classify the extremely thermophilic amoebae in the genus *Echinamoeba*. The isolate OSB1 differs from the two known species *E. exundans* and *E. silvestris* by being distinctly larger, with an average length of 21.5 μm compared to 14 μm and 11 μm, respectively (Page 1975). Furthermore, the subpseudopodia are not

Table 4 Uncorrected pairwise distances (changes per 100 nucleotides) between small subunit ribosomal RNA sequences of *E. thermarum* isolates and *E. exundans*

E. thermarum isolate and clone number	OSB1 clone 4	OSB1 clone 5	OSB1 clone 9	AG3 clone 5	KV2 clone 2	Ar1 clone 5	GV9 clone 2	MV2 clone 4
OSB1 clone 5	2.6	–						
OSB1 clone 9	2.5	0.9	–					
AG3 clone 5	4.1	2.3	2.6	–				
KV2 clone 2	3.7	2.4	2.1	1.0	–			
Ar1 clone 5	4.0	2.3	2.4	0.7	0.7	–		
GV9 clone 2	4.1	1.7	2.3	1.6	1.1	1.6	–	
MV2 clone 4	1.3	3.1	3.1	3.5	3.8	3.5	4.0	–
E. exundans	14.9	15.1	14.7	15.7	14.8	15.5	15.6	15.6

as apparent. The cysts (only observed in isolate P3) have about the same size as those of *E. silvestris*, whereas those of *E. exundans* are clearly smaller (Page 1988). Like the new extremely thermophilic isolates, *E. silvestris* has only a reduced ability to form cysts in culture (Page 1988).

The phylogenetic position of the extremely thermophilic amoebae in SSU rRNA sequence comparisons is in accordance with morphological data: *E. exundans* is their next but distinctly separate relative. A clonal culture of amoeba OSB1 harbors at least three different copies of the SSU rRNA gene. Heterogeneity of rRNA gene copies has already been reported for foraminifera (Holzmann et al. 1996) and parasitic protists (Gunderson et al. 1987; Xiao et al. 1999).

The most obvious difference between the new amoebae and the other *Echinamoeba* species is their much higher growth temperature. They are even unable to grow or move at ambient temperatures, where *E. exundans* and *E. silvestris* usually thrive. The original strains of the latter were cultured at room temperature and the morphological observations were carried out at 23 °C (Page 1967, 1975). Even isolates that were derived from hospital hot water systems proliferated at 30 °C and below and not at 44 °C (Fields et al. 1989; Rohr et al. 1998). Based on their bigger size, their distinctly different SSU rRNA sequences, and their obligately thermophilic lifestyle, our isolates represent a new species in the genus *Echinamoeba*, which we name *E. thermarum* n. sp.

For monoxenic cultivation and physiological characterization of *E. thermarum*, the isolation of the food bacterium OSrt was important in order to obtain constant growth conditions. *E. thermarum* strain OSB1 did not grow under marine salt conditions or at low pH values. Therefore, it appears to be adapted to neutral terrestrial low salinity hot springs, from where we had isolated it. As shown in this study, *E. thermarum* occurs in hot springs in North and Middle America, Asia, and Europe. In the Agnano Terme area in a more detailed study, we could find it in a great number of hot springs (data not shown). We guess that some of the amoebae from Yellowstone National Park observed by Ramaley et al. (2001) and the isolates of Hindle (1932) and Kahan (1969) may have belonged to this species as well. Most likely *E. thermarum* is distributed worldwide in freshwater hydrothermal areas with neutral pH. Some of the temperatures measured at the sampling sites were above the upper growth temperature observed for *E. thermarum* in the laboratory. This discrepancy reflects local temperature inhomogeneities at the sampling sites rather than an expanded temperature tolerance of the organisms in nature.

E. thermarum is one of the most thermophilic eukaryotes. Only this amoeba and thermophilic fungi such as *Thermomyces lanuginosus* and *Thermoascus aurantiacus* are optimally adapted for living at 50 °C (Tansey and Brock 1978). Due to its close relationship to mesophilic members of the same genus, the extreme thermophily of *E. thermarum* appears to be a secondary adaptation to the hot environment. In view of its pronounced thermostability and its close relationship to mesophiles, *E. thermarum* appears as an ideal organism to study heat adaptation of eukaryotes.

Description of a new species

Echinamoeba thermarum n. sp.

Baumgartner, Yapi, Gröbner-Ferreira & Stetter

Diagnosis: Small free-living amoebae with morphological characteristics of the genus; during locomotion often irregular triangular or elongate, always flattened; typical fine subpseudopodia not always visible; length 15–35 μm (average 22 μm); width 8–16 μm (average 11 μm); cyst diameter 5.8–12 μm (average 8 μm); encystation only rare in culture. Extremely thermophilic; no growth and no typical movement at ambient temperature; fastest growth around 50 °C.

Type locality: Hot brook formed by the outflow of Octopus Spring, Yellowstone National Park, USA.

Type material: Slides with hematoxylin-stained specimens of isolate OSB1 are deposited in the Oberösterreichische Landesmuseum in Linz (accession nos. 2002/940 and 2002/941). Living material of isolate OSB1 is conserved at the American Type Culture Collection (ATCC) as strain PRA-13.

Etymology: Thermae, lat. "hot spring", referring to the typical habitat.

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