Extremophiles (2009) 13:521–531
DOI 10.1007/s00792-009-0239-1

ORIGINAL PAPER

Tolerance of thermophilic and hyperthermophilic microorganisms to desiccation

Kristina Beblo · Elke Rabbow · Reinhard Rachel · Harald Huber · Petra Rettberg

Received: 20 January 2009 / Accepted: 16 March 2009 / Published online: 31 March 2009

Abstract We examined short- and long-term desiccation tolerance of 31 strains of thermophilic and hyperthermophilic Archaea and thermophilic phylogenetically deep-branching Bacteria. Seventeen organisms showed a significant high ability to withstand desiccation. The desiccation tolerance turned out to be species-specific and was influenced by several parameters such as storage temperature, pH, substrate or presence of oxygen. All organisms showed a higher survival rate at low storage temperatures (−20°C or below) than at room temperature. Anaerobic and microaerophilic strains are influenced negatively in their survival by the presence of oxygen during desiccation and storage. The desiccation tolerance of Sulfolobales strains is co-influenced by the pH and the substrate of the pre-culture. The distribution of desiccation tolerance in the phylogenetic tree of life is not domain specific. Surprisingly, there are dramatic differences in desiccation tolerance among organisms from the same order and even from closely related strains of the same genus. Our results show that tolerance of vegetative cells to desiccation is a common phenomenon of thermophilic and hyperthermophilic microorganisms although they originated from quite different non-arid habitats like boiling acidic springs or black smoker chimneys.

Keywords Desiccation · Tolerance · Survival · Hyperthermophilic · Archaea

Communicated by L. Huang.

K. Beblo (✉) · E. Rabbow · P. Rettberg
Radiation Biology Division,
German Aerospace Center (DLR e.V.),
Institute of Aerospace Medicine,
Linder Hoehe, 51147 Cologne, Germany
e-mail: kristina.beblo@dlr.de

K. Beblo · H. Huber
Faculty of Natural Sciences III,
Institute for Microbiology and Archaea Center,
University of Regensburg, Regensburg, Germany

R. Rachel
Faculty of Natural Sciences III, Center for Electron Microscopy,
University of Regensburg, Regensburg, Germany

Introduction

The present-day habitats of many thermophilic and hyperthermophilic microorganisms resemble what we assume to have been the living conditions on early Earth. Ocean temperatures went up to 100°C (Nisbet and Sleep 2001), while the atmosphere contained no oxygen (Chyba 2005). It is therefore not surprising that hypotheses were developed to the effect that thermophilic or hyperthermophilic microorganisms (Stetter 1996; Di Giulio 2000) were the first forms of life on Earth. However, whether life originated on planet Earth or came from elsewhere is another question, which is now being discussed in the scientific community. There is a possibility that Earth was infected by organisms attached to meteorites (Nicholson et al. 2000; Horneck et al. 2001). An exchange of meteorite material between intersolar planets is now regarded as certain (Melosh 2003). Organisms carried by meteorites would have had to survive a long journey through space, one of the harshest known environments an organism could ever encounter (Nicholson et al. 2000). The prevailing vacuum and the resulting absence of water is a huge problem for living organisms. For a long time, it was assumed that only spores could survive desiccation on Earth (Gest and Mandelstam 1987) and in space (Horneck et al. 1994).

With the discovery and analysis of *Deinococcus radiodurans*, the first desiccation experiments with vegetative cells were initiated (Anderson et al. 1956). Desiccation experiments using Archaea have been reported only for methanogenic (Kendrick and Kral 2006; Morozova and Wagner 2007) and halophilic strains (Kottemann et al. 2005). Their tolerance to desiccation and radiation is often compared with that of vegetative *D. radiodurans* cells (Mattimore and Battista 1996). In this study, we investigated vegetative cells from a wide range of thermophilic and hyperthermophilic organisms and their ability to survive short- and long-term desiccation. We also present an overview on the distribution of desiccation tolerance in all phylogenetically deep branching thermophilic/hyperthermophilic Archaea and thermophilic Bacteria.

Materials and methods

Strains and culture conditions

The microorganisms used and their culture conditions are summarized in Table 1.

Desiccation experiments

Cell concentrations were determined by use of a Thoma chamber (depth 0.02 mm).

Approximately 10^7 cells from the late exponential or early stationary phase were spread evenly on glass slides (10 × 26 × 1 mm) (Marienfeld GmbH & Co. KG, Lauda-Koenigshofen, Germany) or on glass fiber filters (MN 85/90, Ø 11 cm, Macherey-Nagel GmbH, Dueren, Germany) and dried 6–8 h under oxic conditions at room temperature (relative humidity of the atmosphere 33 ± 3.5%). After drying, the slides of glass/pieces of filters were either stored under oxic conditions at room temperature, at −20°C or at −70°C. Pre-desiccated cells on glass slides were stored partly in an exsiccator over silica-gel (relative humidity of the atmosphere in the exsiccator 4 ± 0.7%) or in a vacuum chamber. High vacuum (10^{-5} Pa) was produced by an ion getter pumping system (TCP 380; Pfeiffer Vacuum GmbH, Asslar, Germany) and continuously monitored during exposure.

All samples of anaerobic organisms were spread, dried and stored in an anaerobic chamber (Coy Laboratory Products Inc. USA, Arbor, Michigan, USA) at room temperature (relative humidity of the atmosphere in the anaerobic chamber 13 ± 0.5%). The anoxic samples stored at −20°C were gas tight shrink-wrapped in the anaerobic chamber before transferring to −20°C. All anoxic desiccation experiments were only carried out with strictly anaerobic strains.

To test the influence of the pH during desiccation Sulfolobales and Thermoplasmatales strains were tested before and after "neutralization" of the pre-culture. "Neutralization" was carried out by adding $CaCO_3$ to the culture until pH 4.5 to pH 5.5 was reached.

To test the influence of attachment to inorganic surfaces on the desiccation tolerance, some Sulfolobales strains were either cultivated on sulfidic ore mixtures (pyrite, sphalerite, chalcopyrite) (0.33% w/v; grain size <0.125 mm) (Huber et al. 1989), on different sterilized solfatara sands (Pisciarelli Solfatara, Italy; Krisuvik, Iceland) or on elemental sulphur. *Thermoplasmaacidophilum* and *Thermoplasma volcanium* were tested in the presence and absence of sterilized solfatara sand. These adherence materials occur in the original habitat of the microorganisms.

Samples were stored as follows: short-term desiccation 0–24 h, long-term desiccation 1 day to 1 year under normal pressure conditions (relative humidity of the atmosphere 33 ± 3.5%).

Periodical desiccation experiments with *Hydrogenothermus marinus*

To test if organisms exposed to repeated desiccation events acquire a higher tolerance against desiccation, 10^7 cells of *H. marinus* were applied on glass slides and dried over night at room temperature. After reactivation by incubation of the glass plates in the corresponding media at 65°C, 10^7 cells of the culture with the highest dilution factor were desiccated again over night on glass slides. This desiccation reactivation cycle was performed five times.

Determination of the survival rate

The glass slides or glass fibre filters with desiccated cells were transferred into normal culture media of the corresponding strain and incubated as described in Table 1. Growth of the cells was followed by phase-contrast microscopy with 400× or 1,000× magnification.

Detection of viable cells after desiccation treatment was achieved by the most probable number technique (American Public Health Association 1972) via dilution series with tenfold or 100-fold dilution steps, respectively. Plating on solid medium could not be used, because nearly all tested strains do not form colonies on solid surfaces (see strain references in Table 1).

The survival rate (S) was calculated as relative survival after desiccation treatment (N) compared to the untreated control (N_0) ($S = N/N_0$). Survival rates were determined at different periods of time up to 1 year.

Table 1 Strains, media, growth and culture conditions

Organism	Strains	Culture media	Temp. (°C)	pH	Gasphase
A. brierleyi	DSM 1651^T, Zillig et al. (1980)	Allen, Brock (1978)	75	2.0	Air
A. pernix	DSM 11879^T, Sako et al. (1996)	JXT, Sako et al. (1996)	90	6.5	Air
A. fulgidus	DSM 4304^T, Stetter (1988)	MGG, modified Stetter (1988)	85	6.5	80% H_2, 20% CO_2
I. hospitalis with *N. equitans*	KIN4/M, Huber et al. (2002a)	½ SME, modified Huber et al. (2006)	90	6.0	80% H_2, 20% CO_2
I. hospitalis	DSM 18386^T, Paper et al. (2007)	½ SME, modified Huber et al. (2006)	90	6.0	80% H_2, 20% CO_2
I. pacificus	DSM 13166^T, Huber et al. (2000)	½ SME, modified Huber et al. (2006)	90	6.0	80% H_2, 20% CO_2
M. sedula	DSM 5348^T, Huber et al. (1989)	Allen, Brock (1978)	65	2.0	Air
M. prunae	DSM 10039^T, Fuchs et al. (1996)	Allen, Brock (1978)	65	2.0	Air
M. jannaschii	DSM 2661^T, Jones et al. (1984)	MJ, modified Romesser et al. (1979)	85	6.0	80% H_2, 20% CO_2
M. thermoautotrophicus	DSM 1053^T, Zeikus and Wolfe (1972)	MS, Balch et al. (1979)	65	7.0	80% H_2, 20% CO_2
M. kandleri	DSM 6324^T, Kurr et al. (1992)	BSM, Kurr et al. (1992)	100	7.0	80% H_2, 20% CO_2
M. barkeri	DSM 800^T, Kluyver and Schnellen (1947)	MS, Balch et al. (1979)	37	7.0	80% H_2, 20% CO_2
M. mazei	DSM 3318, Barker (1936)	MS, Balch et al. (1979)	37	7.0	80% H_2, 20% CO_2
P. aerophilum	DSM 7523^T, Voelkl et al. (1993)	BS, Voelkl et al. (1993)	100	7.0	79% H_2, 20% CO_2, 1% O_2
P. furiosus	DSM 3638^T, Fiala and Stetter (1986)	SME, Fiala et al. (1986)	95	6.5	80% N_2, 20% CO_2
P. occultum	DSM 2709^T, Stetter et al. (1983)	½ SME, modified Huber et al. (2006)	100	5.5	80% H_2, 20% CO_2
Sulfolobus sp.	Ker 2, Huber and Stetter (1991)	Allen, Brock (1978)	65	2.0	Air
Sulfolobus sp.	VE 2	Allen, Brock (1978)	65	2.0	Air
S. metallicus	DSM 6482^T, Huber and Stetter (1991)	Allen, Brock (1978)	65	2.0	Air
S. solfataricus	Ron 12/III, Zillig et al. (1980)	Allen, Brock (1978)	80	2.0	Air
T. pendens	DSM 2475^T, Zillig et al. (1983)	Allen, Brock (1978)	85	6.0	80% N_2, 20% CO_2
T. acidophilum	DSM 1728^T, Darland et al. (1970)	Modified Segerer et al. (1988)	55	2.0	Air
T. volcanicum	DSM 4299^T, Segerer et al. (1988)	Modified Segerer et al. (1988)	55	2.0	Air
T. tenax	DSM 2978^T, Zillig et al. (1981)	Allen, Brock (1978)	85	6.0	80% N_2, 20% CO_2
A. aeolicus	VF5, Deckert et al. (1998)	SME, Huber and Eder (2006)	85	7.0	79% H_2, 20% CO_2, 1% O_2
A. pyrophilus	DSM 6848^T, Huber et al. (1992)	SME, Huber and Eder (2006)	85	7.0	79% H_2, 20% CO_2, 1% O_2
H. thermophilus	DSM 6534^T, Kawasumi et al. (1984)	Kawasumi et al. (1984)	70	7.0	78% H_2, 20% CO_2, 2% O_2
H. hirschii	DSM 11420^T, Stoehr et al. (2001a)	Modified Huber et al. (1992)	60	7.0	78% H_2, 20% CO_2, 2% O_2
H. marinus	DSM 12046^T, Stoehr et al. (2001b)	VM1, modified ZoBell (1941)	65	7.0	78% H_2, 20% CO_2, 2% O_2
Thermocrinis ruber	DSM 12173^T, Huber et al. (1998)	Synthetic-OS, Brock (1978)	85	7.0	94% N_2, 3% O_2, 3% H_2
Thermovibrio ruber	DSM 14644^T, Huber et al. (2002b)	SME, Huber and Eder (2006)	80	6.0	80% H_2, 20% CO_2

Results

Survival and growth of the tested strains after desiccation treatment

The vegetative cells of *Aeropyrum pernix*, *Methanopyrus kandleri*, *Methanosarcina mazei*, *Nanoarchaeum equitans*, *Pyrobaculum aerophilum*, *Pyrococcus furiosus*, *Pyrodictium occultum*, *Hydrogenophilus hirschii*, *Thermocrinis ruber*, the two tested *Thermoplasma* strains and the Sulfolobales strains (*Metallosphaera prunae*, *Sulfolobus* sp. VE 2, and *Sulfolobus solfataricus*) showed no survival after desiccation.

17 out of the 32 tested strains possess the ability to survive different periods of desiccation (Table 2).

Tables 2 and 3 summarize quantitatively the desiccation tolerance of the 17 desiccation tolerant strains. A few organisms survived only a time period of up to 1 or 3 days like *Acidianus brierleyi* and *Methanothermobacter thermoautotrophicus* (under oxic storage conditions). Besides these two exceptions all other strains exhibited desiccation tolerances of at least 1 week, although significant differences occurred depending on the storage temperature or the presence or absence of oxygen. This was the case for *Archaeoglobus fulgidus*, *Ignicoccus hospitalis*, *Ignicoccus pacificus*, *Metallosphaera sedula*, *Methanocaldococcus jannaschii*, *Methanosarcina barkeri*, *Methanothermobacter thermoautotrophicus*, *Sulfolobus* sp. Ker 2, *Sulfolobus metallicus*, *Thermofilum pendens*, *Thermoproteus tenax*, *Aquifex aeolicus*, *Aquifex pyrophilus*, *Hydrogenobacter thermophilus*, *Hydrogenothermus marinus*, *Thermovibrio ruber*.

To get an overview on the amount of their tolerance, survival rates were qualitatively measured (Fig. 1a–i). In general, it was obvious that within the first hours and days

Table 2 Qualitative analysis of the desiccation tolerance

Organism	Desiccation in days																	
	RT, oxic conditions						−20°C, oxic conditions						RT, anoxic conditions					
	1	3	7	14	21	28	1	3	7	14	21	28	1	3	7	14	21	28
Acidianus brierleyi, ore	+	−	−	−	−	−	+	+	−	−	−	−	ND	ND	ND	ND	ND	ND
Archaeoglobus fulgidus	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+
Metallosphaera sedula, pH 2, S°	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND	ND	ND	ND	ND
Metallosphaera sedula, pH 5, S°	+	+	+	−	−	−	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND
Metallosphaera sedula, pH 2, ore	+	+	−	−	−	−	+	+	+	−	−	−	ND	ND	ND	ND	ND	ND
Metallosphaera sedula, pH 5, ore	+	+	+	+	−	−	+	+	+	+	+	−	ND	ND	ND	ND	ND	ND
Methanocaldococcus jannaschii	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	+	−	−
Methanothermobacter thermoautotrophicus	+	−	−	−	−	−	+	+	−	−	−	−	+	+	+	+	+	+
Methanosarcina barkeri	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulfolobus sp. Ker 2, pH 2, S°	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND	ND	ND	ND	ND
Sulfolobus sp. Ker 2, pH 5, S°	+	+	−	−	−	−	+	+	+	+	−	−	ND	ND	ND	ND	ND	ND
Sulfolobus sp. Ker 2, pH 2, ore	+	+	−	−	−	−	+	+	+	−	−	−	ND	ND	ND	ND	ND	ND
Sulfolobus sp. Ker 2, pH 5, ore	+	+	−	−	−	−	+	+	+	−	−	−	ND	ND	ND	ND	ND	ND
Sulfolobus metallicus, pH 2, S°	−	−	−	−	−	−	−	−	−	−	−	−	ND	ND	ND	ND	ND	ND
Sulfolobus metallicus, pH 5, S°	+	+	+	−	−	−	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND
Sulfolobus metallicus, pH 2, ore	+	−	−	−	−	−	+	+	+	+	+	−	ND	ND	ND	ND	ND	ND
Sulfolobus metallicus, pH 5, ore	+	+	+	−	−	−	+	+	+	+	−	−	ND	ND	ND	ND	ND	ND
Thermofilum pendens	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−
Thermoproteus tenax	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−
Aquifex aeolicus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aquifex pyrophilus	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogenobacter thermophilus	+	−	−	−	−	−	+	+	+	+	−	−	ND	ND	ND	ND	ND	ND
Hydrogenothermus marinus	+	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND
Thermovibrio ruber	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+

Strains were cultivated under species-specific standard conditions (see Table 1). 10^7 cells were applied on glass fiber filter pieces or glass slides. Drying and storage of the cells was carried out under the described conditions. (+) indicates that a culture grew up after transfer of the desiccated cells into fresh media. (−) no growth detectable. *ND* survival after desiccation was not tested under this condition (aerobic organisms were only tested under oxic conditions)

Table 3 Survival of *I. hospitalis*, *I. pacificus* and the co-culture *I. hospitalis* with attached *N. equitans* after desiccation

	Desiccation in days							
	1	2	3	4	7	14	21	28
I. hospitalis								
RT, oxic conditions	−	−	−	−	−	−	−	−
−20°C, oxic conditions	+	−	−	−	−	−	−	−
RT, anoxic conditions	+	+	+	+	−	−	−	−
−20°C, anoxic conditions	+	+	+	+	+	+	+	+
I. hospitalis with *N. equitans*								
RT, oxic conditions	−	−	−	−	−	−	−	−
−20°C, oxic conditions	−	−	−	−	−	−	−	−
RT, anoxic conditions	+	+	−	−	−	−	−	−
−20°C, anoxic conditions	+	+	+	+	+	+	+	+
I. pacificus								
RT, oxic conditions	−	−	−	−	−	−	−	−
−20°C, oxic conditions	+	−	−	−	−	−	−	−
RT, anoxic conditions	+	+	+	−	−	−	−	−

Strains were cultivated under species-specific standard conditions (see Table 1). 10^7 cells were applied on glass fiber filter pieces. Drying and storage of the filters with the applied cells was carried out under the described conditions. (+) indicates that a culture grew up after transfer of the desiccated cells into fresh media and (−) no growth detectable

the survival rates of the cells decreased substantially [S (1 day) = 10^{-2} to 10^{-7}]. In contrast, longer desiccation periods resulted in a significantly lower inactivation (depending on the strain). For instance, 1% of *H. marinus* cells survived only one day of desiccation in the presence of oxygen. The subsequent reduction of viable cells was only about one order of magnitude per week. After 4 weeks of desiccation, about 10 of originally 10^7 cells had survived. To our surprise, this result was also obtained after 112 days of desiccation and even after 196 days at least one cell from the original population in the sample survived the used desiccation treatment (Fig. 1i).

The survival rate of *H. marinus* after desiccation in ultra high vacuum is the same as under normal pressure conditions (Fig. 2). After desiccation in an exsiccator with a low relative humidity (4 ± 0.7%) the survival rate of *H. marinus* decreased about one order of magnitude compared to the survival rates under room conditions with a higher relative humidity (33 ± 3.5%).

The survival rate after desiccation of *H. marinus* did not change after five periodical cycles of desiccation and reactivation. On average 1% of the original 10^7*H. marinus* cells survived the first day of desiccation. After five desiccation reactivation cycles, the survival rate was the same as at the beginning of the experiment.

Desiccation tolerance depends on the storage temperature

After complete drying at room temperature and storage at −20°C, survivability of all strains after desiccation was significantly higher than after storage at room temperature (RT) (Table 2). Remarkably, there is no difference between storage of the cells at −20°C or at −70°C. *M. sedula* cultivated and desiccated on solfatara sand showed no significant difference in its survival at storage temperatures of −20°C or at −70°C (data not shown).

Desiccation tolerance of anaerobic strains depends on the presence of oxygen

The survival rates after desiccation for *A. fulgidus*, *A. aeolicus*, *A. pyrophilus, I. hospitalis*, *I. pacificus,* and *M. barkeri* were significantly lower, when the cells were in contact with oxygen during the desiccation process. The survival rate of *A. pyrophilus* and *A. aeolicus* under oxic desiccation conditions is, on average, at least one order of magnitude lower than under anoxic desiccation conditions. Nevertheless, none of the original 10^7*Aquifex*-cells survived storage for 56 days or longer, in the presence or absence of oxygen (Fig. 1g, h).

Not all anaerobic strains possess a noticeable tolerance against the presence of oxygen during desiccation process. *M. jannaschii*, *M. thermoautotrophicus*, *T. tenax*, *T. pendens* and *Thermovibrio ruber* are highly sensitive to oxygen and survived long periods of desiccation only under anoxic conditions (Fig. 1b–f). *M. thermoautotrophicus* survived four weeks of desiccation, when the cells were dried and stored at room temperature under anoxic conditions; in the presence of oxygen the organism can survive only one day (Fig. 1c).

Influence of pH and substrate on Sulfolobales and Thermoplasma strains

Cultivated in sulphur-containing medium, all tested desiccation-resistant Sulfolobales strains (*M. sedula, S. metallicus,*and *Sulfolobus* sp. Ker 2) showed survival after desiccation only when the pre-culture was "neutralized" before desiccation treatment (Table 2). When stored at low pH, 99% of Sulfolobales cells did not survive desiccation for more than 5 h. After 24 h of desiccation none of the originally 10^7 cells had survived. In this short-term desiccation experiment with wet cells, a storage temperature at −20°C had no positive influence on the survival of *M. sedula*.

In contrast to cultures grown on elemental sulfur, *S. metallicus*, *Sulfolobus* sp. Ker 2 and *M. sedula* survived

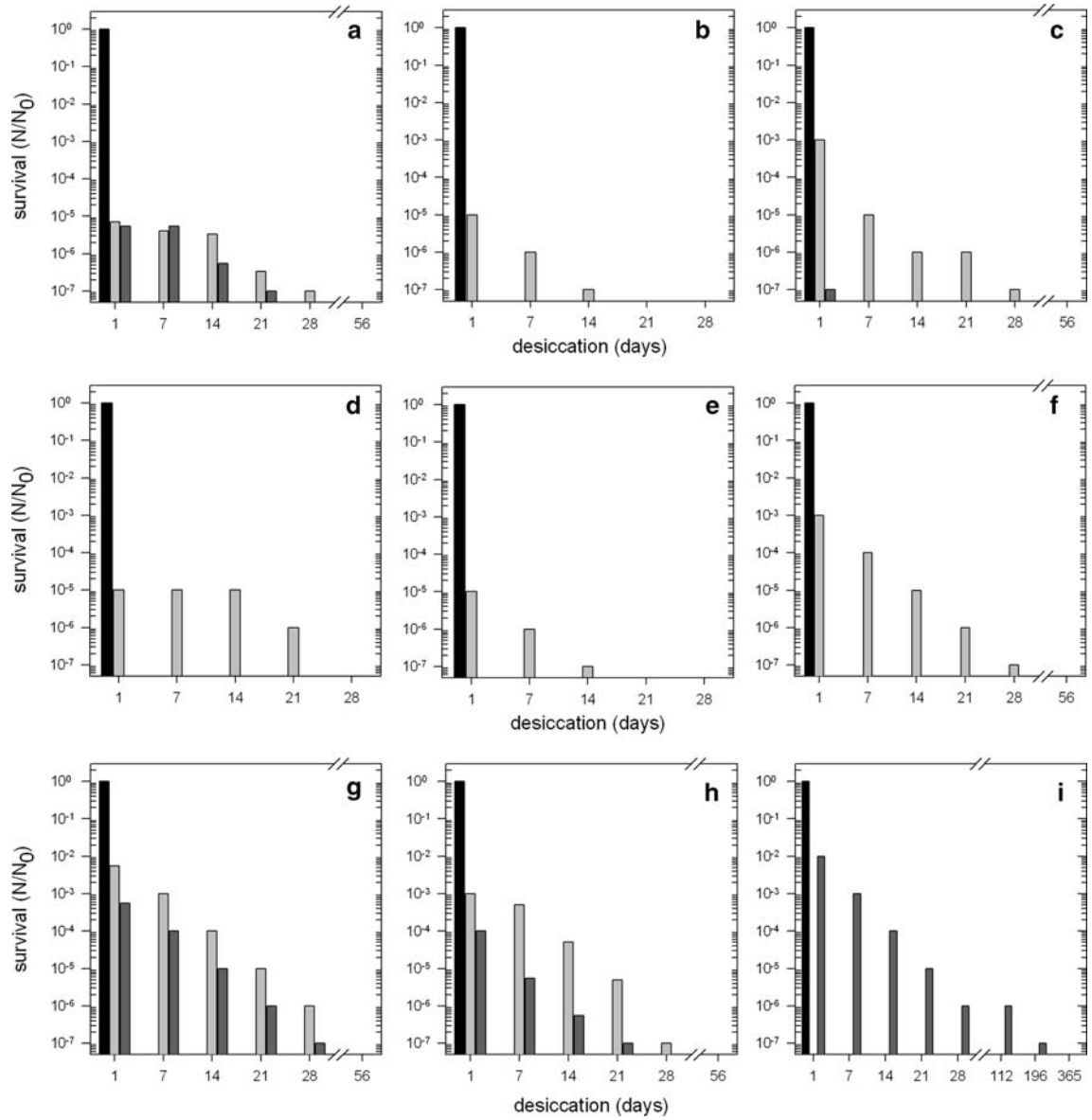

Fig. 1 Survival of *A. fulgidus* (**a**), *M. jannaschii* (**b**), *M. thermoautotrophicus* (**c**), *T. pendens* (**d**), *T. tenax* (**e**), *Thermovibrio ruber* (**f**), *A. aeolicus* (**g**), *A. pyrophilus* (**h**), *H. marinus* (**i**) after desiccation. Strains were cultivated under species-specific standard conditions (see Table 1). 10^7 cells were originally applied on glass slides or glass fiber filters. *Black* survival without desiccation (N_0), *light grey* survival after desiccation in the absence of oxygen (relative humidity $13 \pm 0.5\%$, 10^5 Pa, $\%O_2 < 5$ ppm) (*N*), *dark grey* survival after desiccation in the presence of oxygen under laboratory conditions (*N*) (relative humidity $33 \pm 3.5\%$, 10^5 Pa)

desiccation at pH 2 for at least a few days when they were grown on sulfidic ores (Table 2) or on solfatara sand. In addition, a significant increase of the survival rate was obtained after storage at low temperatures (−20°C) in comparison to room temperature. Higher survival rates were obtained for most of the Sulfolobales strains after neutralization of the culture (final pH 5) (Table 2).

In contrast, the addition of solfatara sand had no influence on the survival rates of the two *Thermoplasma* strains. *T. acidophilum* and *T. volcanium* did not survive any desiccation treatment.

Desiccation tolerance of *I. hospitalis* and *I. pacificus* in pure culture and tolerance of the co-culture *I. hospitalis* with *N. equitans*

The desiccation tolerance of the two tested *Ignicoccus* strains is influenced by two parameters: storage temperature and presence of oxygen. Lower temperature and low oxygen pressure during desiccation yielded higher survival rates. In addition to these parameters we found an influence on the desiccation tolerance for *I. hospitalis* cells, depending on cultivation with or without *N. equitans*.

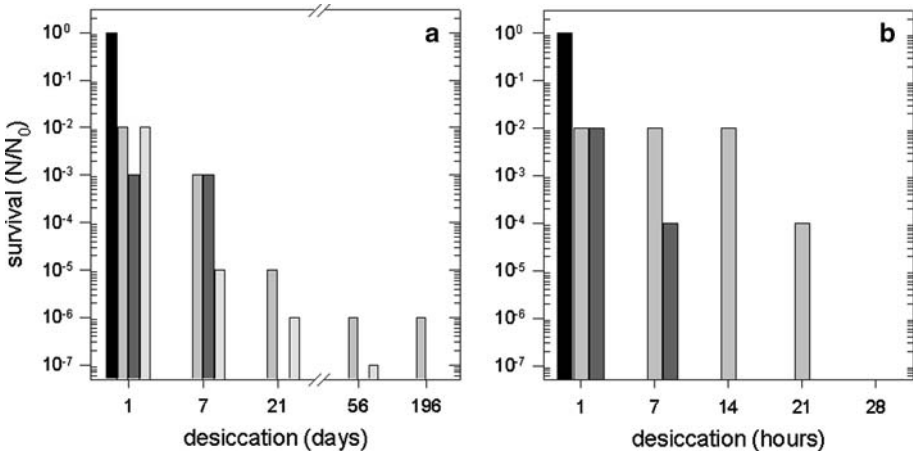

Fig. 2 Survival of *H. marinus* (**a**) and *M. sedula* (**b**) after desiccation. 10^7 cells were originally applied on glass slides or on glass fibre filters. Both organisms were cultivated under species-specific standard conditions (see Table 1). **a** Survival of *H. marinus* under normal pressure conditions, high vacuum and in an exsiccator. *Black* survival without desiccation (N_0), *light grey* survival after desiccation under normal pressure conditions (relative humidity 33 ± 3.5%; 105 Pa) (N), *dark grey* survival after desiccation in high vacuum (relative humidity 0%; 10^{-5} Pa; vacuum tolerance was only tested up to 7 days) (N), *white grey* survival after desiccation in an exsiccator (relative humidity 4 ± 0.7%, 10^5 Pa) (N). **b** Survival of *M. sedula* after short-term desiccation. *M. sedula* was pre-cultivated with elemental sulfur (see Table 1; drying at pH 2, storage under oxic conditions at different temperatures). *Black* survival without desiccation (N_0), *light grey* survival after desiccation at room temperature (N), *dark grey* survival after desiccation at −20°C (N)

Interestingly, the survival rate of *I. hospitalis* was lower in co-culture than it was in a pure culture. *I. pacificus* and *I. hospitalis* showed very similar tolerances against desiccation (Table 3). *N. equitans* showed no desiccation tolerance and we could not detect any *N. equitans* cells attached on *I. hospitalis* cells after desiccation treatment.

Discussion

General remarks on survival after desiccation

Our data demonstrate that desiccation tolerance of vegetative cells seems to be a common phenomenon and is present in nearly all orders of thermophilic and hyperthermophilic Archaea and thermophilic phylogenetically deep-branching Bacteria. The results presented here show great variations in desiccation tolerance of organisms from different orders and also of species from the same order (Aquificales, Desulfurococcales, methanogens, Sulfolobales) or closely related organisms from the same genus (*M. sedula* and *M. prunae*, *M. barkeri and M. mazei* or *S. metallicus,* and *S. solfataricus*). However, there are organisms from the same genus (*A. aeolicus* and *A. pyrophilus* or *I. hospitalis* and *I. pacificus*) with very similar tolerances against desiccation too.

It seems remarkable that most of the tested microorganisms belonging to the order Aquificales and further organisms, gaining their energy with the "knallgas"-reaction (like members of the order Sulfolobales), are quite resistant against desiccation. This is especially true for *A. aeolicus*, *A. pyrophilus*, and *H. marinus*. In contrast, *Thermocrinis ruber* and *H. hirschii*, also belonging to the order Aquificales, are not desiccation resistant.

Although most of the cells die during the first hours of the desiccation process, a constant number of cells can survive long-term desiccation (e.g. *H. marinus*). Nevertheless, in comparison to survival rates of mesophilic desiccation resistant vegetative cells, the survival rates of the thermophilic and hyperthermophilic microorganisms are lower. *D. radiodurans* does not show any changes in its survival rate after 100 h of desiccation, and after 400 h of desiccation, 40% of the cells survived (Rettberg et al. 2004). A decrease of survival by 25% was observed for *Halobacterium* sp. strain NRC-1 after 20 days of desiccation (Kottemann et al. 2005). Our tests show that on average only 0.001% of the original *H. marinus* cells, the organism with the highest desiccation tolerance, survived the period of time between 16 days (∼400 h) and 20 days.

Since the discovery and the first analyses of *D. radiodurans*, a correlation between desiccation tolerance and radiation resistance was discussed. Recently, similar correlations for further organisms e.g. *Halobacterium salinarum* NRC-1 (Kish et al. 2009), *Ustilago maydis* (Holloman et al. 2007) and bdelloid rotifers (Gladyshev and Meselson 2008) were demonstrated. In this context, it was assumed that intracellular protein protection mechanisms against oxidative damages occurring during desiccation as well as during irradiation play a key role (Daly et al. 2007). In our case, it remains unclear at the moment if such cellular protection mechanisms are working in the tested microorganisms, too. Interestingly, we

were already able to detect a high tolerance against ionizing radiation up to 10 kGy for some of the tested organisms, but further experiments are necessary (data not shown).

Effects of storage

The desiccation tolerance of the thermophilic and hyperthermophilic microorganisms is influenced by several parameters: storage temperature, pH, substrate and the presence of oxygen. The positive effect of low temperature storage on the desiccation tolerance is known, and freeze-drying is therefore a commonly used procedure to preserve microorganisms for long periods of times (Miyamoto-Shinohara et al. 2000). In contrast to the positive influence of low temperature storage in long-term desiccation experiments, we could show a negative influence of cold temperatures in the short-term experiments with *M. sedula*. Due to probable intracellular ice crystal formation (Mazur 1963), the survival rate of wet, cold stored cells is lower than the survival rate of the cells which were stored at room temperature until complete drying.

Effects of pH and adherence/growth material

A positive influence on the desiccation tolerance of Sulfolobales strains after neutralization (to about pH 5) is obvious. In all living cells, the intracellular pH is at around pH 7 to pH 8 (Luebben and Schaefer 1989). If an organism lives in an environment with low pH, an effective proton transport from the cytoplasma to the environment is necessary (Booth 1985). Since desiccated cells have nearly no metabolic activity, H^+-ions, which penetrate into the cell due to the huge gradient cannot be exported anymore. Due to the fact that the proton permeability of the *Sulfolobus* membrane is significantly lower at room temperature than at 80°C (Elferink et al. 1994), this may help the cells to survive at unfavourable conditions for several hours or even days. Neutralization minimizes this flux of protons into the cells, thereby explaining the higher survival rates of the strains at pH 5. In principle, the increase of survival on sulfidic ores in comparison to elemental sulfur may have the same origin. On elemental sulfur, the local pH in the microenvironment in or at the sulfur particle is lower than in the supernatant (due to the primary production of sulfuric acid) while the composition of the sulfidic ore leads (in the microenvironment) to a lower degree of acidification. In addition, it may be that the cells, when attached to ore particles, are more protected against the influence of water loss.

Growth on or adherence to solid material had no effect in experiments with other microorganisms. *T. acidophilum* and *T. volcanium* showed no survival independent from added adherence material. Osman and colleagues showed that several non-spore-forming organisms (e.g. several *Microbacterium* strains, *Micrococcus mucilaginosus*, *Staphylococcus epidermidis*) died after prolonged desiccation (75 days) in Atacama desert soil extract (Osman et al. 2008). *M. barkeri*, cultivated and dried in Mars soil analogue material showed lower tolerance against desiccation (no methane-production after 12 days of desiccation) (Kendrick and Kral 2006). Our results showed that *M. barkeri* cultivated and dried in a particle free medium, had a higher ability to survive desiccation up to 28 days under anoxic conditions. These results are in accordance with desiccation experiments with *M. barkeri* which demonstrated survival and methane-production of *M. barkeri* after desiccation periods up to 25 days (Morozova and Wagner 2007).

Effects of oxygen and anoxia

Most anaerobic but also some aerobic microorganisms are oxygen-sensitive when they are metabolically inactive (Grogan 1989). Only organisms possessing a functional superoxide dismutase or other quenching mechanisms can dispose oxygen out of the cell. After desiccation, the cells are in a metabolically inactive state and the superoxide dismutase is not active. Although methanogens are strictly anaerobic and are the most oxygen-sensitive organisms among Archaea (Whitman et al. 2006), *M. barkeri* exhibited in our experiments a distinct, already known tolerance against oxygen exposure and desiccation (Fetzer et al. 1993). *M. barkeri* exhibits an active superoxide dismutase, protecting the organisms against oxidative stress (Brioukhanov et al. 2000). In contrast, *M. thermoautotrophicus* possess a superoxide dismutase (Takao et al. 1990), too, but is very sensitive to oxygen.

Intracellular protectants

Thermophilic and hyperthermophilic microorganisms live under very hostile conditions and in the most extreme environments. These organisms have always to ensure that the intracellular effects of heat are repaired, e.g. by means of the thermosome or class II chaperonin (Phipps et al. 1991; Ditzel et al. 1998). The main effects of heat and of water loss in the cells are very similar and include, e.g. DNA double strand breaks (Dose et al. 1992; Mattimore and Battista 1996) and protein denaturation (Prestrelski et al. 1993). Due to heat adaptation of hyperthermophilic microorganisms, these organisms have acquired mechanisms to overcome DNA damages (Grogan 1998, 2000), which might also be employed in repair of desiccation effects in the DNA.

It is discussed that intracellular compatible solutes could influence the tolerance of microorganisms during hostile

conditions. Hincha and Hagemann (2004) showed a possible positive influence of compatible solutes (like sucrose or trehalose) on membranes during desiccation. In some Archaea, like *A. fulgidus*, *M. kandleri*, *P. furisous*, *T. acidophilum*, *P. occultum*, *P. aerophilum*, *T. tenax*, *S. solfataricusand M. sedula* intracellular compatible solutes have been detected in noticeable amounts under certain growth conditions (Martins et al. 1997). If such components have a significant influence on the desiccation tolerance of the tested organisms remains an open question at the moment. However, it might be that a small part of the cells of a culture possess higher intracellular concentrations of compatible solutes and exhibit therefore a higher tolerance against desiccation.

Relationship of symbiosis to stress tolerance

The hyperthermophilic Archaea *N. equitans* and *I. hospitalis* represent a unique and the only known archaeal host-parasite/symbiosis-system (Huber et al. 2002a; Paper et al. 2007). *N. equitans* can only be grown in co-culture with *I. hospitalis* and not in axenic cultures. It is not known whether the dependence of *N. equitans* on *I. hospitalis* is parasitic or symbiotic. Genome analysis revealed that *N. equitans* lacks nearly all genes for important biosynthetic processes (Waters et al. 2003) and is therefore highly dependent on its host. In our experiments, *I. hospitalis* showed a higher survivability after desiccation in pure culture than in the co-culture. This can in part be explained by the fact that its cytosol contains high amount of a thermosome, the type II chaperonine, against (heat) stress, and the thioredoxin peroxidase, which scavenges peroxides and related radicals (Burghardt et al. 2008). In the presence of *N. equitans*; however, the host cells might be stressed in addition and are therefore liable to the damaging effect of extreme environmental conditions. *N. equitans* obviously has a stressing effect on *I. hospitalis* cells during desiccation. This supports the hypothesis that the *Ignicoccus-Nanoarchaeum*-system is more parasitic than symbiotic.

Conclusions

Vegetative cells of several thermophilic and hyperthermophilic species show a so far unknown significant tolerance against desiccation. In combination with the also demonstrated resistance against high vacuum conditions and first results of a high tolerance against monochromatic UV-C radiation (254 nm) and high doses of gamma radiation (up to 10 kGy) for some of these microorganisms (data not shown) they turn out to be interesting candidates and model organisms not only for simulated space condition experiments but also for future space exposure experiments.

Acknowledgments We want to thank Kerstin Roth for performing desiccation experiments with *Metallosphaera sedula* and Dr. Guenther Reitz, Head of Radiation Biology Department at German Aerospace Center (DLR), for ongoing support.

References

American Public Health Association (1972) Methods for the examination of water and wastewater, 14th edn. American Public Health Association, Washington, DC

Anderson AW, Nordan HC, Cain RF, Parrish G, Duggan DE (1956) Studies on a radiation-resistant micrococcus. Isolation, morphology, cultural characteristics and resistance of γ-radiation. Food Technol 10:575–577

Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: reevaluation of al unique biological group. Microbiol Rev 43:260–296

Barker HA (1936) Studies upon the methane-producing bacteria. Arch Microbiol 7:420–438

Booth IR (1985) Regulation of cytoplasmic pH in bacteria. Microbiol Mol Biol Rev 49:359–378

Brioukhanov A, Netrusov A, Sordel M, Thauer RK, Shima S (2000) Protection of *Methanosarcina barkeri* against oxidative stress: identification and characterization of an iron superoxide dismutase. Arch Microbiol 174:213–216

Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, New York

Burghardt T, Saller M, Guerster S, Mueller D, Meyer C, Jahn U, Hochmuth E, Deutzmann R, Siedler F, Babinger P, Wirth R, Huber H, Rachel R (2008) Insight into the proteome of the hyperthermophilic Crenarchaeon *Ignicoccus hospitalis*: the major cytosolic and membrane proteins. Arch Microbiol 190:379–394

Chyba CF (2005) Rethinking Earth's early atmosphere. Science 308:962–963

Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Leapman RD, Lai B, Ravel B, Li SW, Kemner KM, Fredrickson JK (2007) Protein oxidation implicated as the primary determinant of bacterial radioresistance. PLoS Biol 5:769–779

Darland G, Brock TD, Samsonoff W, Cont SF (1970) A thermophilic, acidophilic *Mycoplasma* isolated from a coal refuse pile. Science 170:1416–1418

Deckert G, Warren PV, Gaasterland T, Young WG, Lenox AL, Graham DE, Overbeek R, Snead MA, Keller M, Aujay M, Huber R, Feldman RA, Short JM, Olsen GJ, Swanson RV (1998) The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. Nature 392:353–358

Di Giulio M (2000) The universal ancestor lives in a thermophilic or hyperthermophilic environment. J Theor Biol 203:203–213

Ditzel L, Loewe J, Stock D, Stetter KO, Huber H, Huber R, Steinbacher S (1998) Crystal structure of the thermosome, the archaeal chaperonin and homolog of CCT. Cell 93:125–138

Dose K, Bieger-Dose A, Labusch M, Gill M (1992) Survival in extreme dryness and DNA-single-strand breaks. Adv Space Res 12:221–229

Elferink MGL, De Wit JG, Driessen AJM, Konings WN (1994) Stability and proton- permeability of liposomes composed of archaeal tetraether lipids. Biochem Biophys Acta 1193:247–254

Fetzer S, Bak F, Conrad R (1993) Sensitivity of methanogenic bacteria from paddy soil to oxygen and desiccation. FEMS Microbiol Ecol 12:107–115

Fiala G, Stetter KO (1986) *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100°C. Arch Microbiol 145:56–61

Fiala G, Stetter KO, Jannasch HW, Langworthy TA, Madon J (1986) *Staphylothermus marinus* sp. nov. respresents a novel genus of extremely thermophilic submarine heterotrophic archaebacteria growing up to 98°C. Syst Appl Microbiol 8:106–113

Fuchs T, Huber H, Teiner K, Burggraf S, Stetter KO (1996) *Metallosphaera prunae*, sp. nov., a novel metal-mobilizing, thermoacidophilic archaeum, isolated from a uranium mine in Germany. Syst Appl Microbiol 18:560–566

Gest H, Mandelstam J (1987) Longevity of microorganisms in natural environments. Microbiol Sci 4:69–71

Gladyshev E, Meselson M (2008) Extreme resistance of bdelloid rotifers to ionizing radiation. Proc Natl Acad Sci USA 105:5139–5144

Grogan DW (1989) Phenotypic characterization of the archaebacterial genus *Sulfolobus*: comparison of five wild-type strains. J Bacteriol 171:6710–6719

Grogan DW (1998) Hyperthermophiles and the problem of DNA instability. Mol Microbiol 28:1043

Grogan DW (2000) The question of DNA-repair in hyperthermophilic archaea. Trends Microbiol 8:180–185

Hincha DK, Hagemann M (2004) Stabilization of model membranes during drying by compatible solutes involved in the stress tolerance of plants and microorganisms. Biochem J 383:277–283

Holloman WK, Schirawski J, Holliday R (2007) Towards understanding the extreme radiation resistance of *Ustilago maydis*. Trends Microbiol 15:525–529

Horneck G, Buecker H, Reitz G (1994) Long-term survival of bacterial spores in space. Adv Space Res 14:1041–1045

Horneck G, Rettberg P, Reitz G, Wehner J, Eschweiler U, Strauch K, Panitz C, Starke V, Baumstark-Khan C (2001) Protection of bacterial spores in space, a contribution to the discussion on panspermia. Orig Life Evol Biosph 31:527–547

Huber R, Eder W (2006) Aquificales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The Prokaryotes: a handbook on the biology of bacteria. Springer, New York, pp 925–938

Huber G, Stetter KO (1991) *Sulfolobusmetallicus*, sp. nov., a novel strictly chemolithotrophic thermophilic archaeal species of metal-mobilizers. Syst Appl Microbiol 14:372–378

Huber G, Spinnler C, Gambacorta A, Stetter KO (1989) *Metallosphaera sedula* gen. and sp. nov. represents a new genus of aerobic, metal-mobilizing, thermoacetophilic archaebacteria. Syst Appl Microbiol 12:38–47

Huber R, Wilharm T, Huber D, Trincone A, Burggraf S, Koenig H, Rachel R, Rockinger I, Fricke H, Stetter KO (1992) *Aquifex pyrophilus* gen. nov. sp. nov., represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacterium. Syst Appl Microbiol 15:340–351

Huber R, Eder W, Heldwein S, Wanner G, Huber H, Rachel R, Stetter KO (1998) *Thermocrinis ruber* gen. nov., sp. nov., a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park. Appl Environ Microbiol 64:3576–3583

Huber H, Burggraf S, Mayer T, Wyschkony I, Rachel R, Stetter KO (2000) *Ignicoccus* gen. nov., a novel genus of hyperthermophilic, chemolithoautotrophic archaea, represented by two new species, *Ignicoccus islandicus* sp. nov. and *Ignicoccus pacificus* sp. nov. Int J Syst Evol Microbiol 50:2093–2100

Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO (2002a) A new phylum of archaea represented by a nanosized hyperthermophilic symbiont. Nature 417:63–67

Huber H, Diller S, Horn C, Rachel R (2002b) *Thermovibrio ruber* gen. nov., sp. nov., an extremely thermophilic, chemolithoautotrophic, nitrate-reducing bacterium that forms a deep branch within the phylum *Aquificae*. Int J Syst Evol Microbiol 52:1859–1865

Huber H, Hohn MJ, Rachel R, Stetter KO (2006) Nanoarchaeota. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The Prokaryotes: a handbook on the biology of bacteria. Springer, New York, pp 274–280

Jones W, Leigh J, Mayer F, Woese C, Wolfe R (1984) *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch Microbiol 136:254–261

Kawasumi T, Igarashi Y, Kodama T, Minoda Y (1984) *Hydrogenobacterthermophilus* gen. nov. sp. nov., an extremely thermophilic, aerobic, hydrogen-oxidizing bacterium. Int J Syst Evol Microbiol 34:5–10

Kendrick MG, Kral TA (2006) Survival of methanogens during desiccation: implications for life on Mars. Astrobiology 6:546–551

Kish A, Kirkali G, Robinson C, Rosenblatt R, Jaruga P, Dizdaroglu M, DiRuggiero J (2009) Salt shield: intracellular salts provide cellular protection against ionizing radiation in the halophilic archaeon, Halobacterium salinarum NRC-1. Environ Microbiol (published online)

Kluyver AJ, Schnellen GTP (1947) Fermentation of carbon monoxide by pure cultures of methane bacteria. Arch Biochem 14:57–70

Kottemann M, Kish A, Iloanusi C, Bjork S, DiRuggiero J (2005) Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation. Extremophiles 9:219–227

Kurr M, Huber R, Koenig H, Jannasch HW, Fricke H, Trincone A, Kristjansson JK, Stetter KO (1992) *Methanopyrus kandleri*, gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110°C. Arch Microbiol 156:239–247

Luebben M, Schaefer G (1989) Chemiosmotic energy conversion of the archaebacterial thermoacidophile *Sulfolobus acidocaldarius*: oxidative phosphorylation and the presence of an F_0-related *N*, *N*′-dicyclohexylcarbodiimide-binding proteolipid. J Bacteriol 171:6106–6116

Martins LO, Huber R, Huber H, Stetter KO, da Costa MS, Santos H (1997) Organic solutes in hyperthermophilic archaea. Appl Environ Microbiol 63:896–902

Mattimore V, Battista R (1996) Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J Bacteriol 78:633–637

Mazur P (1963) Kinetics of water loss from cells at subzero temperatures and the likelihood of intracellular freezing. J Gen Physiol 47:347–369

Melosh HJ (2003) Exchange of meteorites (and life?) between stellar systems. Astrobiology 3:207–215

Miyamoto-Shinohara Y, Imaizumi T, Sukenobe J, Murakami Y, Kawamura S, Komatsu Y (2000) Survival rate of microbes after freeze-drying and long-term storage. Cryobiology 41:251–255

Morozova D, Wagner D (2007) Stress response of methanogenic archaea from siberian permafrost compared with methanogens from non permafrost habitats. FEMS Microbiol Ecol 61:16–25

Nicholson WL, Munakata N, Horneck G, Melosh HL, Setlow P (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 64:548–572

Nisbet EG, Sleep NH (2001) The habitat and nature of the early life. Nature 409:1083–1091

Osman S, Peeters Z, La Duc MT, Mancinelli R, Ehrenfreund P, Venkateswaran K (2008) Effect of shadowing on survival of Bacteria under conditions simulating the martian atmosphere and UV radiation. Appl Environ Microbiol 74:959–970

Paper W, Jahn U, Hohn M, Kronner M, Naether D, Burghardt T, Rachel R, Stetter KO, Huber H (2007) *Ignicoccus hospitalis* sp. nov., the host of '*Nanoarchaeum equitans*'. Int J Syst Evol Microbiol 57:803–808

Phipps BM, Hoffmann A, Stetter KO, Baumeister W (1991) A novel ATPase complex selectively accumulated upon heat shock is a

major cellular component of thermophilic archaebacteria. EMBO J 10:1711–1722

Prestrelski SJ, Tedeschi N, Arakawa T, Carpentert JF (1993) Dehydration-induced conformational transitions in proteins and their inhibition by stabilizers. Biophys J 65:661–671

Rettberg P, Pogoda de La Vega U, Horneck G (2004) *Deinococcus radiodurans*—a model organism for life under martian conditions. In: Proceedings of the third European workshop on exo-astrobiology, pp 59–62

Romesser JA, Wolfe RS, Mayer F, Speiss E, Walther-Mauruschat A (1979) *Methanogenium,* a new genus of marine methanogenic bacteria, and characterization of *Methanogenium cariaci* sp. nov. and *Methanogeniummarisnigri* sp. nov. Arch Microbiol 121:147–153

Sako Y, Nomura N, Uchida A, Ishida Y, Morii H, Koga Y, Koga Y, Hoaki T, Maruyama T (1996) *Aeropyrum pernix* gen. nov., sp. nov., a novel aerobic hyperthermophilic archaeon growing at temperatures up to 100°C. Int J Syst Bacteriol 46:1070–1077

Segerer A, Longworthy TA, Stetter KO (1988) *Thermoplasma acidophilum* and *Thermoplasma volcanium* sp. nov. from solfatara fields. Syst Appl Microbiol 10:161–171

Stetter KO (1988) *Archaeoglobus fulgidus* gen. nov., sp. nov.: a new taxon of extremely thermophilic archaebacteria. Syst Appl Microbiol 10:172–173

Stetter KO (1996) Hyperthermophiles in the history of life. Ciba Found Symp 202:1–10

Stetter KO, Koenig H, Stackebrandt E (1983) *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulfur reducing archaebacteria growing optimally at 105°C. Syst Appl Microbiol 4:535–551

Stoehr R, Waberski A, Liesack W, Voelker H, Wehmeyer U, Thomm M (2001a) *Hydrogenophilus hirschii* sp. nov., a novel thermophilic hydrogen-oxidizing β-proteobacterium isolated from Yellowstone National Park. Int J Syst Evol Microbiol 51:481–488

Stoehr R, Waberski A, Voelker H, Tindall B, Thomm M (2001b) *Hydrogenothermus marinus* gen. nov., sp. nov., a novel thermophilic hydrogen-oxidizing bacterium, recognition of *Calderobacterium hydrogenophilum* as a member of the genus *Hydrogenobacter a*nd proposal of the reclassification of *Hydrogenobacter acidophilus* as *Hydrogenobaculum acidophilum* gen. nov., comb. nov., in the phylum '*Hydrogenobacter/Aquifex*'. Int J Syst Evol Microbiol 51:1853–1862

Takao M, Oikawa A, Yasui A (1990) Characterization of a superoxide dismutase gene from the archaebacterium *Methanobacterium thermoautotrophicum*. Arch Biochem Biophys 283:210–216

Voelkl P, Huber R, Drobner E, Rachel R, Burggraf S, Trincone A, Stetter KO (1993) *Pyrobaculum aerophilum* sp. nov., a novel nitrate-reducing hyperthermophilic archaeum. Appl Environ Microbiol 59:2918–2926

Waters E, Hohn MJ, Ahel I, Graham DE, Adams MD, Barnstead M, Beeson KY, Bibbs L, Bolanos R, Keller M, Kretz K, Lin X, Mathur E, Ni J, Podar M, Richardson T, Sutton GG, Simon M, Soell D, Stetter KO, Short JM, Noordewier M (2003) The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. Proc Natl Acad Sci USA 100:12984–12988

Whitman WB, Bowen TL, Boone DR (2006) The methanogenic Bacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The Prokaryotes: a handbook on the biology of bacteria. Springer, New York, pp 165–207

Zeikus J, Wolfe R (1972) *Methanobacterium thermoautotrophicus* sp. n., an anaerobic, autotrophic, extreme thermophile. J Bacteriol 109:707–713

Zillig W, Stetter KO, Wunderl S, Schulz W, Priess H, Scholz J (1980) The *Sulfolobus*-"*Caldariella*" group: taxonomy on the basis of the structure of DNA-dependent RNA polymerases. Arch Microbiol 125:259–269

Zillig W, Stetter KO, Schaefer W, Janekovic D, Wunderl S, Holz J, Palm P (1981) *Thermoproteales*: a novel type of extremely thermoacidophilic anaerobic archaebacteria isolated from icelandic solfataras. Zentralbl Bakteriol Mikrobiol Hyg I Abt C 2:205–227

Zillig W, Gierl A, Schreiber G, Wunderl S, Janekovic D, Stetter KO, Klenk HP (1983) The archaebacterium *Thermofilum pendens* represents a novel genus of the thermophilic, anaerobic sulfur respiring Thermoproteales. Syst Appl Microbiol 4:79–87

ZoBell CE (1941) Studies on marine bacteria. The cultural requirements of heterotrophic aerobes. J Mar Res 4:42–75