

RESEARCH LETTER – Environmental Microbiology

## Lack of correlation of desiccation and radiation tolerance in microorganisms from diverse extreme environments tested under anoxic conditions

Kristina Beblo-Vranesevic<sup>1,\*</sup>, Maria Bohmeier<sup>1</sup>, Alexandra K. Perras<sup>2,3</sup>, Petra Schwendner<sup>4</sup>, Elke Rabbow<sup>1</sup>, Christine Moissl-Eichinger<sup>2,5</sup>, Charles S. Cockell<sup>4</sup>, Pauline Vannier<sup>6</sup>, Viggo T. Marteinson<sup>6,7</sup>, Euan P. Monaghan<sup>8</sup>, Pascale Ehrenfreund<sup>8,9</sup>, Laura Garcia-Descalzo<sup>10</sup>, Felipe Gómez<sup>10</sup>, Moustafa Malki<sup>11</sup>, Ricardo Amils<sup>11</sup>, Frédéric Gaboyer<sup>12</sup>, Frances Westall<sup>12</sup>, Patricia Cabezas<sup>13</sup>, Nicolas Walter<sup>13</sup> and Petra Rettberg<sup>1</sup>

<sup>1</sup>Radiation Biology Department, Institute of Aerospace Medicine, German Aerospace Center (DLR), Linder Höhe, 51147 Cologne, Germany, <sup>2</sup>Department of Internal Medicine, Medical University of Graz, Auerbruggerplatz 15, 8010 Graz, Austria, <sup>3</sup>Department of Microbiology and Archaea, University of Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany, <sup>4</sup>UK Center for Astrobiology, School of Physics and Astronomy, University of Edinburgh, Peter Guthrie Tait Road, EH9 3FD, Edinburgh, UK, <sup>5</sup>BioTechMed Graz, Mozartgasse 12/II, 8010 Graz, Austria, <sup>6</sup>MATISProkaria, Vinlandsleid 12, 113 Reykjavík, Iceland, <sup>7</sup>Faculty of Food Science and Nutrition, University of Iceland, Vatnsmýrarvegur 16, 101 Reykjavík, Iceland, <sup>8</sup>Leiden Observatory, Universiteit Leiden, Niels Bohrweg 2, 2333 Leiden, Netherland, <sup>9</sup>Space Policy Institute, George Washington University, 1957 E Street, 20052 Washington DC, USA, <sup>10</sup>Instituto Nacional de Técnica Aeroespacial-Centro de Astrobiología (INTA-CAB), Torrejón de Ardoz, 28850 Madrid, Spain, <sup>11</sup>Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid (UAM), Calle Nicolás Cabrera 1, 28049 Madrid, Spain, <sup>12</sup>Centre de Biophysique Moléculaire, Centre National de la Recherche Scientifique (CNRS), Rue Charles Sadron, 45071 Orléans, France and <sup>13</sup>European Science Foundation (ESF), Quai Lezay-Marnésia, 67080 Strasbourg, France

\*Corresponding author: Radiation Biology Department, Institute of Aerospace Medicine, Radiation Biology Department, German Aerospace Center (DLR), Linder Höhe, 51147 Cologne, Germany. Tel: +49-2203-6012194; Fax: +49-2203-61970; E-mail: [kristina.beblo@dlr.de](mailto:kristina.beblo@dlr.de)

One sentence summary: The survival after desiccation and after exposure to ionizing radiation of microorganisms from different extreme environments refutes the previously reported correlation between desiccation tolerance and radiation tolerance.

Editor: Hermann Heipieper

<sup>†</sup>Kristina Beblo-Vranesevic, <http://orcid.org/0000-0002-4834-7121>

### ABSTRACT

Four facultative anaerobic and two obligate anaerobic bacteria were isolated from extreme environments (deep subsurface halite mine, sulfidic anoxic spring, mineral-rich river) in the frame MASE (Mars Analogues for Space Exploration) project. The isolates were investigated under anoxic conditions for their survivability after desiccation up to 6 months and their

Received: 14 December 2017; Accepted: 19 February 2018

© FEMS 2018. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

tolerance to ionizing radiation up to 3000 Gy. The results indicated that tolerances to both stresses are strain-specific features. *Yersinia intermedia* MASE-LG-1 showed a high desiccation tolerance but its radiation tolerance was very low. The most radiation-tolerant strains were *Buttiauxella* sp. MASE-IM-9 and *Halanaerobium* sp. MASE-BB-1. In both cases, cultivable cells were detectable after an exposure to 3 kGy of ionizing radiation, but cells only survived desiccation for 90 and 30 days, respectively. Although a correlation between desiccation and ionizing radiation resistance has been hypothesized for some aerobic microorganisms, our data showed that there was no correlation between tolerance to desiccation and ionizing radiation, suggesting that the physiological basis of both forms of tolerances is not necessarily linked. In addition, these results indicated that facultative and obligate anaerobic organisms living in extreme environments possess varied species-specific tolerances to extremes.

**Keywords:** correlation; desiccation; radiation; survival; anaerobes; extreme environment

## INTRODUCTION

Anaerobic microorganisms are widely distributed in Earth's extreme environments, yet we still know little about their physiology and their capacity to adapt to extreme conditions. In particular, there is a paucity of studies whereby different anaerobic microorganisms from extreme environments are investigated to understand their diverse physiological and metabolic capabilities. In this study, the two stressors of interest were the tolerance to periods of water loss and the exposure to ionizing radiation. These stressors also occur in other extreme environments but their combination is rare. Microorganisms frequently experience periodic desiccation in subaerial environments or during dispersal. Although most natural environments do not experience ionizing radiation beyond the level of naturally occurring background radiation (Thorne 2003), this stress can be explored as a proxy for an organisms' ability to repair general cell damage. Furthermore, there has often been a claimed correlation between desiccation and ionizing radiation resistance, which is of interest to explore further. It is suggested that the physiological basis and repair mechanisms to counteract the stress-induced damage by radiation or desiccation might be linked or might even be the same (Mattimore and Battista 1996).

While there are several studies investigating the survivability of model microorganisms, such as *Escherichia coli* and *Deinococcus radiodurans*, after desiccation and after exposure to ionizing radiation tested under oxic conditions (Clavero et al. 1994; Welsh and Herbert 1999; Daly 2009; Bauermeister et al. 2011), there are only a few examples where facultative and strict anaerobic microorganisms were tested against these stressors under anoxic conditions. The survival capacity after exposure to one stressor in form of ionizing radiation was described for the hyperthermophilic anaerobic microorganisms, *Pyrococcus furiosus* and *Thermococcus gammatolerans* (DiRuggiero et al. 1997; Jolivet et al. 2003). However, there are only very few studies about the tolerance in terms of sensitivity of anaerobic microorganisms to desiccation (Fetzer, Bak and Conrad 1993; Beblo et al. 2009).

The application of both of these stressors gives insights into the abilities that microorganisms have evolved to survive damage to macromolecules such as proteins, membranes and nucleic acids.

During desiccation, initially the free intracellular water and subsequently the hydration shell of different molecules disappear, consequently affecting cellular components in their functions. For example, protein denaturation and perturbation of the lipid membrane by phase changes, e.g. structural conversion of bilayer sheets to spherical micelles, might appear (Cox 1993; Prestrelski et al. 1993; Billi and Potts 2002). The DNA is also affected through loss of water; DNA protein cross-links and strand

breaks can occur (Bieger-Dose et al. 1992; Dose et al. 1992). Additionally, the DNA can change from the B to the A-conformation resulting in DNA single- and double-strand breaks. Dehydration for 6 weeks caused approximately 60 DNA double-strand breaks per genome in *D. radiodurans* (Fredrickson et al. 2008). Nevertheless, the survival of the microorganisms after 6 weeks of desiccation was not even reduced by one order of magnitude (Mattimore and Battista 1996). Another form of damaging stress that occurs during desiccation is the formation of reactive oxygen species (ROS), which, in turn, leads to oxidative stress (França, Panek and Eleutherio 2007). The ROS are mainly superoxide anions ( $\bullet\text{O}_2^-$ ) and hydroxyl radicals ( $\bullet\text{OH}$ ) affecting all macromolecular cellular components (Cabiscol, Tamarit and Ros 2000). However, the exact origin of the radicals during rehydration is unknown, but a possible source could be the metabolism itself (e.g. respiratory chain) when the cells were growing aerobically (González-Flecha and Demple 1995; França, Panek and Eleutherio 2007). They are also formed by indirect effects through the radiation-induced radiolysis of intracellular water and surrounding water and account for approximately 80% of introduced DNA damages (Michaels and Hunt 1978; Jones et al. 1994; Riley 1994). After exposure to ionizing radiation of 5 kGy, approximately 200 double-strand breaks were detected in *D. radiodurans* (Cox and Battista 2005). However, the survivability of *D. radiodurans* was not reduced (Battista, Earl and Park 1999). In addition, ionizing radiation also affects proteins through oxidation processes, lipids through lipid peroxidation and disturbance of membrane permeability in eukaryotic and prokaryotic systems (Leyko and Bartosz 1985; Daly et al. 2007; Krisko and Radman 2010).

MASE (Mars Analogues for Space Exploration), a project funded by the European Union, was initiated to investigate anaerobic microorganisms in terrestrial extreme environments and their physiological adaptations to these extreme environmental conditions (Cockell et al. 2017). In this article, we describe the survivability after prolonged desiccation of up to 4 weeks and exposure to ionizing radiation (up to 3 kGy) of four facultative anaerobic and two strict anaerobic microorganisms isolated from different extreme environments in the frame of this project.

## MATERIALS AND METHODS

### Strains and culture conditions

During the MASE project, over 30 pure cultures were obtained from various extreme environments (Cockell et al. 2017). From this list, six distantly related bacterial strains were picked

**Table 1.** Strains, origins and cultivation conditions.

Strain Phylum Class	Origin	Medium	Supplements (wt/vol)	Gas phase (vol/vol)	Temperature (°C)
<i>Acidiphilium</i> sp. PM Alphaproteobacteria	River Rio Tinto, Spain	MASE-I	0.01% KNO <sub>3</sub> 0.01% C-Org-Mix	80% N <sub>2</sub> , 20% CO <sub>2</sub>	30
<i>Buttiauxella</i> sp. MASE-IM-9, Proteobacteria	Islinger Mühlbach, Germany	MASE-II	0.1% Yeast extract	80% N <sub>2</sub> , 20% CO <sub>2</sub>	30
Gammaproteobacteria					
<i>Clostridium</i> sp. MASE-IM-4 Firmicutes Clostridia	Islinger Mühlbach, Germany	MASE-II—FeCl <sub>2</sub>	0.01% Dimethylamine 0.001% FeCl <sub>2</sub>	15% H <sub>2</sub> , 25% CO <sub>2</sub> , 60% N <sub>2</sub>	30
<i>Halanaerobium</i> sp. MASE-BB-1 Proteobacteria	Boulby Mine, Great Britain	HACE	0.1% Yeast extract	15% H <sub>2</sub> , 25% CO <sub>2</sub> , 60% N <sub>2</sub>	45
Gammaproteobacteria					
<i>Trichococcus</i> sp. MASE-IM-5 Firmicutes, Bacilli	Islinger Mühlbach, Germany	MASE-II—FeCl <sub>2</sub>	0.01% Na <sub>2</sub> SO <sub>4</sub> 0.01% C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> × 2 H <sub>2</sub> O 0.02% KNO <sub>3</sub>	15% H <sub>2</sub> , 25% CO <sub>2</sub> , 60% N <sub>2</sub>	30
<i>Y. intermedia</i> MASE- LG-1 Proteobacteria	Lake Grænavatn, Iceland	MASE-I	0.01% KNO <sub>3</sub> 0.01% C-Org-Mix	80% N <sub>2</sub> , 20% CO <sub>2</sub>	30
Gammaproteobacteria					

for further analysis. The following microorganisms, namely *Acidiphilium* sp. PM (DSM 24941), *Buttiauxella* sp. MASE-IM-9 (DSM 105071), *Clostridium* sp. MASE-IM-4 (DSM 105631), *Halanaerobium* sp. MASE-BB-1 (DSM 105537), *Trichococcus* sp. IM-5 (DSM 105632) and *Yersinia intermedia* MASE-LG-1 (DSM 102845). Media and strain-specific cultivation conditions are summarized in Table 1 and described in detail in Cockell et al. (2017). The incubation was carried out at the indicated cultivation temperature, and cultures were shaken at 50 rpm. Noteworthy, for *Clostridium* sp. MASE-IM-4 only vegetative cells have been observed during the applied cultivation condition.

### Desiccation and irradiation experiments

For the desiccation experiments, the cells were cultivated under optimal growth conditions until stationary growth phase was reached. Desiccation experiments were performed as described by Beblo et al. (2009). Briefly, cell concentrations were determined by counting in a Thoma chamber. One milliliter of cell culture (cell densities ranged from  $\sim 5 \times 10^6$  cells/ml to  $\sim 5 \times 10^7$  cells/ml) was spread evenly on four glass slides and dried under anoxic conditions in an anaerobic chamber (Coy Laboratory Products Inc., [O<sub>2</sub>] < 0.0001%, relative humidity  $13 \pm 0.5\%$ ; both in vol/vol) in the presence of drying agent calcium chloride. Afterwards, the dried cells were stored within the anaerobic chamber.

Exposure to ionizing radiation was carried out according to earlier studies (Beblo et al. 2011). Stationary phase cell cultures, in liquid suspensions, were transferred anoxically into 7 ml glass HPLC vials (WICOM Germany GmbH), which were tightly sealed with rubber stoppers and aluminum caps. Irradiation was conducted with an X-ray source Gulmay RS 225A (Gulmay Medical Ltd) at 200 kV and 15 mA. Cells were irradiated at a distance of 19.5 cm below the X-ray source with  $20 \text{ Gy min}^{-1} \pm 5 \text{ Gy min}^{-1}$  up to 3 kGy. The dose rate was measured with a UNIDOS dosimeter (PTW Freiburg GmbH). All irradiation experiments were performed under anoxic conditions at room temperature.

### Determination of the survival

At dedicated time points, the dried cells on glass slides or the irradiated cells as a liquid suspension were transferred under

anoxic conditions into the strain-specific culture medium and incubated for up to 4 weeks (Table 1, for description of the procedure see Beblo et al. 2009). Growth of the cells in all dilutions was observed visually and by phase-contrast microscopy (Zeiss® Axiolmager TM M2) with 400× or 1000× magnification. Determination of the survival and enumeration of cultivable cells was achieved by the most probable number (MPN) technique via dilution series with 10-fold dilution steps (Franson 1985). The MPN technique was applied for all six strains, since not all strains are able to grow on solid surfaces.

All experiments were repeated independently at least three times, representing biological replicates. The data shown within graphs represent mean values with standard deviations. The survival (S) was calculated as relative survival after cell damaging treatment (N) compared to the non-treated control (N<sub>0</sub>) ( $S = N/N_0$ ).

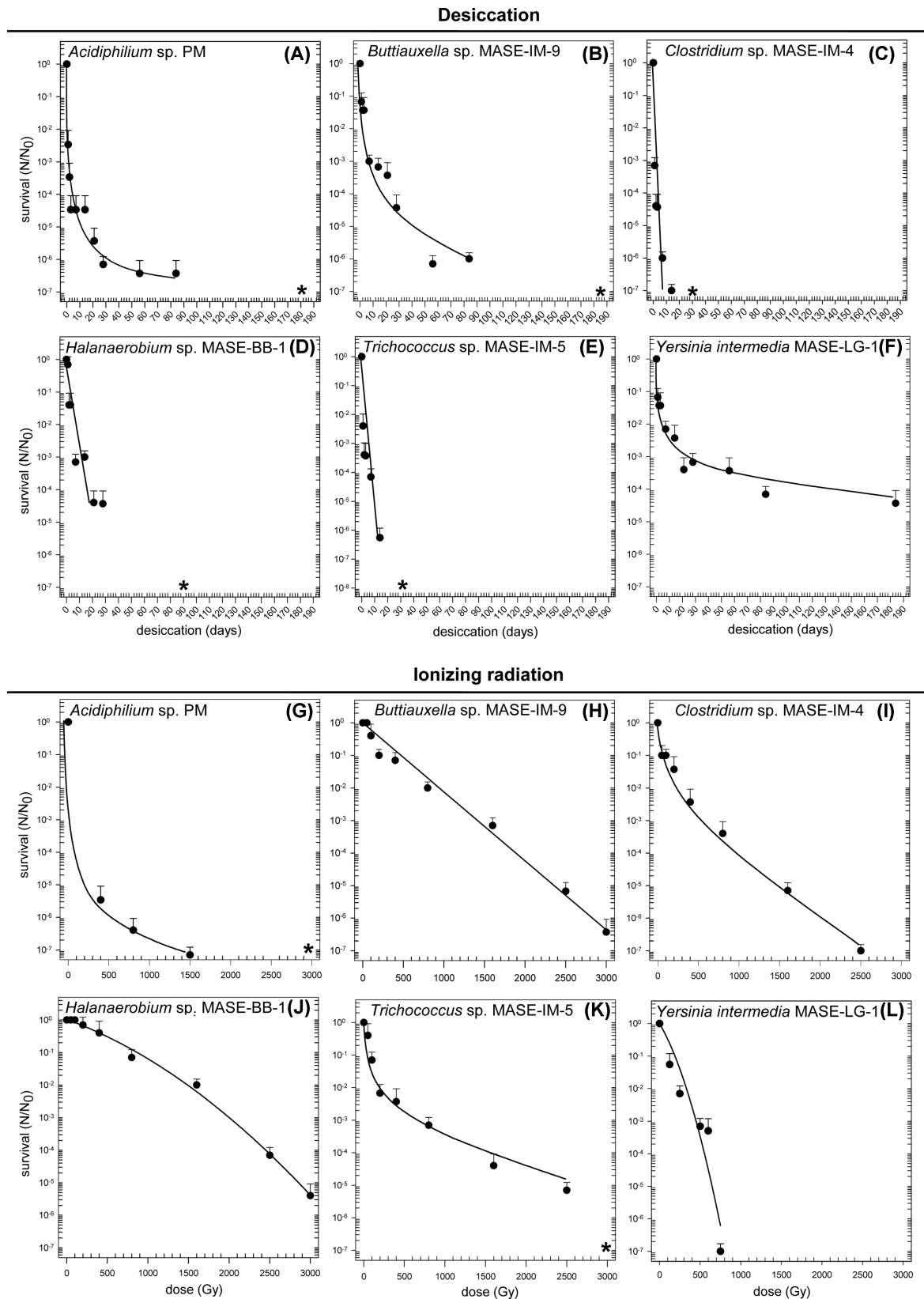
Due to the applied MPN technique and depending on the growth density of the specific strain ( $\sim 5 \times 10^6$  cells/ml to  $\sim 5 \times 10^7$  cells/ml), the detection limit of the determination of survival was  $\sim 1 \times 10^{-8}$ .

## RESULTS

The six vegetative strains *Acidiphilium* sp. PM, *Buttiauxella* sp. MASE-IM-9, *Clostridium* sp. MASE-IM-4, *Halanaerobium* sp. MASE-BB-1, *Trichococcus* sp. MASE-IM-5, and *Y. intermedia* MASE-LG-1 showed different levels of survival after desiccation and after exposure to radiation (Fig. 1).

### Tolerance to desiccation

The survival curves of all tested organisms showed an exponential decay as described by Chen and Alexander (1973). Thereby, the survival rate decreased substantially within the first days of desiccation and the survival decreased until it plateaued. Only *Y. intermedia* MASE-LG-1 was able to survive the maximum tested time period of desiccation (184 days). After 184 days, the survival of this organism was  $S(184 \text{ days}) = 3.7 \times 10^{-5}$  (Beblo-Vranesevic et al. 2017a). In contrast to this high tolerance to water loss, *Clostridium* sp. MASE-IM-4, *Trichococcus* sp. MASE-IM-5 and *Halanaerobium* sp. MASE-BB-1 were more sensitive to desic-



**Figure 1.** Survival of the MASE isolates after anoxic desiccation (A–F) and after exposure to ionizing radiation under anoxic conditions (G–L). For desiccation experiments, the cells were applied to glass slides, dried and stored under anoxic conditions up to 184 days. For anoxic irradiation experiments, the cells were exposed to ionizing radiation up to 3 kGy in liquid culture medium under anoxic conditions. *Acidiphilium* sp. PM (A, G), *Buttiauxella* sp. MASE-IM-9 (B, H), *Clostridium* sp. MASE-IM-4 (C, I), *Halanaerobium* sp. MASE-BB-1 (D, J), *Trichococcus* sp. MASE-IM-5 (E, K), *Y. intermedia* MASE-LG-1 (F, L). Solid lines are the survival curves fitted by hand based on the survival data; N<sub>0</sub>, viable cells without desiccation or without irradiation; N, viable cells after desiccation or without irradiation (n = 3 with standard deviation); \*no viable cells detected.

cation. After 4 weeks in a dry state, no living cells of *Clostridium* sp. MASE-IM-4 and *Trichococcus* sp. MASE-IM-5 were detectable (Fig. 1C and E). The survival of *Halanaerobium* sp. MASE-BB-1 after 4 weeks of desiccation was reduced by more than four orders of magnitude (Fig. 1D) to  $S(28 \text{ days}) = 3.7 \times 10^{-5}$  and no living cells were found after 56 days of anoxic storage in a dry state. The survival of *Acidiphilium* sp. PM and *Buttiauxella* sp. MASE-IM-9 after 4 weeks were  $S(28 \text{ days}) = 7.0 \times 10^{-7}$  and  $S(28 \text{ days}) = 3.7 \times 10^{-5}$  respectively, and these strains could outlast 84 days in a desiccated form (Fig. 1A and B). All these results summarized, the organisms can be ranked in terms of their tolerance to desiccation: *Clostridium* sp. MASE-IM-4 < *Trichococcus* sp. MASE-IM-5 < *Halanaerobium* sp. MASE-BB-1 < *Acidiphilium* sp. PM < *Buttiauxella* sp. MASE-IM-9 < *Y. intermedia* MASE-LG-1.

### Tolerance to ionizing radiation

Survival after exposure to ionizing radiation of the tested MASE isolates varied greatly between isolates. Different types of curves were obtained and not all organisms showed the typically shouldered survival curve as it is described by Kiefer (1990). Only two organisms (*Buttiauxella* sp. MASE-IM-9 and *Halanaerobium* sp. MASE-BB-1; Fig. 1H and J) were able to multiply after an exposure to 3 kGy ( $S(\textit{Buttiauxella} \text{ sp. MASE-IM-9, 3 kGy}) = 3.7 \times 10^{-7}$ ;  $S(\textit{Halanaerobium} \text{ sp. BB-1, 3 kGy}) = 4 \times 10^{-6}$ ). For *Clostridium* sp. MASE-IM-4 and *Trichococcus* sp. MASE-IM-5, the highest dose at which culturable cells were detected after reinoculation was 2.5 kGy (Fig. 1I and K). *Acidiphilium* sp. PM was more sensitive to ionizing radiation and only survived exposures up to 1.5 kGy (Fig. 1G). The most sensitive organism amongst this tested group of microorganisms was *Y. intermedia* MASE-LG-1. Multiplying cells were only observed to a dose of 0.8 kGy (Fig. 1L). Concluding these data, the tolerance to an exposure to ionizing radiation can be ranked as follows: *Y. intermedia* MASE-LG-1 < *Acidiphilium* sp. PM < *Clostridium* sp. MASE-IM-4 < *Trichococcus* sp. MASE-IM-5 < *Buttiauxella* sp. MASE-IM-9 < *Halanaerobium* sp. MASE-BB-1.

### Survival data for various microorganisms from literature data

Table 2 lists organisms from other studies and their desiccation and radiation tolerance in which both parameters have been measured for a given organism. For comparability with previous studies, desiccation tolerance was defined as the tolerance to survive four weeks (28 days) in a dried state at room temperature and if applicable under anoxic conditions. Radiotolerance was defined as the ability to survive an exposure to ionizing radiation with a dose of 3 kGy. Additionally, there are many microorganisms for which only one treatment was tested and the list could be enlarged tremendously (e.g. Jolivet et al. 2003; Walsh and Camilli 2011).

All combinations of tolerance to desiccation and to ionizing radiation were prevalent within the MASE strains (Tables 2 and 3). *Yersinia intermedia* MASE-LG-1 and *Acidiphilium* sp. MASE-PM showed tolerance to desiccation, but not to ionizing radiation. *Halanaerobium* sp. MASE-BB-1 and *Buttiauxella* sp. MASE-IM-9 showed tolerances to both treatments (28 days desiccation; 3 kGy); *Trichococcus* sp. MASE-IM-5 and *Clostridium* sp. MASE-IM-4 did not survive either 4 weeks of desiccation, or exposure to 3 kGy of ionizing radiation. In general, there is a trend of correlation between tolerance to desiccation and to ionizing radiation; 50% of the organisms possessed the ability to survive both stresses. However, 33% of organisms showed no correlation and

can outlast only one of the applied stresses. Additionally, 17% could not survive even one stress (Table 3). Most importantly, as visible in the rankings of the investigated organisms, the idea that ionizing radiation resistance is caused by desiccation resistance is not supported since some strains have high ionizing radiation resistance, but not desiccation resistance and vice versa.

## DISCUSSION

Tolerance of vegetative cells to desiccation and to ionizing radiation seems to be a common phenomenon present in all domains in the tree of life including Bacteria and Archaea (Potts 1994; DiRuggiero et al. 1997; Beblo et al. 2009, 2011; Confalonieri and Sommer 2011). However, the response of anaerobic microorganisms to different extremes, such as drought and radiation, is still poorly understood and a systematic comparison of survival cannot be made due to the different experimental setups. In this study, the survival of six facultative or obligate anaerobic microorganisms after exposure to desiccation and to ionizing radiation under anoxic conditions was examined and compared to data in the literature. The tolerance to desiccation and to radiation of the tested microorganisms was found to vary substantially. These variations were found within genera as in the case of the genus *Yersinia*, indicating that both tolerances are a species-specific feature. Several previous works reported on the distribution of desiccation and radiation tolerance within phylogenetically diverse microorganisms (Thomas et al. 2006; LaDuc et al. 2007; Musilova et al. 2015). Especially, La Duc and colleagues (2007) showed that there is no general correlation between short desiccation periods up to 7 days and radiation tolerance: 34 strains withstood desiccation, but surprisingly none of these strains tested strains survived a treatment of 5 kGy. *Yersinia intermedia* MASE-LG-1 tested here was highly desiccation tolerant which is in contrast with the desiccation-sensitive strain *Y. pestis* which is not able to survive desiccation on glass for 24 h (Rose et al. 2003). Similar species-specific specificities have been shown for the aerobic deinococcal radiation-sensitive representatives (Callegan et al. 2008)

It has been postulated that tolerance to desiccation is correlated with tolerance to radiation, since desiccation would select for repair capabilities that serendipitously allow for radiation tolerance, even though these organisms do not grow in naturally high-radiation environments (e.g. Mattimore and Battista 1996). Our data allow us to compare the desiccation and radiation tolerance of our selected anoxically grown isolates, and thus to investigate whether the postulated correlation is true for all organisms. There are different theories which try to explain the presumed correlation or relationship between tolerance to desiccation and radiotolerance. There are some reasons which are associated with the natural environment and with the cell as a whole like (i) habitat and (ii) cell aggregates and biofilms. Additionally, there are some intracellular factors which play a role inside the cells like (iii) specific enzymes and (iv) compatible solutes. Nevertheless, if other factors like a toroidal genome structure (Levin-Zaidman et al. 2003; Cox and Battista 2005), or the intracellular ion content (Daly et al. 2004; Daly 2009) as it is described for *D. radiodurans* play a role in the investigated strains remains speculative.

### Habitat

One explanation is based on the assumption that the organisms' original habitat influences their tolerance against stressful conditions. This has been observed for microorganisms that grow in

**Table 2.** Overview of microbial survival after desiccation (28 days) and ionizing radiation (3 kGy) from literature data.

Organism	Oxidative state of the experimental setup	Desiccation (28 days)	Radiation (3 kGy)	Reference
<i>Acidiphilium</i> sp. PM	Anoxic	+	-	In this study
<i>Buttiauxella</i> sp. MASE-IM-10	Anoxic	+	+	In this study
<i>Clostridium</i> sp. MASE-IM-4	Anoxic	-	-	In this study
<i>Halanaerobium</i> sp. MASE-BB-1	Anoxic	+	+	In this study
<i>Trichococcus</i> sp. MASE-IM-5	Anoxic	-	-	In this study
<i>Yersinia intermedia</i> MASE-LG-1	Anoxic	+	-	In this study
<i>Archaeoglobus fulgidus</i>	Anoxic	+	+	Beblo et al. 2009; Beblo et al. 2011
<i>Ignicoccus hospitalis</i>	Anoxic	-	+	Beblo et al. 2009; Koschnitzki 2016
<i>Methanocaldococcus jannaschii</i>	Anoxic	-	+	Beblo et al. 2009; Beblo et al. 2011
<i>Methanosarcina barkeri</i>	Anoxic	+	+ <sup>b</sup>	Morozova and Wagner 2007; Anderson, Apolinario and Sowers 2012
<i>Methanothermobacter thermoautotrophicus</i>	Anoxic	+	- <sup>c</sup>	Beblo et al. 2009; Beblo et al. 2011
<i>Nanoarchaeum equitans</i>	Anoxic	-	- <sup>c</sup>	Beblo et al. 2009; Beblo et al. 2011
<i>Pyrococcus furiosus</i>	Anoxic	-	+	DiRuggiero et al. 1997; Beblo et al. 2009
<i>Thermoproteus tenax</i>	Anoxic	-	+	Beblo et al. 2009; Beblo et al. 2011
<i>Thermofilum pendens</i>	Anoxic	-	- <sup>c</sup>	Beblo et al. 2009; Beblo et al. 2011
<i>Aquifex pyrophilus</i>	Microoxic	+	+	Beblo et al. 2009; Beblo et al. 2011
<i>Hydrogenothermus marinus</i>	Microoxic	+	+	Beblo et al. 2009; Beblo et al. 2011
<i>Metallosphaera sedula</i>	Microoxic/oxic	-	+	Beblo et al. 2009; Beblo et al. 2011
<i>Sulfolobus metallicus</i>	Microoxic/oxic	-	+	Beblo et al. 2009; Beblo et al. 2011
<i>Acinetobacter radioresistens</i>	Oxic	+	+	Nishimura, Ino and Iizuka 1988; Jawad et al. 1998
<i>Brevundimonas</i> sp.	Oxic	+	+	Dartnell et al. 2010; Musilova et al. 2015
<i>Chroococcidiopsis</i> sp.	Oxic	+	+	Caiola et al. 1996; Billi et al. 2000
<i>Deinococcus radiodurans</i>	Oxic	+	+	Daly 2009; Bauermeister et al. 2011
<i>Deinococcus geothermalis</i>	Oxic	+	+	Ferreira et al. 1997; Frösler et al. 2012
<i>Escherichia coli</i>	Oxic	-	-	Clavero et al. 1994; Welsh and Herbert 1999
<i>Geodermatophilus poikilotropi</i>	Oxic	+	+	Montero-Calasanz et al. 2014
<i>Halobacterium salinarum</i>	Oxic	+ <sup>a</sup>	+	Kottemann et al. 2005; Leuko and Rettberg 2017
<i>Halococcus hamelinensis</i>	Oxic	+	+	Leuko and Rettberg 2017
<i>Halococcus morrhuae</i>	Oxic	+	+	Leuko and Rettberg 2017
<i>Halomonas</i> sp.	Oxic	+	+	Musilova et al. 2015
<i>Kocuria polaris</i>	Oxic	-	+	Shirsalimian et al. 2016
<i>Listeria monocytogenes</i>	Oxic	+ <sup>a</sup>	-	Niemira et al. 2003; Hingston et al. 2013
<i>Methylobacterium extorquens</i>	Oxic	+	+	Romanovskaya et al. 2002
<i>Rhodococcus</i> sp.	Oxic	-	+	Dartnell et al. 2010; Musilova et al. 2015
<i>Salmonella typhimurium</i>	Oxic	+ <sup>a</sup>	+	Thayer and Boyd 1991; Li et al. 2012
<i>Yersinia pestis</i>	Oxic	-	-	Rose et al. 2003; Sommers and Cooke 2009

Survival was tested at a maximum of 20 days of desiccation.

<sup>b</sup>Survival was tested after exposure to 2.5 kGy.

<sup>c</sup>Survival was tested after exposure to 5 kGy.

**Table 3.** Distribution of resistance to desiccation and ionizing radiation amongst the investigated organisms from Table 2.

Desiccation (28 days)	Radiation (3 kGy)	Percentage
-	-	17%
-	+	22%
+	-	11%
+	+	50%

dry habitats like deserts or highly saline areas, such as various deinococci, *Chroococcidiopsis* and some haloarchaea (Caiola, Billi and Friedmann 1996; Billi et al. 2000; Stan-Lotter and Fendrihan 2015). Additionally, different microbial strains were isolated around Chernobyl. Those strains are able to tolerate better the exposure to different doses of radiation than bacterial communities from other sites with lower background radiation lev-

els (Ruiz-González et al. 2016). However, some deep-sea organisms such as *Archaeoglobus fulgidus* and *Aquifex pyrophilus* also show correlation between radiation and desiccation resistance (Stetter 1988; Huber et al. 1992; Beblo et al. 2009, 2011). The same correlation was shown in this work for *Buttiauxella* sp. MASE-IM-9 and *Halanaerobium* sp. MASE-BB-1. For both organisms, it is unlikely that they experience desiccation or high levels of radiation in their natural (aqueous) environment (Cockell et al. 2017). Furthermore, in the Boulby mine located 1100 m below ground the habitat of *Halanaerobium* sp. MASE-BB-1, the background radiation level was determined to be lower than on the surface (Malczewski, Kisiel and Dorda 2013).

### Cell aggregates and biofilm

The capability of cells to form aggregates or to live in biofilms may also enable them to survive desiccation and radiation. Filament-forming cyanobacteria and tetrad-forming strains

such as *D. radiodurans* and *Chroococcidiopsis* sp. are tolerant to desiccation (de Winder, Matthijs and Mur 1989; Jena et al. 2006; Thomas et al. 2006; Baqué et al. 2013). It was hypothesized that cells attached to each other in a biofilm help each other during repair processes, for example with the exchange of genetic material (Cvitkovitch 2004). For *D. geothermalis*, *Chroococcidiopsis* sp. and two *Streptococcus* strains, it was shown that these microorganisms are able to form biofilms. As a part of a biofilm, they are more tolerant to cell damaging treatment compared to planktonic cells (Baqué et al. 2013; Marks, Reddinger and Hakansson 2014; Frösler et al. 2017). In our case, all MASE isolates grow as single cells under applied optimal growth conditions. At suboptimal growth conditions *Halanaerobium* sp. MASE-BB-1, and at high-sulfate concentrations *Y. intermedia* MASE-LG-1 grow in chains (Schwendner et al. 2018). The first, *Halanaerobium* sp. MASE-BB-1, is able to survive desiccation and ionizing radiation. In contrast, *Y. intermedia* MASE-LG-1 was the only strain to show a tolerance to long-term desiccation being able to survive up to half a year while being sensitive to radiation.

### Specific enzymes

There are additional factors to expect a correlation between desiccation and radiation tolerance especially in (facultative) anaerobic microorganisms. The strains tested here were facultative and obligate anaerobes, and some of the other listed representatives are strictly anaerobes and consequently oxygen-sensitive. During and after desiccation and exposure to ionizing radiation, ROS production has been demonstrated (Jones et al. 1994; França, Panek and Eleutherio 2007). The capacity of these anaerobic strains to effectively protect their intracellular components and to eliminate ROS is of crucial importance for their survival. One strategy is the elimination of ROS by the superoxide dismutase or the superoxide reductase (Cannio et al. 2000). This enzymatic system produces  $H_2O_2$  which is later eliminated by peroxidases, catalases or hydroperoxide reductases (Seaver and Imlay 2001). Nevertheless, not all facultative or obligate anaerobic strains were tolerant to desiccation and ionizing irradiation and a general protection by superoxide dismutase/reductase system can be neglected.

### Compatible solutes

Water loss and high salinity have similar effects on a microbial cell. To counteract osmotic stress, several microorganisms take up or produce intracellular compatible solutes or follow the salt-in strategy which is most commonly detected in halophiles (Galinski 1995; Kempf and Bremer 1998). It has been shown that compatible solutes, due to their radical scavenging capacity, their ability to stabilize proteins and membranes, positively influence the desiccation tolerance of microorganisms but the desiccation itself is not inducing compatible solute accumulation (Smirnoff and Cumbes 1989; Lippert and Galinski 1992; Hinch and Hagemann 2004). Recently, it has been reported that the response of *Y. intermedia* MASE-LG-1 to salt stress (e.g. NaCl) involves an accumulation of L-asparagine and sucrose which might be one explanation for its tolerance to desiccation (Schwendner et al. 2017). For *Halanaerobium praevalens*, a close relative to the MASE strain *Halanaerobium* sp. MASE-BB-1, it was shown that the organisms is using the salt-in strategy and KCl is accumulated to respond to changes in the osmotic balance (Oren, Heldal and Norland 1997).

A possible link between intracellular osmoadaptation compounds and microbial tolerance to ionizing radiation has also

been discussed (Kish et al. 2009; Webb and DiRuggiero 2012). Additionally, for the compatible solute ectoine, a protective influence on isolated DNA was shown (Hahn et al. 2017). However, in *Hydrogenothermus marinus* and *A. fulgidus* only an enhanced desiccation tolerance but no improvement of the radiation tolerance due to cultivation at hyper optimal salinity (NaCl) has been observed (Beblo-Vranesevic et al. 2017b). In *Y. intermedia* MASE-LG-1, L-asparagine and sucrose are produced due to high osmolality, but the radiation sensitivity was not altered.

## CONCLUSION

Our data demonstrated that (facultative) anaerobes from extreme environments showed different response to desiccation and ionizing radiation. We did not observe an obvious correlation between desiccation and radiation tolerance, suggesting that although some of the biochemical basis behind desiccation and radiation tolerance, such as in the quenching of ROS, may be similar, the pathways determining desiccation and radiation tolerance in microorganisms are likely different to involve distinct biochemical pathways. Indeed, the diversity of possible responses that microorganisms can deploy to cope with these extremes may explain why high tolerance to one stress does not imply high tolerance to the other. Although the matter remains open as to whether desiccation stress can select for high radiation stress in some organisms, our data showed that microorganisms can possess tolerance to ionizing radiation and yet be sensitive to desiccation stress. Further work to elucidate the pathways of ionizing and radiation stress in microorganisms is merited.

## FUNDING

This work was supported by European Community's Seventh Framework Program (FP7/2007 - 2013) [grant agreement no. 607297].

**Conflict of interest.** None declared.

## REFERENCES

- Anderson KL, Apolinario EE, Sowers KR. Desiccation as a long-term survival mechanism for the archaeon *Methanosarcina barkeri*. *Appl Environ Microb* 2012;78:1473–9.
- Battista JR, Earl AM, Park MJ. Why is *Deinococcus radiodurans* so resistant to ionizing radiation? *Trends Microbiol* 1999;7:362–5.
- Bauermeister A, Moeller R, Reitz G et al. Effect of relative humidity on *Deinococcus radiodurans*' resistance to prolonged desiccation, heat, ionizing, germicidal, and environmentally relevant UV radiation. *Microb Ecol* 2011;61:715–22.
- Baqué M, Scalzi G, Rabbow E et al. Biofilm and planktonic lifestyles differently support the resistance of the desert cyanobacterium *Chroococcidiopsis* under space and Martian simulations. *Orig Life Evol B* 2013;43:377–89.
- Beblo K, Rabbow E, Rachel R et al. Tolerance of thermophilic and hyperthermophilic microorganisms to desiccation. *Extremophiles* 2009;13:521–31.
- Beblo K, Douki T, Schmalz G et al. Survival of thermophilic and hyperthermophilic microorganisms after exposure to UV-C, ionizing radiation and desiccation. *Arch Microbiol* 2011;193:797–809.
- Beblo-Vranesevic K, Bohmeier M, Perras AK et al. The responses of an anaerobic microorganism, *Yersinia intermedia* MASE-

- LG-1 to individual and combined simulated Martian stresses. *PLoS One* 2017a;12:e0185178.
- Beblo-Vranesevic K, Galinski EA, Rachel R et al. Influence of osmotic stress on desiccation and irradiation tolerance of (hyper)-thermophilic microorganisms. *Arch Microbiol* 2017b;199:17–28.
- Bieger-Dose A, Dose K, Meffert R et al. Extreme dryness and DNA-protein cross-links. *Adv Space Res* 1992;12:265–70.
- Billi D, Friedmann EI, Hofer KG et al. Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Appl Environ Microb* 2000;66:1489–92.
- Billi D, Potts M. Life and death of dried prokaryotes. *Res Microbiol* 2002;153:7–12.
- Cabiscol E, Tamarit J, Ros J. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol* 2000;3:3–8.
- Caiola MG, Billi D, Friedmann EI. Effect of desiccation on envelopes of the cyanobacterium *Chroococcidiopsis* sp. (Chroococcales). *Eur J Phycol* 1996;31:97–105.
- Callegan RP, Nobre MF, McTernan PM et al. Description of four novel psychrophilic, ionizing radiation-sensitive *Deinococcus* species from alpine environments. *Int J Syst Evol Microbiol* 2008;58:1252–8.
- Cannio R, Fiorentino G, Morana A et al. Oxygen: friend or foe? archaeal superoxide dismutases in the protection of intra- and extracellular oxidative stress. *Front Biosci* 2000;5:D768–79.
- Chen M, Alexander M. Survival of soil bacteria during prolonged desiccation. *Soil Biol Biochem* 1973;5:213–21.
- Clavero MR, Monk JD, Beuchat LR et al. Inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Appl Environ Microb* 1994;60:2069–75.
- Cockell CS, Schwendner P, Perras A et al. Anaerobic microorganisms in astrobiological analog environments: from field site to culture collection. *Int J Astrobiol* 2017;1–15. DOI: 10.1017/S1473550417000246.
- Confalonieri F, Sommer S. Bacterial and archaeal resistance to ionizing radiation. *J Phys Conf Ser* 2011;261:012005.
- Cox CS. Roles of water molecules in bacteria and viruses. *Origins Life Evol B* 1993;23:29–36.
- Cox MM, Battista JR. *Deinococcus radiodurans* - the consummate survivor. *Nat Rev Microbiol* 2005;3:882–92.
- Cvitkovitch D. Genetic exchange in biofilms. In: Ghannoum M, O'Toole G (ed). *Microbial Biofilms*. Washington, DC: ASM Press, 2004, 192–205. DOI: 10.1128/9781555817718.ch11.
- Daly MJ. A new perspective on radiation resistance based on *Deinococcus radiodurans*. *Nat Rev Microbiol* 2009;7:237–45.
- Daly MJ, Gaidamakova EK, Matrosova VY et al. Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. *Science* 2004;306:1025–8.
- Daly MJ, Gaidamakova EK, Matrosova VY et al. Protein oxidation implicated as the primary determinant of bacterial radioreistance. *PLoS Biol* 2007;5:e92.
- Dartnell LR, Hunter SJ, Lovell KV et al. Low-temperature ionizing radiation resistance of *Deinococcus radiodurans* and antarctic dry valley bacteria. *Astrobiology* 2010;10:717–32.
- De Winder B, Matthijs HC, Mur LR. The role of water retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestrial habitat. *Arch Microbiol* 1989;152:458–62.
- DiRuggiero J, Santangelo N, Nackerdien Z et al. Repair of extensive ionizing-radiation DNA damage at 95 degrees C in the hyperthermophilic archaeon *Pyrococcus furiosus*. *J Bacteriol* 1997;179:4643–5.
- Dose K, Bieger-Dose A, Labusch M et al. Survival in extreme dryness and DNA-single-strand breaks. *Adv Space Res* 1992;12:221–9.
- Ferreira AC, Nobre MF, Rainey FA et al. *Deinococcus geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. *Int J Syst Evol Microbiol* 1997;47:939–47.
- Fetzer S, Bak F, Conrad R. Sensitivity of methanogenic bacteria from paddy soil to oxygen and desiccation. *FEMS Microbiol Ecol* 1993;12:107–15.
- França MB, Panek AD, Eleutherio EC. Oxidative stress and its effects during dehydration. *Comp Biochem Physiol A Mol Integr Physiol* 2007;146:621–31.
- Franson MAH (ed). *Standard Methods for the Examination of Water and Wastewater*, 16th edn. Washington DC: American Public Health Association, 1985.
- Fredrickson JK, Shu-mei WL, Gaidamakova EK et al. Protein oxidation: key to bacterial desiccation resistance? *ISME J* 2008;2:393–403.
- Frösler J, Panitz C, Wingender J et al. Survival of *Deinococcus geothermalis* in biofilms under desiccation and simulated space and martian conditions. *Astrobiology* 2017;17:431–47.
- Galinski EA. Osmoadaptation in bacteria. *Adv Microb Physiol* 1995;37:273–328.
- González-Flecha B, Demple B. Metabolic sources of hydrogen peroxide in aerobically growing *Escherichia coli*. *J Biol Chem* 1995;270:13681–7.
- Hahn MB, Meyer S, Schröter MA et al. DNA protection by ectoine from ionizing radiation: molecular mechanisms. *Phys Chem Chem Phys* 2017;19:25717–22.
- Hincha DK, Hagemann M. Stabilization of model membranes during drying by compatible solutes involved in the stress tolerance of plants and microorganisms. *Biochem J* 2004;383:277–83.
- Hingston PA, Stea EC, Knöchel S et al. Role of initial contamination levels, biofilm maturity and presence of salt and fat on desiccation survival of *Listeria monocytogenes* on stainless steel surfaces. *Food Microbiol* 2013;36:46–56.
- Huber R, Wilharm T, Huber D et al. *Aquifex pyrophilus* gen. nov. sp. nov., represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacteria. *Syst Appl Microbiol* 1992;15:340–51.
- Jawad A, Snelling AM, Heritage J et al. Exceptional desiccation tolerance of *Acinetobacter radioresistens*. *J Hosp Infect* 1998;39:235–40.
- Jena SS, Joshi HM, Sabareesh KVP et al. Dynamics of *Deinococcus radiodurans* under controlled growth conditions. *Biophys J* 2006;91:2699–707.
- Jolivet E, L'Haridon S, Corre E et al. *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *Int J Syst Evol Microbiol* 2003;53:847–51.
- Jones GDD, Boswell TV, Lee J et al. A comparison of DNA damages produced under conditions of direct and indirect action of radiation. *Int J Radiat Biol* 1994;66:441–5.
- Kempf B, Bremer E. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch Microbiol* 1998;170:319–30.
- Kiefer J. *Biological Radiation Effects*. Springer-Verlag, 1990. DOI: 10.1007/978-3-642-83769-2.
- Kish A, Kirkali G, Robinson C et al. Salt shield: intracellular salts provide cellular protection against ionizing radiation in the



- halophilic archaeon, *Halobacterium salinarum* NRC-1. *Environ Microbiol* 2009;11:1066–78.
- Koschnitzki D. The radiation tolerance of *Ignicoccus*; species – their astrobiological relevance and implications to DNA repair processes. Ph.D. Thesis. Department for Microbiology, University Regensburg 2016.
- Kottemann M, Kish A, Iloanusi C et al. Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation. *Extremophiles* 2005;9:219–27.
- Krisko A, Radman M. Protein damage and death by radiation in *Escherichia coli* and *Deinococcus radiodurans*. *P Natl Acad Sci USA* 2010;107:14373–7.
- La Duc MT, Benardini JN, Kempf MJ et al. Microbial diversity of Indian ocean hydrothermal vent plumes: microbes tolerant of desiccation, peroxide exposure, and ultraviolet and  $\gamma$ -irradiation. *Astrobiology* 2007;7:416–31.
- Leuko S, Rettberg P. The effects of HZE Particles,  $\gamma$  and X-ray radiation on the survival and genetic integrity of *Halobacterium salinarum* NRC-1, *Halococcus hamelinensis*, and *Halococcus morhuae*. *Astrobiology* 2017;17:110–7.
- Levin-Zaidman S, Englander J, Shimoni E et al. Ringlike structure of the *Deinococcus radiodurans* genome: a key to radioresistance? *Science* 2003;299:254–6.
- Leyko W, Bartosz G. Membrane effects of ionizing radiation and hyperthermia. *Int J Radiat Biol Relat Stud Phys Chem Med* 1985;49:743–70.
- Li H, Bhaskara A, Megalis C et al. Transcriptomic analysis of *Salmonella* desiccation resistance. *Foodborne Pathog Dis* 2012;9:1143–51.
- Lippert K, Galinski EA. Enzyme stabilization by ectoine-type compatible solutes: protection against heating, freezing and drying. *Appl Microbiol Biot* 1992;37:61–5.
- Malczewski D, Kisiel J, Dorda J. Gamma background measurements in the Boulby Underground Laboratory. *J Radioanal Nucl Ch* 2013;298:1483–9.
- Marks LR, Reddinger RM, Hakansson AP. Biofilm formation enhances fomite survival of *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *Infect Immun* 2014;82:1141–6.
- Mattimore V, Battista JR. Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J Bacteriol* 1996;178:633–7.
- Michaels HB, Hunt JW. A model for radiation damage in cells by direct effect and by indirect effect: a radiation chemistry approach. *Rad Res* 1978;74:23–34.
- Montero-Calasan MD, Hofner B, Göker M et al. *Geodermatophilus poikilotrophi* sp. nov.: a multitolerant actinomycete isolated from dolomitic marble. *BioMed Res Int* 2014;2014:1–11.
- Morozova D, Wagner D. Stress response of methanogenic archaea from Siberian permafrost compared with methanogens from nonpermafrost habitats. *FEMS Microbiol Ecol* 2007;61:16–25.
- Musilova M, Wright G, Ward JM et al. Isolation of radiation-resistant bacteria from Mars analog Antarctic dry valleys by preselection, and the correlation between radiation and desiccation resistance. *Astrobiology* 2015;15:1076–90.
- Niemira BA, Fan X, Sokorai KJ et al. Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and *Listeria innocua* ATCC 51742 inoculated on endive (*Cichorium endiva*). *J Food Prot* 2003;66:993–8.
- Nishimura Y, Ino T, Iizuka H. *Acinetobacter radioresistens* sp. nov. isolated from cotton and soil. *Int J Syst Evol Microbiol* 1988;38:209–11.
- Oren A, Heldal M, Norland S. X-ray microanalysis of intracellular ions in the anaerobic halophilic eubacterium *Haloanaerobium praevalens*. *Can J Microbiol* 1997;43:588–92.
- Potts M. Desiccation tolerance of prokaryotes. *Microbiol Rev* 1994;58:755–805.
- Prestrelski SJ, Tedeschi N, Arakawa T et al. Dehydration-induced conformational transitions in proteins and their inhibition by stabilizers. *Biophys J* 1993;65:661–71.
- Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol* 1994;65:27–33.
- Romanovskaya VA, Rokitko PV, Mikheev AN et al. The effect of  $\gamma$ -radiation and desiccation on the viability of the soil bacteria isolated from the alienated zone around the Chernobyl Nuclear Power Plant. *Mikrobiologiya* 2002;71:608–13.
- Rose LJ, Donlan R, Banerjee SN et al. Survival of *Yersinia pestis* on environmental surfaces. *Appl Environ Microb* 2003;69:2166–71.
- Ruiz-González MX, Czirják GÁ, Genevoux P et al. Resistance of feather-associated bacteria to intermediate levels of ionizing radiation near Chernobyl. *Sci Rep* 2016;6:22969.
- Schwendner P, Bohmeier M, Rettberg P et al. Variable metabolomic responses in the anaerobic organism *Yersinia intermedia* MASELG-1 to NaCl and MgSO<sub>4</sub> at identical water activity. *Front Microbiol* 2018, DOI: 10.3389/fmicb.2018.00335.
- Seaver LC, Imlay JA. Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. *J Bacteriol* 2001;183:7173–81.
- Shirsalimian MS, Sepahy AA, Amoozegar MA et al. Isolation of a mesophilic and halotolerant strain of *Kocuriapolaris* from Gandom Beryan area in the Lut Desert of Iran, moderately resistant to gamma radiation and desiccation. *Biosci Biotechnol Res Asia* 2016;13:2343–50.
- Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* 1989;28:1057–60.
- Sommers CH, Cooke PH. Inactivation of a virulent *Yersinia pestis* in butterfield's phosphate buffer and frankfurters by UVC (254 nm) and gamma radiation. *J Food Prot* 2009;72:755–9.
- Stan-Lotter H, Fendrihan S. *Halophilic archaea*: life with desiccation, radiation and oligotrophy over geological times. *Life* 2015;5:1487–96.
- Stetter KO. *Archaeoglobus fulgidus* gen. nov., sp. nov.: a new taxon of extremely thermophilic archaebacteria. *Syst Appl Microbiol* 1988;10:172–3.
- Thayer DW, Boyd G. Effect of ionizing radiation dose, temperature, and atmosphere on the survival of *Salmonella typhimurium* in sterile, mechanically deboned chicken meat. *Poult Sci* 1991;70:381–8.
- Thomas DJ, Boling J, Boston PJ et al. Extremophiles for ecopoiesis: desirable traits for and survivability of pioneer Martian organisms. *Gravit Space Res* 2006;19:91–104.
- Thorne MC. Background radiation: natural and man-made. *J Radiol Prot* 2003;23:29–42.
- Walsh RL, Camilli A. *Streptococcus pneumoniae* is desiccation tolerant and infectious upon rehydration. *mBio* 2011;2:e00092–11.
- Webb KM, DiRuggiero J. Role of Mn<sup>2+</sup> and compatible solutes in the radiation resistance of thermophilic bacteria and archaea. *Archaea* 2012;2012:1–11.
- Welsh DT, Herbert RA. Osmotically induced intracellular trehalose, but not glycine betaine accumulation promotes desiccation tolerance in *Escherichia coli*. *FEMS Microbiol Lett* 1999;174:57–63.