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

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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 six months and their 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.

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 (Welsh and Herbert 1999; Clavero *et al.* 1994; 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 *et al.* 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 (Billi and Potts 2002; Prestrelski *et al.* 1993; Cox 1993). 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 six weeks caused approximately 60 DNA double-strand breaks per genome in *D. radiodurans* (Fredrickson *et al.* 2008). Nevertheless, the survival of the microorganisms after six 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 *et al.* 2007). The ROS are mainly superoxide anions ($\bullet O_2^-$), and hydroxyl radicals (•OH)

affecting all macromolecular cellular components (Cabiscol *et al.* 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 *et al.* 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 (Jones *et al.* 1994; Michaels and Hunt 1978; 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 *et al.* 1999). In addition, ionizing radiation also affects proteins through oxidation processes, lipids through lipid peroxidation and disturbance of membrane permeability in eu- 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 theses extreme environmental conditions (Cockell *et al.* 2017). In this article, we describe the survivability after prolonged desiccation of up to four 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 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 ml of cell culture (cell densities ranged from ~ 5 x 10^6 cells / ml to ~ 5 x 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_2] < 0.0001%, relative humidity 13 ± 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 Gy min^{-1} ± 5 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 four 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 ten-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_0) ($S = N/N_0$).

Due to the applied MPN-technique and depending on the growth density of the specific strain (~ 5×10^6 cells / ml to ~ 5×10^7 cells / ml) the detection limit of the determination of survival was ~ 1×10^{-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 d) = 3.7 x 10^{-5} (Beblo-Vranesevic *et al.* 2017). 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 desiccation. After four weeks in a dry state no living cells of *Clostridium* sp. MASE-IM-4 and *Trichococcus* sp. MASE-IM-5 were detectable (Fig. 1C, 1E). The survival of *Halanaerobium* sp. MASE-BB-1 after four weeks of desiccation was reduced by more than four orders of magnitude (Fig. 1D) to S (28 d) = 3.7 x 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 four weeks were S (28 d) = 7.0 x 10^{-7} and S (28 d) = 3.7 x 10^{-5} respectively and these strains could outlast 84 days in a desiccated form (Fig. 1A, 1B). 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, 1J) were able to multiply after an exposure to 3 kGy (S (*Buttiauxella* sp. MASE-IM-9, 3 kGy) = 3.7 x 10^{-7}; S (*Halanaerobium* sp. BB-1, 3 kGy) = 4 x 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, 1K). *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 followed: *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. Walsh and Camilli 2011; Jolivet *et al.* 2003).

All combinations of tolerance to desiccation and to ionizing radiation were prevalent within the MASE strains (Table 2, 3). *Y. 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 four 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 isn't 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; Beblo *et al*. 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 seven days and radiation tolerance: thirty-four strains withstood desiccation, but surprisingly none of these strains tested strains survived a treatment of 5 kGy. *Y. intermedia* MASE-LG-1 tested here was highly desiccation tolerant which is in contrast with the desiccation sensitive strain *Yersinia pestis* which is not able to survive desiccation on glass for 24 hours (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, (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.

(i) 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 dry habitats like deserts or highly saline areas, such as various deinococci, *Chroococcidiopsis* and some haloarchaea (Caiola *et al.* 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 levels (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 (Beblo *et al.* 2009, Beblo *et al.* 2011; Stetter 1988; Huber *et al.* 1992). The same correlation was shown in this work for *Buttiauxella*

sp. MASE-IM-9 and *Halanerobium* 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 *et al.* 2013).

(ii) 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 *et al.* 1989; Thomas *et al.* 2006; Jena *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 (Frösler *et al.* 2017; Baqué *et al.* 2013, Marks *et al.* 2014). 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.* 2017). 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.

(iii) 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 *et al.* 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.

(iv) 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 (Kempf and Bremer 1998; Galinski 1995). 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 *et al.* 1989; Lippert and Galinski 1992; Hincha 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 *et al.* 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.

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Conflicts of interests

The authors declare no conflict of interest.

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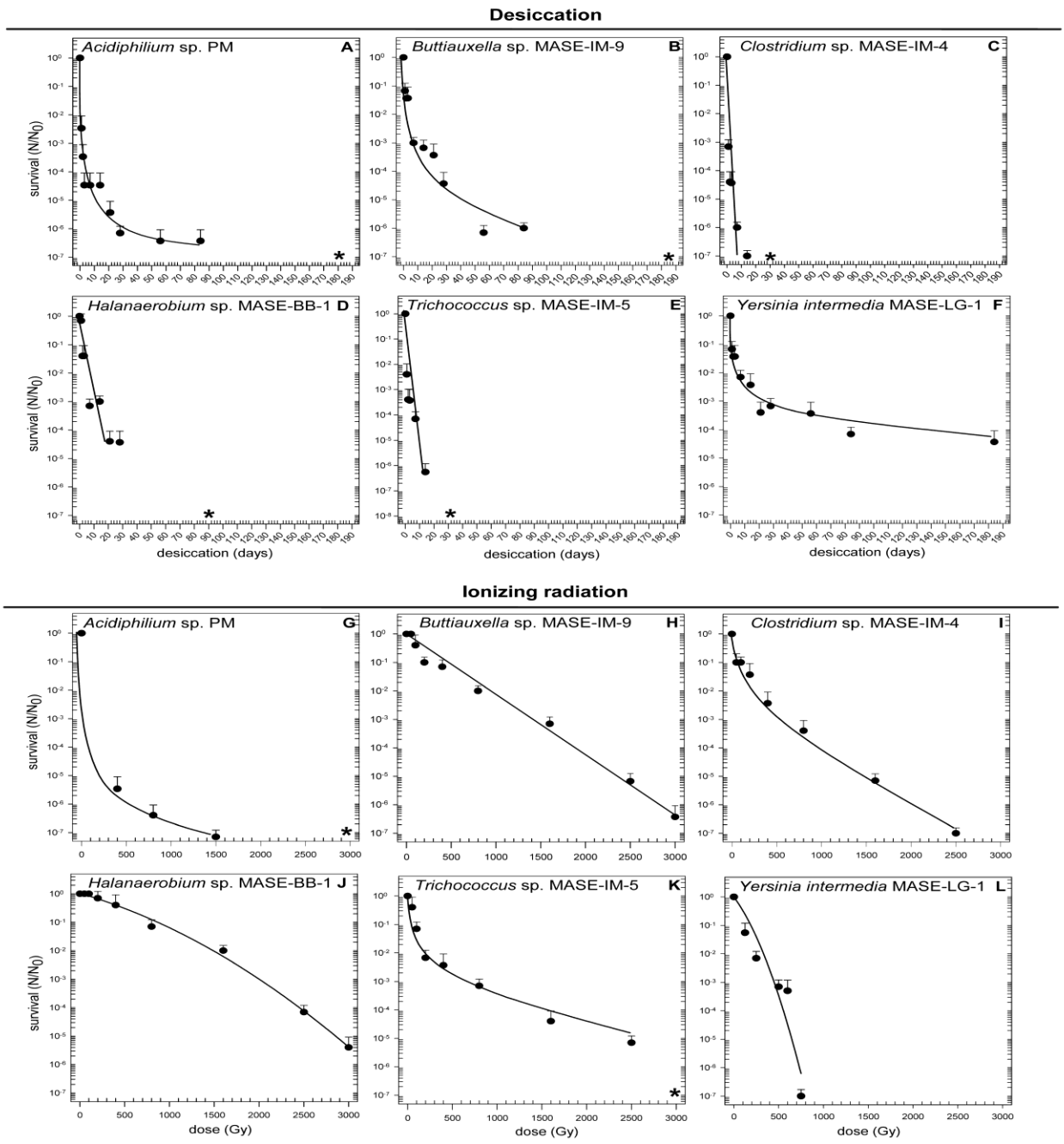


Fig. 1 Survival of the MASE isolates after anoxic desiccation (A-F) and after exposure to ionizing radiation under anoxic conditions (G-L). For desiccation experiments, the cells were applied to glass slides, dried and stored under anoxic conditions up to 184 days. For anoxic irradiation experiments, 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; $N_{0:}$ viable cells without desiccation or without

irradiation; N: viable cells after desiccation or without irradiation (n=3 with standard deviation); *: no viable cells detected.

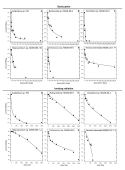
Table 1 Strains, origins and cultivation conditions

Strain **Phylum** **Class**	**Origin**	**Medium**	**Supplements (wt/vol)**	**Gas phase (vol/vol)**	**Temp. (°C)**
Acidiphilium sp. PM Proteobacteria Alphaproteiobacteria	River Rio Tinto, Spain	MASE-I	0.01% KNO_3 0.01% C-Org-Mix	80% N_2, 20% CO_2	30
Buttiauxella sp. MASE-IM-9, Proteobacteria Gammaproteobacteria	Islinger Mühlbach, Germany	MASE-II	0.1% Yeast extract	80% N_2, 20% CO_2	30
Clostridium sp. MASE-IM-4 Firmicutes Clostridia	Islinger Mühlbach, Germany	MASE-II - $FeCl_2$	0.01% Dimethylamine 0.001% $FeCl_2$	15% H_2, 25% CO_2, 60% N_2	30
Halanaerobium sp. MASE-BB-1 Proteobacteria Gammaproteobacteria	Boulby Mine, Great Britain	HACE	0.1% Yeast extract	15% H_2, 25% CO_2, 60% N_2	45
Trichococcus sp. MASE-IM-5 Firmicutes, Bacilli	Islinger Mühlbach, Germany	MASE-II - $FeCl_2$	0.01% Na_2SO_4 0.01% $C_6H_5Na_3O_7$ x 2 H_2O 0.02% KNO_3	15% H_2, 25% CO_2, 60% N_2	30
Y. intermedia MASE-LG-1 Proteobacteria Gammaproteobacteria	Lake Grænavatn, Iceland	MASE-I	0.01% KNO_3 0.01% C-Org-Mix	80% N_2, 20% CO_2	30

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**
Acidiphilium sp. PM	anoxic	+	-	In this study
Buttiauxella sp. MASE-IM-10	anoxic	**+**	**+**	In this study
Clostridium sp. MASE-IM-4	anoxic	**-**	**-**	In this study
Halanaerobium sp. MASE-BB-1	anoxic	**+**	**+**	In this study
Trichococcus sp. MASE-IM-5	anoxic	**-**	**-**	In this study
Yersinia intermedia MASE-LG-1	anoxic	**+**	**-**	In this study
Archaeoglobus fulgidus	anoxic	**+**	**+**	Beblo *et al.* 2009; Beblo *et al.* 2011
Ignicoccus hospitalis	anoxic	**-**	**+**	Beblo *et al.* 2009, Koschnitzki 2016
Methanocaldococcus jannaschii	anoxic	**-**	**+**	Beblo *et al.* 2009; Beblo *et al.* 2011
Methanosarcina barkeri	anoxic	**+**	**+**[b]	Morozova and Wagner 2007; Anderson *et al.* 2012
Methanothermobacter thermoautotrophicus	anoxic	**+**	**-**[c]	Beblo *et al.* 2009; Beblo *et al.* 2011
Nanoarchaeum equitans	anoxic	**-**	**-**[c]	Beblo *et al.*

				2009; Beblo *et al.* 2011
Pyrococcus furiosus	anoxic	-	+	Beblo *et al.* 2009; DiRuggiero *et al.* 1997
Thermoproteus tenax	anoxic	-	+	Beblo *et al.* 2009; Beblo *et al.* 2011
Thermofilum pendens	anoxic	-	-[c]	Beblo *et al.* 2009; Beblo *et al.* 2011
Aquifex pyrophilus	microoxic	+	+	Beblo *et al.* 2009; Beblo *et al.* 2011
Hydrogenothermus marinus	microoxic	+	+	Beblo *et al.* 2009; Beblo *et al.* 2011
Metallosphaera sedula	microoxic / oxic	-	+	Beblo *et al.* 2009; Beblo *et al.* 2011
Sulfolobus metallicus	microoxic / oxic	-	+	Beblo *et al.* 2009; Beblo *et al.* 2011
Acinetobacter radioresistens	oxic	+	+	Jawad *et al.* 1998; Nishimura *et al.* 1988
Brevundimonas sp.	oxic	+	+	Dartnell *et al.* 2010; Musilova *et al.* 2015
Chroococcidiopsis sp.	oxic	+	+	Caiola *et al.* 1996; Billi *et al.* 2000
Deinococcus radiodurans	oxic	+	+	Daly 2009; Bauermeister

				et al. 2011
Deinococcus geothermalis	oxic	+	+	Frösler *et al.* 2017; Ferreira *et al.* 1997
Escherichia coli	oxic	-	-	Welsh and Herbert 1999; Clavero *et al.* 1994
Geodermatophilus poikilotrophi	oxic	+	+	Montero-Calasanz *et al.* 2014
Halobacterium salinarum	oxic	+[a]	+	Kottemann *et al.* 2005; Leuko and Rettberg 2017
Halococcus hamelinensis	oxic	+	+	Leuko and Rettberg 2017
Halococcus morrhuae	oxic	+	+	Leuko and Rettberg 2017
Halomonas sp.	oxic	+	+	Musilova *et al.* 2015
Kocuria polaris	oxic	-	+ 	Shirsalimian *et al.* 2016
Listeria monocytogenes	oxic	+[a]	-	Hingston *et al.* 2013; Niemira *et al.* 2003
Methylobacterium extorquens	oxic	+	+	Romanovskaya *et al.* 2002
Rhodococcus sp.	oxic	-	+	Dartnell *et al.* 2010; Musilova *et al.* 2015

Salmonella typhimurium	oxic	+[a]	+	Li *et al.* 2012; Thayer and Boyd 1991
Yersinia pestis	oxic	-	-	Rose *et al.* 2003; Sommers and Cooke 2009

[a] survival was tested at a maximum of 20 days of desiccation; [b] survival was tested after exposure to 2.5 kGy; [c] 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%