Extremophiles (2009) 13:49–57
DOI 10.1007/s00792-008-0196-0

ORIGINAL PAPER

Subcellular integrities in *Chroococcidiopsis* sp. CCMEE 029 survivors after prolonged desiccation revealed by molecular probes and genome stability assays

Daniela Billi

Received: 4 June 2008 / Accepted: 19 September 2008 / Published online: 18 October 2008
© Springer 2008

Abstract Desiccation-tolerant cells must either protect their cellular components from desiccation-induced damage and/or repair it upon rewetting. Subcellular damage to the anhydrobiotic cyanobacterium *Chroococcidiopsis* sp. CCMEE 029 stored in the desiccated state for 4 years was evaluated at the single-cell level using fluorescent DNA strand breakage labelling, membrane integrity and potential related molecular probes, oxidant-sensing fluorochrome and redox dye. Covalent modifications of dried genomes were assessed by testing their suitability as PCR template. Results suggest that desiccation survivors avoid/and or limit genome fragmentation and genome covalent modifications, preserve intact plasma membranes and phycobiliprotein autofluorescence, exhibit spatially-reduced ROS accumulation and dehydrogenase activity upon rewetting. Damaged cells undergo genome fragmentation, loss of plasma membrane potential and integrity, phycobiliprotein bleaching, whole-cell ROS accumulation and lack respiratory activity upon rewetting. The co-occurrence of live and dead cells within dried aggregates of *Chroococcidiopsis* confirms that desiccation resistance is not a simple process and that subtle modifications to the cellular milieu are required to dry without dying. It rises also intriguing questions about the triggers of dead cells in response to drying. The capability of desiccation survivors to avoid and/or reduce subcellular damage, shows that protection mechanisms are relevant in the desiccation tolerance of this cyanobacterium.

Keywords Anhydrobiosis · *Chroococcidiopsis* · Desiccation · Dried genome · Oxidative stress · PCD

Abbreviations

CM-H_2DCFDA	5-(and-6)-Chloromethyl-2′, dichlorodihydrofluorescein diacetate, acetyl ester
DAPI	4′,6-Diamidino-2-phenylindole
DCF	2′,7′-Dichlorofluorescein
DiBAC4(3)	Bis-(1,3-dibutylbarbituric acid) trimethine oxonol
FITC	Fluorescein isothiocyanate
HIP1	Highly iterated palindromic sequences, type1
INT	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride
PCD	Programmed cell death
STRR	Short tandemly repeated repetitive
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling

Introduction

Water and life are inseparable, nevertheless a small but diverse group of organisms, including animals, plants and microbes, can dry without dying, originating a phenomenon known as anhydrobiosis partially understood until recently (Alpert 2006). Cyanobacteria of the genus *Chroococcidiopsis* constantly appear in the most extreme

This paper is dedicated to the memory of E. Imre Friedmann and his wife Roseli, who pioneered researches on *Chroococcidiopsis* and life in desert environments.

Communicated by A. Driessen.

D. Billi (✉)
Department of Biology, University of Rome "Tor Vergata",
Via della Ricerca Scientifica 1, 00133 Rome, Italy
e-mail: billi@uniroma2.it

and dry habitats on earth. Two such environments, the McMurdo Dry Valleys in Antarctica and the Atacama Desert in Chile, are also considered close terrestrial analogs of two Mars environmental extremes: cold and aridity (Warren-Rhodes et al. 2006; Wierzchos et al. 2006). In its natural environment, to escape the harsh outside climate *Chroococcidiopsis* occupies the last refuges for life inside porous rocks or at the stone-soil interfaces, where it survives in a dry, ametabolic state for prolonged periods (Friedmann et al. 1993; Warren-Rhodes et al. 2006). Hence in these environments the time scales of biological and geological processes overlap, and this cyanobacterium is considered an extant representative of ancient desiccation-tolerant cells, the "eoanhydrobiotes" (Billi and Potts 2000, 2002). Thanks to their lifestyle and capability to thrive in extreme conditions, desert strains of *Chroococcidiopsis* provide proper phototrophic models for exobiological research (Grilli Caiola and Billi 2007).

In desiccation-sensitive cells water removal is lethal because of the damage it induces at every level of cellular organization, including membrane phase transition and oxidative damage to proteins, lipids and nucleic acids (Potts 2001). Reduced protein hydration can have a severe effect on enzyme activity and/or electron transport chains, resulting in oxidative stress when radicals and reactive oxygen species (ROS) production exceeds the antioxidant system (França et al. 2007). Desiccation-induced damage to macromolecules results in metal-catalyzed Haber–Weiss and Fenton reactions, while other potential modifications of proteins and nucleic acids via Maillard (browning) reaction can occur, over time leading to heterogeneous products referred to as advanced glycosylation end products (AGEs) (Potts et al. 2005). To understand how *Chroococcidiopsis* withstands desiccation, it is essential to evaluate whether it prevents desiccation-induced damage or it repairs it upon rewetting. Interesting hints about the mechanisms underlying its desiccation tolerance arise from its capability to cope with extremes exceeding those it currently meets in nature. Hot and cold desert isolates, including isolate CCMEE 029, here investigated, in the hydrated state, survive to exposure to ionizing radiation doses, as high as 15 kGy (Billi et al. 2000), while dried cells of CCMEE 029 remain viable during the first few minutes of exposure to simulated Martian UV flux (Cockell et al. 2005). It has been proposed that an efficient DNA repair system which repairs the genome fragmentation induced by ionizing radiation exists in *Chroococcidiopsis* that might contribute both to its desiccation tolerance (Billi et al. 2000) and recovery after exposure to a simulated Martian UV flux (Cockell et al. 2005). An efficient DNA repair system has also been shown for *Deinococcus radiodurans*, and considered a consequence of desiccation tolerance (Mattimore and Battista 1996). However, unlike *D. radiodurans*, the anhydrobiotic cyanobacterium *Nostoc commune* does not undergo genome degradation and oxidative DNA damage after prolonged desiccation (Shirkey et al. 2003).

Previous cytological and ultrastructural studies on two desert strains of *Chroococcidiopsis*, CCMEE 029 and CCMEE 034, subjected to over 5 years of desiccation, identified that within dried aggregates, few surviving cells retained typical ultrastructural features, whereas varying degrees of degenerations occurred in others (Grilli Caiola et al. 1993, 1996a). Still, no further attempts have been undertaken to investigate the macromolecular integrity in desiccation-tolerant cells of *Chroococcidiopsis*.

In this work, subcellular damage to the anhydrobiotic cyanobacterium *Chroococcidiopsis* sp. CCMEE 029 (hereafter *Chroococcidiopsis*) after 4 years of storage in the desiccated state was investigated at the single-cell level, employing fluorescent DNA breakage labelling, membrane integrity and membrane potential related molecular probes, oxidant-sensing fluorochrome and redox dye. Genomic PCR fingerprinting was employed to assess the presence of covalent modifications since they prevent DNA polymerases activity (Potts et al. 2005). Within dried aggregates of *Chroococcidiopsis,* surviving cells, which avoided plasma membrane destabilization, maintaining genome stability, as well as a subset of functional proteins, were discriminated from desiccation injured cells.

Materials and methods

Cyanobacterial strain, drying and rewetting conditions

Chroococcidiopsis sp. CCMEE 029 (N6904) was isolated by Roseli Ocampo–Friedmann from cryptoendolithic growth in Nubian sandstone collected by E. Imre Friedmann in the Negev desert, and currently maintained at the University of Rome "Tor Vergata". The strain was grown in BG-11 medium for 1 month, then ten aliquots of 50-µl culture were spotted into each one of four Petri dishes. With the lid removed they were allowed to dry overnight under a stream of sterile air, at room temperature (RT), then sealed in plastic envelopes and stored at RT in the dark at 44% relative humidity. After more than 4 years of storage, the dried cells were rehydrated by adding 50 µl of distilled water (to maintain solute concentration prior to the desiccation) and stained either immediately or after 30 min for viability tests or 24 h of rewetting for genome stability assay.

Cell viability and ROS level indicators

(1) Rehydrated cells were stained with the cell-impermeant nucleic acid dye SYTOX Green (molecular probes, S-7020) at a final concentration of 50 µM for

5 min in the dark, at RT; after washing, the cell-permeant nucleic acid stain DAPI (molecular probes, D1306) was added at a final concentration of 5 μg/ml for 10 min, in the dark at RT;

(2) 30-min rewetted cells were treated with the membrane potential-sensitive probe $DiBAC_4(3)$ (molecular probes, B-438) at a final concentration of 1 μM for 10 min in the dark at RT;

(3) Rehydrated cells were loaded for 30 min at RT with the oxidant-sensing probe CM-H_2DCFDA (C6827, molecular probes) at a final concentration of 1 μM;

(4) 30-min rewetted cells were stained with the redox INT dye (Sigma) at a final concentration of 0.01% (w/v), for 1 h in the dark at RT.

Controls were 1-month-old liquid cultures of *Chroococcidiopsis*, with or without treatment of 0.1 mM of H_2O_2 for 15 min.

Images were taken at a constant exposure time, gain, and offset using a Nikon TE200 inverted microscope and an Olympus IX70 microscope equipped with Delta Vision Imaging Workstation (Applied Precision). The software package Image-Pro Plus (Media Cybernetics) was used in detection and quantitative analyses of fluorescent areas.

Genome stability assays

(1) *TUNEL* Dried cells were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) at 4°C for 1 h, then washed in PBS and finally incubated in the permeabilization solution (0.1% Triton X-100 in PBS) for 30 min on ice. Labelling of strand breaks with fluorescein dUTP was performed on cell suspensions, according to the TUNEL kit manual (In situ Cell Death Detection Kit, Fluorescein, Roche). After the TUNEL reaction, cells were counterstained with DAPI.

(2) *Genomic PCR fingerprinting* Dried cells were rewetted with sterile distilled water, then either immediately or after 24 h, were subjected to three cycles of freeze-thawing and finally boiled for 5 min. After centrifugation, the supernatant was used for genomic PCR amplifications using the primers HIP1-CA (5′-GCGATCGCCA-3′), STRR3 (5′-CAACAGTCAA CAGT-3′) (Bruno et al. 2005), and six random decameric primers (5′-GGTGCGGGAA-3′, 5′-GTTT CGCTCC-3′, 5′-GTAGACCCGT-3′, 5′-AAGAGCCC GT-3′, 5′-AACGCGCAAC-3′ and 5′-CCCGTCAGC A-3′) (Shirkey et al. 2003). PCR conditions were 1 cycle at 94°C for 3 min; 30 cycles at 94°C for 30 s, 35°C for 30 s and 72°C for 1 min and 1 cycle at 72°C for 7 min. Genomic DNA extracted from one-month *Chroococcidiopsis* liquid cultures was used as a control.

Results

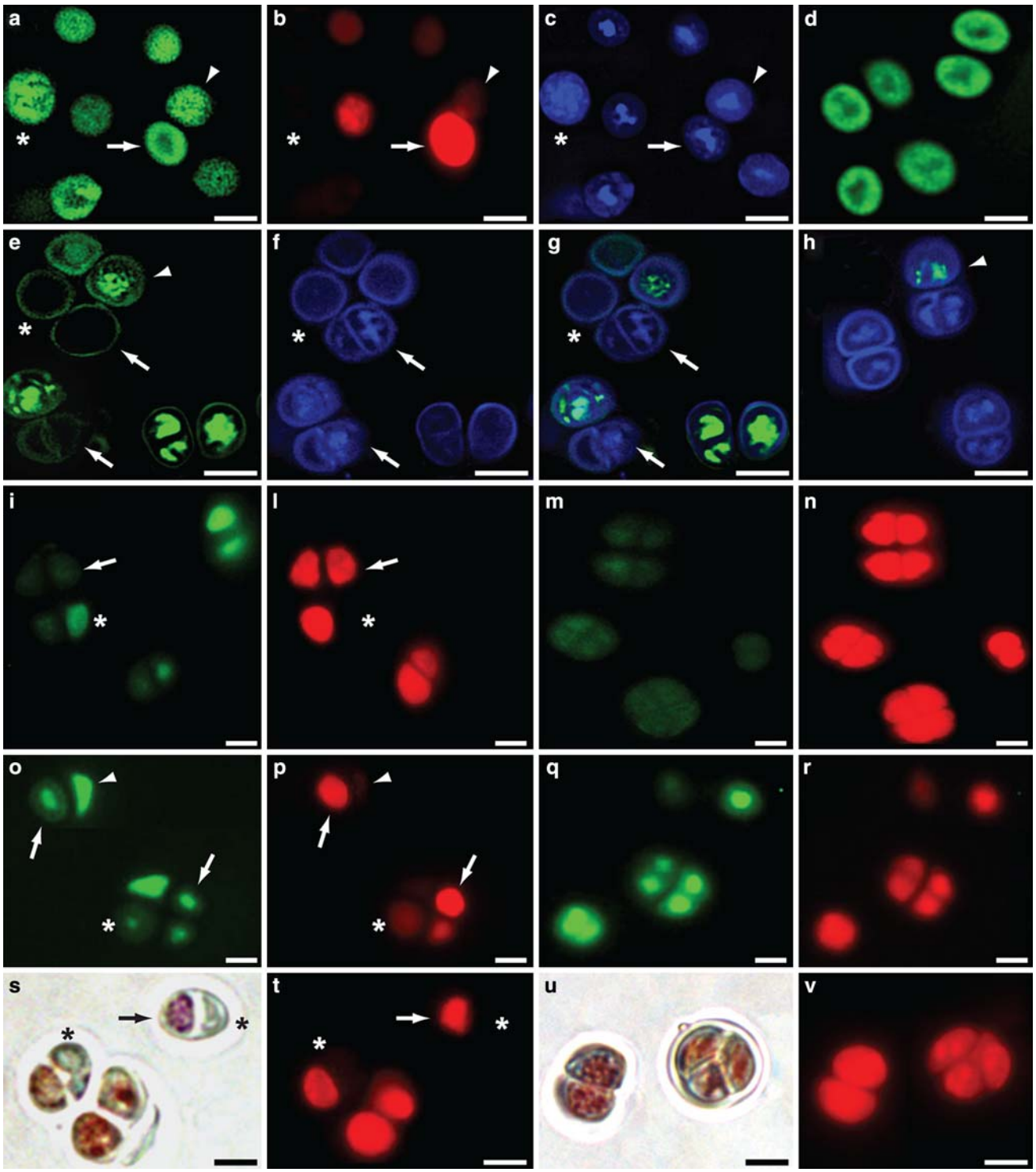
Genome integrity in desiccation survivors

DNA strand breaks were detected in *Chroococcidiopsis* cells after 4 years of storage in the desiccated state, using FITC-dUTP labelling of 3′-hydroxyl ends. A background TUNEL signal was present in the peripheral cytoplasm and absent in the nucleoid area of a few dried cells (Fig. 1a, arrow), which also exhibited phycobiliprotein autofluorescence (Fig. 1b, arrow). A TUNEL signal accounting for a large population of genomic breaks occurred throughout the cytoplasm of most of the dried cells (Fig. 1a, arrowhead, asterisk), in which the autofluorescence of photosynthetic pigments was very weak or completely lacking (Fig. 1b, arrowhead, asterisk). The severe DNA fragmentation of dried, damaged cells was revealed by an eightfold increase of the intensity of FITC-positive areas when compared with that of cells with background TUNEL signal. DAPI counterstaining confirmed the presence of nucleoids in the central cytoplasmic area of dried cells (Fig. 1c), while a whole-cell DAPI signal occurred in dried cells, indicating extensive degradation (Fig. 1c, asterisk). Healthy cells from 1-month-old liquid culture showed a background TUNEL signal in the peripheral cytoplasm (Fig. 1d).

Intact plasma membrane in desiccation survivors

The integrity of plasma membranes in dried cells of *Chroococcidiopsis* was investigated after their rewetting by staining with SYTOX Green, a DNA-specific molecular probe which enters cells with compromised membranes. Within dried aggregates cells with undamaged plasma membranes appeared as SYTOX Green-negative (Fig. 1e, arrows), with DAPI-stained nucleoids (Fig. 1f, arrows) and red phycobiliprotein autofluorescence (not shown). Whereas cells with permealized membranes were SYTOX Green-positive (Fig. 1e, arrow), while dried cells with extensive cellular degradation, resulted both SYTOX Green-negative and DAPI-negative (Fig. 1e, f, asterisk). As a consequence, cells with intact plasma membranes were identified by merge analysis as SYTOX Green-negative and DAPI-positive (Fig. 1g, arrows). Merge analysis of 1-month-old liquid culture revealed few SYTOX Green-positive cells (Fig. 1h, arrowhead).

Alterations in the plasma membrane potential, were investigated by staining with $DiBAC_4(3)$, a membrane potential-sensitive molecular probe, whose uptake is restricted to depolarized cells (e.g. dead or metabolically inactive cells). In 30-min rewetted samples, a weak green fluorescence indicative of a reduced $DiBAC_4(3)$ influx, occurred in cells (Fig. 1i, arrow) which also maintained



phycobiliprotein autofluorescence (Fig. 1l, arrow). While in rewetted cells, the loss of the plasma membrane potential resulted in an intense green fluorescence, due to a greater $DiBAC_4(3)$ influx (Fig. 1i, asterisk). Furthermore, cells with depolarized membranes also exhibited evidences of extensive bleaching of phycobiliproteins (Fig. 1l, asterisk). In 1-month-old liquid culture, most of the cells had polarized plasma membranes and showed a weak green fluorescence (Fig. 1m) and intense autofluorescence of phycobiliproteins (Fig. 1n).

◀ **Fig. 1** In situ detection of damage in the anhydrobiotic cyanobacterium *Chroococcidiopsis* after 4 years of storage in the desiccated state. **a** Background TUNEL signal in a dried cell (*arrow*) with unbleached phycobiliproteins (**b**, *arrow*); TUNEL signal in dried cells (*arrowhead*, *asterisk*) with bleached phycobiliproteins (**b**, *arrowhead*, *asterisk*). **c** DAPI-stained nucleoids in dried cells (*arrow, arrowhead*); diffused DAPI-stained nucleoid in an extensively damaged cell (*asterisk*). **d** Background TUNEL signal in healthy cells from liquid culture. **e** Lack of SYTOX Green-staining of nucleoids in cells with intact plasma membrane (*arrows*) and in a deeply injured cell (*asterisk*). **f** DAPI-stained nucleoids of SYTOX Green-negative cells (*arrows*); lack of DAPI-stained nucleoid in a deeply injured cell (*asterisk*). **g** Merge analysis showing SYTOX Green-negative and DAPI-positive undamaged cells (*arrows*). **h** Merge analysis showing a dead cell (*arrowhead*) in control liquid culture. **i** Low DiBAC4(3) fluorescence in dried cells with intact plasma membrane potential (*arrow*) and phycobiliprotein autofluorescence (**l**, *arrow*); intense DiBAC4(3) fluorescence in an injured cell (*asterisk*) with bleached pigments (**l**, *asterisk*). **m–n** Low DiBAC4(3) fluorescence and intense photosynthetic pigment autofluorescence in liquid control culture. **o** CM-H_2DCFDA oxidation into the inner cytoplasm of dried cells (*arrows*) showing pigment autofluorescence (**p**, *arrows*); whole-cell ROS accumulation in cells (*arrowhead*) with bleached pigments (**p**, *arrowhead*); reduced DCF fluorescence in extensively damaged cells (*asterix)* with bleached pigments (**p**, *asterisk*). **q–r** ROS accumulation and pigment autofluorescence in control liquid culture after oxidative stress. **s–t** Red formazan spots in respiring rewetted cells (*arrow*) with phycobiliprotein autofluorescence (*arrow*); dead cells lacking formazan spots (*asterisks*) and showing bleached phycobiliproteins (*asterisks*). **u–v** Dehydrogenase activity and pigment autofluorescence in liquid control culture. *Scale bars* are 5 μm

Table 1 Percentage of dried cells of *Chroococcidiopsis* avoiding subcellular damage according to different methodologies

Assay	Cellular feature	Results and percentage[a]
TUNEL	DNA fragmentation	No, 10 ± 4
SYTOX Green	Loss of membrane integrity	No, 20 ± 5[b]
$DiBAC_4(3)$	Loss of membrane potential	No, 26 ± 7[d]
CM-H_2DCFDA	ROS accumulation	No, 12 ± 2[c]
INT	Respiration recovery	Yes, 28 ± 6[d]

[a] No (negative) and yes (positive) results for cellular feature being evaluated. A total number of about 400 cells (positive or negative) was counted for each assay in four trails

[b] Cells were SYTOX Green-negative and DAPI-positive

[c] Cells with spatially limited DCF fluorescence and unbleached autofluorescence of photosynthetic pigments

[d] Cells were rewetted for 30 min before the assay

Spatially reduced ROS accumulation in desiccation survivors

The presence of ROS was investigated in dried aggregates of *Chroococcidiopsis* after their rewetting by using the cell-permeant, oxidation-sensitive dye CM-H_2DCFDA, which remains non-fluorescent until acetate groups are removed by cellular esterases and oxidized to highly fluorescent DCF. An intense green fluorescence, spatially limited to the inner cytoplasm, occurred in cells (Fig. 1o, arrows) which also exhibited pigment autofluorescence (Fig. 1p, arrows). An intense DCF fluorescence instead, occurred through the whole-cytoplasm of the rewetted cells (Fig. 1o, arrowhead) with bleached pigments (Fig. 1p, arrowhead). In addition, a spatially reduced DCF fluorescence (Fig. 1o, asterisk) occurred in cells with bleached pigments (Fig. 1p, asterisk), indicating damaged esterase and/or cell leakage. The spatial reduction of ROS accumulation into the inner cytoplasm of rewetted cells with intact pigment autofluorescence, was supported by the twofold decrease of the ratio between the DCF fluorescence and the whole-cytoplasm autofluorescence, because of the presence of photosynthetic pigments. In 1-month-old liquid culture, CM-H_2DCFDA oxidative products were not observed (not shown) but after a hydrogen peroxide treatment, an intense DCF fluorescence occurred in the whole-cytoplasm (Fig. 1q) which spatially overlapped with the whole-cytoplasm pigment autofluorescence (Fig. 1r).

Dehydrogenase activity in rewetted cells

Dried cells of *Chroococcidiopsis* were tested for their capability for respiration upon rewetting, by monitoring the INT reduction into insoluble red formazan, whose size corresponds to the intensity of respiratory activity (Zimmermann et al. 1978). Red formazan spots occurred in the cytoplasm of rewetted cells (Fig. 1s, arrow) which exhibited phycobiliprotein autofluorescence (Fig. 1t, arrow). While INT reduction lacked in dead cells (Fig. 1s, asterisks) with bleached pigments (Fig. 1t, asterisks). In 1-month-old liquid culture, the dehydrogenase activity was observed in all cells (Fig. 1u) which also showed photosynthetic pigment autofluorescence (Fig. 1v).

The percentage of desiccation survivors according to the employed assays is summarized in Table 1.

Genome stability in desiccated and rewetted cells

The genome of *Chroococcidiopsis* cells after 4 years of storage in the desiccated state was investigated for the presence of covalent modifications by assessing its suitability as PCR template. Virtually identical genomic HIP1-CA (Fig. 2a) and very similar STTR3-PCR (Fig. 2b) fingerprints were obtained from dried and control cells. In addition, these fingerprints were identical to those obtained from 24-h rewetted cells (Fig. 2a, b). Different genomic PCR fingerprints were obtained from either dried and 24-h rewetted cells as well as from liquid control cultures, when using six different random decameric primers. As in

multiple trials only the primer number 5 (RN5) yielded reproducible and consistent band profiles, it is the only one shown (Fig. 2c).

Discussion

Anhydrobiotes must either protect their cellular structures from desiccation-induced damage and/or repair it upon rewetting. Within cell aggregates of the anhydrobiotic cyanobacterium *Chroococcidiopsis* stored in the desiccated state for 4 years, a few survivors showed total or limited avoidance of desiccation-induced damage. These cells were identified by means of fluorescent DNA breakage labelling, membrane integrity and membrane potential related probes, oxidant-sensing probe and redox dye. The lack of genome fragmentation in these survivors matched the absence of phycobiliprotein bleaching, the maintenance of intact and polarized plasma membranes, spatially-restricted ROS accumulation into central cytoplasm and dehydrogenase activity upon rewetting. Whereas, damaged cells underwent genome fragmentation, plasma membrane destabilization, phycobiliprotein bleaching, whole-cell ROS accumulation and absence of respiratory activity upon rewetting.

The lack of genome fragmentation in *Chroococcidiopsis* desiccation survivors resembles that occurring in cells of *N. commune* after prolonged desiccation (Shirkey et al. 2003). This genomic response is fundamentally different from that of *D. radiodurans* where desiccation leads to extensive genome damage (Mattimore and Battista 1996). Remarkably, the genome of dried cells of *Chroococcidiopsis* is also protected from covalent modifications as showed by the high quality of genomic HIP1- and STTR3-PCR fingerprints obtained without using AGE disrupting reagents. Such a reagent was used for the HIP1-PCR fingerprinting of the dried cells of *N. commune* (Shirkey et al. 2003). The virtually identical or very similar HIP1- and STRR- PCR fingerprints of dried, rewetted and liquid culture of *Chroococcidiopsis* revealed that HIP and STRR sequences remain accessible after desiccation and that they are involved in promoting genome reorganization and DNA repair upon rewetting (Shirkey et al. 2003; Robinson et al. 2000). On the other hand, the different PCR fingerprints obtained with random decameric primers from dried, rewetted and liquid culture of *Chroococcidiopsis* may be related to conformational changes because of the cell hydration state and/or DNA damage repair as reported for *N. commune* (Shirkey et al. 2003). The avoidance and/or limitation of desiccation-induced damage in the genome of *Chroococcidiopsis* desiccation survivors was further confirmed by the TUNEL assay. The TUNEL signal occurring in the survivors was comparable to that of the healthy ones, whereas that of damaged cells was indicative of a large population of DNA strand breaks, similar to those observed in *Trichodesmium* IMS101 subjected to oxidative stress (Berman-Frank et al. 2004). Although the presence of oxidative DNA damage was not investigated here, it possibly did occur in desiccation survivors, at least after rewetting, as indicated by the ROS accumulation in their inner cytoplasmic areas.

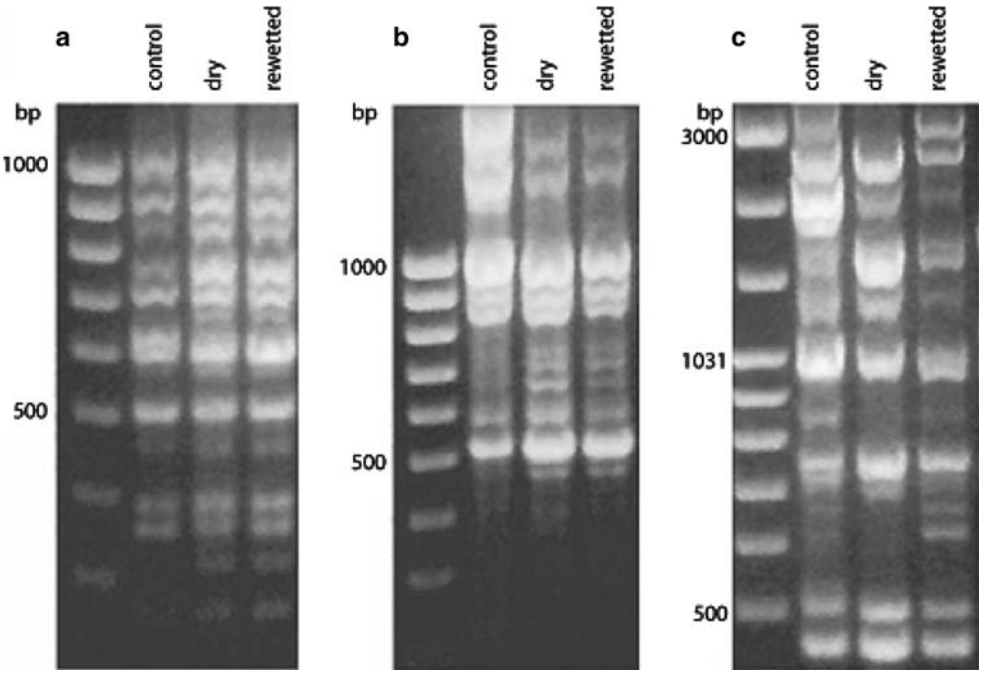


Fig. 2 Genomic PCR fingerprinting of the anhydrobiotic cyanobacterium *Chroococcidiopsis* after 4 years of storage in the desiccated state. **a** HIP1-CA fingerprints of control cells from liquid culture (*lane b*), dried cells (*lane c*) and 24-h rewetted cells (*lane d*). **b** STRR-3 fingerprints of liquid culture (*lane b*), dried cells (*lane c*) and 24-h rewetted cells (*lane d*). **c** RN5 fingerprints of control cells from liquid culture (*lane b*), dried cells (*lane c*) and 24-h rewetted cells (*lane d*). Molecular weight markers (*lanes a*)

Despite the ROS accumulation in *Chroococcidiopsis* desiccation survivors, it seems that at least a subset of proteins were protected from oxidative damage. Here, the phycobilisomes did not bleach in the survivors as they did in the damaged cells; it has been shown that in *N. commune* the light-harvesting complexes are readily degraded after short-term drying, even in the dark (Potts 1985), and the photosynthetic recovery is impaired if extracellular polysaccharides are removed (Tamaru et al. 2005). However, it is difficult to make generalization about macromolecule stability in dry cytoplasm, for example, a stable, unusual phycoerythrin was detected in 2-year-dried mats of the terrestrial cyanobacterium *Lyngbya arboricola* (Tripathi et al. 2007). Also esterases were shown to be functionally stable in the *Chroococcidiopsis* desiccation survivors as the CM-H_2DCFDA probe used in ROS detection does not function in cells were they are damaged (He and Häder 2002). It was previously reported by Cockell et al. (2005) that in dried cells of *Chroococcidiopsis* exposed to a Martian UV flux, the esterase activity lasted long after the culturability loss and degraded at a much faster rate than the loss of pigment autofluorescence.

The employment of viability indicators, revealing different cellular properties and physiological functions, allowed the identification of desiccation survivors. The permanence of phycobiliprotein autofluorescence provided a survival marker which was associated with genome stability, undamaged plasma membranes and dehydrogenase activity upon rewetting. In addition, even if membrane integrity and membrane potential related probes showed that desiccation survivors avoid membrane destabilization, it is not known if upon drying, *Chroococcidiopsis* accumulates trehalose, like most anhydrobiotes (Crowe 2007) including the cyanobacterium *N. commune* (Potts 1999; Shirkey et al. 2003). Genes for trehalose metabolism are up-regulated in 3-h desiccated cells of *Anabaena* PCC7120, supporting the relevance of this disaccharide in the initial stages of desiccation (Katoh et al. 2004). Indeed, even though trehalose is present in small quantities in dried cells of *Anabaena* PCC7120, it induces a chaperone system crucial for dehydration tolerance (Higo et al. 2006).

These outcomes provide a new framework for evaluating the desiccation tolerance of *Chroococcidiopsis*, where oxidative stress defence plays a key role. Proteins represent the initial target of oxidative damage and their oxidation precedes any detectable DNA damage (Du and Gebicki 2004). The role of elevated Mn/Fe concentration ratios in protecting *D. radiodurans* proteins, including those involved in DNA repair, from oxidative damage which is induced by ionizing radiation has been reported (Daly 2006). Recently, close correlations between high Mn/Fe concentration ratios, high levels of ionizing radiation resistance and low susceptibility to desiccation-induced protein oxidation were detected in bacterial isolates from dry climate soils (Fredrickson et al. 2008).

How *Chroococcidiopsis* faces oxidative stress upon desiccation, prolonged periods in a dry state and subsequent rewetting, remains largely unknown. Although, Fe-superoxide dismutase was found to accumulate in the peripheral cytoplasm of 1-year-dried cells of *Chroococcidiopsis,* whereas in 1-month control cells it mainly occurred in the nucleoplasm (Grilli Caiola et al. 1996b). If this ROS scavenging enzyme is functionally stable in dried cells of *Chroococcidiopsis*, then its accumulation might account for the spatially restricted ROS accumulation observed only in the inner cytoplasm of *Chroococcidiopsis* desiccation survivors. In *N. commune,* cells dried for 13 years showed an active Fe-superoxide dismutase, which was found between rehydrating cells and the extracellular fluid (Shirkey et al. 2000). The whole-cell ROS accumulation observed here in the *Chroococcidiopsis* desiccation-damaged cells, might therefore represent a failure of antioxidant defence leading to cell death.

The occurrence within dried aggregates of *Chroococcidiopsis* of live and dead cells poses intriguing questions about its survival after air drying. If death is the outcome of a passive externally driven process (necrosis), then it seems that desiccation resistance is not a simple process. Furthermore, subtle modifications at the cellular milieu level are required to dry without dying. Indeed, within a given *Chroococcidiopsis* aggregate, cells occur in different physiological states, which may influence their desiccation tolerance (Billi and Potts 2002). Otherwise, if death is the outcome of an active, programmed cell death (PCD) process, then typical markers could be scored in dry, dead cells of *Chroococcidiopsis*. Distinctive morphological and biochemical PCD markers such as cell membrane integrity, nucleic acid fragmentation and increased caspsase activity, were identified during autocatalytic PCD of planktonic cyanobacteria in response to various environmental stressors, including oxidative stress (Berman-Frank et al. 2004; Bidle and Falkowski 2004). Hence, in dried cells of *Chroococcidiopsis* the occurrence of damaged plasma membranes could be related to late PCD phases, which lead to cellular dissolution as reported in *Anabaena* PCC7120 (Ning et al. 2002). Evidence is still needed to confirm the existence of a desiccation-triggered caspase-dependent PCD in *Chroococcidiopsis*, but, it is generally accepted that antioxidant failures during drying could cause changes in the cellular redox status which triggers PCD (Kranner and Birtić 2005). However, the existence of other possible causes for genetically driven PCD in *Chroococcidiopsis* aggregates upon desiccation must also be considered. In *Escherichia coli* a stress-induced toxin–antitoxin module, which requires a quorum-sensing molecule for communication between individuals, causes death

of a bacterial subpopulation, which become a resource to permit the survival of the others (Kolodkin-Gal et al. 2007). While in sporulating bacteria a cannibalism system induced by nutrient limitation occurs and the death of a subpopulation of cells delays the sporulation of other individuals by releasing nutrients, and hence avoiding what could be a disadvantage process, if committed in response to a brief fluctuation in nutrient availability (Bassler and Losick 2006).

Whatever the mechanisms causing death of only few *Chroococcidiopsis* cells within a dried aggregate are, it may be assumed that they contribute to the survival of the others by providing physical protection and/or nutrients supply upon rewetting.

While it seems sensible to speculate that even though the subcellular integrities of *Chroococcidiopsis* desiccation survivors clearly indicate protection mechanisms as playing a key role in desiccation tolerance, equally, an interplay with repair mechanisms must take place. These processes can guarantee survival after prolonged dry storage when oxidative processes continue even in absence of metabolic activity, as well as when dried cells experience additional environmental stressors.

Acknowledgments I thank anonymous reviewers for helpful comments. This work was funded by the Italian Space Agency (MoMa project) and the Italian Ministry of Foreign Affairs (Direzione Generale per la Promozione e Cooperazione Culturale). Thanks are due to Dr. Palma Mattioli for image analysis and Roberto Targa for skilful assistance in image editing.

References

Alpert P (2006) Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? J Exp Biol 209:1575–1584

Bassler BL, Losick R (2006) Bacterially speaking. Cell 125:237–246

Berman-Frank I, Bidle KD, Haramaty L, Falkowski PG (2004) The demise of the marine cyanobacterium, *Trichodesmium* spp., via an autocatalyzed cell death pathway. Limnol Oceanogr 49:997–1005

Bidle KD, Falkowski PG (2004) Cell death in planktonic, photosynthetic microorganisms. Nat Rev Microbiol 2:643–655

Billi D, Potts M (2000) Life without water: responses of prokaryotes to desiccation. In: Storey KB, Storey JM (eds) Cell and molecular response to stress: environmental stressors and gene responses. Elsevier, Amsterdam, pp 181–192

Billi D, Potts M (2002) Life and death of dried prokaryotes. Res Microbiol 153:7–12

Billi D, Friedmann EI, Hofer KG, Grilli Caiola M, Ocampo-Friedmann R (2000) Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. Appl Environ Microbiol 66:1489–1492

Bruno L, Billi D, Albertano P (2005) Optimization of molecular techniques applied to the taxonomy of epilithic *Leptolyngbya* strains. Arch Hydrobiol Suppl Algol Stud 117:197–207

Cockell CS, Schuerger AC, Billi D, Friedmann EI, Panitz C (2005) Effects of a Simulated Martian UV Flux on the cyanobacterium, *Chroococcidiopsis* sp. 029. Astrobiology 5:127–140

Crowe JH (2007) Trehalose as a "chemical chaperone": fact and fantasy. Adv Exp Med Biol 94:143–158

Daly MJ (2006) Modulating radiation resistance: insights based on defenses against reactive oxygen species in the radioresistant bacterium *Deinococcus radiodurans*. Clin Lab Med 26:491–504

Du J, Gebicki JM (2004) Proteins are major initial cell targets of hydroxyl free radicals. Int J Biochem Cell Biol 36:2334–2343

França MB, Panek AD, Eleutherio EC (2007) Oxidative stress and its effects during dehydration. Comp Biochem Physiol A Mol Integr Physiol 146:621–631

Fredrickson JK, Li SMW, Gaidamakova EK, Matrosova VY, Zhai M, Sulloway HM, Scholten JC, Brown MG, Balkwill DL, Daly MJ (2008) Protein oxidation: key to bacterial desiccation resistance? Int J Syst Evol Microbiol 2:393–403

Friedmann EI, Kappen L, Meyer MA, Nienow JA (1993) Long-term productivity in the cryptoendolithic communities of the Ross desert, Antarctica. Microb Ecol 25:51–69

Grilli Caiola M, Billi D (2007) *Chroococcidiopsis* from desert to Mars. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Cellular origin, life in extreme habitats and astrobiology. Springer, Berlin, pp 553–568

Grilli Caiola M, Ocampo-Friedmann R, Friedmann EI (1993) Cytology of long-term desiccation in the cyanobacterium *Chroococcidiopsis* (Chroococcales). Phycologia 32:315–322

Grilli Caiola M, Billi D, Friedmann EI (1996a) Effect of desiccation on envelopes of the cyanobacterium *Chroococcidiopsis* sp. (Chroococcales). Eur J Phycol 31:97–105

Grilli Caiola M, Canini A, Ocampo-Friedmann R (1996b) Iron superoxide dismutase (Fe-SOD) localization in *Chroococcidiopsis* (Chroococcales, Cyanobacteria). Phycologia 35:90–94

He YY, Häder DP (2002) Reactive oxygen species and UV-B: effect on cyanobacteria. Photochem Photobiol Sci 1:729–736

Higo A, Katoh H, Ohmori K, Ikeuchi M, Ohmori M (2006) The role of a gene cluster for trehalose metabolism in dehydration tolerance of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. J Microbiol 152:979–987

Katoh H, Asthana RK, Ohmori M (2004) Gene expression in the cyanobacterium *Anabaena* sp. PCC7120 under desiccation. Microb Ecol 47:164–174

Kolodkin-Gal I, Hazan R, Gaathon A, Carmeli S, Engelberg-Kulka H (2007) A linear pentapeptide is a quorum-sensing factor required for *mazEF*-mediated cell death in *Escherichia coli*. Science 318:652–655

Kranner I, Birtić S (2005) A modulating role for antioxidants in desiccation tolerance. Integr Comp Biol 45:734–740

Mattimore V, Battista JR (1996) Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J Bacteriol 178:633–637

Ning SB, Guo HL, Wang L, Song YC (2002) Salt stress induces programmed cell death in prokaryotic organism *Anabaena*. J Appl Microbiol 93:15–28

Potts M (1985) Protein synthesis and proteolysis in immobilized cells of cyanobacterium *Nostoc commune* UTEX 584 exposed to matric water stress. J Bacteriol 164:1025–1031

Potts M (1999) Mechanisms of desiccation tolerance in cyanobacteria. Eur J Phycol 34:319–328

Potts M (2001) Desiccation tolerance: a simple process? Trends Microbiol 9:553–559

Potts M, Slaughter SM, Hunneke FU, Garst JF, Helm RF (2005) Desiccation tolerance of prokaryotes: application of principles to human cells. Integr Comp Biol 45:800–809

Robinson NJ, Rutherford JC, Pocock MR, Cavet JS (2000) Metal metabolism and toxicity: repetitive DNA. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer, Dordrecht, pp 443–463

Shirkey B, Kovarcik DP, Wtight DJ, Wilmoth G, Prickett TF, Helm RF, Gregory EM, Potts M (2000) Active Fe-containing superoxide dismutase and abundant *sodF* mRNA in *Nostoc commune* (Cyanobacteria) after years of desiccation. J Bacteriol 182:189–197

Shirkey B, McMaster NJ, Smith SC, Wright DJ, Rodriguez H, Jaruga P, Birincioglu M, Helm RF, Potts M (2003) Genomic DNA of *Nostoc commune* (Cyanobacteria) becomes covalently modified during long-term (decades) desiccation but is protected from oxidative damage and degradation. Nucleic Acids Res 31:2995–3005

Tamaru Y, Takani Y, Yoshida T, Sakamoto T (2005) Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. Appl Environ Microbiol 71:7327–7333

Tripathi SN, Shivali K, Shrivastava A (2007) Extraction and purification of an unusual phycoerythrin in a terrestrial desiccation tolerant cyanobacterium *Lyngbya arboricola*. J Appl Phycol 19:441–447

Warren-Rhodes KA, Rhodes KL, Pointing SB, Ewing SA, Lacap DC, Gómez-Silva B, Amundson R, Friedmann EI, McKay CP (2006) Hypolithic cyanobacteria, dry limit of photosynthesis, and microbial ecology in the hyperarid Atacama Desert. Microb Ecol 52:389–398

Wierzchos J, Ascaso C, McKay CP (2006) Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. Astrobiology 6:415–422

Zimmermann R, Iturriaga R, Becker-Birck J (1978) Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl Environ Microbiol 36:926–935