Insights into the mechanisms of desiccation resistance of the Patagonian PAH-degrading strain *Sphingobium* sp. 22B

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**Abstract**

**Aim:** To analyse the physiological response of *Sphingobium* sp. 22B to water stress.

**Methods and Results:** The strain was grown under excess of carbon source and then subjected to low (60RH) and high (18RH) water stress conditions for 96 h. Quantification of trehalose, glycogen, polyhydroxybutyrate (PHB) and transmission electron microscopy (TEM) was studied. Genes linked with desiccation were searched in *Sphingobium* sp. 22B and *Sphingomonas* sensu latu genomes and their transcripts were quantified by real-time PCR. Results showed that, in the absence of water stress, strain 22B accumulated 47.6% of glycogen, 0.84% of trehalose and 44.9% of PHB per cellular dry weight. Glycogen and trehalose were mobilized under water stressed conditions, this mobilization was significantly higher in 60RH in comparison to 18RH. Gene *treY* was upregulated sixfold in 60RH relative to control condition. TEM and quantification of PHB revealed that PHB was mobilized under 60RH condition accompanied by the downregulation of the phbB gene. TEM images showed an extracellular amorphous matrix in 18RH and 60RH. Major differences were found in the presence of *aqpZ* and trehalose genes between strain 22B and *Sphingomonas* genomes.

**Conclusion:** Strain 22B showed a carbon conservative metabolism capable of accumulation of three types of endogenous carbon sources. The strain responds to water stress by changing the expression pattern of genes related to desiccation, formation of an extracellular amorphous matrix and mobilization of the carbon sources according to the degree of water stress. Trehalose, glycogen and PHB may have multiple functions in different degrees of desiccation. The robust endowment of molecular responses to desiccation shown in *Sphingobium* sp. 22B could explain its survival in semi-arid soil.

**Significance and Impact of the Study:** Understanding the physiology implicated in the toleration of the PAH-degrading strain *Sphingobium* sp 22B to environmental desiccation may improve the bioaugmentation technologies in semi-arid hydrocarbon-contaminated soils.

**Introduction**

Patagonia is a semi-arid scrub plateau located at the southern end of South America, shared by Argentina and Chile. It constitutes a vast area of steppe and desert that extends south from latitude 37° to 51°S occupying 673 000 square kilometres. This region has low precipitation concentrated in winter, strong water deficits in spring and summer, and persistent and intense western winds (Paruelo *et al.* 1998).

Indigenous micro-organisms are regularly exposed to desiccation, this being the limiting factor of life in semi-
wide range of natural and anthropogenic aromatic compounds (Stolz 2009). Members of this group are broadly distributed in nature and have been isolated from a variety of environments such as pristine (Lin et al. 2012) and contaminated (Vacca et al. 2005) soil, in cold (Margesin et al. 2012), extreme (Farias et al. 2011) and arid environments (Reddy and García-Pichel 2007). Although there are some reports on physiology and responses to environmental factors in bacteria of the genus Sphingomonas (Fegatella and Cavicchioli 2000; Fida et al. 2012), little is known about the responses used by these microorganisms against the fluctuating availability of water in semi-arid Patagonia.

In this work, we researched some physiological properties that allow strain 22B to survive the environmental conditions of Patagonia. The occurrence, in strain 22B, of mechanisms involved in the tolerance of cells to desiccation reported for other bacteria, such as the ability to produce reserve compounds, compatible solutes and extracellular polymeric substances (EPS) were examined. In addition, the recently obtained genomic information of strain 22B (Madueño et al. 2016) provided the possibility of analysing the occurrence, expression and distribution of key genes involved in those physiological processes.

**Materials and methods**

**Culture conditions**

*Sphingobium* sp. 22B (DDBJ/ENA/GenBank under the accession number LTAB000000000) was grown in liquid mineral medium (LMM) with 1% glucose at 28°C and 150 rev min⁻¹ for 48 h. The concentration of ammonium chloride in LMM was reduced to 0.1 g l⁻¹ to allow the accumulation of reserve compounds (5 g l⁻¹ ClNa, 1 g l⁻¹ K₃PO₄H₂O, 1.7 g l⁻¹ (NH₄)₂H₂PO₄, 0.1 g l⁻¹ (NH₄)₂SO₄, 0.2 g l⁻¹ SO₄Mg). Cells were harvested at 48 h, washed with NaCl solution (0.85%, w/v) and lyophilized for subsequent analyses.

**Water stress resistance test in Sphingobium sp. 22B**

*Sphingobium* sp. 22B was cultivated for 48 h with an excess of carbon source (culture conditions) in three Erlenmeyer flasks. Total culture volume (400 ml) of each flask was filtered under sterile conditions in fractions of 20 ml onto 0-45 μm and 60-μm pore-size nitrocellulose filters (Millipore, Darmstadt, Germany) by a vacuum pump. The filters with harvested cells on their surface were subjected to the following conditions:

- High water stress condition (18RH): 20 filters were placed separately in independent sterile petri dishes and...
laid together into desiccator at 18% of relative humidity (RH). RH was measured with a thermohygrometer with Max/Min function (Gesa, Urduzil, Vizcaya, Spain) for 96 h at 28°C. Finally, all filters were taken by tweezer under sterilized conditions and resuspended in 400 ml of physiological solution (PS). Different fractions of this volume were used for cell survival determination, extraction and quantification of intracellular polyhydroxybutyrate (PHB), glycogen and trehalose, and for transmission electron microscopy (TEM) (see below).

iiLow water stress condition (60RH): 20 filters were placed separately in independent sterile Petri dishes and, afterwards, all of them were placed together into a container at 60% RH measured with a thermohygrometer with Max/Min function (Gesa) for 96 h at 28°C. Finally, all filters were taken by a tweezer under sterilized conditions and resuspended in 400 ml of PS. Different fractions of this volume were used for cell survival determination, extraction and quantification of intracellular PHB, glycogen and trehalose, and for TEM.

iiiControl: 20 filters were obtained and resuspended all together immediately in 400 ml of sterile PS without any stress condition.

Independent Erlenmeyer flasks were used for each condition. The assay was performed in triplicate.

Determination of cell survival

The survival of the strain 22B under each condition was performed by viable count (CFU) with R2 medium (Reasoner and Geldreich 1985) using 1/10 serial dilutions of 1 ml of cell suspension obtained after water stress treatments. Survival rates were calculated as (log (CFU in low (60RH) or high (18RH) water stress condition))/log (CFU in the control condition) × 100 (Alvarez et al. 2004). The percentage of lost water was calculated weighting filters before and after being subjected to stress condition.

Transmission electron microscopy

A cell suspension of 1.5 ml in PS was spun down at 800 g for 5 min and fixed in 2% glutaraldehyde in phosphate buffer 0.2 mol l⁻¹ (pH 7.2–7.4) for 2 h at 4°C. Secondary fixation was performed using osmium tetroxide 1% for 1 h at 4°C and subsequently, the samples were dehydrated in a growing series of alcohols and embedded in epoxy resin. Ultrathin sections (90 nm) were contrasted with uranyl acetate and lead citrate and 10–15 fields were examined under each condition in a transmission electron microscope JEM 1200 EX II (JEOL Ltd, Tokyo, Japan) and photographed with a camera Erlangshen ES1000W, Model 785 (Gatan Inc., Pleasanton, CA) in Servicio Central de Microscopía Electrónica de la Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.

Extraction and quantification of intracellular PHB

For quantitative determination of PHB, 5–10 mg of lyophilized cells were subjected to methanolysis in the presence of 15% (v/v) sulphuric acid, and 3-hydroxybutyrate-methylester was analysed by HP 5890 gas chromatograph with flame ionization detector and VF-23 ms column 30 m × 0.25 mm × 0.39 mm (Varian, Palo Alto, CA, USA). The injection volume was 0.2 µl. Helium (13 mm min⁻¹) was used as carrier gas. The temperature of the injector and detector was 270°C. Tridecanoic acid was used as internal standard.

Extraction and quantification of intracellular glycogen

Total polysaccharide was extracted from lyophilized cells by classical alkaline hydrolysis (Elbein and Mitchell 1973). Glycogen quantification was performed by enzymatic hydrolysis. Alkaline extracts were digested with 2 µl of alpha-amylase (519 IU) and 15 µl of amyloglucosidase (129 IU) in 50 mmol l⁻¹ sodium acetate buffer pH 5 in final volume of 1 ml at 55°C for 4 h. Glucose was determined by a specific glucose oxidase method (Hernández et al. 2008).

Extraction and quantification of intracellular trehalose

Trehalose was extracted from 10 to 30 mg of lyophilized cells with 15 ml of 80% ethanol for 3 h at 65°C. After centrifugation, each pellet was washed with 5 ml of 80% ethanol and centrifuged. Both supernatants were combined and vacuum dried. The residue was dissolved in 0.5–1 ml water (Zhang and Yan 2012). The water-soluble residue containing trehalose was analysed by isocratic HPLC. Trehalose was separated on a Carbopac PA1 (Dionex) column (4 × 250 mm) and NaOH/AcNa (0.2 mol l⁻¹/0.3 mol l⁻¹) pH 12 was used as eluent and detected with electrochemical PAD (Pulse Amperometric Detector) Water 2465.

Primer design, RNA isolation and quantitative real-time PCR (qRT-PCR)

The sequences of single-copy Sphingobium sp. 22B genes (DDBJ/ENA/GenBank; LTAB00000000) (Table 3) were used as template for the design of primers related to physiological key genes by PRIMER 3 Software (ver. 0.4.0). Specificity of primers was verified in silico by RAST (Rapid Annotation using Subsystem Technology ver. 2.0) and by Artemis Software and by PCR with the same program.
detailed below, followed by gel electrophoresis. The used primers are shown in Table 1. Cell cultures for RNA extraction were obtained, as detailed before, from a water stress resistance test, using a filter area representing approximately 5 ml of culture for each sample, which was resuspended in RNA later© (Sigma Aldrich, St. Louis, MI, USA) solution and stored at −80°C to prevent RNA degradation. RNA extraction was carried out with RNeasy Mini Kit (Qiagen, Venlo, Netherlands) following the manufacturer’s instructions. For cDNA synthesis, 1 mg of each RNA sample was treated with DNase I (Promega, Fitchburg, WI, USA) for 1 h at 37°C. The DNA-free RNA was then used as template to synthesize the cDNA with M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and Random Hexamer Primer (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer’s instructions. RNA extraction and cDNA synthesis were carried out in triplicate from independent cultures of each condition. The efficiencies of the selected primer pairs (Table 1) were checked by real-time PCR (Stratagene Mx3000P) with serial dilutions of an equimolar mixture of cDNA of the three conditions tested in this work as templates. PCR efficiency was near 1. The reaction mix contained 1 μl of DNA template, 1 μmol l⁻¹ of the forward and reverse primer, 0.2 μl of BSA (Sigma Aldrich) 2× SYBR Green PCR Master Mix (Promega). Total reaction volume of 10 μl was reached with PCR-grade water. The program started with a hold at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 55°C for 15 s and elongation at 72°C for 15 s. qPCR assays were performed in three technical replicates on samples and negative controls. The negative controls consisted of PCR blanks with only the reaction mix and PCR blanks containing the mix and 1 μl of PCR-grade water. Threshold cycles (Ct) were measured in separate tubes, in triplicate. Identity and purity of the amplified product were checked by analysing the melting curve at the end of amplification. The differences between Cts were calculated in every sample for each gene of interest as follows: Ct gene of interest—Ct 16SrRNA gene as reference gene marker. Relative changes in the expression level of one specific gene (ΔΔCt) were calculated by the ΔCt method. The expression ratios for the different genes were obtained dividing the normalized power values under desiccation (18RH) and humid (60RH) conditions calculated from the ΔΔCt method, using the 16s as reporter gene.

‘In silico’ search of stress-related genes in draft genome of Sphingobium sp. 22B and in complete genomic projects in Sphingomonas strains

RAST (SEED Viewer ver. 2.0) and NCBI annotation server was used for the selection of genes related to osmotic stress, trehalose and glycogen biosynthesis and utilization, PHB metabolism and exopolysaccharide synthesis in Sphingobium sp. 22B. The sequence of the coding proteins of each gene of the strain 22B (DDBJ/ENA/GenBank; LTAB00000000) was compared in November 2017 by BLAST genome (http://www.ncbi.nlm.nih.gov/genome) with 10 strains of Sphingobium and Novosphingobium with complete genome projects available at that moment (Table 4). In addition, each gene name and EC number was searched in Sphingomonas complete genome projects.

Statistical analysis

All experiments in this study were performed in triplicate. Results are expressed as mean values ± SEM. Data were

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### Table 1 qPCR primers used in this study

| Primer name | EC number | Sequence (5’–3’) | Pb | Reference |
|-------------|-----------|-----------------|----|-----------|
| glgC_F      | 2.7.7.27  | 5’-GTC CATGGGCATCTACGCT-3’ | 244 | This work |
| glgC_R      |           | 5’-GTCGTCAGGGCAGATTTGG-3’ |    | This work |
| glgP_F      | 2.4.1.1   | 5’-ATCTGGTGCAGGGCATCTAC-3’ | 187 | This work |
| glgP_R      |           | 5’-GTCGATCTACCCGATGATCT-3’ |    | This work |
| AqpZ_F      |           | 5’-CCGTTCAGCCTGTTATGC-3’ | 150 | This work |
| AqpZ_R      |           | 5’-GGATCTGGCCGACATCG-3’ |    | This work |
| phbB_F      | 1.1.136   | 5’-GACGACTGGAACGAGGTGAT-3’ | 231 | This work |
| phbB_R      |           | 5’-ATTGACGGGGAACGACATAT-3’ |    | This work |
| lgt1_F      |           | 5’-TTCACAGTCACCAGATCAG-3’ | 188 | This work |
| lgt1_R      |           | 5’-GGGCCAAATATGATGTC-3’ |    | This work |
| OtsA_F      |           | 5’-GTTTACGTCAGCGGTCAG-3’ | 163 | This work |
| OtsA_R      |           | 5’-CTTCTTCACCAAGTGGAGC-3’ |    | This work |
| treY_F      | 5.4.99.15 | 5’-GCAGAACCTGTGAACTG-3’ | 250 | This work |
| treY_R      |           | 5’-TCCTGACCCATATGTCGAG-3’ |    | This work |
| 1055F       |           | 5’-ATGGCTGCTGTCAGCT-3’ | 337 | Harms (2003) |
| 1392r       |           | 5’-ACGGGCGGTGCTAC-3’ |    | Harms (2003) |
analysed by ANOVA (one-way) followed by Fisher’s test. In all cases, P values were calculated with Student’s t test and those lower than 0.05 were considered statistically significant. All statistical tests were performed using GRAPHPAD PRISM 6.0.

Results

Physiological responses of strain *Sphingobium* sp. 22B to water stress conditions

The physiological responses of the strain 22B to water stress were compared during incubation of cells under high water stress condition (18RH) and low water stress condition (60RH). In agreement with previous results (Madueño et al. 2011), *Sphingobium* sp. 22B exhibited high cell culturability (80-6 ± 2.6%) after 96 h under 18RH (with 94.5 ± 0.85% water loss). A cell culturability of 99.2 ± 0.45% was observed in 60RH where 58.8 ± 5.1% of water loss occurred.

*Sphingobium* sp. 22B was able to produce and accumulate 44.9 ± 6.4% of PHB, 4.8 ± 1.4% of glycogen and 0.84 ± 0.16% of trehalose per cellular dry weight (CDW) during its growth with an excess of carbon source after 96 h of incubation at 28°C (Table 2) (control condition). The content of trehalose and glycogen varied significantly (P < 0.05) during cell incubation under 18RH and 60RH conditions. In comparison to the control condition, after 96 h under 18RH, cells mobilized approximately 77% of glycogen (2.0 ± 0.3%) and 65% of trehalose (0.28 ± 0.1) content, but PHB was not mobilized (45.7 ± 28.4) (Table 2). In contrast, under 60RH, cells mobilized the three store carbon compounds studied in this work, approximately 50% of PHB (22.1 ± 15.9), 85% of glycogen (0.7 ± 0.3) and 88% of trehalose (0.1 ± 0.04) (Table 2).

The ultrastructure of *Sphingobium* sp. 22B cells was analysed by TEM under control, 18RH and 60RH conditions (Fig. 1). Transmission electron micrographs of cells under the 60RH condition (Fig. 1a) showed intracytoplasmic inclusion bodies with smaller size in comparison with those shown in control cells and cells under the 18RH condition (Fig. 1c,e). Interestingly, TEM showed the production of an amorphous matrix which could represent an EPS around the cells after 96 h of incubation under 60RH (Fig. 1b) and 18RH (Fig. 1d) conditions, in contrast to control cells and cells grown in R3 broth (Fig. 1g).

Genomic features linked to survival under desiccation

The availability of the *Sphingobium* sp. 22B genome (DDBJ/ENA/GenBank; LTAB00000000) (Madueño et al. 2016) allowed us to analyse the occurrence of genes hypothetically involved in water stress response. Since 22B strain was able to produce PHB, glycogen, trehalose and probably an EPS, and because there are many reports that showed the relationship of these compounds with desiccation, we searched for putative genes related to their metabolism. As it is a draft genome, still incomplete, we regarded the apparent absence or low copy number of a given gene with caution. Results of this search are summarized in Table 3.

Gene *aqpZ* is present in the genome of strain 22B (Table 3) and encodes a water channel belonging to the major intrinsic protein family (King et al. 2004) and has been involved in environmental stress response in bacteria (Wang 2002; Sinetova et al. 2015; Wood 2015). Putative genes for two different pathways of trehalose biosynthesis (*otsAB* and *treYZ*) and two genes for its degradation (*tre, tp*) were found in *Sphingobium* sp. 22B (Table 3). As expected, 22B strain genome contained genes for the entire glycogen biosynthesis and degradation pathways, such as *glgC, glgA, glgB, glgP* and *glgX*, (Table 3). Multiple genes involved in PHB metabolism were identified with at least three of them coding for polyhydroxyalkanoic acid synthase enzymes, one for acetocetyl-CoA reductase and five for 3-ketoacyl-CoA thiolase enzymes. The presence of diverse genes for transferases and glycosyltransferases (*tfbP, epsF, lgt1, lgt2*) related to the synthesis of extracellular polysaccharides were also found in 22B strain genome (Table 3).

Comparative analyses of stress-related genes in draft genome of *Sphingobium* sp. 22B and in *Sphingomonas* complete genomic projects

The genetic endowment of *Sphingobium* sp. 22B related with the responses to environmental stresses was compared to those of the other 10 *Sphingomonas* complete genome projects available in National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) (Table 4). In most of the studied strains, the complete pathway for biosynthesis and degradation of glycogen and for biosynthesis of trehalose from glucose (*otsAB*) were found (Table 4). An aquaporin family gene related to osmotic stress (*aqpZ*) and *epsF* gene involved in the biosynthesis.

### Table 2 Intracellular percentage of glycogen, trehalose and PHB per cellular dry weight (CDW) in the strain *Sphingobium* sp. 22B under control, 18RH (18% of relative humidity) and 60RH (60% of relative humidity) conditions. Values are means ± SEM

| Conditions | Glycogen % CDW | Trehalose % CDW | PHB % CDW |
|------------|----------------|----------------|-----------|
| Control    | 4.8 ± 1.4      | 0.84 ± 0.16    | 44.9 ± 6.4|
| 18RH       | 2.0 ± 0.3      | 0.28 ± 0.1     | 45.7 ± 28.4|
| 60RH       | 0.7 ± 0.3      | 0.10 ± 0.04    | 22.1 ± 15.9|
of an EPS were found in *Sphingobium* sp. 22B and also in four other *Sphingobium* sp. strains (Table 4). Major differences between *Sphingomonas* ‘sensu lato’ strains and strain 22B were found in trehalose metabolism. Gene *tre*, which encodes trehalase, and *treY* and *treZ* genes for the biosynthesis of trehalose from glycogen, were only found in two *Sphingomonas* strains (*Novosphingobium* sp. PPIY, *Sphingobium* sp. YBL2 for *tre* gene, and *Sphingobium* sp. SYK-6 Ac, *Sphingobium* sp. YBL2 *treY* and *treZ* genes) (Table 4), and also *tp* gene which encodes trehalose phosphorylase enzyme was found in three complete genome sequences of *Sphingomonas* (*Sphingobium* sp. YBL2, *Sphingobium* sp. EP60837, *Sphingobium* sp. MI1205).

**qRT-PCR**

To analyse the expression of genes hypothetically related to water stress response in *Sphingobium* sp. 22B, specific primers were designed. Those primers were related to single-copy genes, involved in osmotic stress (*aqpZ*), trehalose biosynthesis (*otsA, otsB, treY, treZ*), trehalase utilization (*tre, ga*), glycogen biosynthesis and utilization (*glgA, glgB, glgC glgP*), PHB (*phbB*) and exopolysaccharide biosynthesis (*epsF, lgt1*). Only those that showed clear specificity and efficiencies between 0·95 to 1 were used in this assay. Figure 2 shows the fold changes in mRNA levels of the genes *glgC, glgP, aqpZ, phbB, otsA, treY, lgt1* under 18RH, 60RH and control conditions calculated from the ΔΔCt method. *AqpZ* gene expression showed no difference between 18RH, 60RH and control conditions. The glycogen degradation and biosynthesis genes *glgC and glgP* were significantly downregulated under 18RH and 60RH conditions in comparison to control, although no differences in the expression under both water stress conditions studied were found (Fig. 2). The expression of *phbB* gene, which is involved in polyhydroxybutyrate biosynthetic process, was downregulated in 60RH and was not significantly different in 18RH in
comparison to the control condition (Fig. 2). Putative gene \(lgt1\), encoding a glycosyl transferase involved in EPS biosynthesis, and \(otsA\) gene which encodes for an alpha,alpha-trehalose-phosphate synthase, were downregulated in 60RH in comparison with the control, and not detected under the high water stress condition (18RH). The gene \(treY\) which encodes a malto-oligosyltrehalose synthase was sixfold upregulated under the low water stress condition (60RH) in comparison with the control condition (Fig. 2).

### Table 3
Putative genes hypothetically linked to survival under desiccation in Sphingobium sp. 22B draft genome (http://www.ncbi.nlm.nih.gov/genome). Genes shown are related to osmotic stress, trehalose and glycogen biosynthesis and utilization; PHB and exopolysaccharide biosynthesis in Sphingobium sp. 22B genome

| Product name | Gene | EC number | Reactions | Scenario reactions | GO | No. copy |
|--------------|------|-----------|-----------|--------------------|----|----------|
| Osmotic stress | Aquaporin family protein | aqpZ | – | – | R02737 | GO:0005215 | 1 |
| Trehalose biosynthesis and utilization | Alpha,alpha-trehalose-phosphate synthase | otsA | 2.4.1.15 | R00836 R06043 | R02737 | GO:0003825 | 1 |
| Trehalose-phosphatase | otsB | 3.1.3.12 | R02778 R06228 | -- | 1 |
| Malto-oligosyltrehalose synthase | treY | 5.4.99.15 | R06243 R01824 | -- | GO:0047470 | 1 |
| Malto-oligosyltrehalose trehalohydrolase | treZ | 3.2.1.141 | -- | -- | -- | 1 |
| Glycoside hydrolase family 15 protein (glucoamylase) | ga | 3.2.1.3 | -- | -- | -- | 1 |
| Trehalase | treA | 3.2.1.28 | R00010 R06103 | R00010 | GO:0004555 | 1 |
| Glycoside hydrolase family 65 protein (trehalose phosphorylase) | tp | 2.4.1.64 | R02727 R06053 | R02727 | GO:0047656 | 1 |
| Glycogen biosynthesis and utilization | Glycogen synthase | glgA | 2.4.1.21 | R02421 | -- | R02421 | GO:0009011 | 1 |
| Glycogen branching enzyme | glgB | 2.4.1.18 | R02110 | R02110 | -- | 1 |
| Glucose-1-phosphate adenytransferase | glgC | 2.7.7.27 | R00948 | R00948 | -- | GO:0008878 | 1 |
| Glycogen/starch/alpha-glucan phosphorylase | glgP | 2.4.1.11 | R01821 | R02111 | GO:004645 | 1 |
| Glycogen debranching enzyme | glgX | 3.2.1.- | -- | -- | -- | 1 |
| PHB synthesis | Acetyl-CoA acetyltransferase | phbA | 2.3.1.9 | -- | -- | R04254 | GO:0003985 | 5 |
| Beta-ketoacyl-ACP reductase | phbB | 1.1.1.36 | R01779 | R01977 | GO:0008454 | 1 |
| Poly-beta-hydroxybutyrate polymerase | phbC | – | -- | -- | -- | 2 |
| Class I poly(R)-hydroxyalkanoic acid synthase | phaC | – | – | – | -- | 1 |
| Exopolysaccharide biosynthesis | Exopolysaccharide biosynthesis glycosyltransferase ExsF | epsF | 2.4.1.- | -- | -- | -- | GO:0009058 | 1 |
| Glycosyl transferase, family 4 protein | lgt1 | – | -- | -- | -- | 2 |
| Glycosyl transferase, family 2 protein | lgt2 | – | -- | -- | -- | 1 |
| Undecaprenyl-phosphate galactosephosphotransferase | rfbP | 2.7.8.6 | -- | -- | GO:0047360 | 1 |
Table 4 Comparative genome analysis between Sphingobium sp. 22B draft genome and Sphingomonas ‘sensu lato’ complete genome projects showing the presence of at least one (+) of putative genes linked with osmotic stress; trehalose and glycogen biosynthesis and utilization; PHB and exopolysaccharide biosynthesis. NF: putative genes not found.

| Gene                  | Encoding gene                                      | EC number          | Sphingobium sp. C1 | Sphingobium sp. RAC03 | Sphingobium sp. EP08037 | Sphingobium sp. YBL2 | Sphingobium sp. SYK-6 | Sphingobium sp. TK5 | Sphingobium japonicum UT85 | Sphingobium chlorophenolicum L-1 | Sphingobium sp. MI205 | Novosphingobium aromatovorans DSM 12444 | 822 sp. aromaticivorans |
|-----------------------|---------------------------------------------------|---------------------|--------------------|-----------------------|-------------------------|--------------------|-----------------------|-----------------------|--------------------------------------|--------------------------------------|-------------------------|--------------------------------------|------------------------|
| **Osmotic stress**    |                                                   |                     |                    |                       |                         |                    |                       |                       |                                      |                                      |                         |                                      |                        |
| aqpZ                  | Aquaporin family protein                          | --                  | NF                 | NF                    | NF                      | +                  | +                     | ++                    | +                                    | +                                    | NF                      | NF                      | NF                      |
| **Trehalose biosynthesis and utilization** | | | | | | | | | | | | | | |
| otsA                  | Alpha, alpha-trehalose-phosphate synthase         | 2.4.1.15            | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| otsB                  | Trehalose-phosphatase                             | 3.1.3.12            | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| treY                  | Malto-oligosyltrehalosylsynthase                  | 5.4.99.15           | NF                  | NF                    | NF+                    | NF                 | NF                    | NF                    | NF+                                  | NF+                                  | NF                      | NF                      | NF                      |
| treZ                  | Malto-oligosyltrehalose trehalohydrolase          | 3.2.1.141           | NF                  | NF                    | NF+                    | NF                 | NF                    | NF                    | NF+                                  | NF+                                  | NF                      | NF                      | NF                      |
| ga                    | Glycosyldehydrodase family 15 protein (glucoamylase) | 3.2.1.3             | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| tre                   | Trehalase                                         | 3.2.1.287           | NF                  | NF                    | NF+                    | NF                 | NF                    | NF                    | NF+                                  | NF+                                  | NF                      | NF                      | NF                      |
| tp                    | Glycoside hydrolase family 65 protein (trehalose phosphorolysase) | 2.4.1.64           | NF                  | NF                    | NF+                    | NF                 | NF                    | NF                    | NF+                                  | NF+                                  | NF                      | NF                      | NF                      |
| **Glycogen biosynthesis and utilization** | | | | | | | | | | | | | | |
| gglA                  | Glycogen synthase                                 | 2.4.1.21            | NF                  | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| gglB                  | Glycogen-branching enzyme                          | 2.4.1.18            | NF                  | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| gglC                  | Glucose-1-phosphate adenytransferase              | 2.7.7.73            | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| gglP                  | Glycogen/starch/alpha-glucan phosphorylase        | 2.4.1.11            | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| gglX                  | Glycogen-debranching enzyme                        | 3.2.1.1             | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| **PHB biosynthesis**  |                                                   |                     |                    |                       |                         |                    |                       |                       |                                      |                                      |                         |                         |                         |
| phbA                  | Acetyl-CoA acetyltransferase                       | 2.3.1.19            | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| phbB                  | Beta-ketoacyl-ACP reductase                        | 1.1.1.36            | NF                  | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| phbC                  | Poly-beta-hydroxybutyrate polymerase              | --                  | NF+                 | NF                    | +                      | +                  | +                     | ++                    | NF+                                  | NF+                                  | NF                      | NF                      | NF                      |
| phaC                  | Class I poly(R)-hydroxylalkanoic acid synthase    | --                  | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| **Exopolysaccharide biosynthesis** | | | | | | | | | | | | | | |
| epsF                  | Exopolysaccharide biosynthesis glycosyltransferase EpsF | 2.4.1.36           | NF                  | NF                    | NF                    | NF                 | NF                    | NF                    | NF+                                  | NF+                                  | NF                      | NF                      | NF                      |
| tgt1                  | Glycosyltransferase, family 4 protein             | --                  | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| tgt2                  | Glycosyltransferase, family 2 protein             | --                  | +                   | +                     | +                      | +                  | +                     | ++                    | +                                    | +                                    | +                       | +                       | +                       |
| rfbP                  | Undecaprenyl-phosphogalactose phosphotransferase   | 2.7.8.6             | NF                  | NF                    | NF                    | NF                 | NF                    | NF                    | NF+                                  | NF+                                  | NF                      | NF                      | NF                      |
Desiccation resistance in *Sphingobium* sp. 22B

**Discussion**

Micro-organisms have the capacity to utilize a huge variety of nutrients and adapt to continuously changing environmental conditions. Many micro-organisms, including yeast and bacteria, accumulate carbon and energy reserves to cope with starvation conditions temporarily present in the environment (Wilson et al. 2010). *Sphingobium* sp. 22B strain shows a carbon conservative metabolism which allows the cells to store carbon and energy within different compounds, such as PHB, glycogen and trehalose (Table 2). These compounds may play different roles in cells and may be part of the complex metabolic network present in strain 22B to cope with the adaption to environmental stresses.

The synthesis and accumulation of PHB is a known and widely distributed property in prokaryotes and depends on either the type of strain or the carbon source used in the process (Verlinden et al. 2007; Getachew and Woldesenbet 2016). PHB seems to be relevant for 22B strain physiology, since it accumulates significant amounts of these lipids (Table 2, Fig. 1e) and has in its genome a robust endowment of genes/proteins for their metabolism (Table 3). As a comparison with strain 22B, which stores 45% CDW of PHB under the control condition, other bacteria like *Bacillus cereus* CFR06 and *Caulobacter crescentus* accumulate 50 and 18% CDW of PHB, respectively, from glucose as carbon source (Qi and Rehm 2001; Halami 2008). In particular, other strains of *Sphingomonas* showed an accumulation of PHA from glucose in percentages that ranged from 2.9 to 70-2% (w/w) (Godoy 2003). In strain 22B, PHB may serve as an endogenous carbon and energy source, providing a temporal nutritional independence from the environment and the maintenance of the metabolic activity during starvation under low water stress conditions (60RH) (Table 2). The results obtained in this study, however, showed that under the high water stress condition (18RH), *Sphingobium* sp. 22B maintained the same intracellular PHB concentration as the control (Table 2), kept the integrity of intracytoplasmic inclusion bodies (Fig. 1) and showed the same expression level of *phbB* gene under 18RH condition in comparison with the control condition (Fig. 2). Therefore, the endogenous mobilization of PHB did not seem to be a process related to high water stress condition (18RH) in strain 22B. Recently, Goh et al. (2014) provided evidence that in *Delftia acidovorans* the enhancement of stress tolerance can be achieved without mobilization of previously accumulated PHA. The authors argue that intracytoplasmic PHA granules could act as a specific site for binding a stress-resistant protein reducing the stress-resistant protein cytoplasmic concentration and consequently the expression of more proteins. Obruca et al. (2016) analysed the PHB monomer 3-hydroxybutyrate (3HB) as a chemical chaperone capable of protecting model enzymes from different environmental stresses finding that 3HB exhibits a greater protective effect than that of the trehalose. In concordance with those authors’ arguments, PHB might play diverse roles under different degrees of water stress: it can serve as an endogenous carbon source under 60RH and can protect cells under 18RH.

Cells of the 22B strain actively metabolized carbohydrates under both conditions of water stress tested in this work, suggested by the mobilization of intracellular glycogen and trehalose (Table 2), the upregulation of *treY* gene under low water stress condition, the absence of significant differences between the expression of the glycogen synthesis and degradation genes *glgC* and *glgP*, and the downregulation of *otsA* gene under water stress conditions (Fig. 2).

Glycogen biosynthesis is another mechanism for carbon storage in bacteria and *Sphingobium* sp. 22B accumulates approximately 5% CDW (Table 2) under excess of the carbon source. Beer et al. (2004) indirectly suggested that members of the genus *Sphingomonas* may have the ability to produce glycogen, and are able to reach a biomass glycogen level of 5–24 (% w/w). Glycogen has been detected in higher concentrations in other bacteria such as 18% of the dry weight in Cyanobacteria after nitrogen withdrawal (Klotz and Forchhammer 2017) and up to 60

**Figure 2** Gene expression of key genes/markers (glgP, glgC, aqpZ, phbB, lgt1, otsA and treY) regulated during 18RH (high water stress) and 60RH (low water stress) conditions. Values expressed as fold change are means ± SEM of three independent RNA preparations and indicate the change in the mRNA levels of genes studied in comparison with the control condition (value of 1). The significance of gene expression between conditions are shown with different letters (a,b) and was determined by Student’s *t* test using a *P* value of ±0.05 as the threshold (control: ■ 60RH; ■ 18RH).
CDW% in Clostridia just prior to sporulation (Preis et al. 1983). According to our knowledge this is the first report of the production of glycogen in a strain of Sphingobium. Under the water stress conditions studied in this work (18RH and 60RH), endogenous mobilization of glycogen in strain 22B may occur in order to attenuate carbon starvation, which is a consequence of desiccation (Table 2). In concordance with the greatest mobilization of glycogen under the 60RH condition (Table 2), treY gene, which is involved in the conversion of endogenous glycogen to trehalose, was upregulated (Fig. 2). However, the concentration of trehalose was less (0.1 ± 0.04% CDW) under the 60RH condition in comparison with 18RH (0.28 ± 0.1% CDW). Some authors have reported that genes which are involved in the metabolism of trehalose were overexpressed under osmotic stress and desiccation (Cytyn et al. 2007; Johnson et al. 2011) and they assigned biological functions to trehalose, such as carbon source or compatible solute under osmotic stress conditions. (Omar et al. 2014; Shleeva et al. 2017). In Rhodococcus opacus PD630, the accumulation of trehalose was 0.48% CDW from glucose as the carbon source after 7 days in dehydration (Alvarez et al. 2004). Comparative genome analysis revealed that the strain 22B could be synthesizing trehalose through two different pathways (otsAB and treYZ) while only in two complete genomes of Sphingomonas ‘sensu latu’ included in this study (Sphingobium sp. SYK-6 and YBL2) the treYZ pathway was present. Diverse trehalose synthesis pathways could provide Sphingobium sp. 22B the necessary metabolic flexibility to respond to environmental stress with this disaccharide, giving to the strain competitive advantages over other bacteria under desiccation. Additionally, glycogen and trehalose in Sphingobium 22B may serve as an endogenous source of carbon and energy and may provide sugar residues for the biosynthesis of extracellular polymer, which is produced under 60RH and 18RH conditions, as the TEM analysis revealed (Fig. 1b,d).

Sphingobium sp. 22B has at least four putative genes that encode for glycosyltransferases involved in the synthesis of EPS (lgt1, lgt2, epsF, rfbP) (Table 4). EPS has been associated with the responses of Sphingomonas wittichii RW1 to osmotic stress, since a transcriptomic study demonstrated the induction of genes involved in the polysaccharide synthesis, assembly and export of EPS (Roggo et al. 2013). Although in strain 22B subjected to water stress conditions, an extracellular amorphous matrix was seen in TEM, lgt1 gene expression (Fig. 2) was downregulated indicating that this gene may not be involved in the synthesis of EPS. The production of an extracellular amorphous matrix by strain 22B in response to water stress may offer a significant mechanical protection to cells, preventing intracellular water loss (Ophir and Gutnick 1994), and allowing the cells to make metabolic adjustments to survive water stress (Roberson and Firestone 1992; Tribelli and López 2011).

In 4 of the 10 complete genomes of Sphingomonas ‘sensu latu’ and in the genome of Sphingobium sp. 22B, the gene aqpZ coding for aquaporin protein, was present (Table 4). Aquaporins are water channels mediating transmembrane water flux in E. coli (Calamita 2000) and the absence or even mutations of this gene in many living microorganisms indicates that aquaporins may have nonessential functions and are not always related to microbial survival (Tanghe et al. 2006). Many authors studying aquaporins found inconsistencies (Tanghe et al. 2006) in results during hypoosmotic (Booth and Louis 1999) and hyperosmotic stress in bacteria (Hernández-Castro et al. 2003). The results obtained in this study suggested that aquaporins has no evident function in Sphingobium sp. 22B under water stress conditions, since aqpZ was not differentially expressed between the water stress conditions studied (Fig. 2).

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**Conflict of Interest**

The authors have no conflict of interest to declare.

**REFERENCES**

Alvarez, H.M., Silva, R., Cesari, A.C., Zamit, A.L., Peressutti, S.R., Reichelt, R., Keller, U., Malkus, U. et al. (2004) Physiological and morphological responses of the soil bacterium Rhodococcus opacus strain PD630 to water stress. FEMS Microbiol Ecol 50, 75–86.

Ayub, N.D., Pettinari, M.J., Ruiz, J.A. and López, N.I. (2004) A polyhydroxybutyrate-producing Pseudomonas sp. isolated from Antarctic environments with high stress resistance. Curr Microbiol 49, 170–174.
Baraniecki, C., Aislabie, J. and Foght, J.M. (2002) Characterization of Sphingomonas sp. Ant 17, an aromatic hydrocarbon-degrading bacterium isolated from Antarctic soil. Microb Ecol 43, 44–54.

Beer, M., Kong, Y.H. and Seward, R.J. (2004) Are some putative glycogen accumulating organisms (GAO) in anaerobic: aerobic activated sludge systems members of the alpha-Proteobacteria? Microbiology 150, 2267–2275.

Booth, I.R. and Louis, P. (1999) Managing hyposmotic stress: aquaporins and medianosensitive channels in Escherichia coli. Curr Opin Microbiol 2, 166–169.

Calamita, G. (2000) The Escherichia coli aquaporin-Z water channel. Mol Microbiol 37, 254–262.

Crowe, J.H., Crowe, L.M., Carpenter, J.F. and Aurrel Wistrom, C. (1987) Stabilization of dry phospholipid bilayers and proteins by sugars. Biochim Biophys Acta 242, 1–10.

Cytryn, E.J., Sangurdekar, D.P., Streeter, J.G., Franck, W.L., Chang, W.S., Stacey, G., Emerich, D.W., Joshi, T. et al. (2007) Transcriptional and physiological responses of Bradyrhizobium japonicum to desiccation-induced stress. J Bacteriol 189, 6751–6762.

Elbein, A.D. and Mitchell, M. (1973) Levels of glycogen and trehalose in Mycobacterium smegmatis and the purification and properties of the glycogen synthetase. J Bacteriol 113, 863–873.

Farias, M.E., Revale, S., Mancini, E., Ordoñez, O., Turjanski, A., Cortez, N. and Vazquez, M.P. (2011) Genome sequence of Sphingomonas sp. S17, isolated from an alkaline, hyperarcesnic, and hypersaline volcano-associated lake at high altitude in the Argentinean Puna. J Bacteriol 193, 3686–3687.

Fegatella, F. and Cavichioli, R. (2000) Physiological responses to starvation in the marine oligotrophic ultramicrobacterium Sphingomonas sp. strain RB2256. Appl Environ Microbiol 66, 2037–2044.

Festa, S., Coppotelli, B.M. and Morelli, I.S. (2013) Bacterial diversity and functional interactions between bacterial strains from a phenanthrene-degrading consortium obtained from a chronically contaminated soil. Int Biodeterior Biodegradation 85, 42–51.

Fida, T.T., Breugelmans, P., Lavigne, R., Coronado, E., Johnson, D.R., van de Beer, J.R., Mayer, A.P., Heipieper, H.J. et al. (2012) Exposure to solute stress affects genome-wide expression but not the PAH-degrading activity of Sphingomonas sp. LH128 in biofilms. Appl Environ Microbiol 78, 8311–8320.

Getachew, A. and Woldesenbet, F. (2016) Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low cost agricultural waste material. BMC Res Notes 9, 509.

Godoy, F. (2003) Sphingopyxischilensis sp. nov., a chlorophenol-degrading bacterium that accumulates polyhydroxyalkanoate, and transfer of Sphingomonas alaskensis to Sphingopyxis alaskensis comb. nov. Int J Syst Evol Microbiol 53, 473–477.

Goh, L., Purama, R.K. and Sudesh, K. (2014) Enhancement of stress tolerance in the polyhydroxyalkanoate producers without mobilization of the accumulated granules. Appl Biochem Biotechnol 172, 1585–1598.

Halami, P.M. (2008) Production of polyhydroxyalkanoate from starch by the native isolate Bacillus circulatus CFR06. World J Microbiol Biotechnol 24, 805–812.

Halverson, L.J. and Firestone, M.K. (2000) Differential effects of permeating and nonpermeating solutes on the fatty acid composition of Pseudomonas putida. Appl Environ Microbiol 66, 2414–2421.

Harms, G., Layton, A.C., Dionisi, H.M., Gregory, Y.G., Garrett, V.M., Hawkins, S.A., Robinson, K.G. and Gayler, G.S. (2003) Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. Environ Sci Technol 37, 343–351.

Helmann, J.D. and Chamberlin, M.J. (1988) Structure and function of bacterial sigma factors. Annu Rev Biochem 57, 839–872.

Hernández, M.A., Mohn, W.W., Martínez, E., Rost, E., Alvarez, A.F. and Alvarez, H.M. (2008) Biosynthesis of storage compounds by Rhodococcus jostii RHA1 and global identification of genes involved in their metabolism. BMC Genom 9, 1–13.

Hernández-Castro, R., Rodríguez, M.C., Seoane, A. and García Lobo, J.M. (2003) The aquaporin gene aqpX of Brucella abortus is induced in hyperosmotic conditions. Microbiol 149, 3185–3192.

Johnson, D.R., Coronado, E., Moreno-Forero, S.K., Heipieper, H.J. and van der Meer, J.R. (2011) Transcriptome and membrane fatty acid analyses reveal different strategies for responding to permeating and non-permeating solutes in the bacterium Sphingomonas wittichii. BMC Microbiol 11, 250.

King, L.S., Kozono, D. and Agre, P. (2004) From structure to disease: the evolving tale of aquaporin biology. Nat Rev Mol Cell Biol 5, 687–698.

Klotz, A. and Forchhammer, K. (2017) Glycogen, a major player for bacterial survival and awakening from dormancy. Future Microbiol 12, 10–13.

Lin, S.-Y., Shen, F.-T., Lai, W.-A., Zhu, Z.-L., Chen, W.-M., Chou, J.-H., Lin, Z. and Young, C.-C. (2012) Sphingomonas formosensis sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from agricultural soil. Int J Syst Evol Microbiol 62, 1581–1586.

Madueño, L., Coppotelli, B.M., Alvarez, H.M. and Morelli, I.S. (2011) Isolation and characterization of indigenous soil bacteria for bioaugmentation of PAH contaminated soil of semi-arid Patagonia, Argentina. Int Biodeterior Biodegradation 65, 345–351.

Madueño, L., Alvarez, H.M. and Morelli, I.S. (2015) Autochthonous bioaugmentation to enhance phenanthrene degradation in soil microcosms under arid conditions. Int J Environ Sci Technol 12, 2317–2326.
Madueño, L., Macchi, M., Morelli, I.S. and Coppotelli, B.M. (2016) Draft whole-genome sequence of Sphingobium sp. 22B, a polycyclic aromatic hydrocarbon–degrading bacterium from semi-arid Patagonia, Argentina. Genome Announce 4, e00488–16.

Margesin, R., Zhang, D.-C. and Busse, H.-J. (2012) Sphingomonas alpina sp. nov., a psychrophilic bacterium isolated from alpine soil. Int J Syst Evol Microbiol 62, 1558–1563.

Matin, A., Veldhuis, C., Stegeman, V. and Veenhuis, M. (1994) Selective advantage of a Spirillum sp. in a carbon–limited environment. Accumulation of poly-beta-hydroxybutyric acid and its role in starvation. J Gen Microbiol 112, 349–355.

Obrucu, S., Sedlacek, P., Mravec, F., Samek, O. and Marova, I. (2016) Evaluation of 3-hydroxybutyrate as an enzyme-protective agent against heating and oxidative damage and its potential role in stress response of poly (3-hydroxybutyrate) accumulating cells. Appl Microbiol Biotechnol 100, 1365–1376.

Omar, H.S., El-assal, S.E., Hussein, E.H.A. and Soliman, M.H. (2014) Identification and characterization of the trehalase gene (OtSA) in some pathogenic bacteria using an in silico approach. Int J Adv Res 2, 403–412.

Ophir, T. and Gutnick, D.L. (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. Appl Environ Microbiol 60, 740–745.

Paruelo, J.M., Beltrán, A., Jobbágy, E., Sala, O.E. and Golluscio, R.A. (1998) The climate of Patagonia: general patterns and controls on biotic processes. Ecología Austral 8, 85–101.

Potts, M. and Webb, S.J. (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58, 755–805.

Preiss, J., Yung, S. and Baecker, P. (1983) Regulation of bacterial glycogen synthesis. Mol Cell Biochem 57, 61–80.

Qi, Q. and Rehm, B.H.A. (2001) Polyhydroxybutyrate biosynthesis in Caulobacter crescentus: molecular characterization of the polyhydroxybutyrate synthase. Microbiology 147, 3353–3358.

Ramos, J.L., Gallegos, M., Marqués, S., Espinosa-urgel, M. and Segura, A. (2001) Responses of Gram-negative bacteria to certain environmental stressors. Curr Opin Microbiol 4, 166–171.

Reasoner, D.J. and Geldreich, E.E. (1985) A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49, 1–7.

Reddy, G.S.N. and García-Pichel, F. (2007) Sphingomonas mucosissima sp. nov. and Sphingomonas desiccabilis sp. nov., from biological soil crusts in the Colorado Plateau, USA. Int J Syst Evol Microbiol 57, 1028–1034.

Roberson, E.B. and Firestone, M.K. (1992) Relationship between desiccation and exopolysaccharide production in a soil Pseudomonas sp. Appl Environ Microbiol 58, 1284–1291.

Roggo, C., Coronado, E., Moreno-Forero, S.K., Harshman, K., Weber, J. and Van der Meer, J.R. (2013) Genome-wide transposon insertion scanning of environmental survival functions in the polycyclic aromatic hydrocarbon degrading bacterium Sphingomonas wittichii RW1. Environ Microbiol 15, 2681–2695.

Setlow, P. (1995) Mechanisms for the prevention of damage to DNA in spores of Bacillus species. Annu Rev Microbiol 49, 29–54.

Shleeva, M.O., Trutneva, K.A., Demina, G.R., Zinin, A.I., Sorokoumova, G.M., Laptinskaya, P.K., Shumkova, E.S. and Kaprelyants, S.A. (2017) Free trehalose accumulation in dormant Mycobacterium smegmatis cells and its breakdown in early resuscitation phase. Front Microbiol 8, 1–12.

Sineto, M.A., Mironov, K.S., Mustard, L., Shapiguzov, A., Bachin, D., Allakhverdiev, S.I. and Los, D.A. (2015) Aquaporin-deficient mutant of Synechocystis is sensitive to salt and high-light stress. J Photochem Photobiol, B 152, 377–382.

Stolz, A. (2009) Molecular characteristics of xenobiotic-degrading spingomonads. Appl Microbiol Biotechnol 81, 793–811.

Takeuchi, M., Hamana, K. and Hiraishi, A. (2001) Proposal of the genus Sphingomonas sensu stricto and three new genera, Sphingobium, Novosphingobium and Sphingopxyxis, on the basis of phylogenetic and chemotaxonomic analyses. Int J Syst Evol Microbiol 51, 1405–1417.

Tanghe, A., Van Dijck, P. and Thevelein, J.M. (2006) Why do microorganisms have aquaporins? Trends Microbiol 14, 78–85.

Trielli, P.M. and López, N.I. (2011) Poly(3-hydroxybutyrate) influences biofilm formation and motility in the novel Antarctic species Pseudomonas extremoaulstralis under cold conditions. Extremophiles 15, 541–547.

Vacca, D.J., Bleam, W.F. and Hickey, W.J. (2005) Isolation of soil bacteria adapted to degrade humid acid-sorbed phenanthrene. Appl Environ Microbiol 71, 3797–3805.

Verlinden, R.A.J., Hill, D.J., Kenward, M.A., Williams, C.D. and Radecka, I. (2007) Bacterial synthesis of biodegradable polyhydroxyalkanoates. J Appl Microbiol 102, 1437–1449.

Wang, Y. (2002) The function of OmpA in Escherichia coli. Biochem Biophys Res Commun 292, 396–401.

Welsh, D.T. (2000) Ecological significance of compatible solute accumulation by microorganisms: from single cells to global climate. FEMS Microbiol Rev 24, 263–290.

Wilson, W.A., Roach, P.J., Montero, M., Baroja-Fernández, E., Munóz, F.I., Eydallin, G., Viale, A.M. and Pozueta-Romero, J. (2010) Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiol Rev 34, 952–985.

Wood, J.M. (2015) Perspectives on the response to osmotic challenges: bacterial responses to osmotic challenges. J Gen Physiol 145, 381–388.

Zhang, Q. and Yan, T. (2012) Correlation of intracellular trehalose concentration with desiccation resistance of soil Escherichia coli populations. Appl Environ Microbiol 78, 7407–7413.