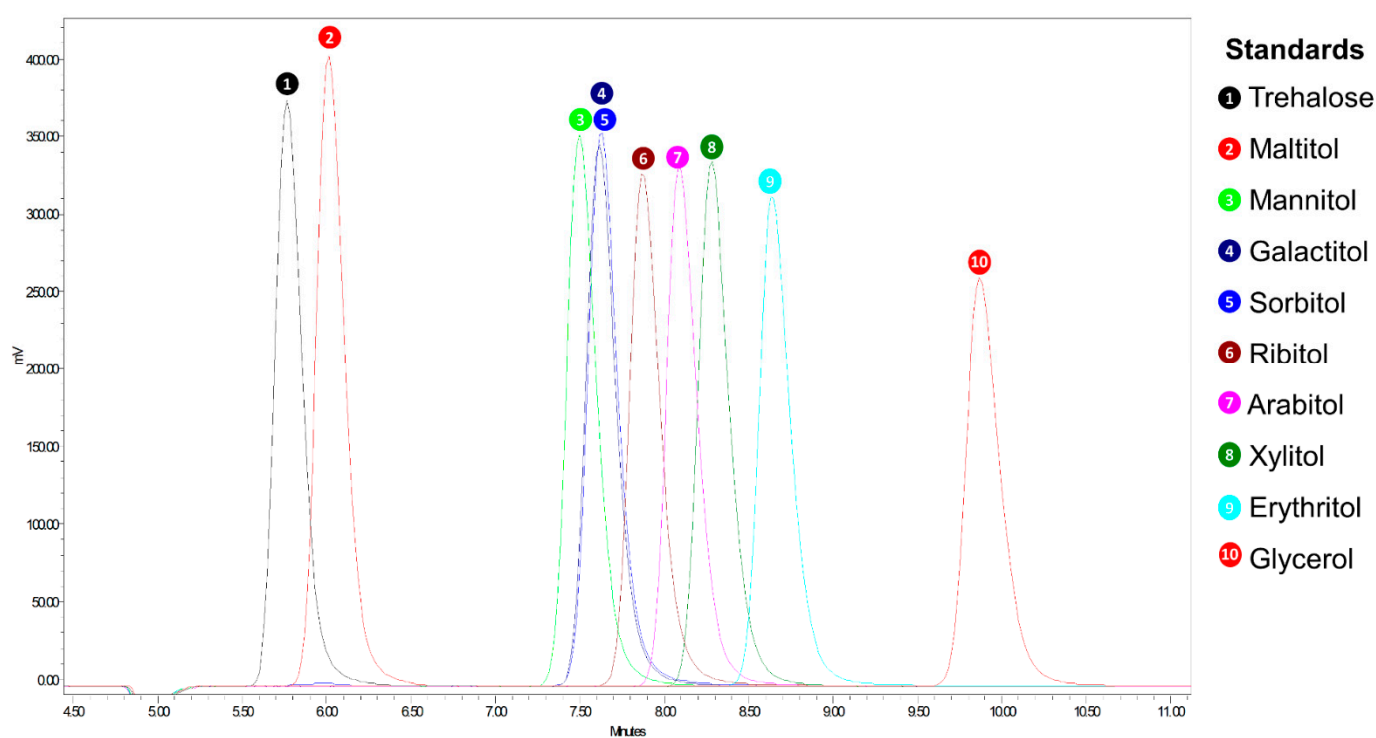


## Supplementary Material

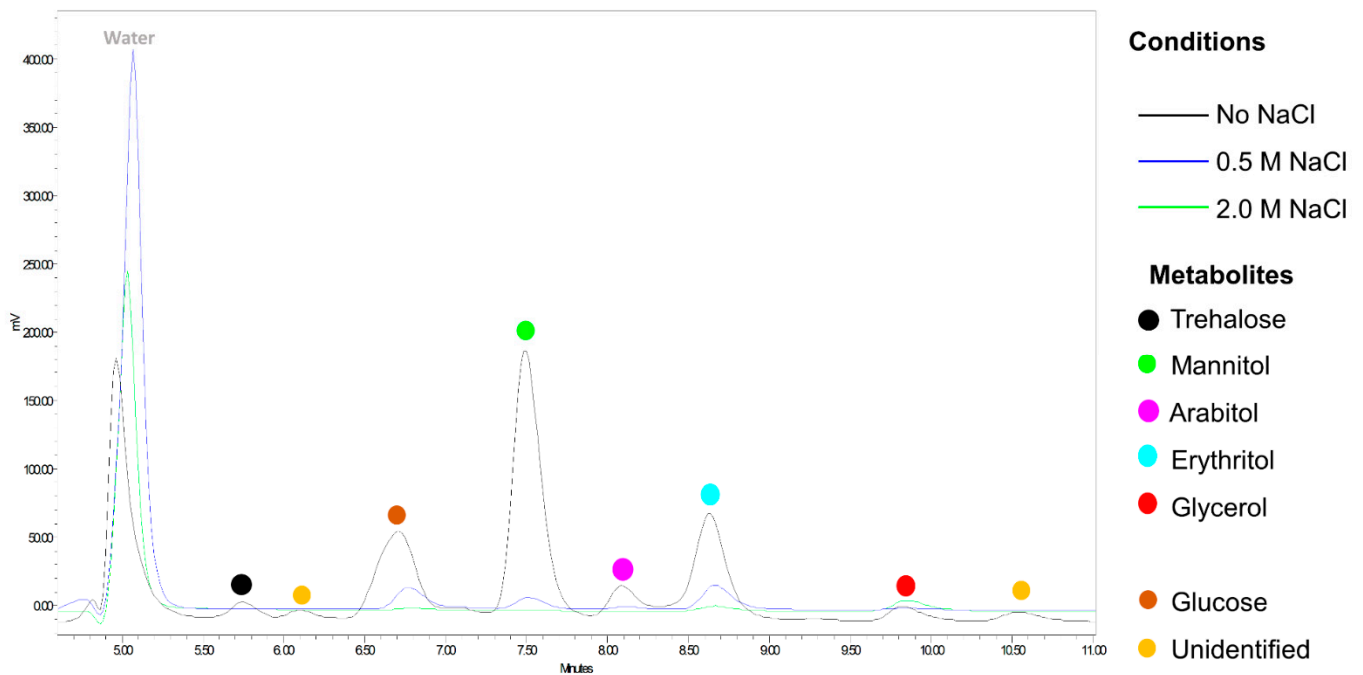
# Osmolyte Signatures for the Protection of *Aspergillus sydowii* cells under Halophilic Conditions and Osmotic Shock

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**Figure S1.** Chromatograms of all used standards. Concentration: 5 mg/mL each



**Figure S2.** Chromatograms of selected samples from all salinity conditions. .

**Table S1.** Primer information for qPCR analysis of enzyme genes involved in the synthesis of compatible solutes.

Metabolite	Gene	Primer Sequence	Genome Location	Tm	Amp. size	Thib	Conc.	Eff.
Trehalose	<i>stps</i>	Fw: TCA ACG ATG GAC CAC TT	scaffold_10:397039-397058	60	109	63	300	0.92
		Rv: GGG TCG GAC TGC TTC GAT AT	scaffold_10:396950-396969					
	<i>ccg-9</i>	Fw: GTC AGC CAC CCT GTG AAA AC	scaffold_3:1753499-1753518	60	84	65	300	1.03
		Rv: CAG CCA ATC TGT CGA TGC AG	scaffold_3:1753435-1753454					
Mannitol	<i>mtld</i>	Fw: CAT CAA CGC CAC CGA TAC AC	scaffold_5:597303-597322	60	97	63	300	0.92
		Rv: ACA TTG AGG CCG TTG TTT GG	scaffold_5:597185-597204					
	<i>m2dh</i>	Fw: GCA TTG TCT CGC TGA CCA TC	scaffold_3:2943327-2943346	60	128	63	300	1.00
		Rv: GGT GGT ACG GGG AGT CTT TT	scaffold_3:2943435-2943454					
Erythritol	<i>tktB</i>	Fw: GCC ACC CAT CGC AGT ATC TA	scaffold_14:515665-515684	60	126	63	700	0.96
		Rv: TGA TAG TCT CGA CCA TGG CG	scaffold_14:515559-515578					
	<i>tktA</i>	Fw: TGC CTT CCA GCA GAT TCA GT	scaffold_2:2845201-2845220	60	126	65	300	1.09

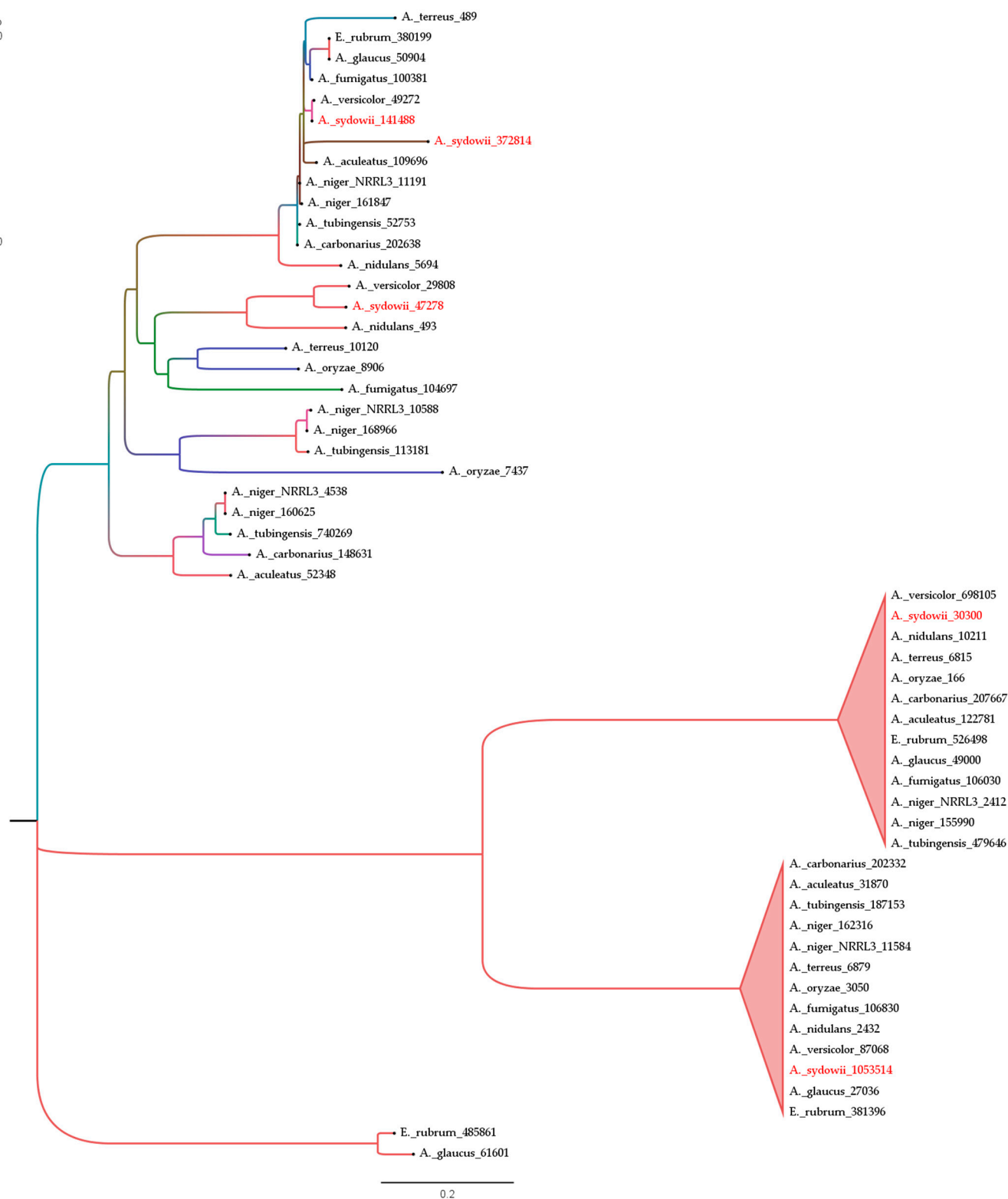
	<i>tad</i>	<b>Rv:</b> CGT ATG GCC GTT TTC CCA TC	scaffold_2:2845095-2845114	60	100	65	300	0.95
		<b>Fw:</b> CTC ATC TCT CCC TTC GTC GG	scaffold_2:1207666-1207685					
		<b>Rv:</b> TCT GGA CAG ACT TGA CAC CG	scaffold_2:1207746-1207765					
		<b>Fw:</b> ACA GGC TCT ACC CTC TGA GT	scaffold_2:1570111-1570130					
Glycerol	<i>gpd</i>	<b>Rv:</b> GAG AGC GCC TCC CAA TGA TA	scaffold_2:1570215-1570234	60	124	60	500	1.06
HOG	<i>hog1</i>	<b>Fw:</b> GTC TTT GCC CTT CCC ATT TTC	scaffold_1:1249970-1249990	60	150	60	500	0.92
		<b>Rv:</b> GAA TTC CGC CAT TTT GAC CG	scaffold_1:1249841-1249860					
	<i>hog2</i>	<b>Fw:</b> CAT GAA TAC CTC GCT CCG TAC	NA	60		63	500	0.96
		<b>Rv:</b> TCA TCT TCC AAG TAT GCA CCG	scaffold_1:4867322-4867342					

**Table S2.** Growth kinetic parameters of *Aspergillus sydowii* grown at different NaCl concentrations.

NaCl Concentration (M)	Specific Growth Rate ( $\mu$ )(h <sup>-1</sup> )	Doubling Time (td)(h)
No NaCl	0.0514	13.48
0.5	0.0683	10.14
1.0	0.0629	11.01
2.0	0.0474	14.62

**Table S3.** *p* values from statistics analysis performed on experiments shown in Figure 1B. Statistical differences were evaluated by one way ANOVA with Kruskal-Wallis and Dunn's multiple comparisons test where *p* < 0.05 is statistically significant.

Solutes	Condition	<i>p</i> Value		
		Day 5	Day 7	Day 11
Trehalose	No NaCl vs. 0.5 M NaCl	0.5172	0.0504	0.019
	No NaCl vs. 2.0 M NaCl	0.5172	0.0504	0.5172
	0.5 M NaCl vs. 2.0 M NaCl	0.019	>0.9999	0.5172
Mannitol	No NaCl vs. 0.5 M NaCl	0.5391	0.5172	0.5172
	No NaCl vs. 2.0 M NaCl	0.5391	0.019	0.019
	0.5 M NaCl vs. 2.0 M NaCl	0.0219	0.5172	0.5172
Arabitol	No NaCl vs. 0.5 M NaCl	0.5391	0.5172	0.5391
	No NaCl vs. 2.0 M NaCl	0.5391	0.5172	0.5391
	0.5 M NaCl vs. 2.0 M NaCl	0.0219	0.019	0.0219
Erythritol	No NaCl vs. 0.5 M NaCl	0.0219	0.5172	0.5172
	No NaCl vs. 2.0 M NaCl	0.5391	0.5172	0.5172
	0.5 M NaCl vs. 2.0 M NaCl	0.5391	0.019	0.019
Glycerol	No NaCl vs. 0.5 M NaCl	>0.9999	>0.9999	>0.9999
	No NaCl vs. 2.0 M NaCl	0.3062	0.0504	0.0504
	0.5 M NaCl vs. 2.0 M NaCl	0.0306	0.0504	0.0504



**Figure S3.** Reconstruction of MAPK phylogeny in selected *Aspergilli*, including Hog1, Hog2, and MpkC genes of *A. sydowii*.

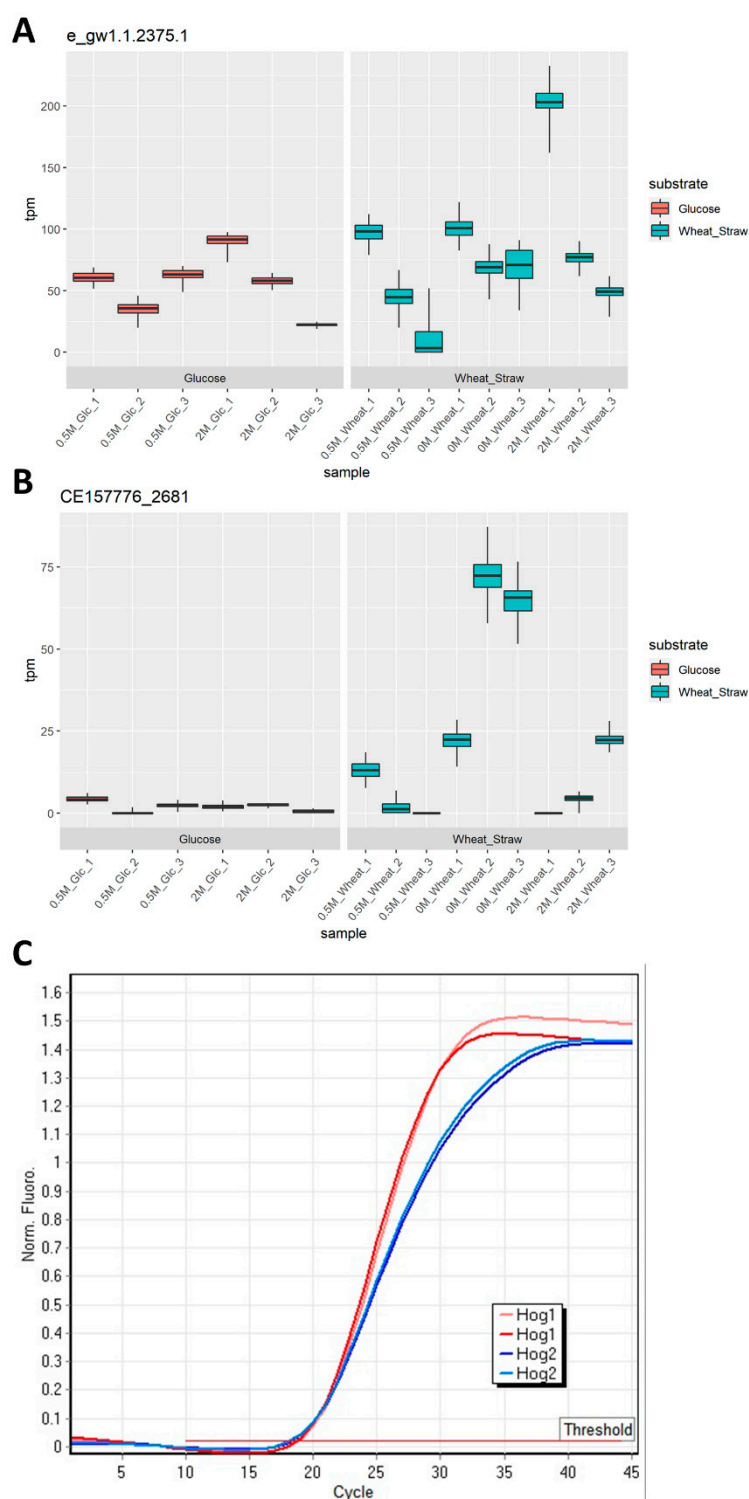
**Table S4.** Genes encoding compatible solute synthesis enzymes in *A. nidulans* and their homologue genes of in *A. sydowii*.

Gene	Enzyme	<i>A. sydowii</i> Gene ID *	Protein ID *	% Ident **
<b>Trehalose Pathway</b>				
<i>tpsA</i>	Trehalose phosphate syn- thase subunit	e_gw1.4.743.1	149182	95
<i>orlA</i>	Trehalose phosphate phosphatase subunit	fgenes1_pm.3_#_972	56871	85
<i>tps3</i>	TPS regulatory subunit	fgenes1_pm.7_#_385	59235	89
<i>stps</i>	Heat shock trehalose phosphate synthase	fgenes1_pm.10_#_111	60104	50
<i>ccg-9</i>	Trehalose phosphate syn- thase	CE565934_14846	780972	83
<i>tpg</i>	Trehalose-6-P phosphatase	gm1.12758_g	96075	87
<i>treb</i>	Neutral tre-halase	e_gw1.4.354.1	149568	90
<i>trea</i>	Acid tre-halase	fgenes1_pm.18_#_129	62558	84
<b>Mannitol Pathway</b>				
<i>mtld</i>	Mannitol-1-phosphate 5- dehydrogenase	e_gw1.5.2043.1	152275	86
<i>m2dh</i>	Mannitol 2-dehydrogenase	fgenes1_pg.3_#_1082	29920	84
<i>mpp</i>	Manitol/Hexitol phosphatase			
<i>hk</i>	Hexokinase			
<b>Glycerol Pathway</b>				
<i>gpd</i>	Glycerol-3-P dehydrogenase	fgenes1_kg.2_#_690_#_L ocus2578v1rpk69.02	40910	89
<i>gpd-m</i>	Glycerol-3-P dehydrogenase mitochondrial	fgenes1_pm.1_#_8	52965	79
<i>gpp</i>	Glycerol-3-P phosphatase	fgenes1_kg.1_#_238_#_L ocus2655v1rpk66.24	38002	91
<i>gut1</i>	Glycerol-kinase	e_gw1.4.2272.1	149605	92
<i>had1</i>	Halo-acid dehalogenase	gm1.5577_g	88894	83
<i>dak1</i>	Dihydroxyacetone kinase			
<i>gld1</i>	Glycerol dehydrogenase	CE68380_3389	283418	90
		fgenes1_pg.5_#_204	31279	86
		fgenes1_pm.11_#_239	60569	56
<b>Pentose phosphate pathway (Arabitol and Erythritol)</b>				
<i>tktA</i>	Transketolase	e_gw1.2.510.1	142846	95
<i>tktB</i>	Transketolase - putative	gm1.10640_g	93957	90
<i>tad</i>	Transaldolase	fgenes1_pm.2_#_386	55064	94
<i>larA / xylA</i>	L-arabinose/D-xylose reductase	e_gw1.2.1363.1	144547	87
<i>ardh</i>	L-arabinitol dehydrogenase	CE897698_12019	1112736	80

\* As in the sequenced genome in Mycocosm \*\* % Identity to *A. nidulans* homologue gene.

**Table S5.** *p* values obtained from statistics analysis performed on experiments shown in Figure 4. (*p* < 0.05 is statistically significant, one-way ANOVA and Dunnett's multiple comparison test).

Solutes	Condition	<i>p</i> Value					
		No NaCl- 0.5M NaCl	No NaCl- 2.0M NaCl	2.0M NaCl- No NaCl	2.0M NaCl- 0.5M NaCl	0.5 M NaCl- No NaCl	0.5 M NaCl- 2.0M NaCl
Trehalose	0 min vs. 10 min	0.9484	<0.0001	<0.0001	0.6028	0.9998	<0.0001
	0 min vs. 30 min	0.9929	<0.0001	<0.0001	0.3974	0.993	<0.0001
	0 min vs. 2 h	0.417	<0.0001	<0.0001	0.2554	0.7194	<0.0001
	0 min vs. 8 h	0.6234	<0.0001	<0.0001	0.1114	0.1675	<0.0001
	0 min vs. 24 h	0.397	<0.0001	<0.0001	0.237	<0.0001	<0.0001
	0 min vs. 48 h	0.8485	<0.0001	<0.0001	<0.0001	0.9925	<0.0001
Mannitol	0 min vs. 10 min	0.0045	<0.0001	0.0004	>0.9999	0.9997	<0.0001
	0 min vs. 30 min	<0.0001	<0.0001	<0.0001	>0.9999	0.9996	<0.0001
	0 min vs. 2 h	<0.0001	<0.0001	0.0021	>0.9999	<0.0001	<0.0001
	0 min vs. 8 h	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	0 min vs. 24 h	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
	0 min vs. 48 h	<0.0001	<0.0001	<0.0001	>0.9999	0.0002	<0.0001
Arabitol	0 min vs. 10 min	0.4791	<0.0001	<0.0001	>0.9999	0.7318	<0.0001
	0 min vs. 30 min	0.0148	<0.0001	0.47	>0.9999	0.9997	<0.0001
	0 min vs. 2 h	<0.0001	<0.0001	>0.9999	>0.9999	<0.0001	<0.0001
	0 min vs. 8 h	0.0052	<0.0001	0.0065	<0.0001	0.0327	<0.0001
	0 min vs. 24 h	0.8105	<0.0001	<0.0001	<0.0001	0.0857	<0.0001
	0 min vs. 48 h	<0.0001	<0.0001	<0.0001	>0.9999	0.2647	<0.0001
Erythritol	0 min vs. 10 min	0.1093	<0.0001	<0.0001	0.0001	0.7	<0.0001
	0 min vs. 30 min	0.0471	0.0354	<0.0001	<0.0001	0.9997	<0.0001
	0 min vs. 2 h	<0.0001	0.9979	<0.0001	<0.0001	0.9997	<0.0001
	0 min vs. 8 h	0.0039	<0.0001	<0.0001	0.3936	<0.0001	<0.0001
	0 min vs. 24 h	<0.0001	0.0149	0.0022	<0.0001	0.0001	<0.0001
	0 min vs. 48 h	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Glycerol	0 min vs. 10 min		>0.9999	<0.0001	<0.0001	>0.9999	>0.9999
	0 min vs. 30 min		>0.9999	<0.0001	<0.0001	>0.9999	>0.9999
	0 min vs. 2 h		>0.9999	<0.0001	<0.0001	>0.9999	<0.0001
	0 min vs. 8 h		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	0 min vs. 24 h		<0.0001	<0.0001	<0.0001	0.0005	<0.0001
	0 min vs. 48 h		<0.0001	<0.0001	<0.0001	0.0011	<0.0001



**Figure S4.** Expression levels of *hog1* and *hog2* genes. Expression of *hog1* (A) and *hog2* (B) genes measured by RNA-Scheme 1. Amplification curves examples of *hog1* and *hog2* (C); (red, C<sub>q</sub> = 18.8) and *Hog2* (blue, C<sub>q</sub> = 18.3) measured by qPCR in a pooled cDNA sample from all tested conditions (0 M, 0.5 M, and 2.0 M NaCl);.

**Table S6.** *p* values obtained in the statistics analysis performed on experiments shown in Figure 8.

Oxidative Markers	Condition	<i>p</i> Value		
		Day 5	Day 7	Day 11
SOD	No NaCl vs. 0.5 M NaCl	0.7352	0.1955	0.0518
	No NaCl vs. 2.0 M NaCl	0.0708	0.0108	0.1890
	0.5 M NaCl vs. 2.0 M NaCl	0.0425	0.0050	0.0186
GSH	No NaCl vs. 0.5 M NaCl	0.3008	0.9327	0.5803
	No NaCl vs. 2.0 M NaCl	0.1212	0.4840	0.1567
	0.5 M NaCl vs. 2.0 M NaCl	0.0288	0.5106	0.0429
MDA	No NaCl vs. 0.5 M NaCl	0.9049	0.0587	0.4844
	No NaCl vs. 2.0 M NaCl	0.3419	0.0029	0.9782
	0.5 M NaCl vs. 2.0 M NaCl	0.0504	0.5400	0.2067
PAOP	No NaCl vs. 0.5 M NaCl	N/A	N/A	N/A
	No NaCl vs. 2.0 M NaCl	N/A	N/A	N/A
	0.5 M NaCl vs. 2.0 M NaCl	N/A	N/A	N/A

**Table S7.** Statistics analysis performed on experiments shown in Figure 9.

Condition	SOD	GSH	HPO	<i>p</i> Value		
				Lipid Peroxidation	MDA	PAOP
No NaCl >>> 0.5 M NaCl						
0 min. vs. 30 min.	0.9638	0.9807	0.9989	0.9763	<0.0001	>0.999
0 min. vs. 2 h	0.1319	*0.0061	0.9989	0.3731	*0.0184	>0.9999
0 min. vs. 8 h	*0.0225	0.9261	0.9989	0.9763	0.1502	>0.9999
No NaCl >>> 2.0 M NaCl						
0 min. vs. 30 min.	*0.0317	0.9649	0.8895	0.4522	*0.0002	*0.0003
0 min. vs. 2 h	*0.034	0.9739	0.9986	0.9958	*0.0042	<0.000
0 min. vs. 8 h	<0.0001	0.8172	>0.9999	0.0898	0.9445	*0.0012
2.0 M NaCl >>> No NaCl						
0 min. vs. 30 min.	0.2782	*0.035	<0.0001	0.4448	<0.0001	0.8622
0 min. vs. 2 h	0.114	0.9903	*0.0016	*0.0003	0.2527	0.8622
0 min. vs. 8 h	*0.0038	<0.0001	*0.032	0.9593	>0.9999	0.4226
2.0 M NaCl >>> 0.5 M NaCl						
0 min. vs. 30 min.	0.499	*0.0005	<0.0001	0.9558	<0.0001	0.8519
0 min. vs. 2 h	0.8543	0.5544	*0.0002	*0.0021	0.9834	0.5478
0 min. vs. 8 h	*0.0001	*0.0016	0.8085	0.9996	0.9626	0.2454
0.5 M NaCl >>> No NaCl						
0 min. vs. 30 min.	*0.0002	0.7412	0.998	0.9782	<0.0001	>0.9999
0 min. vs. 2 h	*0.0026	0.9923	0.9859	0.4957	0.9605	0.9996
0 min. vs. 8 h	0.0835	0.6404	0.3535	*0.0373	*0.006	*0.0005
0.5 M NaCl >>> 2.0 M NaCl						
0 min. vs. 30 min.	0.9434	>0.9999	<0.0001	*0.0201	<0.0001	0.0128
0 min. vs. 2 h	0.9993	0.9996	*0.0015	0.8123	<0.0001	0.0672
0 min. vs. 8 h	0.9991	0.9797	<0.0001	0.0898	0.1462	0.1118

\* *p* < 0.05 is statistically significant using a two-way ANOVA and Dunnett's multiple comparison test.