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Title

Global Transcriptional and Metabolite Analysis of *Desulfovibrio vulgaris* Hildenborough Responses to Long-Term Exposure to Elevated NaCl

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ABSTRACT

The mechanisms of *Desulfovibrio vulgaris* Hildenborough responses to long-term NaCl exposure were studied by global transcriptional and metabolite analyses. The growth of *D. vulgaris* was inhibited by high salinity, and salt inhibition could be relieved by an addition of amino acids (e.g., glutamate, alanine) or yeast extract. Salt shock (sudden increase in salt concentration) and salt adaptation (inoculating cells in the medium containing high concentrations of salt) showed a significant difference in respective transcriptomes. Salt adaptation induced expression of genes involved in amino acid biosynthesis and transportation, electron transfer, hydrogen oxidation, and general stress responses (e.g., heat shock proteins, phage shock proteins, and oxidative stress response proteins). Genes involved in energy metabolism, cell motility, and phage structures were repressed. Genes involved in Na⁺/H⁺ transport, K⁺ uptake and transportation, and proline biosynthesis and transportation were not significantly affected. Metabolite assays and external addition of amino acids into the growth medium of *D. vulgaris* suggest that amino acids, such as glutamate and alanine may accumulate as osmoprotectants in *D. vulgaris*. A conceptual model is proposed to link our observed results to currently available knowledge for further understanding the mechanisms of adaptation of *D. vulgaris* to sodium chloride.

MATERIALS AND METHODS

Cell culture and treatment: *D. vulgaris* cells were grown at the LS4D medium with or without yeast extract. To test the effects of amino acids on *D. vulgaris* growth, yeast extract was removed. NaCl was added into the LS4D medium to make desired concentrations when the LS4D medium was made.

***D. vulgaris* oligonucleotide array:** 70mer oligonucleotide arrays that containing all ORFs were constructed as described (He et al., 2006).

Target preparation, labeling and array hybridization: Total cellular RNA was isolated and purified using TRIzolTM Reagent, and then labeled with Cy5 dye. Genomic DNA was isolated and purified from *D. vulgaris* as described previously (Zhou et al., 1996), and then labeled with Cy3 dye. The labeled RNA and genomic DNA were co-hybridized to the array at 45°C with 50% formamide for 16 hrs in the dark. Image and data analysis were the same as described previously (Chhabra et al., 2006; Mukhopadhyay et al., 2006).

Metabolite determination: *D. vulgaris* cells were grown at the LS4D without added NaCl (the control), or with 250 mM additional NaCl (the treatment). A total of 150 ml of samples were collected for extraction of metabolites. Metabolite assays were conducted with capillary electrophoresis (CE) and mass spectrometric (MS) under optimal conditions.

RESULTS

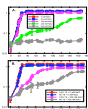


Fig. 1 Effects of yeast extract on the growth of *D. vulgaris* under NaCl stress

- With yeast extract, *D. vulgaris* growth was inhibited ~15% by 250 mM NaCl (A); without yeast extract, its growth was inhibited ~50% by 250 mM NaCl (B).
- The results suggest that yeast extract significantly affected the growth of *D. vulgaris* in the presence of NaCl, which may be because certain substances in yeast extract help *D. vulgaris* cells adapt to high salinity environments.

Transcriptomic analysis of *D. vulgaris* responses to long-term NaCl exposure

Table 4. Expression changes of representative genes of *D. vulgaris* involved in energy metabolism

Gene	Log2(R)	Zscore
DVU011	1.50	2.68
DVU012	1.50	2.68
DVU013	1.49	2.67
DVU014	1.48	2.66
DVU015	1.47	2.65
DVU016	1.46	2.64
DVU017	1.45	2.63
DVU018	1.44	2.62
DVU019	1.43	2.61
DVU020	1.42	2.60
DVU021	1.41	2.59
DVU022	1.40	2.58
DVU023	1.39	2.57
DVU024	1.38	2.56
DVU025	1.37	2.55
DVU026	1.36	2.54
DVU027	1.35	2.53
DVU028	1.34	2.52
DVU029	1.33	2.51
DVU030	1.32	2.50
DVU031	1.31	2.49
DVU032	1.30	2.48
DVU033	1.29	2.47
DVU034	1.28	2.46
DVU035	1.27	2.45
DVU036	1.26	2.44
DVU037	1.25	2.43
DVU038	1.24	2.42
DVU039	1.23	2.41
DVU040	1.22	2.40
DVU041	1.21	2.39
DVU042	1.20	2.38
DVU043	1.19	2.37
DVU044	1.18	2.36
DVU045	1.17	2.35
DVU046	1.16	2.34
DVU047	1.15	2.33
DVU048	1.14	2.32
DVU049	1.13	2.31
DVU050	1.12	2.30
DVU051	1.11	2.29
DVU052	1.10	2.28
DVU053	1.09	2.27
DVU054	1.08	2.26
DVU055	1.07	2.25
DVU056	1.06	2.24
DVU057	1.05	2.23
DVU058	1.04	2.22
DVU059	1.03	2.21
DVU060	1.02	2.20
DVU061	1.01	2.19
DVU062	1.00	2.18
DVU063	0.99	2.17
DVU064	0.98	2.16
DVU065	0.97	2.15
DVU066	0.96	2.14
DVU067	0.95	2.13
DVU068	0.94	2.12
DVU069	0.93	2.11
DVU070	0.92	2.10
DVU071	0.91	2.09
DVU072	0.90	2.08
DVU073	0.89	2.07
DVU074	0.88	2.06
DVU075	0.87	2.05
DVU076	0.86	2.04
DVU077	0.85	2.03
DVU078	0.84	2.02
DVU079	0.83	2.01
DVU080	0.82	2.00
DVU081	0.81	1.99
DVU082	0.80	1.98
DVU083	0.79	1.97
DVU084	0.78	1.96
DVU085	0.77	1.95
DVU086	0.76	1.94
DVU087	0.75	1.93
DVU088	0.74	1.92
DVU089	0.73	1.91
DVU090	0.72	1.90
DVU091	0.71	1.89
DVU092	0.70	1.88
DVU093	0.69	1.87
DVU094	0.68	1.86
DVU095	0.67	1.85
DVU096	0.66	1.84
DVU097	0.65	1.83
DVU098	0.64	1.82
DVU099	0.63	1.81
DVU100	0.62	1.80
DVU101	0.61	1.79
DVU102	0.60	1.78
DVU103	0.59	1.77
DVU104	0.58	1.76
DVU105	0.57	1.75
DVU106	0.56	1.74
DVU107	0.55	1.73
DVU108	0.54	1.72
DVU109	0.53	1.71
DVU110	0.52	1.70
DVU111	0.51	1.69
DVU112	0.50	1.68
DVU113	0.49	1.67
DVU114	0.48	1.66
DVU115	0.47	1.65
DVU116	0.46	1.64
DVU117	0.45	1.63
DVU118	0.44	1.62
DVU119	0.43	1.61
DVU120	0.42	1.60
DVU121	0.41	1.59
DVU122	0.40	1.58
DVU123	0.39	1.57
DVU124	0.38	1.56
DVU125	0.37	1.55
DVU126	0.36	1.54
DVU127	0.35	1.53
DVU128	0.34	1.52
DVU129	0.33	1.51
DVU130	0.32	1.50
DVU131	0.31	1.49
DVU132	0.30	1.48
DVU133	0.29	1.47
DVU134	0.28	1.46
DVU135	0.27	1.45
DVU136	0.26	1.44
DVU137	0.25	1.43
DVU138	0.24	1.42
DVU139	0.23	1.41
DVU140	0.22	1.40
DVU141	0.21	1.39
DVU142	0.20	1.38
DVU143	0.19	1.37
DVU144	0.18	1.36
DVU145	0.17	1.35
DVU146	0.16	1.34
DVU147	0.15	1.33
DVU148	0.14	1.32
DVU149	0.13	1.31
DVU150	0.12	1.30
DVU151	0.11	1.29
DVU152	0.10	1.28
DVU153	0.09	1.27
DVU154	0.08	1.26
DVU155	0.07	1.25
DVU156	0.06	1.24
DVU157	0.05	1.23
DVU158	0.04	1.22
DVU159	0.03	1.21
DVU160	0.02	1.20
DVU161	0.01	1.19
DVU162	0.00	1.18
DVU163	-0.01	1.17
DVU164	-0.02	1.16
DVU165	-0.03	1.15
DVU166	-0.04	1.14
DVU167	-0.05	1.13
DVU168	-0.06	1.12
DVU169	-0.07	1.11
DVU170	-0.08	1.10
DVU171	-0.09	1.09
DVU172	-0.10	1.08
DVU173	-0.11	1.07
DVU174	-0.12	1.06
DVU175	-0.13	1.05
DVU176	-0.14	1.04
DVU177	-0.15	1.03
DVU178	-0.16	1.02
DVU179	-0.17	1.01
DVU180	-0.18	1.00
DVU181	-0.19	0.99
DVU182	-0.20	0.98
DVU183	-0.21	0.97
DVU184	-0.22	0.96
DVU185	-0.23	0.95
DVU186	-0.24	0.94
DVU187	-0.25	0.93
DVU188	-0.26	0.92
DVU189	-0.27	0.91
DVU190	-0.28	0.90
DVU191	-0.29	0.89
DVU192	-0.30	0.88
DVU193	-0.31	0.87
DVU194	-0.32	0.86
DVU195	-0.33	0.85
DVU196	-0.34	0.84
DVU197	-0.35	0.83
DVU198	-0.36	0.82
DVU199	-0.37	0.81
DVU200	-0.38	0.80
DVU201	-0.39	0.79
DVU202	-0.40	0.78
DVU203	-0.41	0.77
DVU204	-0.42	0.76
DVU205	-0.43	0.75
DVU206	-0.44	0.74
DVU207	-0.45	0.73
DVU208	-0.46	0.72
DVU209	-0.47	0.71
DVU210	-0.48	0.70
DVU211	-0.49	0.69
DVU212	-0.50	0.68
DVU213	-0.51	0.67
DVU214	-0.52	0.66
DVU215	-0.53	0.65
DVU216	-0.54	0.64
DVU217	-0.55	0.63
DVU218	-0.56	0.62
DVU219	-0.57	0.61
DVU220	-0.58	0.60
DVU221	-0.59	0.59
DVU222	-0.60	0.58
DVU223	-0.61	0.57
DVU224	-0.62	0.56
DVU225	-0.63	0.55
DVU226	-0.64	0.54
DVU227	-0.65	0.53
DVU228	-0.66	0.52
DVU229	-0.67	0.51
DVU230	-0.68	0.50
DVU231	-0.69	0.49
DVU232	-0.70	0.48
DVU233	-0.71	0.47
DVU234	-0.72	0.46
DVU235	-0.73	0.45
DVU236	-0.74	0.44
DVU237	-0.75	0.43
DVU238	-0.76	0.42
DVU239	-0.77	0.41
DVU240	-0.78	0.40
DVU241	-0.79	0.39
DVU242	-0.80	0.38
DVU243	-0.81	0.37
DVU244	-0.82	0.36
DVU245	-0.83	0.35
DVU246	-0.84	0.34
DVU247	-0.85	0.33
DVU248	-0.86	0.32
DVU249	-0.87	0.31
DVU250	-0.88	0.30
DVU251	-0.89	0.29
DVU252	-0.90	0.28
DVU253	-0.91	0.27
DVU254	-0.92	0.26
DVU255	-0.93	0.25
DVU256	-0.94	0.24
DVU257	-0.95	0.23
DVU258	-0.96	0.22
DVU259	-0.97	0.21
DVU260	-0.98	0.20
DVU261	-0.99	0.19
DVU262	-1.00	0.18
DVU263	-1.01	0.17
DVU264	-1.02	0.16
DVU265	-1.03	0.15
DVU266	-1.04	0.14
DVU267	-1.05	0.13
DVU268	-1.06	0.12
DVU269	-1.07	0.11
DVU270	-1.08	0.10
DVU271	-1.09	0.09
DVU272	-1.10	0.08
DVU273	-1.11	0.07
DVU274	-1.12	0.06
DVU275	-1.13	0.05
DVU276	-1.14	0.04
DVU277	-1.15	0.03
DVU278	-1.16	0.02
DVU279	-1.17	0.01
DVU280	-1.18	0.00
DVU281	-1.19	-0.01
DVU282	-1.20	-0.02
DVU283	-1.21	-0.03
DVU284	-1.22	-0.04
DVU285	-1.23	-0.05
DVU286	-1.24	-0.06
DVU287	-1.25	-0.07
DVU288	-1.26	-0.08
DVU289	-1.27	-0.09
DVU290	-1.28	-0.10
DVU291	-1.29	-0.11
DVU292	-1.30	-0.12
DVU293	-1.31	-0.13
DVU294	-1.32	-0.14
DVU295	-1.33	-0.15
DVU296	-1.34	-0.16
DVU297	-1.35	-0.17
DVU298	-1.36	-0.18
DVU299	-1.37	-0.19
DVU300	-1.38	-0.20
DVU301	-1.39	-0.21
DVU302	-1.40	-0.22
DVU303	-1.41	-0.23
DVU304	-1.42	-0.24
DVU305	-1.43	-0.25
DVU306	-1.44	-0.26
DVU307	-1.45	-0.27
DVU308	-1.46	-0.28