### Proteomic Analysis Identifies Dysregulated Proteins in Butanol

### Tolerant Gram-positive Lactobacillus mucosae BR0713-33

#### Siging Liu<sup>1\*</sup>, Nasib Qureshi<sup>2</sup>, Kenneth Bischoff<sup>1\*\*</sup> and Costel C. Darie<sup>3\*</sup>

<sup>1</sup>Renewable Product Technology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, USA

<sup>2</sup>Bioenery Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, USA

<sup>3</sup>Biochemistry & Proteomics Group, Department of Chemistry & Biomolecular Science, Clarkson University, Potsdam, NY, 13699, USA

- \* Correspondence: Siqing.Liu@usda.gov, <a href="mailto:cdarie@clarkson.edu">cdarie@clarkson.edu</a>
- \*\* The authors dedicate this study to the memory of our colleague Dr. Kenneth Bischoff

#### Supplemental Material:

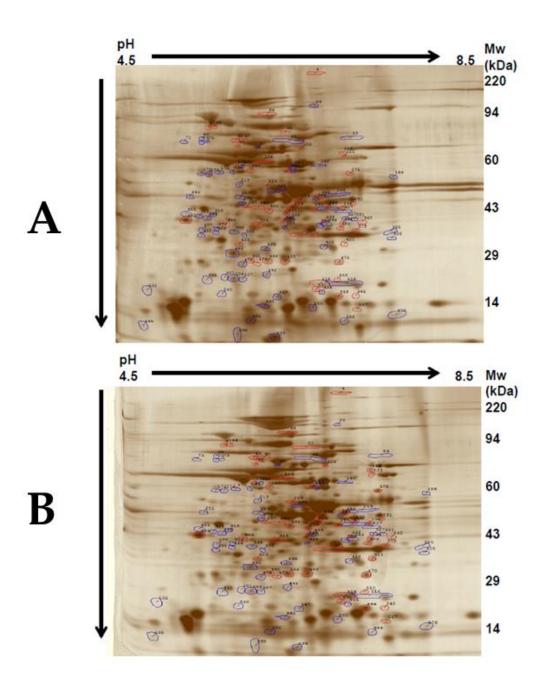
**Figure S1A.** 2D Gel Difference Image (2835 #3) of averaged 2% butanol (gels 2835 #3-4) versus averaged control (gels 2835 #1-2). Polypeptide spots increased in 2% butanol vs control are outlined in blue, while spots decreased in 2% butanol vs control are outlined in red. **Figure S1B**. 2D Gel Difference Image (2835 #2) of control to show spots increased in averaged control (gels 2835 #1-2) versus averaged 2% butanol (gels 2835 #3-4). Polypeptide spots increased in 2% butanol vs control are outlined in blue, while spots decreased in 2% butanol vs control are outlined in red.

**Figure S2A.** 2D Gel Difference Image (2835 #5) of averaged 3% butanol (gels 2835 #5-6) versus averaged control (gels 2835 #1-2). Polypeptide spots increased in 3% butanol vs control are outlined in blue, while spots decreased in 3% butanol vs control are outlined in red. **Figure S2B**. 2D Gel Difference Image (2835 #2) of control to show spots increased in averaged control (gels 2835 #1-2) (in red) versus averaged 3% butanol (gels 2835 #5-6). Polypeptide spots increased in 3% butanol vs control are outlined in blue, while spots decreased in 3% butanol vs control are outlined in red.

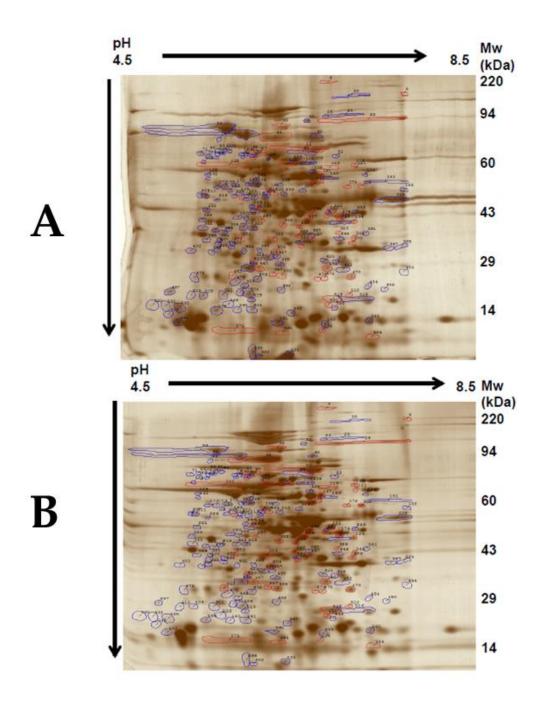
**Table S1.** 2D-gel computational image analyses of protein spots from *L. musosae* with increased expression in response to growth in media containing 2, 3 and 4% butanol versus 0% butanol (39 spots).

**Table S2.** 2D-gel computational image analyses of protein spots from *L. musosae* with decreased expression in response to growth in media containing 2, 3 and 4% butanol versus 0% butanol (14 spots).

# Figure S1



# Figure S2



## Table S1

2D-gel computational image analyses of protein spots from *L. musosae* with increased expression in response to growth in media containing 2, 3 and 4% butanol versus 0% butanol (39 spots).

			2% Butanol	2% Butanol	3% Butanol	3% Butanol	4% Butanol	4% Butanol	Montage
Spot			vs Control	Image					
#	PI	MW	Difference	T-test (p)	Difference	T-test (p)	Difference	T-test (p)	Page #
10	6.1	104,462	0.046	0.069	0.134	0.020	2.3	0.219	3.4
26	5.9	80,477	10.5	0.376	8.9	0.012	5.8	0.011	25
60	5.0	56,123	12.0	0.017	36.9	0.182	13.5	0.048	27
68	5.7	54,220	5.1	0.001	8.9	0.002	5.3	0.166	28
71	4.8	54,200	7.8	800.0	26.7	0.003	28.7	800.0	29
73	5.0	53,904	11.0	0.165	17.2	0.140	8.4	0.015	31
163	5.3	40,114	10.2	0.152	5.5	800.0	10.7	0.025	36
166	5.8	40,054	6.2	0.000	2.6	0.432	18.7	0.000	37
168	5.6	40,054	1.8	0.286	7.3	0.019	9.4	0.019	171
170	5.2	39,934	4.6	0.004	3.8	0.039	3.0	0.046	38
172	5.0	39,639	8.5	800.0	8.3	0.029	7.5	0.006	39
173	5.1	39,639	4.8	800.0	4.1	0.056	3.3	0.021	40
186	6.6	39,233	5.0	0.134	9.5	800.0	9.8	0.097	41
207	4.8	37,525	1.9	0.277	7.2	0.053	8.2	0.006	177
251	4.9	34,083	1.7	0.046	3.1	0.007	4.2	0.007	45
253	5.4	33,891	1.7	0.034	3.1	0.021	3.4	0.043	46
311	4.8	29,795	2.3	0.007	9.1	0.200	6.4	0.018	51
312	5.3	29,762	2.8	0.055	13.4	0.010	4.3	800.0	190
315	5.2	29,553	4.0	0.017	9.2	0.007	5.1	0.031	52
318	5.0	29,251	4.8	0.000	10.7	0.144	7.8	0.186	53
323	5.0	30,438	3.3	0.005	4.7	0.001	4.4	0.101	54
331	6.3	28,871	3.7	0.090	5.8	0.003	5.4	0.010	56
337	5.8	28,428	NC	0.045	10.9	0.522	36.1	0.079	192
372	5.2	29,356	6.2	0.002	9.0	0.121	8.3	0.212	61
385	6.6	26,994	3.3	0.246	6.2	0.001	7.6	0.323	64
386	5.2	28,657	5.5	0.032	14.6	0.005	10.8	0.002	65
387	6.5	26,699	2.4	0.065	3.8	0.003	5.2	0.001	195
395	5.0	26,248	1.9	0.044	5.6	0.031	5.0	0.024	66
435	5.6	24,281	3.4	0.065	3.9	0.044	3.6	0.173	70
451	6.2	23,681	2.3	0.113	1.8	0.270	11.2	0.015	274
497	4.3	20,345	1.6	0.555	5.9	0.052	3.6	0.033	210
501	5.2	20,133	3.5	0.077	10.5	0.018	8.9	0.013	74
511	5.0	19,758	3.2	0.012	11.1	0.074	12.1	0.151	77
516	6.2	19,374	10.4	0.014	6.9	0.029	5.4	0.036	78
536	6.0	18,005	3.4	0.016	4.2	0.010	4.5	0.020	80
540	5.2	17,637	3.9	0.156	4.0	0.086	4.1	0.014	81
548	4.3	16,972	1.4	0.450	4.0	0.091	4.6	0.047	219
598	5.3	5,630	5.2	0.125	11.1	0.016	7.4	0.224	89
599	5.6	4,507	5.7	0.001	5.7	0.022	8.6	0.006	90

## Table S2

2D-gel computational image analyses of protein spots from *L. musosae* with decreased expression in response to growth in media containing 2, 3 and 4% butanol versus 0% butanol (14 spots).

			2% Butanol	2% Butanol	3% Butanol	3% Butanol	4% Butanol	4% Butanol	Montage
Spot			vs Control	Image					
	PI	MW	Difference	T-test (p)	Difference	T-test (p)	Difference	T-test (p)	Page #
4	5.9	156,045	-2.7	0.037	-4.8	0.033	-3.2	0.033	91
33	5.6	73,721	-7.7	0.002	-10.3	0.001	-2.2	0.131	92
46	5.1	64,434	-5.3	0.023	-2.1	0.072	-3.9	0.021	93
65	5.3	54,649	-4.1	0.191	-4.5	0.183	-9.3	0.145	96
111	5.6	47,635	-1.5	0.059	-4.5	0.005	-20.8	0.002	227
113	6.1	48,027	-4.7	0.019	-19.5	0.014	-10.1	0.015	100
121	6.2	45,551	-6.7	0.035	-17.7	0.028	-27.7	0.105	101
175	6.2	39,835	-5.8	0.035	-26.2	0.017	-5.5	0.026	106
252	6.0	34,011	-4.7	0.012	-53.5	0.005	-9.1	0.007	108
270	5.9	32,754	-2.2	0.129	-3.9	0.043	-11.4	0.031	237
302	5.6	30,600	-5.0	0.003	-3.2	0.008	-4.1	0.007	117
462	5.2	22,618	-1.8	0.073	-3.8	0.022	-13.6	0.014	246
463	5.7	22,629	-1.9	0.022	-1.2	0.480	-7.1	0.003	132
466	5.6	22,592	-2.4	0.029	-4.5	0.065	-7.4	0.009	133