metabolites

MDPI

Supplementary Material

Metabolic changes of *Bacillus subtilis* strain 168 according to culture conditions in metabolite pathways including leucine metabolism

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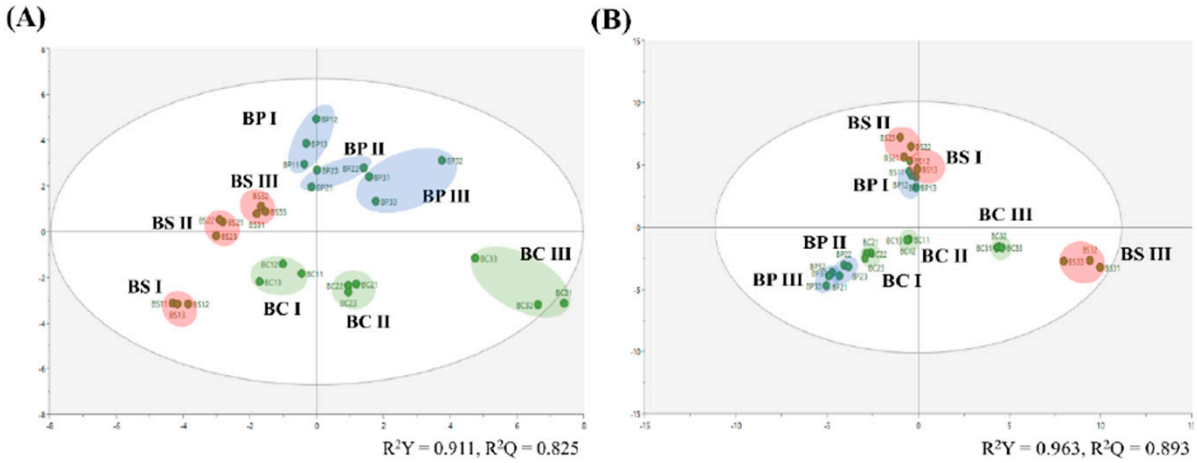

Figure S1. PCA score scatter plot of control (BC), alkaline pH (BP), and high salt concentration (BS) groups based on primary metabolite profiles (A) and secondary volatile metabolite profiles (B).

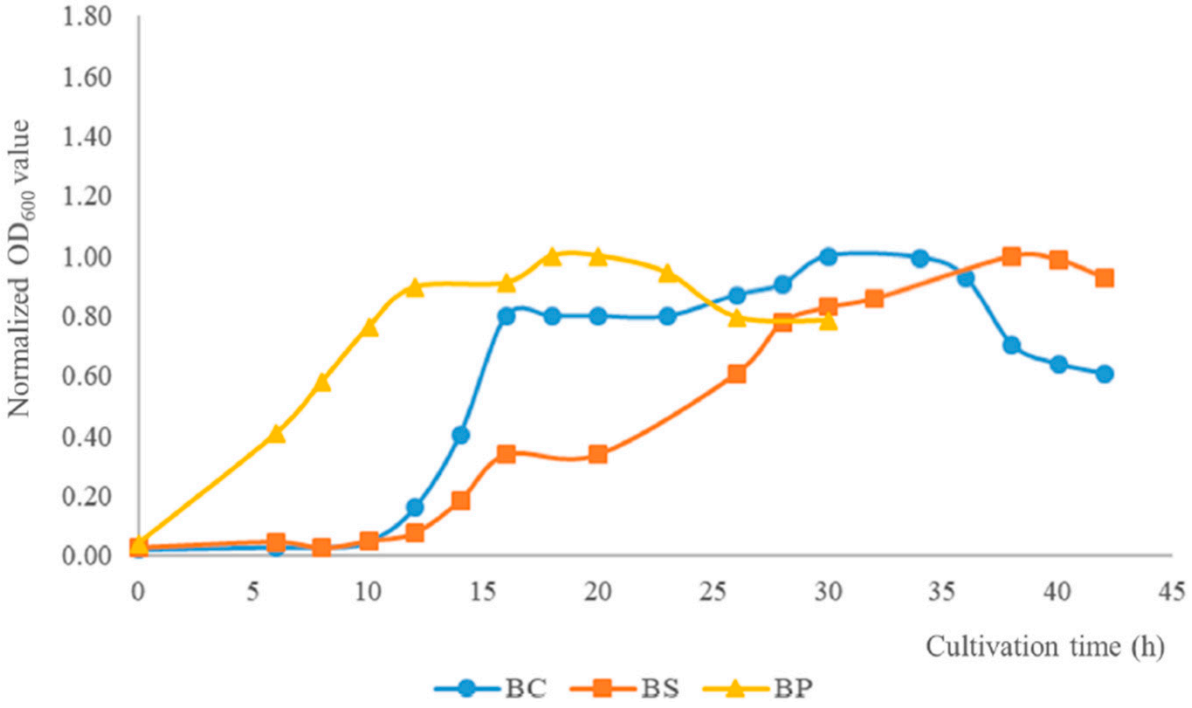

Figure S2. Growth curve of *B. subtilis* strain 168 cultivated under different culture conditions according to cultivation time (BC: control; BP: alkaline pH; BS: high salt concentration).

Table S1. Main variables contributing to discrimination between primary and secondary volatile metabolic profiles of *B. subtilis* fermentations.

No	Metabolite	VIP	Z score values									Possible related metabolism
			BC Ⅰ	BC Ⅱ	BC Ⅲ	BP Ⅰ	BP Ⅱ	BP Ⅲ	BS Ⅰ	BS Ⅱ	BS Ⅲ	
	BP-specific											
C2	Fructose	1.14	0.14	−0.36	−0.62	0.26	0.03	2.58	−0.6	−0.66	−0.76	Carbohydrate metabolism
C4	Galactose	1.36	0.53	0.31	−0.08	−0.69	−0.4	2.4	−0.69	−0.69	−0.69	Carbohydrate metabolism
A1	Alanine	1.07	−0.89	0.57	0.71	0.36	1.29	0.92	−1.65	−0.31	−1.01	Amino acid metabolism
A4	Isoleucine	1.16	−0.69	−0.44	0.46	0.08	0.79	2.12	−1.22	−0.7	−0.41	Amino acid metabolism
A14	Phenylalanine	1.36	−0.46	−0.76	0.48	−0.13	0.92	1.51	−1.4	−0.86	0.72	Amino acid metabolism
v48	Phenylmethanol	1.28	−0.6	−0.6	−0.6	0.13	1.56	1.93	−0.6	−0.6	−0.6	Amino acid metabolism
v12	3-Methylbutyl acetate	1.16	−0.52	−0.52	−0.52	1.88	1.78	−0.52	−0.52	−0.52	−0.52	Amino acid metabolism
v33	Undecan−2-one	1.17	−0.52	−0.52	−0.52	−0.52	2	1.64	−0.52	−0.52	−0.52	Fatty acid metabolism
v43	6-Methyloxan-2-one	1.16	−0.52	−0.52	−0.52	−0.52	1.88	1.74	−0.52	−0.52	−0.52	Fatty acid metabolism
v19	Nonan−2-one	1.15	−0.51	−0.51	−0.51	−0.51	1.94	1.66	−0.51	−0.51	−0.51	Fatty acid metabolism
v41	Undecan-2-ol	1.13	−0.5	−0.5	−0.5	−0.5	1.28	2.25	−0.5	−0.5	−0.5	Fatty acid metabolism
	BS-specific											
v37	Furan-2-ylmethanol	1.33	−0.86	−0.86	−0.86	−0.16	0.14	−0.86	0.81	0.73	1.93	Carbohydrate metabolism
v32	Cyclopent-4-ene-1,3-dione	1.13	−0.62	−0.62	−0.62	0.33	−0.62	−0.62	1.66	1.72	−0.62	Carbohydrate metabolism
v56	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	1.09	−0.79	−0.3	−0.79	−0.13	0.22	−0.79	1.88	1.48	−0.79	Carbohydrate metabolism
v55	4-Hydroxy-2,5-dimethylfuran-3-one	1.11	−0.67	−0.67	−0.67	0.95	−0.67	−0.67	1.75	1.34	−0.67	Carbohydrate metabolism
A5	Proline	1.16	−0.62	−0.02	−0.35	−0.62	−0.64	−0.57	−0.68	1.27	2.22	Amino acid metabolism
A15	Ornithine	1.07	0.61	−0.63	0.12	−0.76	−0.6	−0.74	−0.97	0.92	2.05	Amino acid metabolism
v35	1-Phenylethanone	1.07	−0.67	−0.67	−0.67	1.07	−0.67	−0.67	1.03	1.9	−0.67	Amino acid metabolism
v45	4-Methylpentanoicacid	1.15	−0.5	−0.5	−0.5	−0.5	−0.5	−0.5	1.22	2.28	−0.5	Fatty acid metabolism
v26	2-Ethylhexan−1-ol	1.11	−1.59	−0.31	0.23	0.56	−0.9	−0.93	1.45	0.64	0.85	Fatty acid metabolism
v34	Butanoic acid	1.00	0.05	−0.18	−1.05	0.01	−0.73	−0.56	−0.25	0.2	2.49	Fatty acid metabolism
v11	Butyl acetate	1.08	−0.6	−0.6	−0.6	−0.2	0.12	−0.6	−0.19	0.08	2.6	Fatty acid metabolism
v35	1-Phenylethanone	1.07	−0.67	−0.67	−0.67	1.07	−0.67	−0.67	1.03	1.9	−0.67	Amino acid metabolism
v45	4-Methylpentanoicacid	1.15	−0.5	−0.5	−0.5	−0.5	−0.5	−0.5	1.22	2.28	−0.5	Fatty acid metabolism
v26	2-Ethylhexan-1-ol	1.11	−1.59	−0.31	0.23	0.56	−0.9	−0.93	1.45	0.64	0.85	Fatty acid metabolism

v34	Butanoic acid	1.00	0.05	−0.18	−1.05	0.01	−0.73	−0.56	−0.25	0.2	2.49	Fatty acid metabolism
v11	Butyl acetate	1.08	−0.6	−0.6	−0.6	−0.2	0.12	−0.6	−0.19	0.08	2.6	Fatty acid metabolism

BC: control sample.

BP: cultivation under alkaline pH (pH 8).

BS: cultivation under high salt concentration (1 M salt concentration).

Table S2. Metabolites contributing to significant metabolic changes in metabolic pathways of *B. subtilis.*

Metabolite	Relative contents (average)			Fold change		Metabolite	Relative contents (average)			Fold change	
	BC	BP	BS	BP	BS		BC	BP	BS	BP	BS
Primary metabolites						***Secondary volatile metabolites***					
Leucine	1.399	8.985	1.854	0.81	0.12	Acetaldehyde	14.466	1.682	24.483	−0.93	0.23
Proline	1.873	0.819	14.299	−0.36	0.88	Propan-2-one	2.48	0.614	2.913	−0.61	0.07
Methionine	0.001	0.043	0.008	1.63	0.92	Ethyl 3-methylbutanoate	11.41	2.502	10.012	−0.66	−0.06
Aspartic acid	0.581	0.122	0.121	−0.68	−0.68	Butyl acetate	NDa	ND	5.79	UCb	NeDc
Ornithine	0.113	0.024	0.314	−0.67	0.44	Methyl acetate	ND	0.318	ND	NeD	UC
Glutamine	5.402	2.855	0.695	−0.28	−0.89	Nonan-2-one	ND	1.061	ND	NeD	UC
Glutamic acid	5.405	2.853	0.685	−0.28	−0.9	2,3,5-Trimethylpyrazine	ND	ND	0.163	UC	NeD
Citrulline	0.035	0.001	0.001	−1.54	−1.54	Decan-2-one	ND	0.567	ND	NeD	UC
Cadaverine	0.025	0.001	0.001	−1.4	−1.4	4-Methyl-2-oxovaleric acid	2.391	0.288	1.478	−0.92	−0.21
Lactic acid	2.059	0.513	0.978	−0.6	−0.32	Furan-2-carbaldehyde	0.112	0.125	0.571	0.05	0.71
Fumaric acid	0.103	0.176	0.524	0.23	0.71	2,3,5,6-Tetramethyl-pyrazine	0.751	ND	ND	ND	ND
Phosphoric acid	0.063	0.042	0.001	−0.18	−1.8	3-Methyl-1H-pyrrole	ND	ND	0.738	UC	NeD
Malic acid	0.042	0.584	0.111	1.15	0.42	Ethyl acetate	1.491	1.132	6.48	−0.12	0.64
Succinic acid	1.464	1.691	5.184	0.21	1.82	Propanoic acid	ND	0.429	ND	NeD	UC
Fructose	0.074	1.364	0.013	1.27	−0.74	Undecan-2-one	ND	0.52	ND	NeD	UC
Galactose	0.046	0.694	0.007	1.18	−0.84	Butanoic acid	ND	0.574	4.161	NeD	NeD
Glucose	0.013	0.067	0.001	0.7	−1.12	Dodecan-2-one	ND	1.745	ND	NeD	UC
Maltose	0.019	0.053	0.119	0.45	0.81	Tridecan-2-ol	ND	1.053	ND	NeD	NC
Sucrose	0.053	0.019	0.119	−1.48	1.17	4-Methylpentan-2-one	ND	0.151	ND	NeD	NC
Secondary volatile metabolites				***Secondary volatile metabolites***					***Secondary volatile metabolites***		
Furan-2-ylmethanol	ND	ND	0.183	NC	NeD	Tetradecan-2-ol	ND	3.328	Furan-2-ylmethanol	ND	ND
But-2-enoic acid	ND	0.218	ND	NeD	NC	2-(2-hydroxyethoxy)-Ethanol	3.507	ND	But-2-enoic acid	ND	0.218
Undecan-2-ol	ND	0.412	ND	NeD	NC	Phenol	2.326	0.405	Undecan-2-ol	ND	0.412
Ethyl 2-phenylacetate	ND	1.017	1.287	NeD	NeD	(2E,6E)-3,7,11-Trimethyl-dodeca-2,6,10-trien-1-ol	ND	1.081	Ethyl 2-phenylacetate	ND	1.017
6-Methyloxan-2-one	ND	0.17	ND	NeD	NC	Octadecanoic acid	ND	4.81	6-Methyloxan-2-one	ND	0.17
Tridecan-2-one	ND	1.041	ND	NeD	NC	Tetradecanoic acid	2.475	ND	Tridecan-2-one	ND	1.041

Tetradecan-2-one	ND	2.173	ND	NeD	NC	Ethyl 2-methylpropanoate	13.776	2.105	Tetradecan-2-one	ND	2.173
Phenylmethanol	ND	1.431	ND	NeD	NC	Hexadecanoic acid	25.485	14.58	Phenylmethanol	ND	1.431
2-Phenylethanol	2.215	0.321	2.25	−0.84	0.01	Butane-2,3-dione	23.422	2.455	2-Phenylethanol	2.215	0.321
						4-Methylpentan-2-one	ND	0.151			
Secondary volatile metabolites				***Secondary volatile metabolites***					***Secondary volatile metabolites***		
Furan-2-ylmethanol	ND	ND	0.183	NC	NeD	Tetradecan-2-ol	ND	3.328	Furan-2-ylmethanol	ND	ND
But-2-enoic acid	ND	0.218	ND	NeD	NC	2-(2-hydroxyethoxy)-Ethanol	3.507	ND	But-2-enoic acid	ND	0.218
Undecan-2-ol	ND	0.412	ND	NeD	NC	Phenol	2.326	0.405	Undecan-2-ol	ND	0.412
Ethyl 2-phenylacetate	ND	1.017	1.287	NeD	NeD	(2E,6E)-3,7,11-Trimethyl-dodeca-2,6,10-trien-1-ol	ND	1.081	Ethyl 2-phenylacetate	ND	1.017
6-Methyloxan-2-one	ND	0.17	ND	NeD	NC	Octadecanoic acid	ND	4.81	6-Methyloxan-2-one	ND	0.17
Tridecan-2-one	ND	1.041	ND	NeD	NC	Tetradecanoic acid	2.475	ND	Tridecan-2-one	ND	1.041
Tetradecan-2-one	ND	2.173	ND	NeD	NC	Ethyl 2-methylpropanoate	13.776	2.105	Tetradecan-2-one	ND	2.173
Phenylmethanol	ND	1.431	ND	NeD	NC	Hexadecanoic acid	25.485	14.58	Phenylmethanol	ND	1.431
2-Phenylethanol	2.215	0.321	2.25	−0.84	0.01	Butane-2,3-dione	23.422	2.455	2-Phenylethanol	2.215	0.321
						4-Methylpentan-2-one	ND	0.151			
Secondary volatile metabolites				***Secondary volatile metabolites***					***Secondary volatile metabolites***		
Furan-2-ylmethanol	ND	ND	0.183	NC	NeD	Tetradecan-2-ol	ND	3.328	Furan-2-ylmethanol	ND	ND
But-2-enoic acid	ND	0.218	ND	NeD	NC	2-(2-hydroxyethoxy)-Ethanol	3.507	ND	But-2-enoic acid	ND	0.218
Undecan-2-ol	ND	0.412	ND	NeD	NC	Phenol	2.326	0.405	Undecan-2-ol	ND	0.412
Ethyl 2-phenylacetate	ND	1.017	1.287	NeD	NeD	(2E,6E)-3,7,11-Trimethyl-dodeca-2,6,10-trien-1-ol	ND	1.081	Ethyl 2-phenylacetate	ND	1.017
6-Methyloxan-2-one	ND	0.17	ND	NeD	NC	Octadecanoic acid	ND	4.81	6-Methyloxan-2-one	ND	0.17
Tridecan-2-one	ND	1.041	ND	NeD	NC	Tetradecanoic acid	2.475	ND	Tridecan-2-one	ND	1.041
Tetradecan-2-one	ND	2.173	ND	NeD	NC	Ethyl 2-methylpropanoate	13.776	2.105	Tetradecan-2-one	ND	2.173

Phenylmethanol	ND	1.431	ND	NeD	NC	Hexadecanoic acid	25.485	14.58	Phenylmethanol	ND	1.431
2-Phenylethanol	2.215	0.321	2.25	−0.84	0.01	Butane-2,3-dione	23.422	2.455	2-Phenylethanol	2.215	0.321

(BC: control; BP: alkaline pH; BS: high salt concentration).

a Not detected.

b Unchanged metabolite compared with BC (control).

c Newly detected metabolite compared with BC.

Table S3. The changes of metabolites derived from leucine according to culture conditions, such as alkaline pH and temperature.

Metabolite	**Z-score**								
	BC Ⅰ	**BC Ⅱ**	**BC Ⅲ**	**BP Ⅰ**	**BP Ⅱ**	**BP Ⅲ**	**BS Ⅰ**	**BS Ⅱ**	**BS Ⅲ**
Time-dependent									
Leucine	−0.61	−0.72	1.32	1.24	−0.71	−0.53	−1.32	0.69	0.62
Ketoisocaproate	−0.81	−0.51	1.31	−1.32	0.72	0.60	−0.92	−0.29	1.21
3-Methylbutanal	−0.72	−0.23	0.95	−0.28	1.21	−0.93	−0.71	−0.55	1.26
3-Methylbutanol	0.14	1.07	−1.2	−0.93	1.11	−0.18	−0.66	0.81	−0.15
3-Methylbutanoic acid	−0.66	1.28	−0.62	−1.27	0.50	0.77	−1.26	0.51	0.75
Ethyl 3-methylbutanoate	−1.24	0.94	0.31	−1.27	0.29	0.97	−0.81	−0.49	1.30
3-Methylbutylacetate	0.00	0.00	0.00	0.70	0.62	−1.32	0.00	0.00	0.00
Conditions-dependent									
Leucine	−0.40	−0.56	2.56	0.56	−0.52	−0.42	−0.77	−0.22	−0.24
Ketoisocaproate	−0.47	−0.08	2.27	−1.05	−0.63	−0.65	−0.4	0.01	1.00
3-Methylbutanal	−0.20	0.00	0.48	−0.97	−0.29	−1.26	−0.1	0.09	2.26
3-Methylbutanol	0.52	1.72	−1.21	−1.29	0.04	−0.81	−0.05	0.83	0.25
3-Methylbutanoic acid	−0.48	1.02	−0.45	−0.86	1.29	1.61	−1.11	−0.55	−0.48
Ethyl 3-methylbutanoate	0.39	1.57	1.23	−1.03	−0.73	−0.6	−1.03	−0.73	0.94
3-Methylbutylacetate	−0.52	−0.52	−0.52	1.88	1.78	−0.52	−0.52	−0.52	−0.52

(BC: control; BP: alkaline pH; BS: high salt concentration).