

Supplementary information

***Bacillus* Classification Based on Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry—Effects of Culture Conditions**

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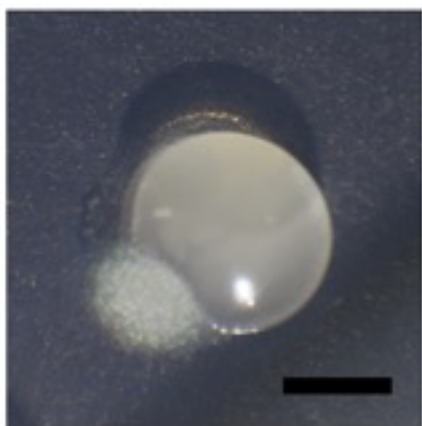
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Table of Contents

Figure S1	The colony morphology of <i>B. vallismortis</i> SFY-3S and <i>B. vallismortis</i> SFY-3W.	3
Table S1	The proportion of <i>Bacillus</i> endospore cell formation (vegetative cells/total cells) after 12, 24, 48, and 72 hours post incubation.	4
Figure S2	The micrographs of (a) <i>B. marisflavi</i> DSM 16204 and (b) <i>B. vallismortis</i> DSM 11031 incubated for 12 hours, 24 hours, 48 hours and 72 hours stained by Schaeffer-Fulton spore staining.	5
Figure S3	MSP dendrograms and spectral gel view of (a) <i>B. cereus</i> and (b) <i>B. subtilis</i> group species cultured for 12, 24, 48, 72 hours.	6
Figure S4	Three- and two-dimensional principal component analysis plots of <i>B. cereus</i> group, <i>B. subtilis</i> group, <i>B. megaterium</i> , <i>B. marisflavi</i> and other <i>Bacillus</i> species cultured for 12, 24, 48, 72 hours.	7
Figure S5	MSP dendrograms and gel-views of (a) <i>B. cereus</i> DSM 31 (b) <i>B. subtilis</i> DSM10 cultured for 12, 24, 48, 72 hours.	8
Figure S6	Biotyper analysis of <i>Bacillus</i> with different culture media.	9-12

(a)



(b)

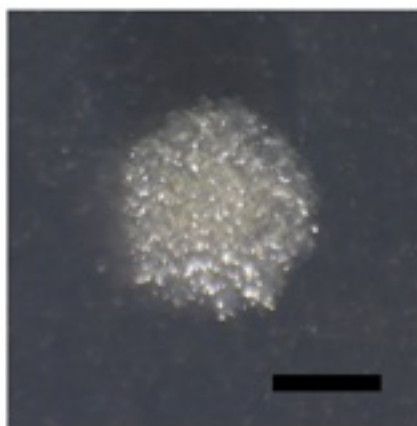


Figure S1. The colony morphology of (a) *B. vallismortis* SFY-3S and (b) *B. vallismortis* SFY-3W at 30°C for 24 hours on LB agar. Scale bars represent 2 mm.

Table S1: The proportion of *Bacillus* endospore cell formation (vegetative cells/total cells) after 12, 24, 48, and 72 hours post incubation.

Species	Endospore Fractions (%)			
	12-hour	24-hour	48-hour	72-hour
<i>B. thuringiensis</i> DSM 2046	0	0	0	0
<i>B. mycooides</i> DSM 2048	0	0	0	0
<i>B. subtilis</i> subsp. <i>subtilis</i> DSM 10	0	0.47	0.63	0.61
<i>B. amyloliquefaciens</i> DSM 7	0	0	10.00	41.33
<i>B. cereus</i> DSM 31	0	0	0	0.33
<i>B. vallismortis</i> DSM 11031	*	*	*	*
<i>B. marisflavi</i> DSM 16204	*	*	*	*
<i>B. cereus</i> 31	0	0.50	0.36	0.17
<i>B. cereus</i> 17	0	0	0.01	0.05
<i>B. cereus</i> 16	0	0	0	0.01
<i>B. thuringiensis</i> subsp. <i>kurstaki</i> ABTS-351	0	0.01	0.50	0.11
<i>B. thuringiensis</i> subsp. <i>aizawai</i> NB-200	0.1	0.01	4.40	5.14
<i>B. mycooides</i> sg5CY	0	0	0	0
<i>B. subtilis</i> 376	0	5	6.67	11.88
<i>B. subtilis</i> No30	0	0	0.16	0.14
<i>B. subtilis</i> fgu5AM	0	0.01	10.00	35.71
<i>B. vallismortis</i> SFY-3W	0	0.01	0.29	5.29
<i>B. vallismortis</i> SFY-3S	0	0.17	10.61	37.5
<i>B. amyloliquefaciens</i> 35	0	0.04	3.44	59.72
<i>Bacillus</i> sp. CRLEF-1A	0	1	7.5	78.05
<i>Bacillus</i> sp. CRLEF-1B	0	1.15	67.14	65.15
<i>Bacillus</i> sp. CRLEF-1C	0	0	33.33	97.96
<i>Bacillus</i> sp. BPRB37-A	0	0.13	11.67	89.47
<i>Bacillus</i> sp. BPRB37-C	0.07	0.91	6.07	95.56
<i>B. licheniformis</i> sp5BS	0	0	0.21	1.69
<i>B. megaterium</i> No56	0	0	0	0
<i>B. megaterium</i> 42	0	0	0	0
<i>Bacillus</i> sp. fgu5FY	0	0	0.01	0.03
<i>B. safensis</i> SC	0.08	2.96	0.02	0.23
<i>Bacillus</i> sp. UOY-1	0	0	0	0

* *B. vallismortis* DSM 11031 and *B. marisflavi* DSM 16204 did not present a fit to this endospore measurement method, substituted by light microscopic observation (Figure S2).

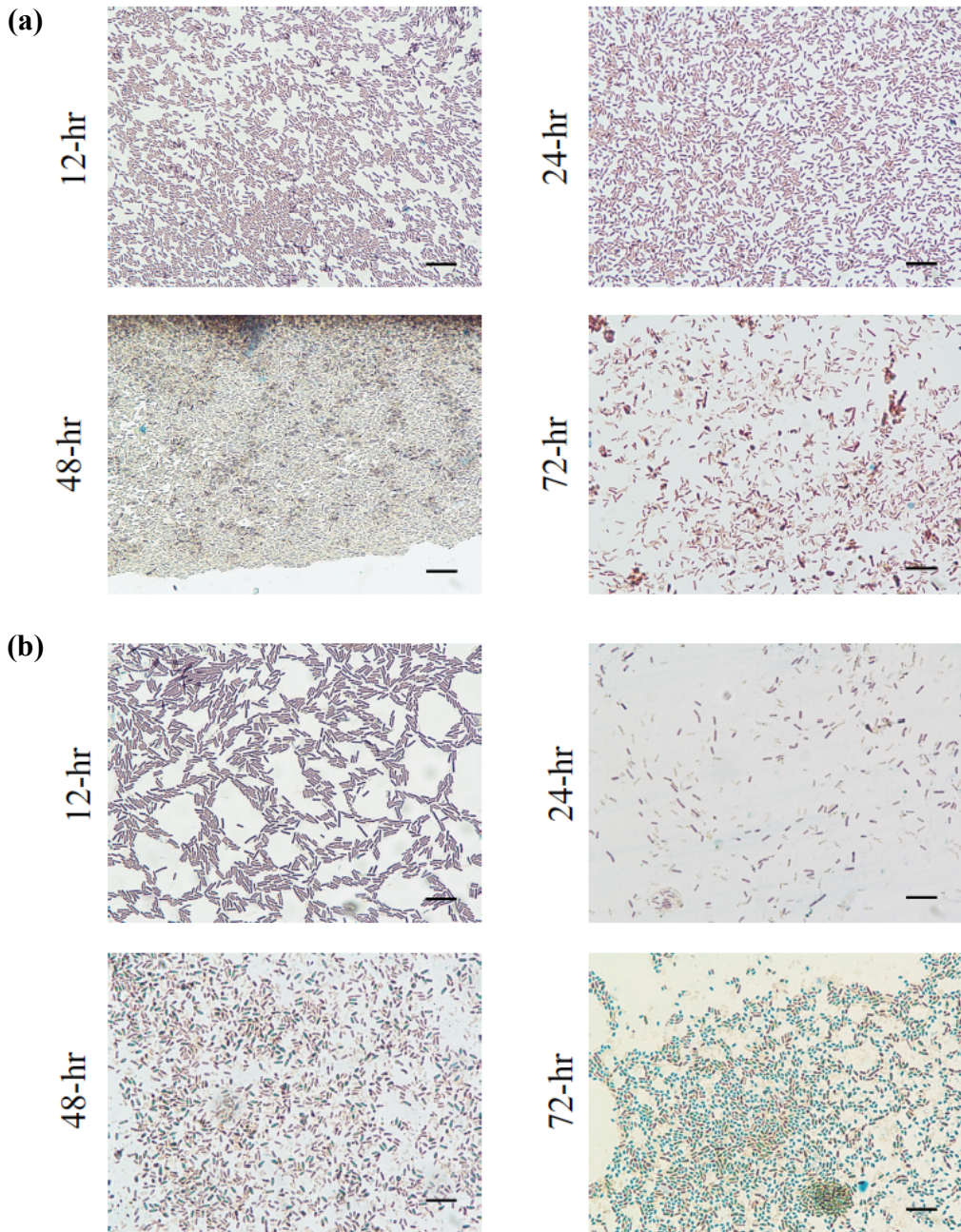


Figure S2. The micrographs of (a) *B. marisflavi* DSM 16204 and (b) *B. vallismortis* DSM 11031 incubated for 12 hours, 24 hours, 48 hours and 72 hours stained by Schaeffer-Fulton spore staining. They were cultured at 30°C on LB agar, and the samples were examined by Eclipse Ci-L microscope (Nikon) with eyepiece lens 10X, objective oil len 100X and software NIS Element D 4.5 (Nikon). Scale bars represent 10 μm .

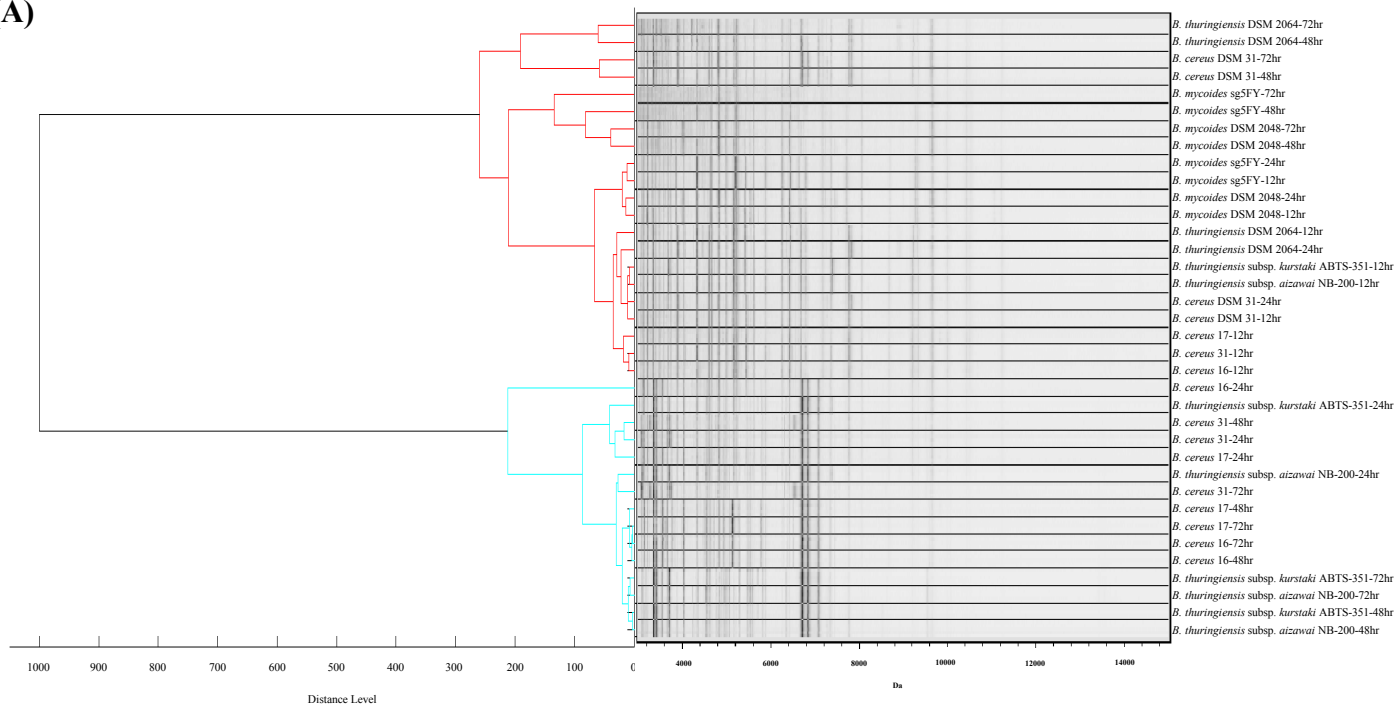
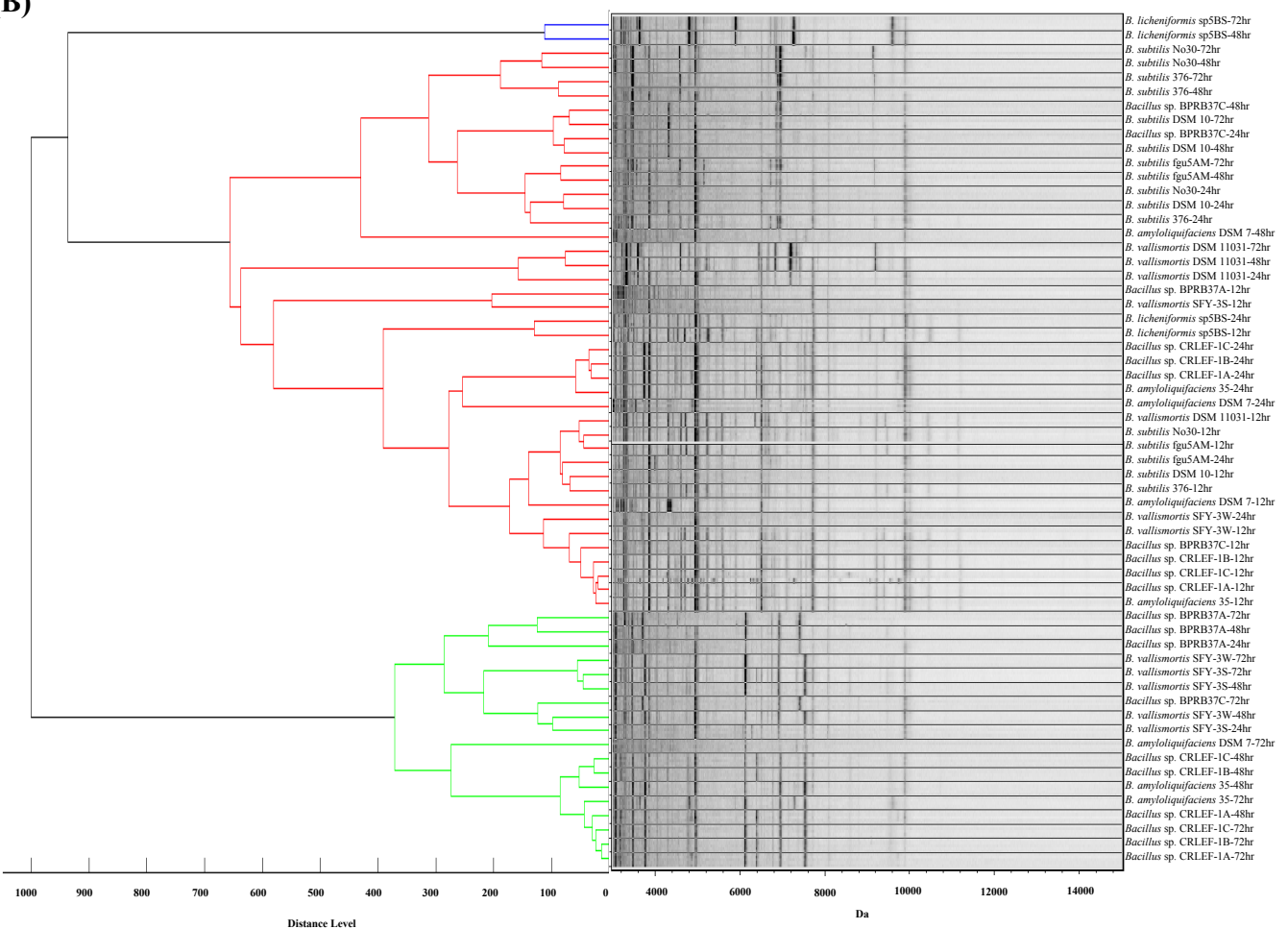
(A)**(B)**

Figure S3. MSP dendrograms and spectral gel view of (a) *B. cereus* and (b) *B. subtilis* group species cultured for 12, 24, 48, 72 hours. Numbers behind the species and strain name indicate growth time.

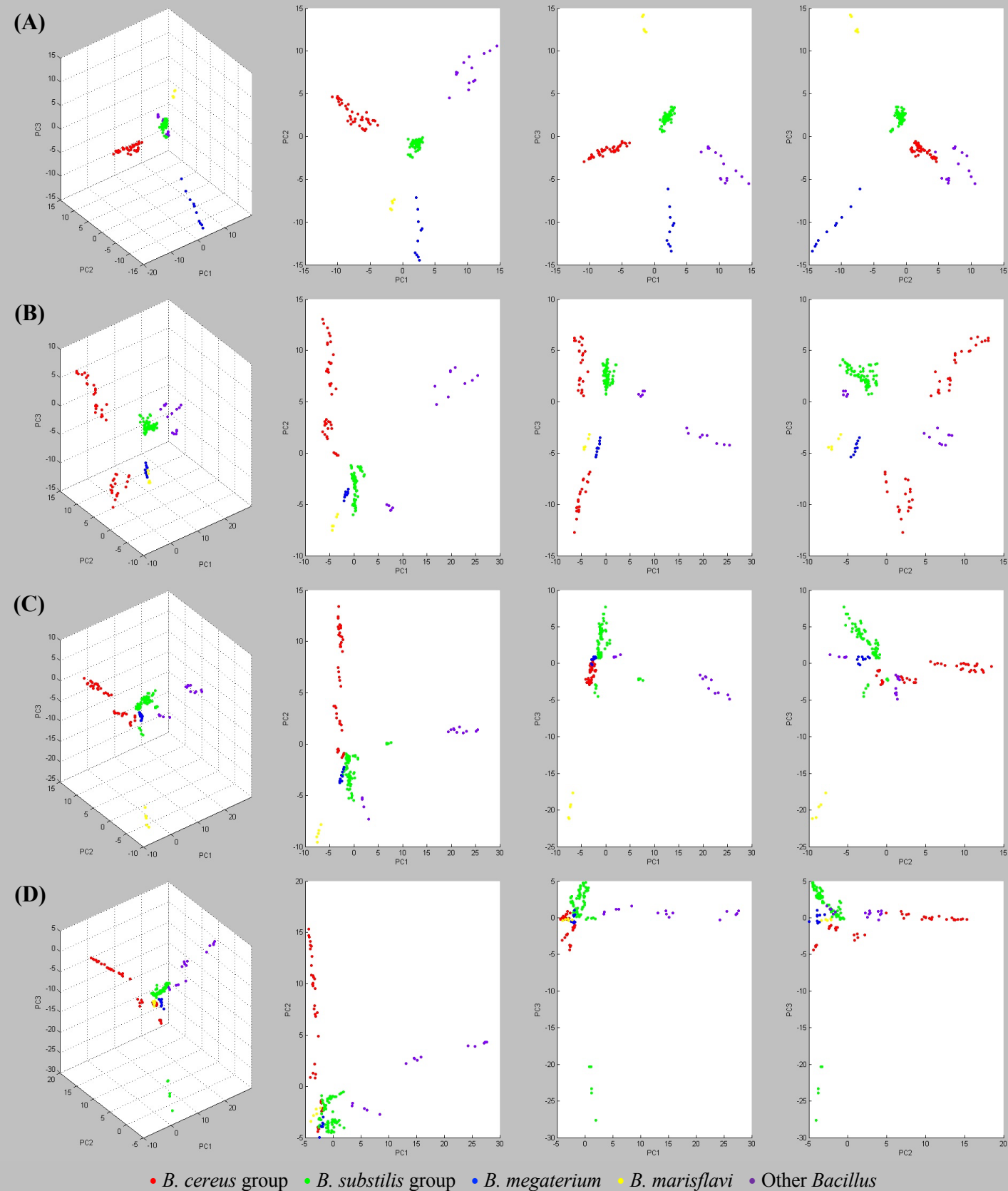


Figure S4. Three- and two-dimensional principal component analysis plots of *B. cereus* group, *B. subtilis* group, *B. megaterium*, *B. marisflavi* and other *Bacillus* species cultured for (A) 12 hours, (B) 24 hours, (C) 48 hours, and (D) 72 hours. The spot presents one spectrum and each *Bacillus* isolate includes five spectra. The plots were generated by first three principal components: PC1, PC2, and PC3.

Endospore proportion

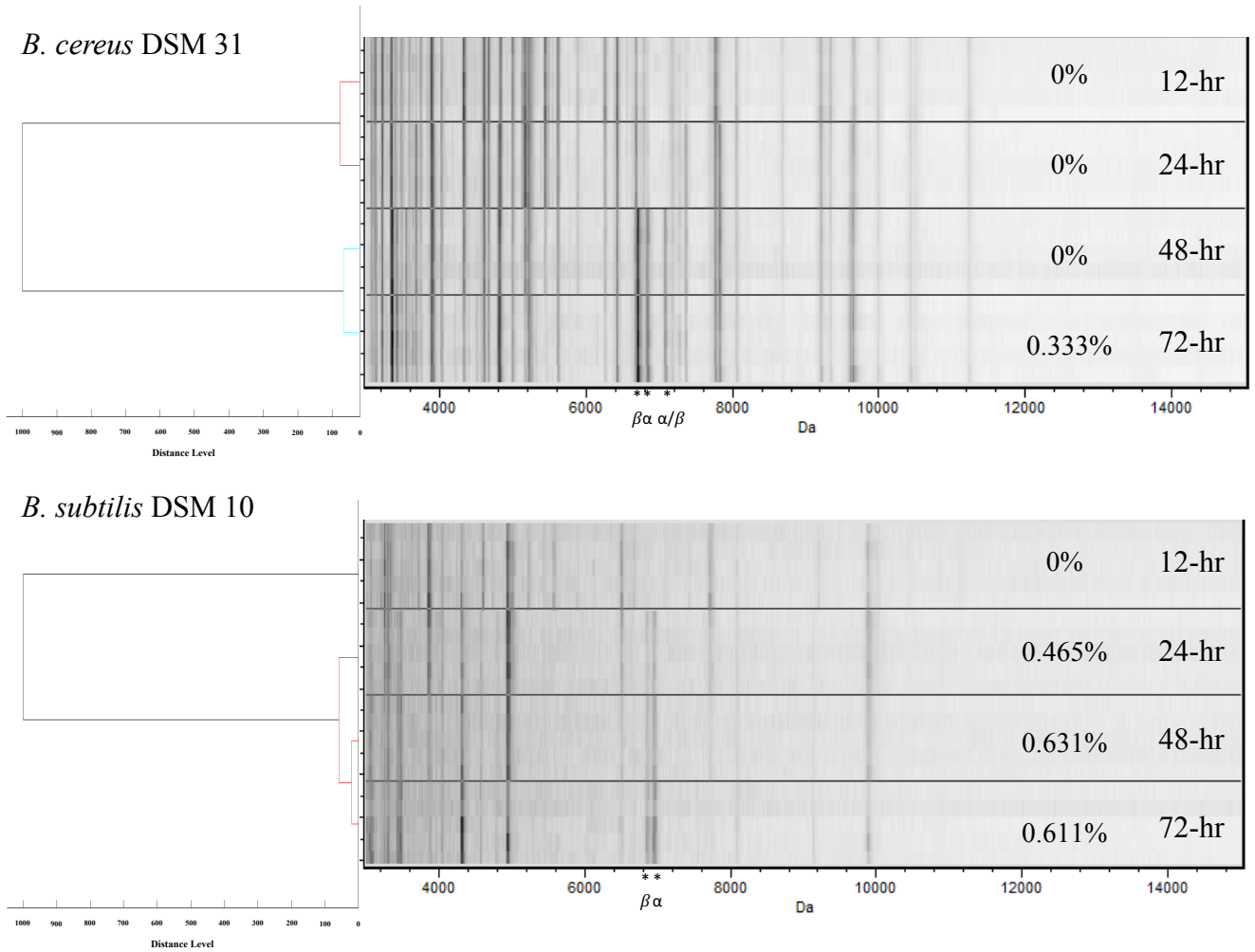


Figure S5. MSP dendrograms and gel-views of (a) *B. cereus* DSM 31 (b) *B. subtilis* DSM10 cultured for 12, 24, 48, 72 hours. The peaks of small acid-soluble proteins (SASPs) are indicated by asterisks. α , β , and α/β indicate the α -SASP, β -SASP, and α/β -SASP, respectively. Endospore proportions of samples are showed on the gel view, according to Table S1.

(a)

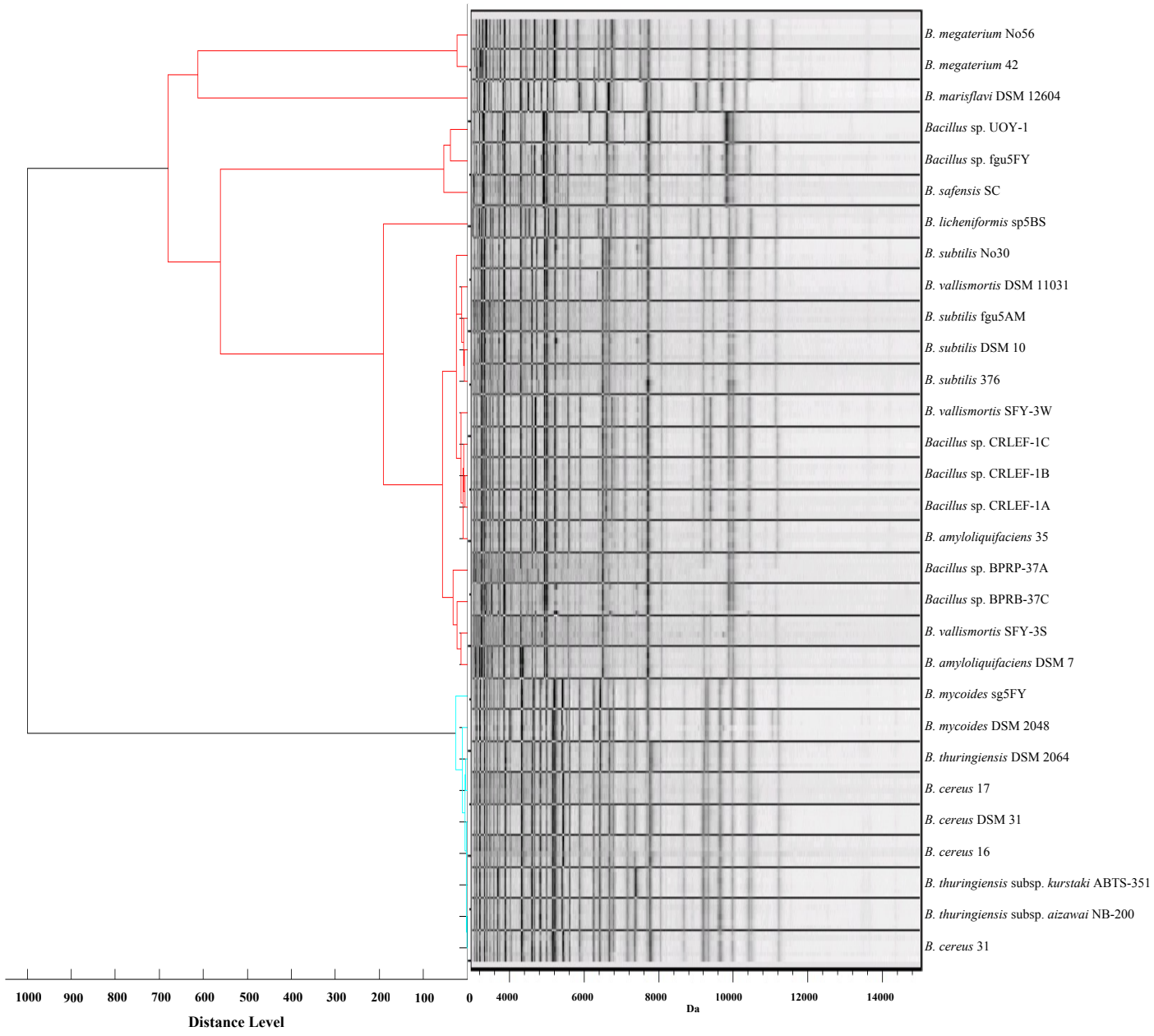


Figure S6. Biotyper analysis of *Bacillus* with different culture media. MSP dendrograms and spectral gel views of *Bacillus* cultured on (a) LB agar, (b) NA, (c) BHI agar and (d) PCA at 30°C for 12 hours. (Continued)

(b)

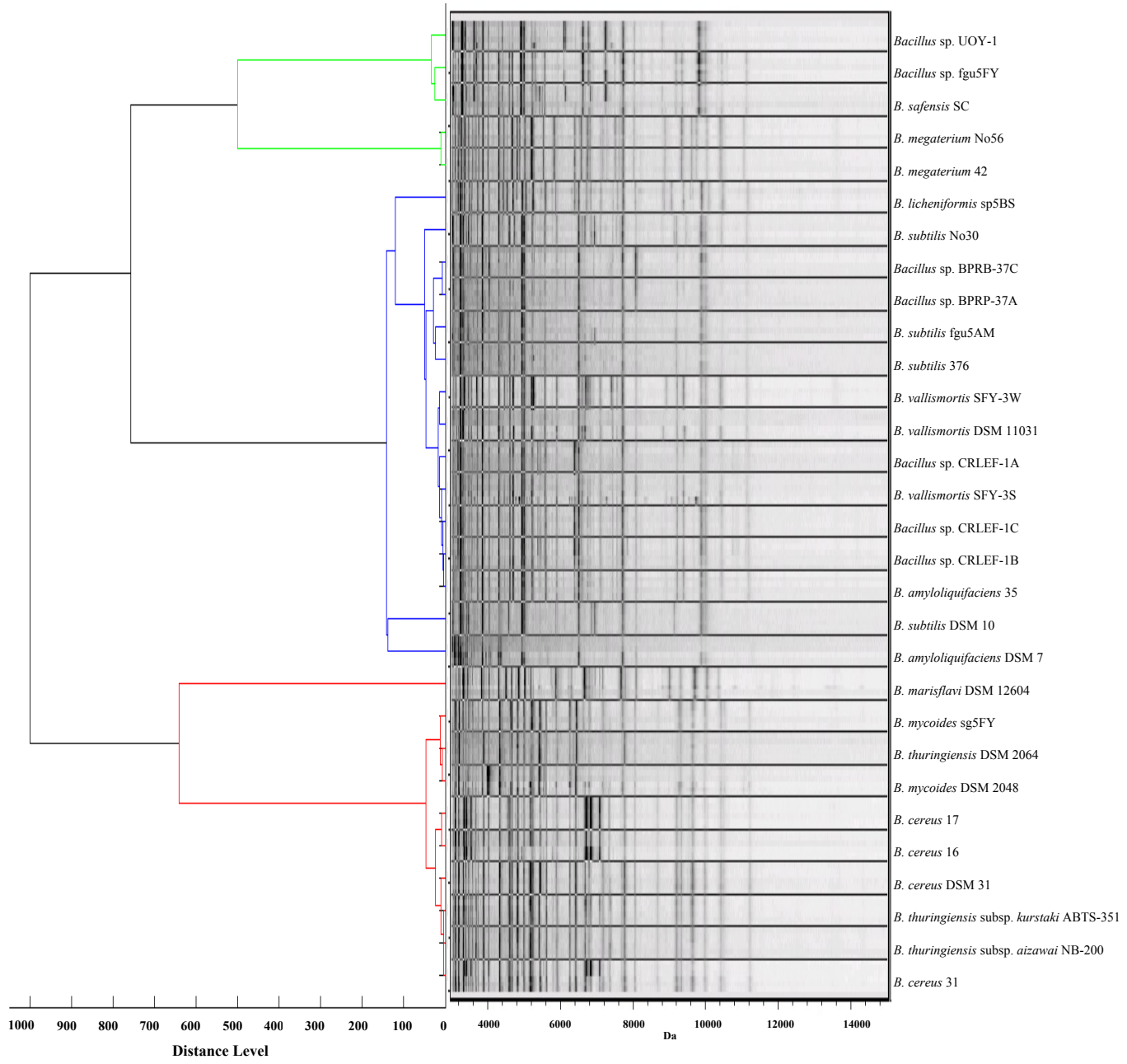


Figure S6. (Continued) Biotyper analysis of *Bacillus* with different culture media. MSP dendrograms and spectral gel views of *Bacillus* cultured on (a) LB agar, (b) NA, (c) BHI agar and (d) PCA at 30°C for 12 hours.

(c)

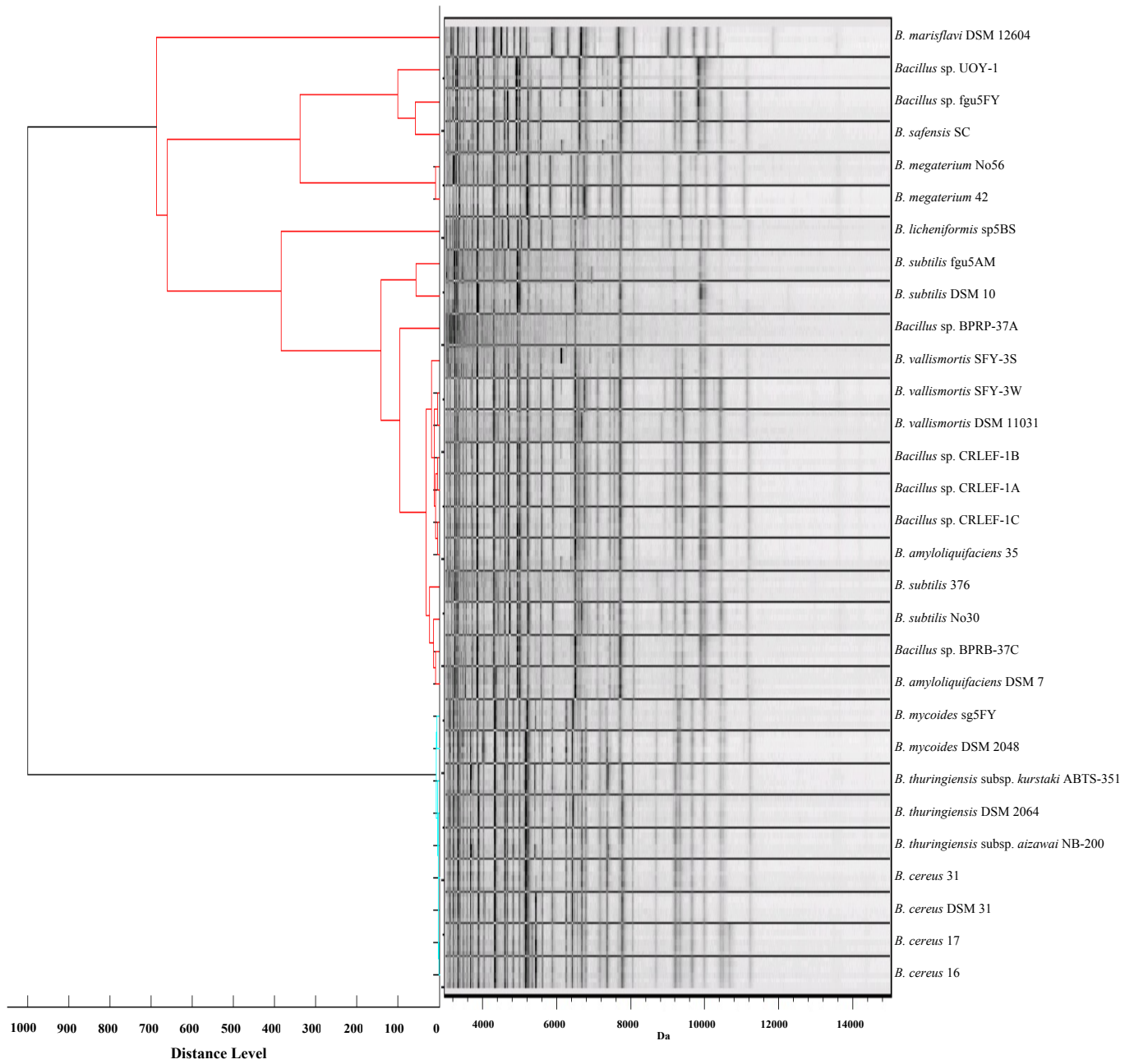


Figure S6. (Continued) Biotyper analysis of *Bacillus* with different culture media. MSP dendrograms and spectral gel views of *Bacillus* cultured on (a) LB agar, (b) NA, (c) BHI agar and (d) PCA at 30°C for 12 hours.

(d)

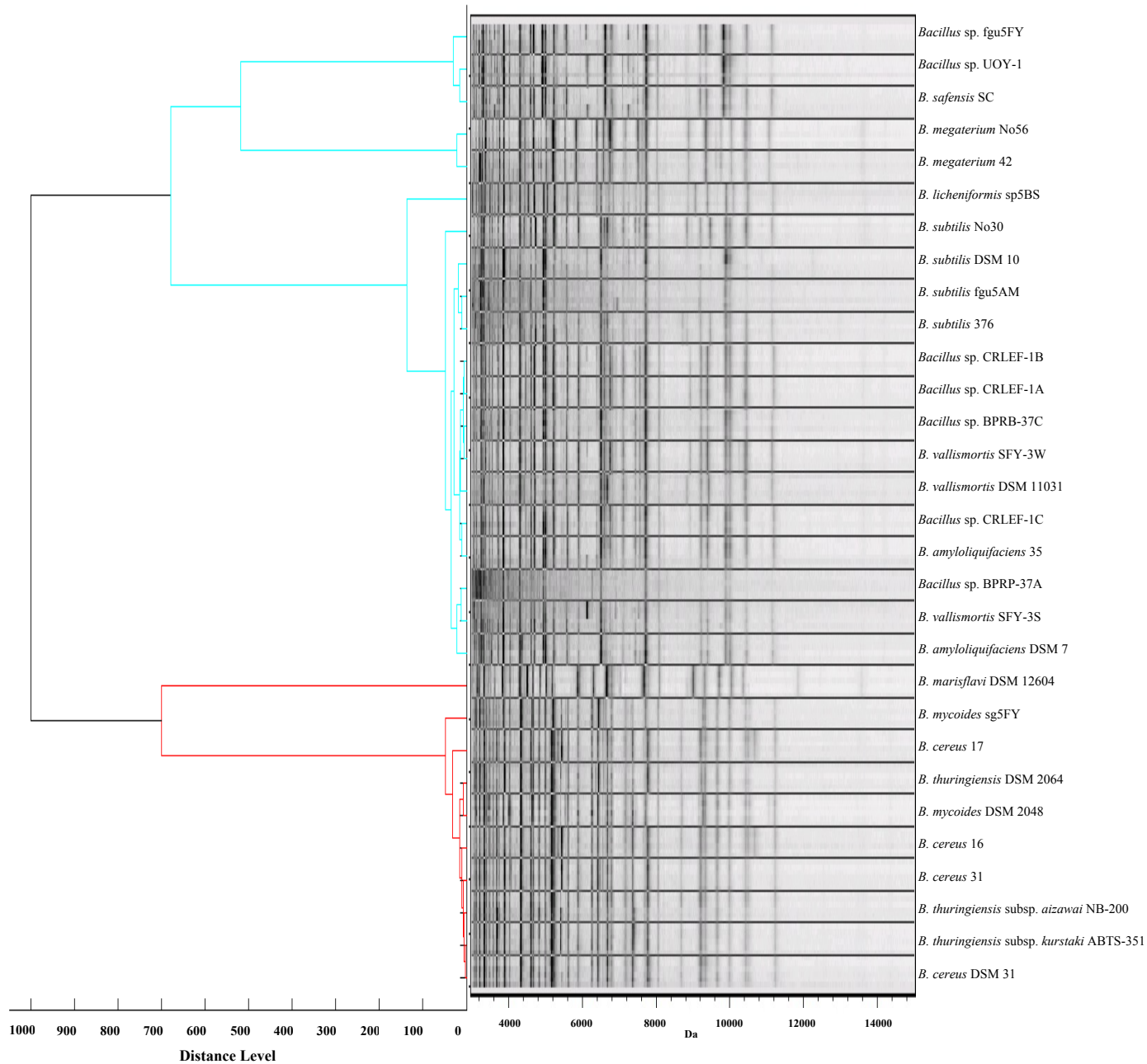


Figure S6. (Continued) Biotyper analysis of *Bacillus* with different culture media. MSP dendrograms and spectral gel views of *Bacillus* cultured on (a) LB agar, (b) NA, (c) BHI agar and (d) PCA at 30°C for 12 hours.