Raman Spectroscopy as a Comprehensive Tool for Profiling Endospore-forming Bacteria

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Table S1: Clostridioides, Paraclostridium, Clostridium and Bacillus species and strains used and their individual growth conditions

Species	Strain	Growth condition		
	Classification and validation			
Bacillus cereus	DSM345, DSM351	Aerobic; NA+MgSO ₄		
Bacillus mycoides	ATCC6462	Aerobic; NA+MgSO ₄		
Bacillus spizizenii	DSM6399	Aerobic; NA+MgSO ₄		
Bacillus thuringiensis	DSM350	Aerobic; NA+MgSO ₄		
Lysinibacillus sphaericus	DSM28	Aerobic; NA+MgSO ₄		
Bacillus licheniformis	DSM13	Aerobic; NA+MgSO ₄		
Paraclostridium sordellii	ATCC9714	Anaerobic; BHI		
Clostridioides difficile	DSM27638, DSM27640, DSM28196, DSM28645, DSM27147, DSM27543, DSM27544, DSM27639, UK062, UK127	Anaerobic; BHI		
Clostridium perfringens	UK063, ATCC13124	Anaerobic; TSC		
Clostridium sporogenes	DSM1664	Anaerobic; BHI		
Clostridium butyricum	DSM2478	Anaerobic; NA+CaCl ₂		
Clostridium tertium	DSM662	Anaerobic; NA+CaCl ₂		
Clostridium beijerinckii	DSM53	Anaerobic; BHI+MnSO ₄		
Clostridium felsineum	DSM51	Anaerobic; BHI+MnSO ₄		
Clostridium cadaveris	DSM1284	Anaerobic; BHI+MnSO ₄		
Clostridium septicum	DSM7534	Anaerobic; BHI+MnSO ₄		

DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; UK Universitätsklinikum Jena, Germany; ATCC, American Type Culture Collection, Manassas, Virginia, USA; NA, Nutrient Agar; MgSO₄, magnesium sulfate; BHI, brain heart infusion; TSC, tryptose sulfite cycloserine agar with egg yolk; CaCl₂, calcium chloride.

Bacillus band position / cm-1	Clostridium band position / cm-1	Band position according to reference / cm-1	Assignment	Reference
3071	3065	3078	Aromatic CH stretching vibration	1, 2
2936	2933	2909-2937	CH stretching vibration	3
1658	1664	1650–1680	Amide I	4, 5
1583		1578	Cytochrome c	6,7
1577	1574	1578	CaDPA COO ⁻ asymmetric stretch; Pyridine ring vibration; v _{ring} (C=C, C=N) of Guanine, Adenine	1, 2, 4, 8
1448	1448	1445 1433-1468 1431-1481	CaDPA pyridine ring vibration; CH ₂ /CH ₃ deformation vibration of lipids and proteins	1, 2, 9-11
1397	1397	1398	CaDPA COO ⁻ symmetric stretch; Cytochrome c	1, 2, 6, 7, 12
1337	1337	1310-1348	CH-deformation vibration	13
1313		1311-1314	Cytochrome c	6,7
1250	1250	1240-1265	Amide III	13
1127		1130	Cytochrome c	7, 14
1016	1016	1016	CaDPA ring breathing of pyridine ring	1, 2, 12
1004	1004	1004	Phenylalanine ring breathing vibration	11
857	854	854 850	C–C stretching vibration of proline, Out-of-plane ring deformation vibration of tyrosine	8, 13
824	824	824	CaDPA C-COO ⁻ stretch Second ring breathing mode of tyrosine	1, 2, 12, 15
749		749	Cytochrome c	6, 7, 14
659	659	657	CaDPA	1

Table S2: Assignment of the major Raman bands for the endospore spectra according to literature.

Table S3: Confusion table of the PCA-LDA classification model of bacteria on a morphological level (xy PCs).

Predicted	(ES)	(VC)	Accuracy / %	Sensitivity /%	Specificity / %
Endospore (ES)	4447	283	02.0	94.0	91.8
Vegetative cells (VC)	398	4477	92.9	91.8	94.0

Table S4: Confusion table of the PCA-LDA classification model of bacteria on a genus level.

Predicted	(B)	(CS)	(CM)	(P)	Accuracy / %	Sensitivity /%	Specificity / %
Bacillus (B)	1120	3	25	5		97.1	99.1
Clostridioides (CS)	6	1605	13	15		97.9	99.6
Clostridium (CM)	26	4	1621	37	97.0	96.0	98.6
Paraclostridium (P)	0	4	4	242		96.8	98.7

Table S5: Confusion table of the PCA-SVM classification of *Bacillus* species on a species level.

Predicted	(Bc)	(Bl)	(Bm)	(Ls)	(Bs)	(Bt)	Accuracy / %	Sensitivity /%	Specificity / %
B. cereus (Bc)	289	0	3	4	0	2		97.0	98.5
B. licheniformis (Bl)	2	147	0	1	0	0		98.0	100.0
B. mycoides (Bm)	5	0	202	0	0	1	07.7	97.1	99.7
L. sphaericus (Ls)	0	0	0	128	0	1	97.7	99.2	99.3
B. spizizenii (Bs)	5	0	0	0	213	0		97.7	100.0
B. thuringiensis (Bt)	1	0	0	2	0	147		98.0	99.6

Table S6: Confusion table of the PCA-SVM classification of *Clostridium* species on a species level.

Predicted	(Cbe)	(Cbu)	(Cc)	(Cf)	(Cp)	(Cse)	(Csp)	(Ct)	Accuracy / %	Sensitivity /%	Specificity /%
C. beijerinckii (Cbe)	129	4	8	1	0	3	1	0		88.4	100.0
C. butyricum (Cbu)	0	259	11	0	2	1	0	3	-	93.8	98.4
C. cadaveris (Cc)	0	0	208	1	0	1	0	1		98.6	97.5
<i>C. felsineum</i> (Cf)	0	1	6	218	0	16	0	0		90.5	98.8
<i>C. perfringens</i> (Cp)	0	10	0	0	239	0	0	3	95.2	94.8	99.8
C. septicum (Cse)	0	2	0	16	0	157	2	3	-	87.2	98.6
C. sporogenes (Csp)	0	1	4	0	0	0	194	0		97.5	99.8
C. tertium (Ct)	0	5	8	0	1	0	0	169		92.3	99.3

Table S7: Confusion table of the PCA-LDA validation model of bacteria on a morphological level.

Predicted	(ES)	(VC)	Accuracy / %	Sensitivity / %	Specificity / %
Endospore (ES)	1140	19	07.7	98.4	97.1
Vegetative cells (VC)	36	1214	97.7	97.1	98.4

Table S8: Confusion table of the PCA-LDA validation model of bacteria on a genus level.

Predicted	(B)	(CS)	(CM)	(P)	Accuracy / %	Sensitivity /%	Specificity / %
Bacillus (B)	351	1	2	5		97.8	100.0
Clostridioides (CS)	0	234	46	13	02.7	79.9	99.4
Clostridium (CM)	0	4	443	0	93./	99.1	93.0
Paraclostridium (P)	0	0	2	58		96.7	98.4

Table S9: Confusion table of the PCA-SVM validation of Bacillus species on a species level.

Predicted	(Bc)	(Bl)	(Bm)	(Ls)	(Bs)	(Bt)	Accuracy / %	Sensitivity /%	Specificity / %
B. cereus (Bc)	48	0	52	0	0	0	-	48.0	98.5
B. licheniformis (Bl)	0	50	0	0	0	0		100.0	100.0
B. mycoides (Bm)	1	0	42	0	8	0	071	82.4	82.8
L. sphaericus (Ls)	0	0	0	49	0	0	0/.1	100.0	100.0
B. spizizenii (Bs)	0	0	0	0	60	0		100.0	97.3
B. thuringiensis (Bt)	3	0	1	0	0	46		92.0	100.0

Table S10: Confusion table of the PCA-SVM validation of *Clostridium* species on a species level.

Predicted	(Cbe)	(Cbu)	(Cc)	(Cf)	(Cp)	(Cse)	(Csp)	(Ct)	Accuracy / %	Sensitivity / %	Specificity /%
C. beijerinckii	27	0	0	0	0	2	0	0		93.1	99.8
(Cbe)											
C. butyricum	0	60	0	0	0	0	0	0		100.0	93.3
(Cbu)											
C. cadaveris	0	0	60	0	0	0	0	0		100.0	100.0
(Cc)											
C. felsineum	0	0	0	53	0	7	0	0		88.3	93.8
(Cf)									01.0		
C. perfringens	0	1	0	0	59	0	0	0	81.8	98.3	93.5
(Cp)											
C. septicum	1	0	0	24	0	35	0	0		58.3	97.7
(Cse)											
C. sporogenes	0	0	0	0	0	0	58	0		100.0	100.0
(Csp)											
C. tertium	0	25	0	0	25	0	0	10		16.7	100.0
(Ct)								[



Figure S1: Mean Raman spectra of the pure substances or salts, including manganese dipicolinate (Mn-DPA, black), calcium dipicolinate (Ca-DPA, red), 2,6-pyridinedicarboxylic acid (DPA, blue), and cytochrome c (Cyt c, green), acquired using 532 nm excitation.



Figure S2: PCA–LDA results of classification of *Clostridioides* (red), *Clostridium* (blue), *Bacillus* (magenta), and *Paraclostridium* (green) measured at 532 nm.

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