

TABLE S1 Demographic characteristics of patients with and without CDI recurrence

Variables	rCDI (N=66)	Non-rCDI (N=177)	Univariate analysis	
			OR (95% CI)	<i>P</i> value
Gender, male [n, (%)]	33 (50.00)	89 (50.28)	0.989 (0.562-1.740)	0.969
Age (years, mean ± SD)	70.82±15.77	63.25±19.38	1.024 (1.007-1.041)	0.006
BMI (mean ± SD)	22.92±4.08	23.30±3.48	0.972 (0.899-1.050)	0.468
Hospital stays before CDI (≥10 days) [n, (%)]	36 (54.55)	57 (32.20)	2.526 (1.417-4.503)	0.002
Medical history [n, (%)]				
1) Smoking	20 (30.30)	42 (23.73)	1.398 (0.745-2.621)	0.297
2) ICU admission	15 (22.73)	21 (11.86)	2.185 (1.049-4.553)	0.037
3) Abdominal surgery	12 (18.18)	17 (9.60)	2.092 (0.939-4.658)	0.071
4) Mechanical ventilation	13 (19.70)	19 (10.73)	2.040 (0.943-4.410)	0.070
5) Cardio-cerebrovascular diseases	19 (28.79)	50 (28.25)	1.027 (0.549-1.919)	0.934
6) Peptic ulcer	10 (15.15)	11 (6.21)	2.695 (1.087-6.684)	0.032
7) Malignant tumor	13 (19.70)	25 (14.12)	1.491 (0.712-3.124)	0.290
8) Chronic kidney failure	11 (16.67)	17 (9.60)	1.882 (0.831-4.265)	0.130
9) Diabetes	10 (15.15)	24 (13.56)	1.138 (0.512-2.530)	0.750
10) Connective tissue disorder	2 (3.03)	5 (2.82)	1.075 (0.203-5.681)	0.932
Drug usage [n, (%)]				
1) Immunosuppressive agents	9 (13.64)	10 (5.65)	2.637 (1.446-5.958)	0.045
2) Aminosalicylic acid	14 (21.21)	21 (11.86)	2.000 (0.949-4.215)	0.068
3) Chemotherapeutic drugs	13 (19.70)	23 (12.99)	1.642 (0.777-3.471)	0.194
4) Glucocorticoids	27 (40.91)	55 (31.07)	1.536 (0.856-2.756)	0.151
5) Antifungal agents	11 (16.67)	22 (12.43)	1.409 (0.642-3.094)	0.393
6) Antibacterial agents				
β-lactams	48 (72.73)	127 (71.75)	1.050 (0.558-1.977)	0.880
Aminoglycosides	4 (6.06)	7 (3.95)	1.567 (0.443-5.537)	0.486
Macrolides	8 (12.12)	13 (7.34)	1.740 (0.686-4.411)	0.243
Tetracyclines	0 (0.00)	2 (1.13)	0.000 (0.000-)	0.999
Fluoroquinolones	23 (34.85)	50 (28.25)	1.359 (0.743-2.483)	0.319
Glycopeptides	16 (24.24)	28 (15.82)	1.703 (0.852-3.404)	0.132
Sulfonamides	1 (1.52)	3 (1.69)	0.892 (0.091-8.733)	0.922
7) PPIs				
Omeprazole	33 (50.00)	49 (27.68)	2.612 (1.457-4.685)	0.001
Pantoprazole	13 (19.70)	24 (13.56)	1.564 (0.743-3.290)	0.239
Esomeprazole	12 (18.18)	19 (10.73)	1.848 (0.842-4.055)	0.126
Rabeprazole	6 (9.09)	11 (6.21)	1.509 (0.535-4.259)	0.437
Accumulated duration of antibacterial agents use (≥4 weeks) [n, (%)]	12 (18.18)	14 (7.91)	2.587 (1.128-5.935)	0.025
Accumulated duration of PPIs use (≥8 weeks) [n, (%)]	19 (28.79)	24 (13.56)	2.577 (1.299-5.112)	0.007
Fever (≥38 °C) [n, (%)]	25 (37.88)	47 (26.55)	1.687 (0.927-3.070)	0.087
Blood tests (mean ± SD)				
1) WBC (×10 ⁹ /L)	8.24±5.56	7.99±5.09	1.009 (0.957-1.065)	0.736
2) PCT (ng/mL)	2.03±5.01	1.85±4.35	1.009 (0.949-1.072)	0.780
3) Cr (umol/L)	78.93±49.83	79.05±40.76	1.000 (0.993-1.007)	0.985

4) ALB (g/L)	29.96±5.42	32.58±6.68	0.935 (0.892-0.981)	0.005
5) CRP (mg/L)	47.88±49.61	43.97±54.08	1.001 (0.996-1.007)	0.608
FOBT positive [n, (%)]	31 (46.97)	62 (35.03)	1.643 (0.926-2.916)	0.090
STs [n, (%)]				0.028
1) Other ST	11 (16.67)	51 (28.81)	Reference	-
2) ST42	7 (10.61)	28 (15.82)	1.159 (0.404-3.325)	0.784
3) ST2	6 (9.09)	25 (14.12)	1.113 (0.369-3.356)	0.850
4) ST3	12 (18.18)	14 (7.91)	3.974 (1.448-10.905)	0.007
5) ST54	4 (6.06)	15 (8.47)	1.236 (0.343-4.451)	0.745
6) ST1	2 (3.03)	1 (0.56)	9.273 (0.771-111.541)	0.079
7) ST5	1 (1.52)	5 (2.82)	0.927 (0.098-8.743)	0.947
8) ST81	21 (31.82)	29 (16.38)	3.357 (1.420-7.936)	0.006
9) ST37	2 (3.03)	9 (5.08)	1.030 (0.195-5.446)	0.972
CCI (mean ± SD)	6.92±4.18	7.25±3.34	0.974 (1.642-9.481)	0.522
SSI (mean ± SD)	3.61±1.92	2.92±1.58	1.270 (1.071-1.505)	0.006
ATLAS Score (mean ± SD)	4.03±1.60	3.83±1.82	1.067 (0.908-1.254)	0.431

Abbreviations: rCDI, recurrent *Clostridioides difficile* infection; BMI, body mass index; PPI, proton pump inhibitor; WBC, white blood cell; PCT, procalcitonin; Cr, creatinine; ALB, albumin; CRP, C-reactive protein; FOBT, fecal occult blood test; ST, sequence type; CCI, Charlson comorbidity index; SSI, severity score index; OR, odds ratio; CI, confidence interval

TABLE S2 Antibiotic susceptibility profiles of 50 ST81 strains to 14 antibacterial agents

Antibacterial agents	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	MIC range (µg/mL)	MIC mode (µg/mL)	Resistance rate (%)
MET	0.5	1	0.125-1	1	0.00
VAN	0.5	0.5	0.25-1	0.5	0.00
CIP	64	>128	64->128	64	100.00
LEV	64	128	4-128	64	92.00
MXF	64	128	2-128	64	80.00
AMP	0.5	2	0.25-2	0.25	12.00
TZP	8/4	8/4	2/4-8/4	8/4	0.00
CLI	128	128	64-128	128	100.00
MEM	2	4	1-4	2	0.00
CRO	64	>128	32->128	64	88.00
CTT	64	>128	32->128	32	64.00
FDX	0.125	0.25	0.125-0.5	0.125	0.00
RFP	≤0.004	≤0.004	≤0.004-0.016	≤0.004	0.00
TGC	0.063	0.063	0.031-0.063	0.063	0.00

Abbreviations: MET, metronidazole; VAN, vancomycin; CIP, ciprofloxacin; LEV, levofloxacin; MXF, moxifloxacin; AMP, ampicillin; TZP, piperacillin/tazobactam; CLI, clindamycin; MEM, meropenem; CRO, ceftriaxone; CTT, cefotetan; FDX, fidaxomicin; RFP, rifaximin; TGC, tigecycline.

TABLE S3 Primers sequences used in this study

Lab notation	Sequence (5'-3')	Use	Product size (bp)
QP01F	TTGAGTCTCTTGAAGTGGTCTAGG	<i>spo0A</i> qRT-PCR primer	143
QP01R	CTCAAAGCGCAATAAATCTAGGAGC		
QP02F	TGACTTTACACTTTCATCTGTTTCTAGC	<i>sigE</i> qRT-PCR primer	86
QP02R	GGGCAAATATACTTCCTCCTCCAT		
QP03F	CGCTCCTAACTAGACCTAAATTGC	<i>sigF</i> qRT-PCR primer	140
QP03R	GGAAGTAACTGTTGCCAGAGAAGA		
QP04F	ATCTTGAACCTGATCCATAACGA	<i>sigG</i> qRT-PCR primer	104
QP04R	CAGTTGTAATGGCACTTGATGC		
QP05F	TCCAAGCCCTGCACATATCC	<i>cspBA</i> qRT-PCR primer	87
QP05R	CGAGCTATCGCAGAAAATGACC		
QP06F	TGTGCTCCCACTTATCTGGAC	<i>cspC</i> qRT-PCR primer	150
QP06R	TGGTGCAGGAAATCAAGGGA		
QP07F	CACCTAAGTCTTTACTGCCCCA	<i>sleC</i> qRT-PCR primer	100
QP07R	CCTCCTACATCAAGACAGCCA		
QP08F	GCATCAATCAATCCAATGACTCCAC	<i>sigD</i> qRT-PCR primer	100
QP08R	TGCCTCTGTAAAGAGTATAGCA		
QP09F	GTTGTTATAACCAGCTGAAGCCATTA	<i>fliC</i> qRT-PCR primer	115
QP09R	TACAAGTTGGAGCAAGTTATGGAAC		
QP10F	GCAACTAATCTAAGAAGTCAGACAA	<i>flgB</i> qPCR primer	112
QP10R	TAGGCATAGTATCATTTAGTGTTC		
QP11F	CTCTAGTTCTAAGATGGACCTTATCTC	<i>motA</i> qRT-PCR primer	110
QP11R	CAATAGAAAGTGATGTAATGGGAATAGAAG		
QP12F	AGTAAGAAATAACTCAGTAGATGATT	<i>tcdR</i> qRT-PCR primer	113
QP12R	TTATTAAATCTGTTTCTCCCTCTTCA		
QP13F	TGGTGAAGATGGTGTTCATGC	<i>tcdB</i> qRT-PCR primer	100
QP13R	TTCTCCCTCAAATTTCTCATCC		
QP14F	TTTGATTTACTGGCCGGAGCATTG	<i>codY</i> qRT-PCR primer	126
QP14R	CTCATCTTCTATAACTGAACTGTCTTGAGC		
QP15F	AAATGGGATAGAAGAGTTGCTAAA	<i>ccpA</i> qRT-PCR primer	153
QP15R	TCTTGTTCAACTATCCATGAAATCATAAC		
QP16F	AGTGAAAGGCTACGGCTCAA	<i>rrn</i> qRT-PCR primer	100
QP16R	CTACGCATTTACCGCTACA		
OS01F	GAGCAACTTTTCGAAGAAATA	OS-PCR primer for 630 reference strain	ON: 401
OS02F	TATTTCTTCGAAAAGTTGCTC		OFF: /
OS03F	AAGGCAACTTATAAAGAAATATTT	OS-PCR primer for R20291 reference strain	ON: 403
OS04F	AAATATTTCTTTATAAAGTTGCCTT		OFF: 272
OS05F	AGAGCAACTTTTTGAAGAAAT	OS-PCR primer for 1470 reference strain and ST81 clinical strain	ON: 403
OS06F	ATTTCTTCAAAAAGTTGCTCT		OFF: 268
OS07F	GAGCAACTTTTTGAAGAAATAT	OS-PCR primer for DSM 28669 reference strain	ON: /
OS08F	ATATTTCTTCAAAAAGTTGCTC		OFF: 269
OS00R	AGAATTGTCTGTTTTTCTTCTA	A common reverse primer used in all OS-PCR	/

QOS01F	TTCATGGGACTTCATTGAA	qOS-PCR primer for ST81 clinical strain	ON: 194 OFF: 161
QOS02F	TTCAATGAAGTCCCATGAA		
QOS00R	ATGTCATATGTATTCACT		

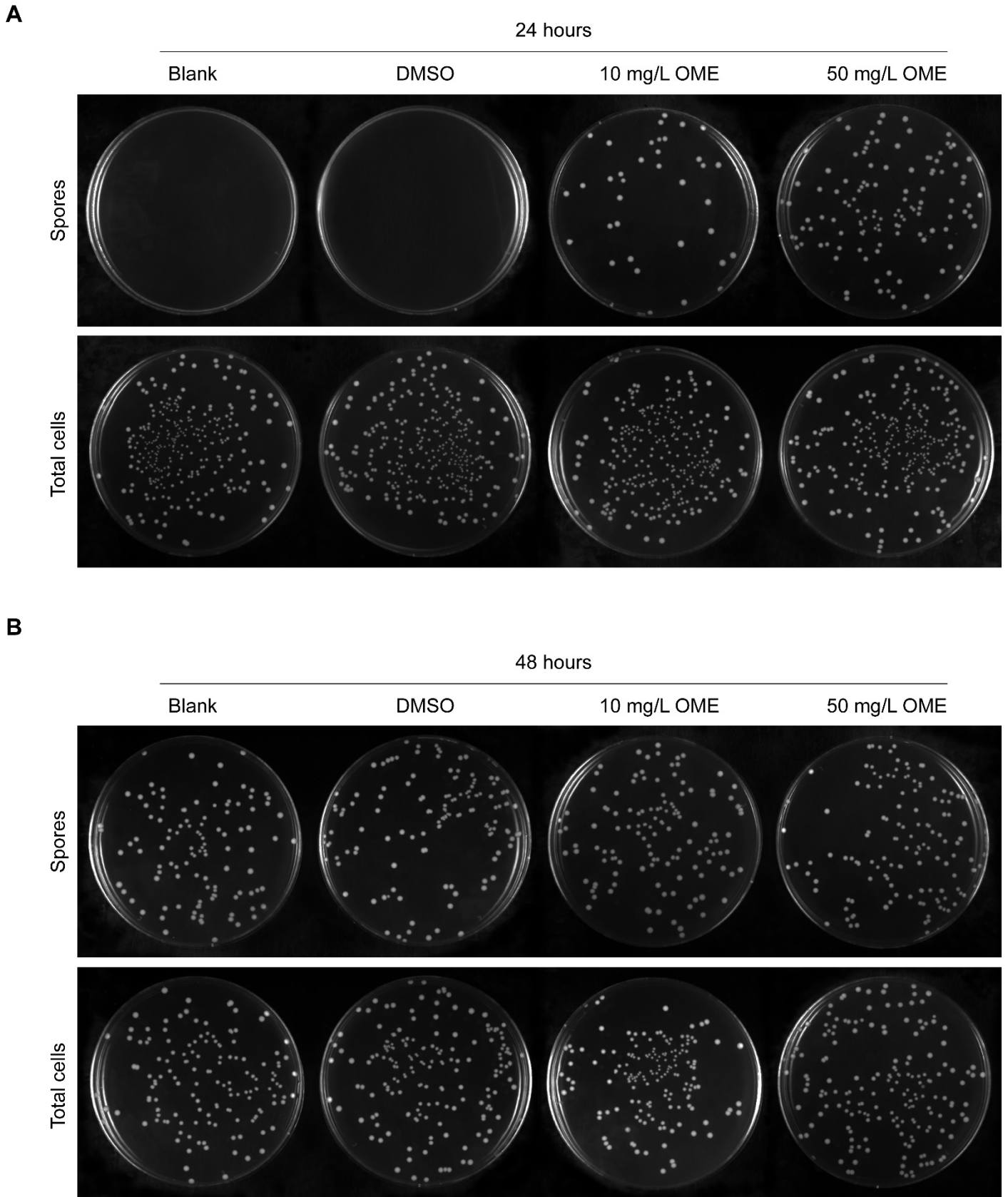


FIG S1 Omeprazole increases the sporulation efficiency of ST81 strains. Representative images of the colony forming unit on the brain heart infusion-supplemented agar supplemented with 0.1% sodium taurocholate represent the numbers of heat-resistant spores and total cells after (A) 24 and (B) 48 h of omeprazole treatment.

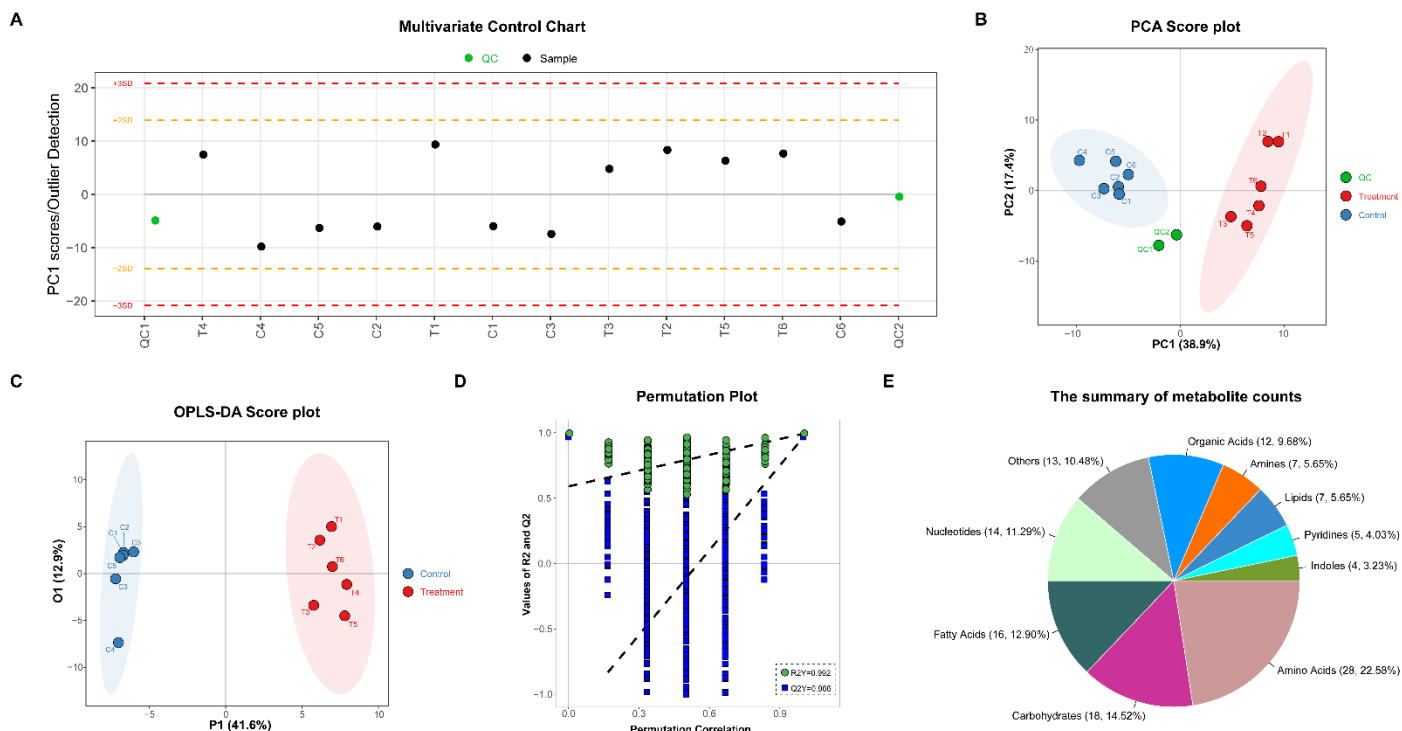


FIG S2 Overview of metabolite classification and quality control in the metabonomics study. (A) Each dot in the multivariate control chart represents an individual sample or quality control (QC). Dots with score values exceeding yellow ($\pm 2SD$) and red ($\pm 3SD$) dashed lines are considered as warning results and outliers, respectively. (B, C) The score plots show group separation in principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA). (D) The Permutation plot displays the correlation coefficient between the original y-variable and the permuted y-variable on the x-axis versus the cumulative R2 and Q2 on the y-axis with regression lines. The cumulative values of R2Y and Q2Y of the permuted model are represented on the bottom right of the plot. (E) The number and percentage of the classes of identified metabolite in all samples are shown in a pie chart.

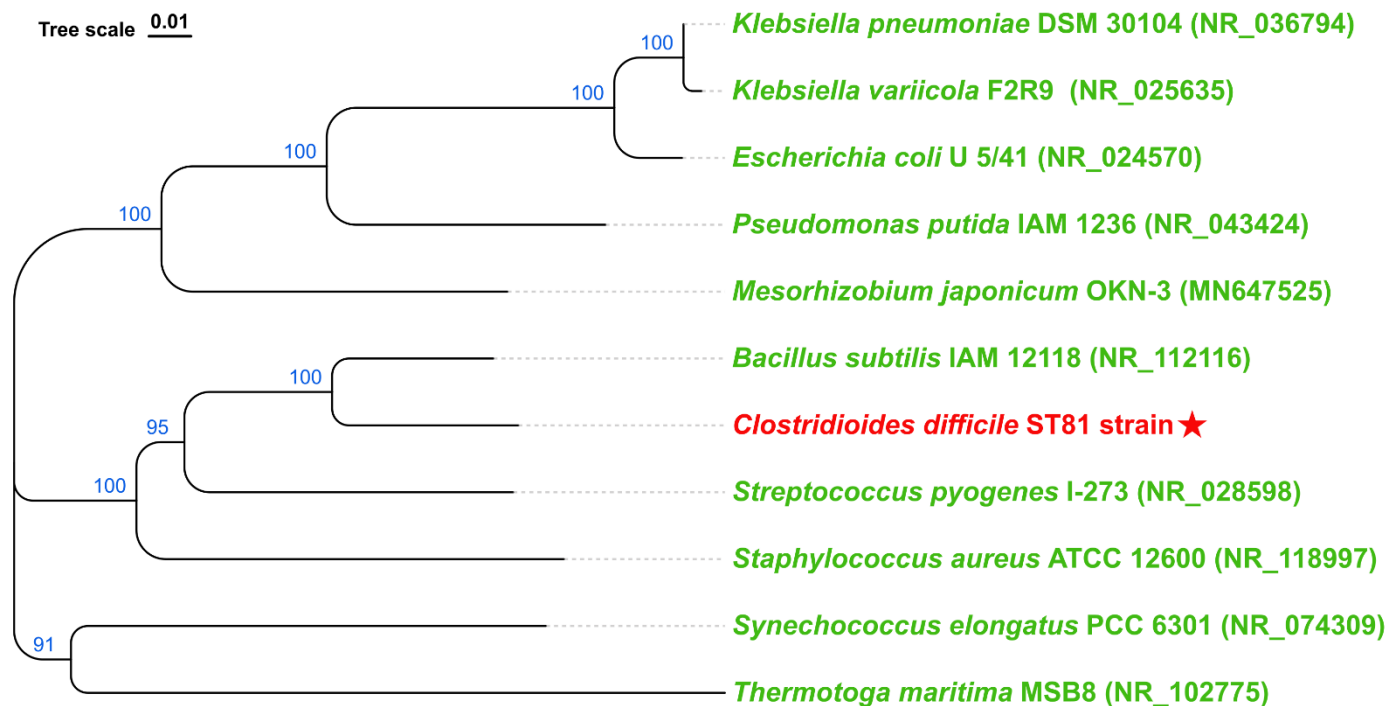


FIG S3 Evolutionary relationships of strains based on 16S rRNA gene sequencing. The 16S rRNA sequence of *Clostridioides difficile* ST81 strain is obtained in this study, which is highlighted with red and star in the tree. Accession numbers of reference sequences in GenBank are given in brackets behind the strain names. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Scale bar indicates 0.01 substitutes per nucleotide position.