Supplemental Figures S3-S6

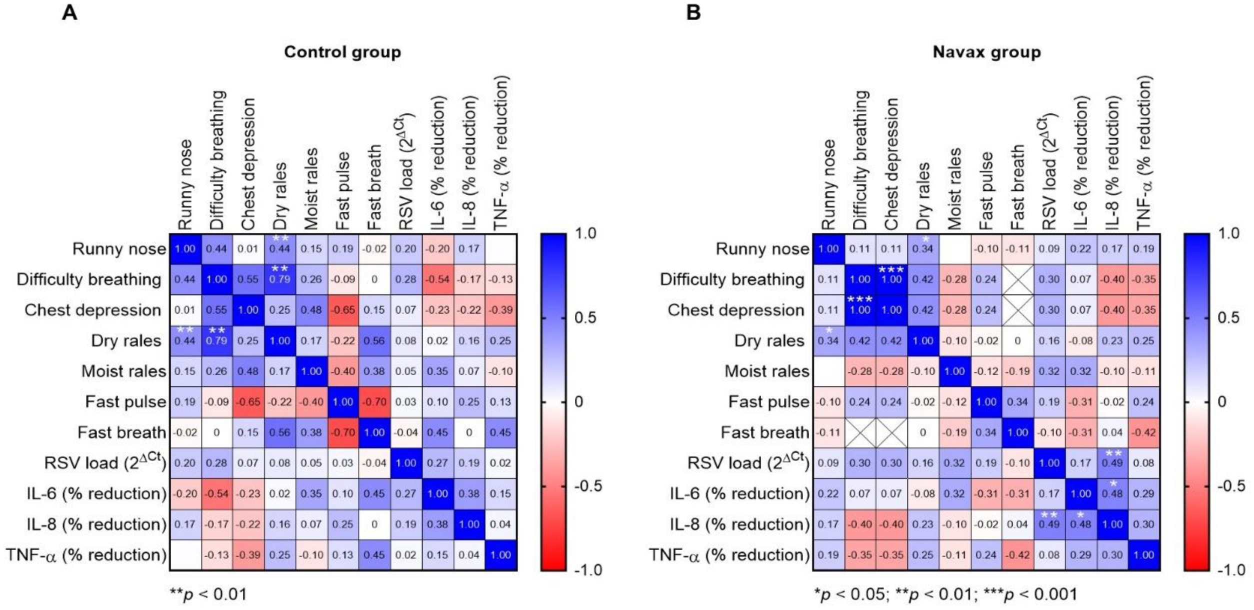
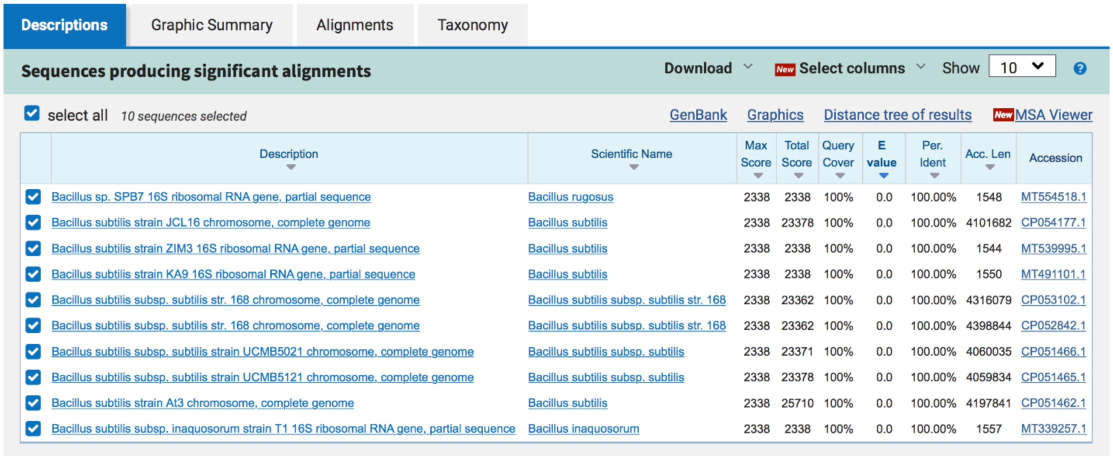


Fig. S3. Associations between symptom improvements and reductions in RSV load and in cytokine levels (IL-6, IL-8, TNF-α) in Control (A) and Navax (B) groups were assessed by Spearman's correlation analysis. Colors and numbers represent correlation factor values (blue: r>0 shows proportional correlation, red: r<0 shows inverse correlation). Some results are left blank either because only one row contains data for both variables or because one of the variables has the same value in all rows.

A.

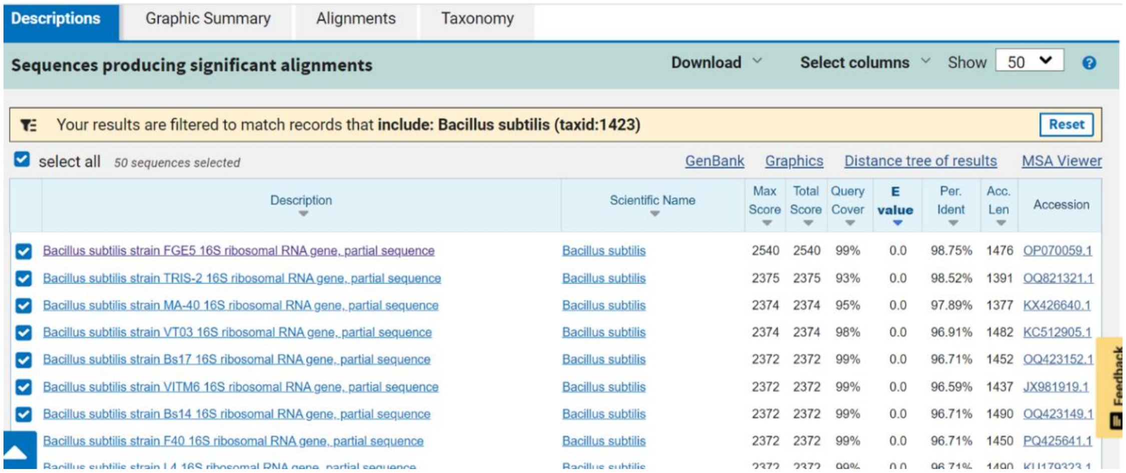


Sequences producing significant alignments

☑ select all *10 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Bacillus sp. SPB7 16S ribosomal RNA gene, partial sequence	Bacillus rugosus	2338	2338	100%	0.0	100.00%	1548	MT554518.1
☑	Bacillus subtilis strain JCL16 chromosome, complete genome	Bacillus subtilis	2338	23378	100%	0.0	100.00%	4101682	CP054177.1
☑	Bacillus subtilis strain ZIM3 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2338	2338	100%	0.0	100.00%	1544	MT539995.1
☑	Bacillus subtilis strain KA9 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2338	2338	100%	0.0	100.00%	1550	MT491101.1
☑	Bacillus subtilis subsp. subtilis str. 168 chromosome, complete genome	Bacillus subtilis subsp. subtilis str. 168	2338	23362	100%	0.0	100.00%	4316079	CP053102.1
☑	Bacillus subtilis subsp. subtilis str. 168 chromosome, complete genome	Bacillus subtilis subsp. subtilis str. 168	2338	23362	100%	0.0	100.00%	4398844	CP052842.1
☑	Bacillus subtilis subsp. subtilis strain UCMB5021 chromosome, complete genome	Bacillus subtilis subsp. subtilis	2338	23371	100%	0.0	100.00%	4060035	CP051466.1
☑	Bacillus subtilis subsp. subtilis strain UCMB5121 chromosome, complete genome	Bacillus subtilis subsp. subtilis	2338	23378	100%	0.0	100.00%	4059834	CP051465.1
☑	Bacillus subtilis strain At3 chromosome, complete genome	Bacillus subtilis	2338	25710	100%	0.0	100.00%	4197841	CP051462.1
☑	Bacillus subtilis subsp. inaquosorum strain T1 16S ribosomal RNA gene, partial sequence	Bacillus inaquosorum	2338	2338	100%	0.0	100.00%	1557	MT339257.1

A.1



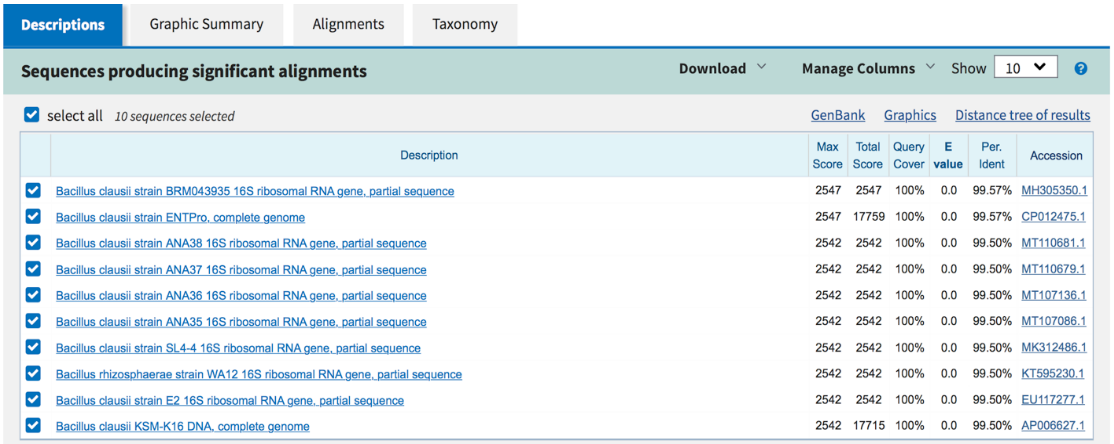
Sequences producing significant alignments

Your results are filtered to match records that **include: Bacillus subtilis (taxid:1423)**

☑ select all *50 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Bacillus subtilis strain FGE5 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2540	2540	99%	0.0	98.75%	1476	OP070059.1
☑	Bacillus subtilis strain TRIS-2 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2375	2375	93%	0.0	98.52%	1391	OQ821321.1
☑	Bacillus subtilis strain MA-40 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2374	2374	95%	0.0	97.89%	1377	KX426640.1
☑	Bacillus subtilis strain VT03 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2374	2374	98%	0.0	96.91%	1482	KC512905.1
☑	Bacillus subtilis strain Bs17 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2372	2372	99%	0.0	96.71%	1452	OQ423152.1
☑	Bacillus subtilis strain VITM6 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2372	2372	99%	0.0	96.59%	1437	JX981919.1
☑	Bacillus subtilis strain Bs14 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2372	2372	99%	0.0	96.71%	1490	OQ423149.1
	Bacillus subtilis strain F40 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2372	2372	99%	0.0	96.71%	1450	PQ425641.1
	Bacillus subtilis strain L4 16S ribosomal RNA gene, partial sequence	Bacillus subtilis	2372	2372	99%	0.0	96.71%	1490	KU179323.1

B.



Sequences producing significant alignments

☑ select all *10 sequences selected*

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Bacillus clausii strain BRM043935 16S ribosomal RNA gene, partial sequence	2547	2547	100%	0.0	99.57%	MH305350.1
☑	Bacillus clausii strain ENTPro, complete genome	2547	17759	100%	0.0	99.57%	CP012475.1
☑	Bacillus clausii strain ANA38 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	99.50%	MT110681.1
☑	Bacillus clausii strain ANA37 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	99.50%	MT110679.1
☑	Bacillus clausii strain ANA36 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	99.50%	MT107136.1
☑	Bacillus clausii strain ANA35 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	99.50%	MT107086.1
☑	Bacillus clausii strain SL4-4 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	99.50%	MK312486.1
☑	Bacillus rhizosphaerae strain WA12 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	99.50%	KT595230.1
☑	Bacillus clausii strain E2 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	99.50%	EU117277.1
☑	Bacillus clausii KSM-K16 DNA, complete genome	2542	17715	100%	0.0	99.50%	AP006627.1

Fig S4. BLAST analysis of *Bacillus subtilis* ANA4 (A) and *B. clausii* ANA39 (B).

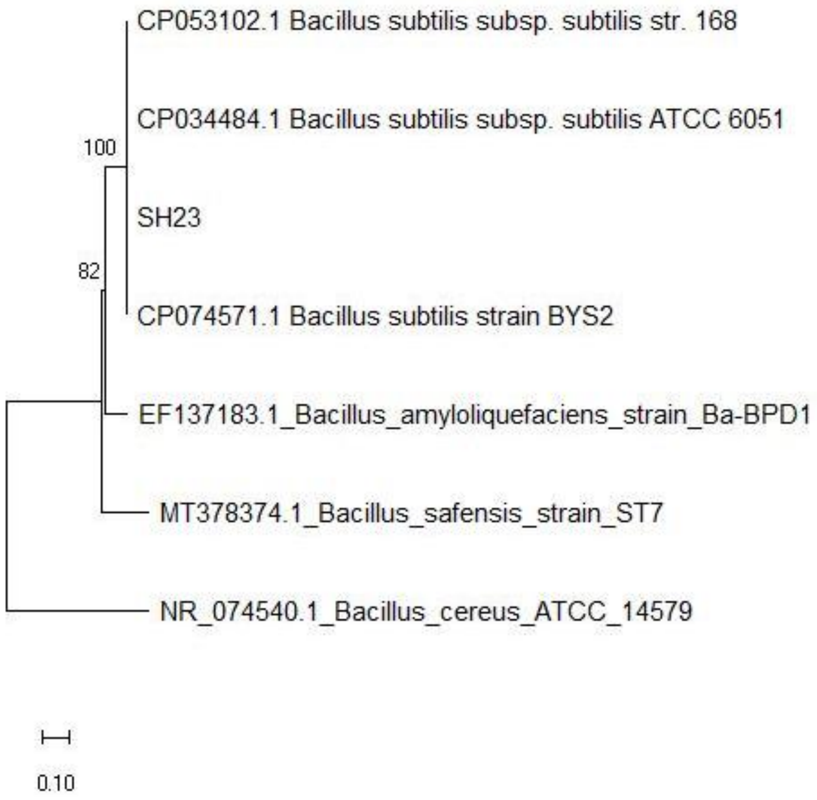


Fig. S5. Phylogenetic tree for *B. subtilis* ANA4

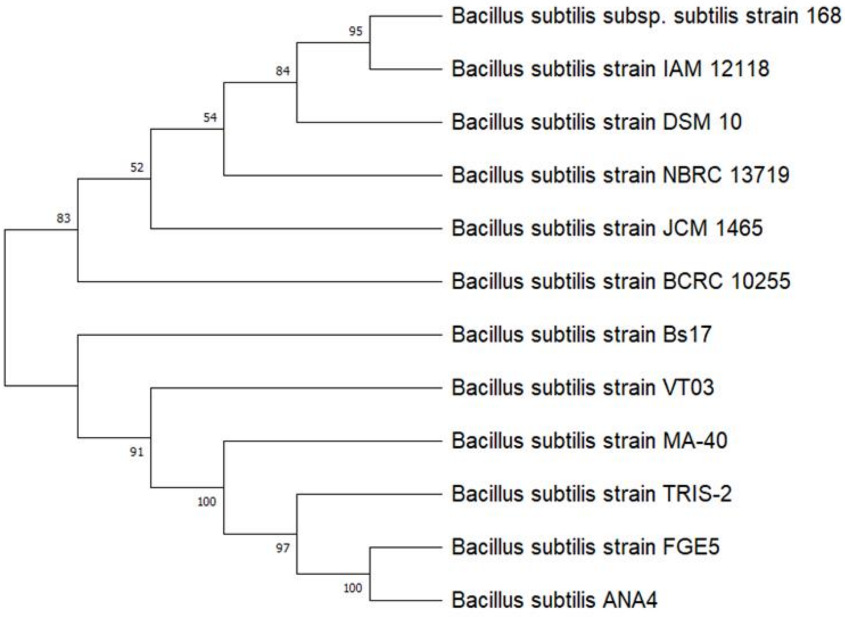


Fig. S5.1. Phylogenetic tree for *B. subtilis* ANA4

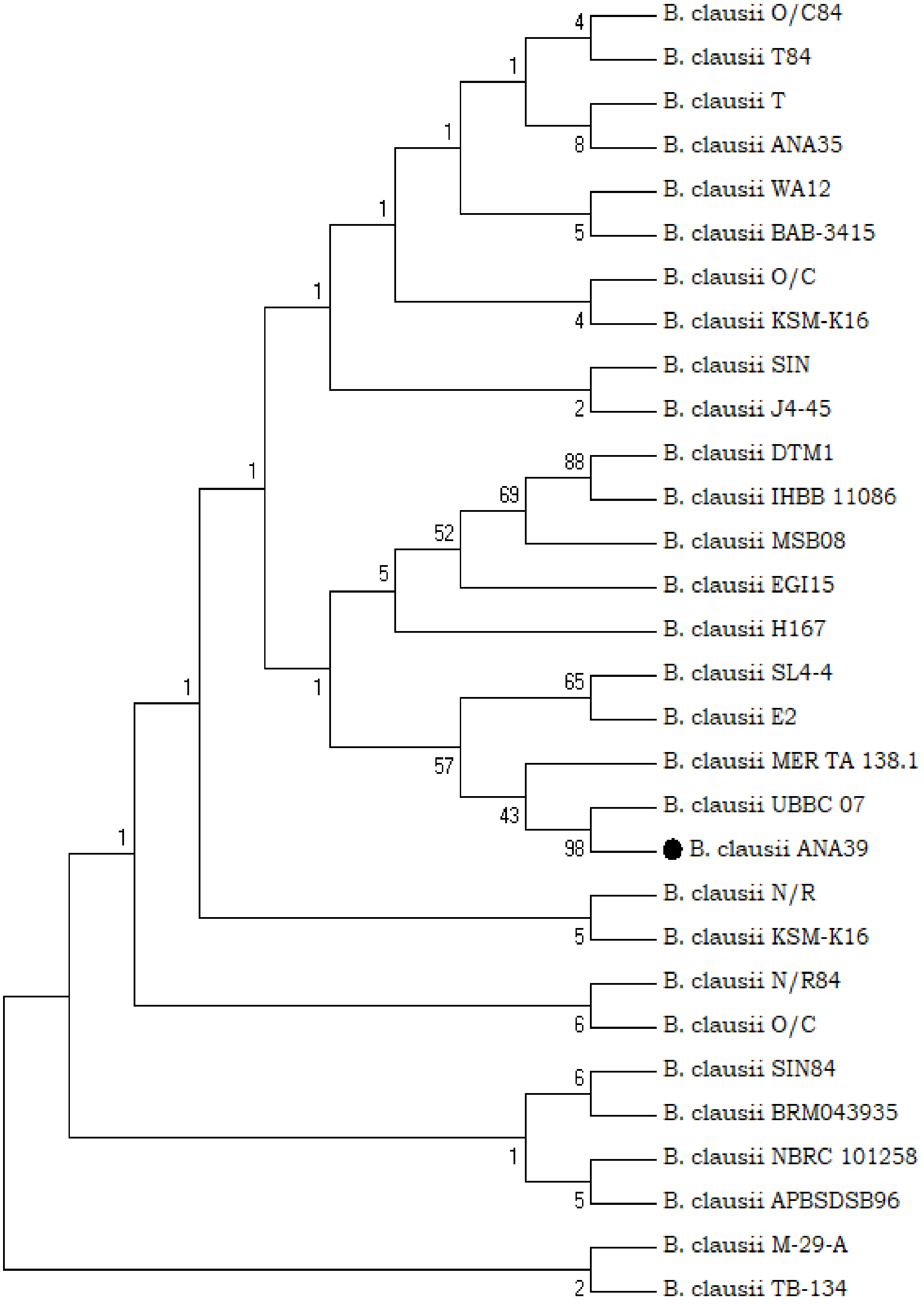


Fig. S6. Phylogenetic tree for *B. clausii* ANA39

Supplemental Tables S1-S5

Table S1. Characterization of *B. subtilis* ANA4 and *B. clausii* ANA39

Characteristics	**ANA4**	**ANA39**
Sporulation efficiency (%)	100	90
Heat stability of spores (°C)	80	65
Width size of vegetative cell (µm)	< 1 µm	< 1 µm
Amylase	++++	++++
Caseinase	++++	+++
Lipase	+	++
Catalase	+	+
Gelatinase	++	+
Optimal temperature (°C)	37	37
Optimal pH	7.4	8.0
6.5% NaCl, 50°C	+++	-
8% NaCl, 37°C	-	+++
Aerobic	++++	+++
Anerobic	+	+++
Hemolysis	γ (No)	γ (No)
VP Test	+	-
Closest match*	*B. subtilis* (100%)	*B. clausii* (99.5%)

-, negative; +, weak or positive; ++, average; +++, good/high; ++++, very good/very high.

**Using 16S rDNA sequence analysis in this work. The similarity score is shown in brackets.*

Table S2. Antibiotic susceptibility of *Bacillus subtilis* ANA4 and *B. clausii* ANA39

Antibiotic discs (µg)[*]	***B. subtilis* ANA4**[+]	***B. clausii* ANA39**[+]
Ampicillin (10)	24.34 ± 0.51 (S)	27.04 ± 1.3 (S)
Chloramphenicol (30)	26.41 ± 0.57 (S)	18.47 ± 0.20 (S)
Ciprofloxacin (5)	0 (R)	30.89 ± 0.61 (S)
Clindamycin (2)	17.05 ± 0.24 (S)	0 (R)
Cotrimoxazol (25)	28.43 ± 0.45 (S)	34.50 ± 0.95 (S)
Erythromycin (15)	22.33 ± 0.31 (S)	0 (R)
Gentamicin (10)	21.52 ± 0.10 (S)	28.22 ± 0.39 (S)
Kanamycin (30)	25.48 ± 0.24 (S)	24.84 ± 0.04 (S)
Neomycin (30)	20.29 ± 0.24 (S)	24.91 ± 0.13 (S)
Rifampicin (30)	19.68 ± 0.57 (S)	39.06 ± 0.68 (S)
Streptomycin (10)	15.17 ± 0.23 (S)	6.51 ± 0.46 (R)
Tetracycline (30)	0 (R)	27.99 ± 0.14 (S)
Trimethoprim (5)	21.13 ± 0.25 (S)	39.83 ± 0.72 (S)
Vancomycin (30)	17.67 ± 0.70 (S)	22.41 0.22 (S)

[*] *Antibiotic-impregnated discs (6 mm) with amount in µg shown in brackets.*

[+] *Diameter of inhibition zones from three individual experiments. S, sensitive; I, intermediate resistant; R, resistant.*

Table S3. Sequence analysis of antibiotic resistance genes in *B. subtilis* ANA4 genome

Resistance gene	% Coverage	% Identity	Start	End	Predicted phenotype	Accession number
rifamycin-inactivating phosphotransferase *rphc*	99.58	82.45	139044	139044	Rifamycin resistance	ng_063825.1
aminoglycoside 6-adenylyltransferase *aadk*	100	98.83	624584	625438	Aminoglycoside resistance	ng_047379.1
streptothricin n-acetyltransferase *sata*	100	95.59	38226	38747	Streptothricin resistance	ng_064662.1
abc-f type ribosomal protection protein *vmlr*	100	98.54	190934	192580	Lincosamide, Streptogramin, Tiamulin resistance	ng_063831.1
macrolide 2'-phosphotransferase *mphk*	100	99.35	108040	108960	Macrolide resistance	ng_065846.1

Notes: The five genes including *rphc, aadk, sata, vmlr, mphk* classified in Ansamycin, Aminoglycoside, Lincosamide/Streptogramin/Tiamulin, and Macrolide antibiotic groups, respectively, were found. They may belong to acquired antibiotic resistant genes. Although those genes are available in the genome, their expression levels may be low so that the strain is still sensitive to tested antibiotics such as streptomycin and erythromycin… Interestingly, tetracyclin and fluoroquinolones resistance genes were not found in the genome of *B. subtilis* ANA4, suggesting that the strain is intrinsic resistance to tetracyclin and ciprofloxacin.

Table S4. Sequence analysis of antibiotic resistance genes in *B. clausii* ANA39 genome

Resistance gene	% Identity	Query / Template length	Contig	Position in contig	Predicted phenotype	Accession number
ant(4')- Ib	98.83	771 / 771	000000F |arrow	81510..82280	Aminoglycoside resistance	AJ506108
erm(34)	96.04	833 / 846	000000F |arrow	1033445..1034277	Macrolide resistance	AY234334
cat	96.79	685 / 687	000000F |arrow	2589949..2590625	Phenicol resistance	AY238971

Notes: The three genes including ant(4')-Ib classified in Aminoglycoside antibiotic group, erm(34) classified in Macrolide antibiotic group, and cat classified in Phenicol antibiotic group were found. They may belong to acquired antibiotic resistant genes. The presence of the two genes ant(4')-Ib and erm(34) are consistent with the streptomycin and erythromycin resistant phenotype of *B. clausii* ANA39 indicated by the diffusion dics assay. Although cat is available in the genome, its expression level may be low so that the strain is still sensitive to chloramphenicol. Interestingly, clindamycin resistance gene was not found in the genome of *B. clausii* ANA39, suggesting that the strain is intrinsic resistance to clindamycin.

Table S5. Sequence analysis of toxin genes in *B. subtilis* ANA4 and *B. clausii* ANA39 genomes

No	**Genes**	**Detection method**			
		Specific sequence amplified by PCR		***Number of gene detected by whole genome sequencing***	
		B. subtilis ANA4	*B. clausii* ANA39	*B. subtilis* ANA4	*B. clausii* ANA39
1	Hemolysin B (*hbl*B)	Not Detectable	Not Detectable	0	0
2	Non-hemolytic enterotoxin A (*nhe*A)	Not Detectable	Not Detectable	0	0
3	*nhe*B	Not Detectable	Not Detectable	0	0
4	*nhe*C	Not Detectable	Not Detectable	0	0
5	Cytotoxin K (*cyt*K)	Not Detectable	Not Detectable	0	0