

**Table S1. Primers, Plasmids, and Strains used in this study**

**Primers used in this study**

<u>Primer Name</u>	<u>Description</u>	<u>Sequence (5' to 3')</u>
ES315	pES036_mTurqR	ATTGGATCCTTATTTGTACAGTTCGTCATG
ES395	pES036_mTurqF	ATTAAGCTTACATAAGGAGGAAGTACTATGGTTTCAAAG
ES429	pES045_mYPetF	ATTAAGCTTACATAAGGAGGAAGTACTATGTCTAAAGGTGAAGAA
ES430	pES045_mYPetR	ATTGGATCCTTTGTACAATTCATTCATAC
ES579	himar 1 for sequencing	CATTTAATACTAGCGACGCCATCT
ES630	himar 2 for sequencing	CCTTCTTGACGAGTTCTTCTGAG
ES871	amsC-UPST REV	TAAAGCCAGTCGGTAAGGCGAA
ES872	amsC-DWST FWD	TCTGACGCGTTATAAGCCGAA
ES880	pSR47S-amsC_UPST FWD	AAAGGATCGATCCTCTAGAGCGATGCCTCTATCATCGG
ES881	amsC_DWST-amsC_UPST REV	GTCAGATTGAGGTGTGATTCTTAATTCC
ES882	amsC_UPST-amsC_DWST FWD	GAATCACACCTCAATCTGACGACCGCCTC
ES883	pSR47S-amsC_DWST REV	CGGCCGCTCTAGAACTAGTGAATGACGAAATCTTTTTGCCCTG
ES896	pSR47S-MCS-FWD	GGATTTGCAGACTACGGGCCTA
ES897	pSR47S-MCS-REV	ATTAGGCACCCAGGCTTTACA
ES898	pBBR1MCS-amsC FWD	GGTCGACGGTATCGATAAGCTTTTAGTGGATCAGCCAAAAC
ES900	pBBR1MCS-amsC+300bp REV	GGGCGAATTGGAGCTCTCATTGAGCAATAAACAGG
ES915	amsC-DWST FWD for sequencing	CCGATTCGCCCTTTGTGTTTCCA
ES916	amsC-UPST REV for sequencing	GCTGGGCGCAGATAACAAATCA
ES981	<i>flgM::tet</i> insertion-deletion allele	AGGAGATGCAGAAATCATAAATGCATT
ES982	<i>flgM::tet</i> insertion-deletion allele	GAACAACCTGCACCATTGCAAGAATTGATTGATTTTCATAGGATTCCTCT
ES983	<i>flgM::tet</i> insertion-deletion allele	TTGATCCTTTTTTATAACAGGAATTCAATATGATTAATTTTTATAAAAAGCAATAAAA
ES984	<i>flgM::tet</i> insertion-deletion allele	TTCAGAAATATAATTAACGTTTCAGCT

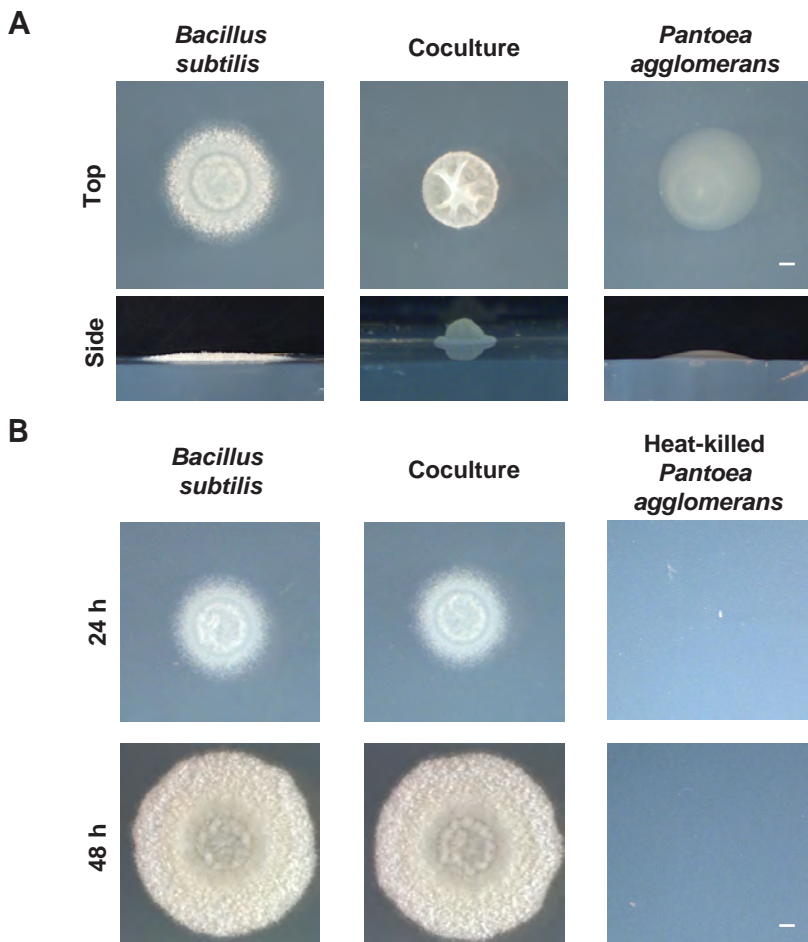
**Plasmids used in this study**

<u>Plasmid</u>	<u>Genotype</u>	<u>Reference</u>
pSR47S	oriTRP4 oriR6K <i>kan</i> <sup>R</sup>	[1]
pSR47S-amsC	oriTRP4 oriR6K <i>kan</i> <sup>R</sup> $\Delta$ <i>amsC</i>	This study
pBBR1MCS	<i>cam</i> <sup>R</sup>	[2]
pBBR1MCS-amsC	<i>PamsC-amsC</i> ( <i>cam</i> <sup>R</sup> )	This study
pKMW3	<i>mariner</i> transposon vector	[3]
pDG1515	<i>amp</i> <sup>R</sup> , <i>tet</i> <sup>R</sup>	[4]
pEA003	<i>amyE::P<sub>spacC</sub>-cfp</i> ( <i>cam</i> <sup>R</sup> )	Kolter lab collection
GL-FP-31	<i>mTurquoise2</i>	Garner lab collection
pES036	<i>amyE::P<sub>spacC</sub>-mTurq</i> ( <i>cam</i> <sup>R</sup> )	This study
pES045	<i>amyE::P<sub>spacC</sub>-Ypet</i> ( <i>cam</i> <sup>R</sup> )	This study

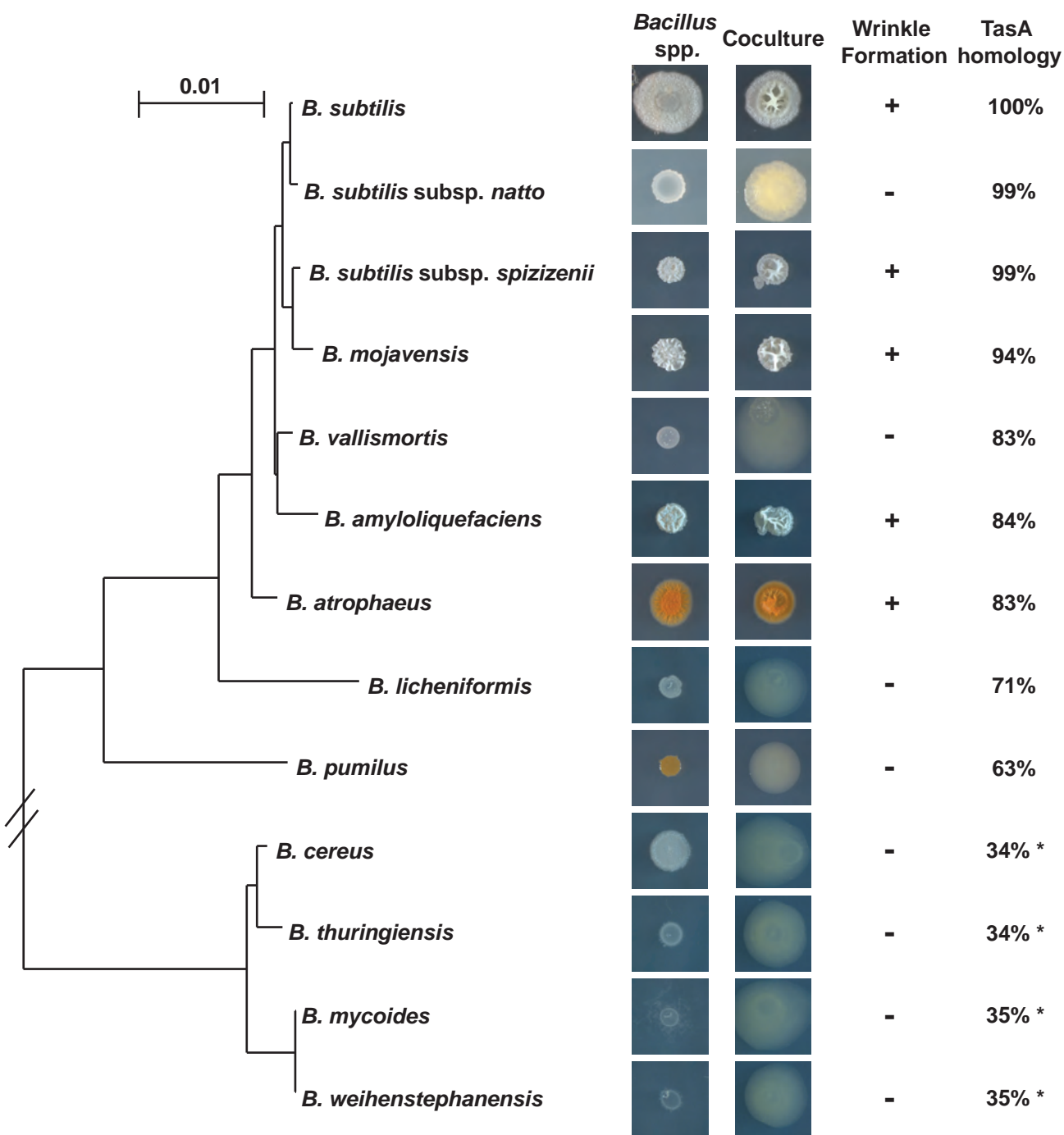
**Strains used in this study**

<u>Strain name (Pseudonym)</u>	<u>Bacterial species/strain</u>	<u>Genotype</u>	<u>Source (Reference)</u>
NCIB3610	<i>Bacillus subtilis</i> 3610	Wild type	Shank lab collection
ES418	<i>Pantoea agglomerans</i>	Wild type	Shank lab collection
ES1154	<i>Bacillus subtilis</i> 3610	<i>tasA::kan</i> <sup>R</sup> ; <i>P<sub>tapA</sub>-yfp</i>	This study
ES6	<i>Bacillus subtilis</i> 3610	<i>epsA-O::tet</i> ; <i>amyE::P<sub>tapA</sub>-yfp</i>	Shank lab collection
ES787	<i>Bacillus subtilis</i> 3610	<i>bsIA::erm</i> <sup>R</sup>	<i>Bacillus</i> Genus Stock Center
ES150 (SSB46)	<i>Bacillus subtilis</i> 3610	<i>srfA::erm</i> <sup>R</sup>	Shank lab collection [5]
ES148 (HV1152)	<i>Bacillus subtilis</i> 3610	<i>hag::tet</i> <sup>R</sup> ; <i>amyE::P<sub>tapA</sub>-yfp</i>	Shank lab collection
ES105	<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> (TU-B-0)	Wild type	<i>Bacillus</i> Genus Stock Center
ES107	<i>Bacillus mojavensis</i> (ROH-1)	Wild type	<i>Bacillus</i> Genus Stock Center
ES108	<i>Bacillus atrophaeus</i> (BGSC10-11A1)	Wild type	<i>Bacillus</i> Genus Stock Center
ES109	<i>Bacillus pumilus</i> (ATCC7061)	Wild type	<i>Bacillus</i> Genus Stock Center
ES111	<i>Bacillus subtilis</i> subsp. <i>natto</i>	Wild type	<i>Bacillus</i> Genus Stock Center
ES113	<i>Bacillus vallismortis</i> (DV1-F-3)	Wild type	<i>Bacillus</i> Genus Stock Center
ES329	<i>Bacillus thuringiensis</i> subsp. <i>Darmstadiensis</i> (BGC4M6)	Wild type	<i>Bacillus</i> Genus Stock Center
ES330	<i>Bacillus licheniformis</i> (ATCC14580)	Wild type	<i>Bacillus</i> Genus Stock Center
ES331	<i>Bacillus mycoides</i> (BGSC6A47)	Wild type	<i>Bacillus</i> Genus Stock Center
ES332	<i>Bacillus weihenstephanensis</i> (BGSC6A50)	Wild type	<i>Bacillus</i> Genus Stock Center
ES334	<i>Bacillus amyloliquefaciens</i> (BGSC10A5)	Wild type	<i>Bacillus</i> Genus Stock Center
ES348	<i>Bacillus cereus</i> (ATCC14579)	Wild type	<i>Bacillus</i> Genus Stock Center
ES747	<i>Bacillus subtilis</i> 3610	<i>amyE::P<sub>spacC</sub>-mTurq cam</i> <sup>R</sup>	This study

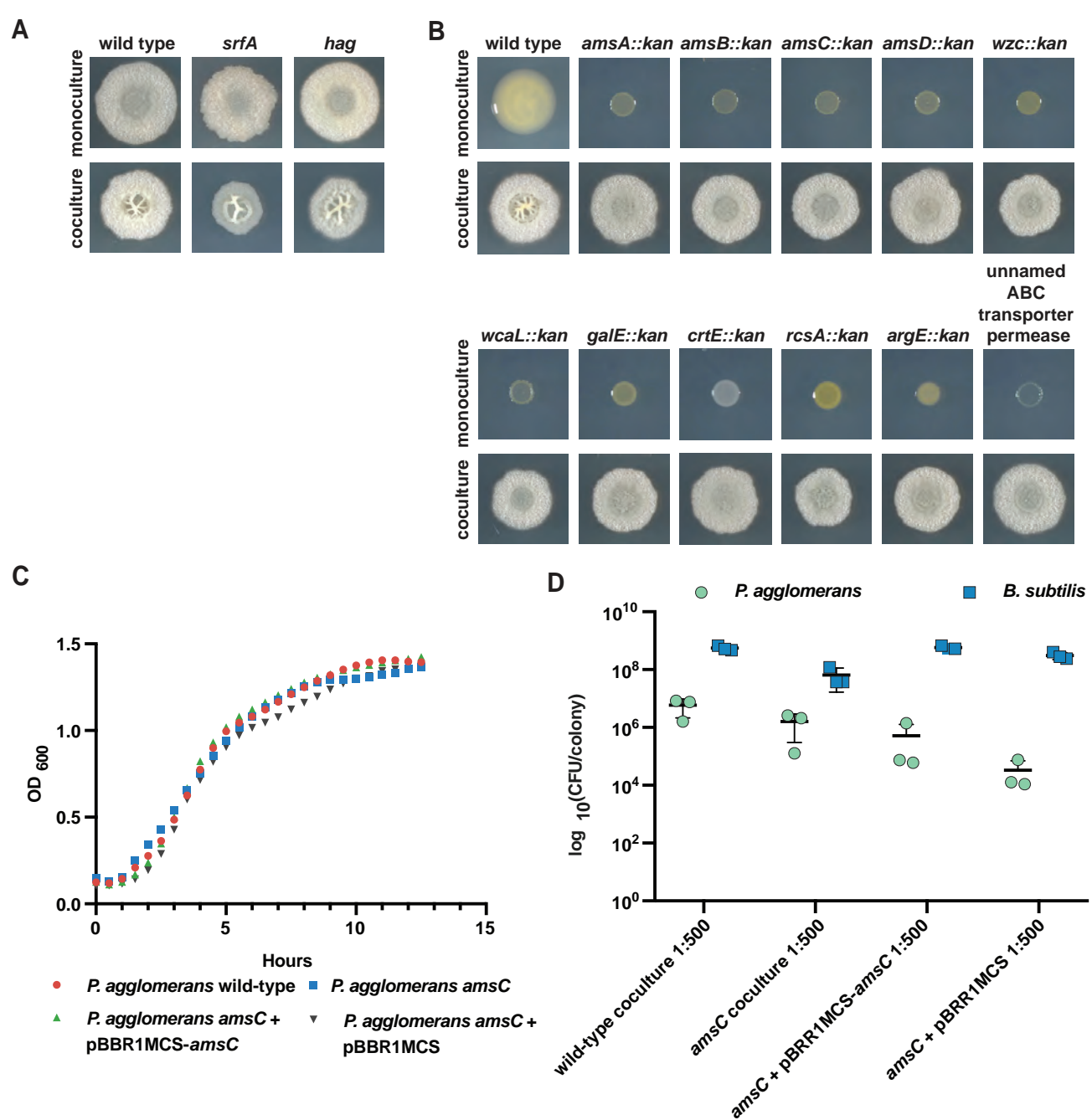
ES943	<i>Escherichia coli</i>	F' delta lac169 rpoS(Am) robA1 creC510 hsdR514endA recA1 u <i>Arabidopsis</i>	Biological Resource Center
ES1261	<i>Pantoea agglomerans</i>	<i>asmA::kan<sup>R</sup></i>	This study
ES1262	<i>Pantoea agglomerans</i>	<i>galE::kan<sup>R</sup></i>	This study
ES1263	<i>Pantoea agglomerans</i>	<i>wcaL::kan<sup>R</sup></i>	This study
ES1264	<i>Pantoea agglomerans</i>	<i>rcaA::kan<sup>R</sup></i>	This study
ES1265	<i>Pantoea agglomerans</i>	ABC transporter permease:: <i>kan<sup>R</sup></i>	This study
ES1266	<i>Pantoea agglomerans</i>	<i>argE::kan<sup>R</sup></i>	This study
ES1267	<i>Pantoea agglomerans</i>	<i>wzc::kan<sup>R</sup></i>	This study
ES1268	<i>Pantoea agglomerans</i>	<i>amsC::kan<sup>R</sup></i>	This study
ES1269	<i>Pantoea agglomerans</i>	<i>amsD::kan<sup>R</sup></i>	This study
ES1270	<i>Pantoea agglomerans</i>	<i>crtE::kan<sup>R</sup></i>	This study
ES1271	<i>Pantoea agglomerans</i>	<i>amsB::kan<sup>R</sup></i>	This study
ES1272	<i>Pantoea agglomerans</i>	<i>amsC::kan<sup>R</sup></i>	This study
ES1147 (DS322)	<i>Bacillus subtilis</i> 3610	<i>flgM::tet<sup>R</sup></i>	Kearns lab collection
ES1153 (DS2447)	<i>Bacillus subtilis</i> 3610	<i>lytA-C::spec<sup>R</sup>, lytD::mIs<sup>R</sup>, lytF::tet<sup>R</sup>, P<sub>hag</sub>-yfp cam<sup>R</sup></i>	Kearns lab collection [6]
ES1172	<i>Bacillus subtilis</i> 3610	<i>flgM::tet<sup>R</sup>, tasA::kan<sup>R</sup></i>	This study
ES2103	<i>Bacillus subtilis</i> 3610	<i>flgM::tet<sup>R</sup>, P<sub>spacC</sub>-mTurq cam<sup>R</sup></i>	This study
ES2104	<i>Bacillus subtilis</i> 3610	<i>lytA-C::spec<sup>R</sup>, lytD::mIs<sup>R</sup>, lytF::tet<sup>R</sup>, P<sub>spacC</sub>-mTurq cam<sup>R</sup></i>	This study
ES2088	<i>Pantoea agglomerans</i>	pES045, <i>amyE::P<sub>spacC</sub>-YPet cam<sup>R</sup></i>	This study
ES2125	<i>Pantoea agglomerans</i>	<i>amsC</i>	This study
ES2105	<i>Pantoea agglomerans</i>	<i>amsC, pBBR1MCS-amsC, cam<sup>R</sup></i>	This study
ES2120	<i>Pantoea agglomerans</i>	<i>amsC, pBBR1MCS, cam<sup>R</sup></i>	This study



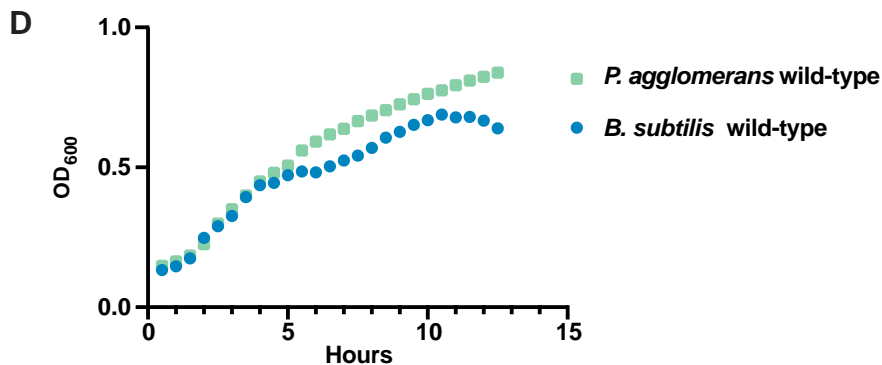
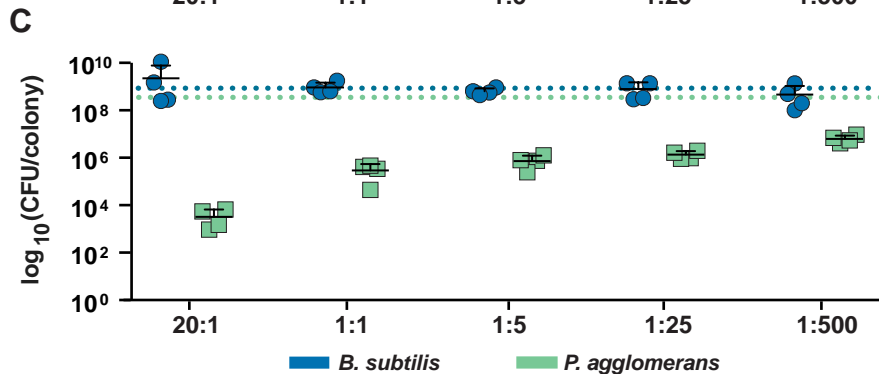
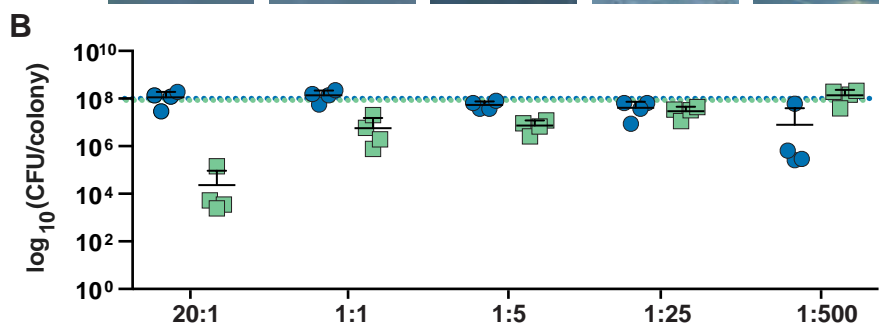
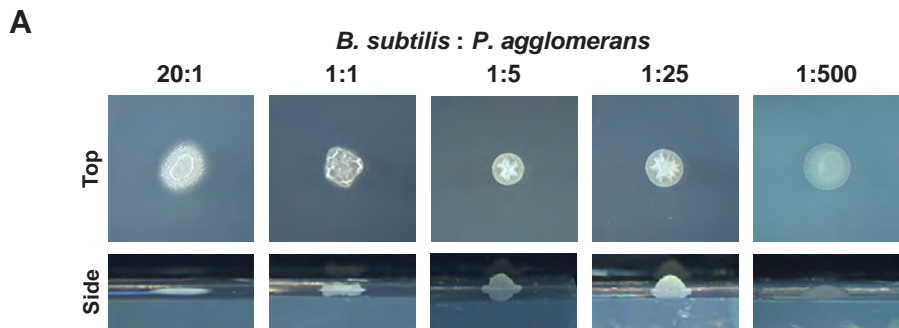
**Figure S1.** (A) Colony morphology of *B. subtilis* and *P. agglomerans* in monoculture and coculture after 24 h of growth on biofilm-inducing medium MSgg. Scale bar is 1 mm. (B) *B. subtilis* monoculture (left) in coculture with heat-killed *P. agglomerans* (middle) and heat-killed *P. agglomerans* (right) grown on MSgg for 24 h and 48 h. Scale bar is 1 mm.



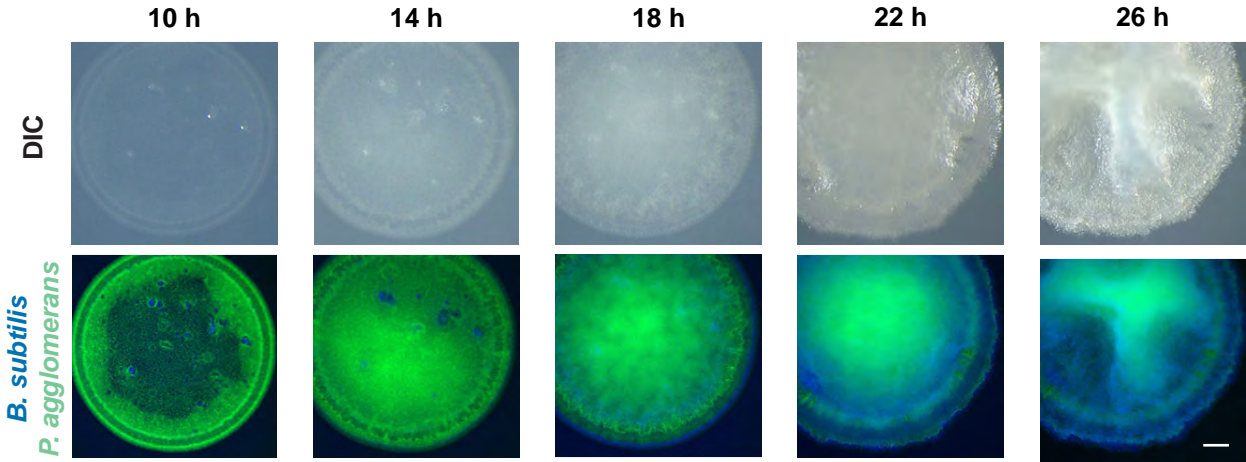
**Figure S2.** Multiple *Bacillus* species form a wrinkled biofilm structure in coculture with *P. agglomerans*. Phylogenetic tree of *Bacillus* species along with images of their colony morphology in monoculture and in coculture with *P. agglomerans* grown on MSgg for 48 h. In the 'wrinkle formation' column, (+) indicates the formation of wrinkles in coculture and (-) indicates no wrinkles in coculture. In the 'TasA homology' column, the number indicates percent amino acid identity between the *B. subtilis* protein TasA and the BLAST-identified gene in the other *Bacillus* species that shows the closest homology to TasA. Numbers marked with an (\*) indicate that this protein is annotated as the cell division protein FtsN in those bacterial genomes.



**Figure S3.** (A) *B. subtilis* wild-type and *srfA* and *hag* mutants in monoculture (top) and in coculture with *P. agglomerans* (bottom) grown on MSg for 48 h (B) *P. agglomerans* mutants containing transposon insertions grown in monoculture (top) and mixed with *B. subtilis* in coculture (bottom) that do not form the characteristic biofilm phenotype when grown on MSg for 48 h. Scale bar is 1 mm. (C) Growth curve of *P. agglomerans* strains (wild-type, *amsC*, *amsC* + pBRR1MCS-*amsC* (complemented *amsC*), and *amsC* + pBRR1MCS (empty vector control) grown in shaking LB liquid culture at 30 °C. (D) CFU/colony of coculture colonies at 1:500 initial *B. subtilis* : *P. agglomerans* ratio harvested at 48 h.

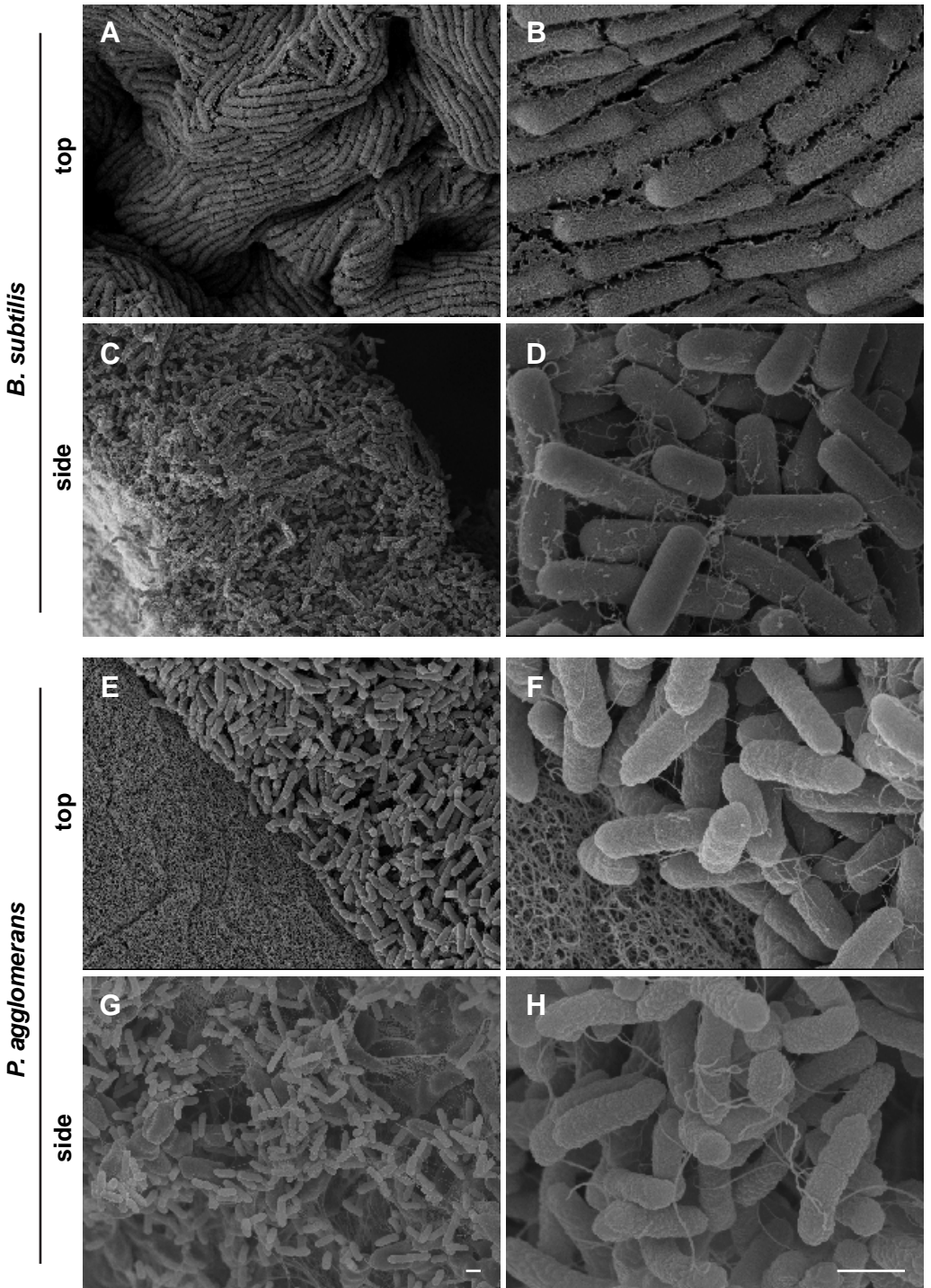


**Figure S4.** Images and CFU/colony quantification of *B. subtilis* and *P. agglomerans* at varying initial ratios in coculture biofilm. (A) Top and side view of *B. subtilis* and *P. agglomerans* coculture biofilms with increasing initial ratios of *P. agglomerans* grown on MSgg for 24 h at 24 °C. (B) CFU/colony of coculture colonies at noted initial ratios harvested at 24 h. Blue and green dotted lines represent CFU/colony of *B. subtilis* monoculture and *P. agglomerans* monoculture harvested at 24 h, respectively. (C) CFU/colony of coculture colonies at noted initial ratios harvested at 48 h. Blue and green dotted lines represent CFU/colony of *B. subtilis* monoculture and *P. agglomerans* monoculture harvested at 48 h, respectively. Error bars indicate standard deviation. (D) Growth curve of monoculture *P. agglomerans* and *B. subtilis* wild-type strains in shaking MSgg liquid culture at 24 °C.



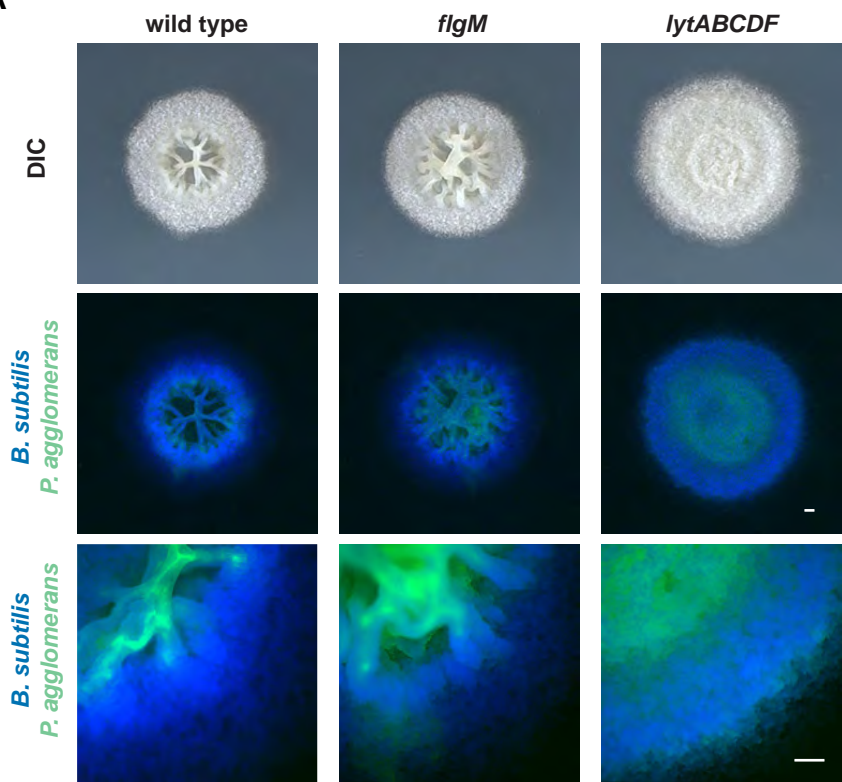
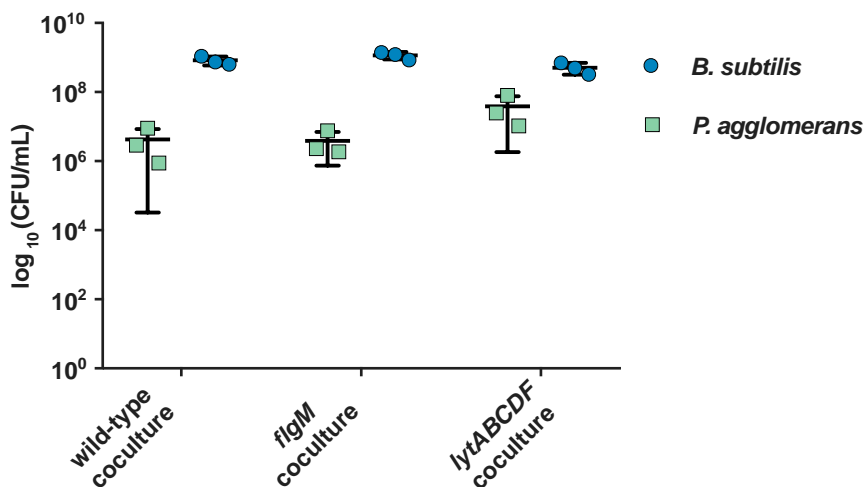
**Figure S5.** Development of a *B. subtilis* and *P. agglomerans* coculture biofilm. *B. subtilis*  $P_{\text{spacC}}\text{-mTurq}$  (false-colored blue) and *P. agglomerans*  $P_{\text{spacC}}\text{-Ypet}$  (false-colored green) in coculture grown on MSgg and imaged over time. Colocalization of two species is indicated in cyan. Phase contrast (top) and fluorescence (bottom) coculture images at 8X magnification. Scale bar is 0.5 mm.

monoculture

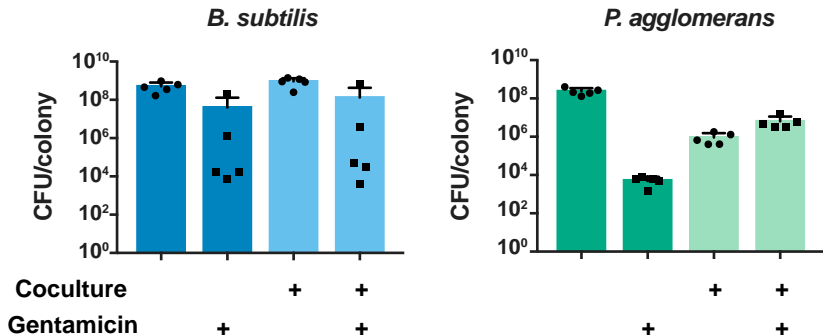


**Figure S6.** Scanning electron microscopy of monoculture *B. subtilis* and *P. agglomerans* biofilms. Top (A, B) and side (D, E) views of *B. subtilis* monoculture colony. Top (E, F) and side (G, H) views of *P. agglomerans* monoculture colony. Scale bars are 1  $\mu\text{m}$  and apply to all images within that column.



**A****B**

**Figure S7.** Colony morphology and localization of *B. subtilis* *flgM* and *lytABCDF* and *P. agglomerans* in coculture. (A) *B. subtilis* (false-colored blue) and *P. agglomerans* (false-colored green) in coculture at increasing initial ratios of *P. agglomerans* grown on MSgg after 48 h of growth. Colocalization of two species is indicated in cyan. Coculture images of phase contrast (top) at 1X magnification, fluorescence (middle) at 1X magnification, and fluorescence (bottom) at 8X magnification. Scale bars are 0.5 mm. (B) CFU/colony of *B. subtilis* wildtype, *flgM*, and *lytABCDF* mutants in coculture with *P. agglomerans* grown on MSgg for 48 h. There are no statistically significant differences between CFU/colony measured for each of the two bacterial species.



**Figure S8.** CFU/colony counts of *B. subtilis* monoculture (dark blue), *B. subtilis* coculture (light blue), *P. agglomerans* monoculture (dark green), and *P. agglomerans* coculture (light green) biofilms either untreated or treated (indicated by +) with gentamicin for 24 h after 24 h of growth. Untreated and treated colonies were harvested 48 h after cultures were initially spotted. Error bars indicate standard deviation. \*,  $P < 0.05$ . Statistical significance was determined by a two-tailed T-test.

### Supplemental References

1. Merriam JJ, Mathur R, Maxfield-Boumil R, Isberg RR. 1997. Analysis of the *Legionella pneumophila flil* gene: intracellular growth of a defined mutant defective for flagellum biosynthesis. *Infect Immun* 65:2497–501.
2. Kovach ME, Phillips RW, Elzer PH, Roop RM, Peterson KM. 1994. pBBR1MCS: a broad-host-range cloning vector. *Biotechniques* 16:800–2.
3. Wetmore KM, Price MN, Waters RJ, Lamson JS, He J, Hoover CA, Blow MJ, Bristow J, Butland G, Arkin AP, Deutschbauer A. 2015. Rapid quantification of mutant fitness in diverse bacteria by sequencing randomly bar-coded transposons. *MBio* 6:e00306-15.
4. Guérout-Fleury A-M, Shazand K, Frandsen N, Stragier P. 1995. Antibiotic-resistance cassettes for *Bacillus subtilis*. *Gene* 1:335–336.
5. Branda SS, González-Pastor JE, Ben-Yehuda S, Losick R, Kolter R. 2001. Fruiting body formation by *Bacillus subtilis*. *Proc Natl Acad Sci USA* 98:11621–11626.
6. Cozy LM, Kearns DB. 2010. Gene position in a long operon governs motility development in *Bacillus subtilis*. *Mol Microbiol* 76:273–285.

**Supplemental Movie 1.** Spatial organization of *B. subtilis* and *P. agglomerans* within a coculture biofilm. *B. subtilis* P<sub>spacC</sub>-mTurq (false-colored blue) and *P. agglomerans* P<sub>spacC</sub>-Ypet (false-colored yellow) grown in coculture on glass coverslips embedded in MSgg agar and imaged at 48 h using confocal laser scanning microscopy. The Z-stack series is composed of 12-overlapping Z-stacks taken upward from the agar surface.