Supplementary methods

**Construction of surfactin reporter**

psrfAA-GFP was constructed by replacing the *rrnB* gene with the *srfAA* promoter in the pGFP-rrnB vector using1 prolonged overlap extension PCR (POE-PCR) 2. *B. subtilis srfAA* promoter region was PCR-amplified with the primers srfAA forward (5`AGCTGTCAAACATGAGAATTGAAAGAATCGTTGTAAGACGC 3`) and srfAA reverse (5` AGTTCTTCTCCTTTGCTAGCTTATTTCCATATTGTCATACCTCC 3`) from P5\_B1. pGFP-rrnB was linearized via PCR with pGFP forward

(5` GTATGACAATATGGAAATAAGCTAGCAAAGGAGAAGAACT 3`) and pGFP reverse (5` CGTCTTACAACGATTCTTTCAATTCTCATGTTTGACAGCTT 3`). The reaction mixture was 1x Q5 reaction buffer, 1x Q5 DNA polymerase, 200 µM dNTP, 4 ng/µL vector and the equimolar amount of the promoter DNA in a total volume of 50 µL. The PCR program was 98oC denaturation 30 s, 30x cycles of 98oC denaturation 15 s, 60oC annealing 30 s, 72oC extension 4:30 min, followed by 5 min extension at 72oC and cooling to 4oC. POE-PCR product was directly transformed into competent *E. coli*, from which the construct was introduced into *B. subtilis* P5\_B1 at the *amyE* locus using natural competence, selecting for Chl resistance and verified by Sanger sequencing.

Table S 1. List of strains used in this study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
|  | **Strain** | **Description** | | **Reference** |  |
|  | P5\_B1 | *B. subtilis* soil isolate from sampling site 55.788800, 12.558300 | | 3 |  |
|  | DTUB38 | P5\_B1 *amyE*::Phyperspank-*gfp* (ChlR) | | 4 |  |
|  | DTUB186 | P5\_B1 *amyE*::Phyperspank-*gfp* (ChlR); *sfp*::mls | |  |
|  | DTUB148 | P5\_B1 *amyE*::Phyperspank-*gfp* (ChlR); *srfAC*::Tn*10* (SpecR) | |  |
|  | DTUB187 | P5\_B1 *amyE*::Phyperspank-*gfp* (ChlR); D*ppsC* (TetR) | |  |
|  | DTUB188 | P5\_B1 *amyE*::Phyperspank-*gfp* (ChlR); D*pksL* (ChlR) | |  |
|  |  | P5\_B1 *amyE*::Phyperspank-*mKate* (SpecR) | |  |
|  |  | P5\_B1 *amyE*::*PsrfAA*-*gfp* (ChlR) | | This study |  |
|  | D749 | *Pedobacter* sp. soil isolate from sampling site 55.788800, 12.558300 | **CemiSt SynCom** | 5 |  |
|  | D757 | *Rhodococcus globerulus s*oil isolate from sampling site 55.788800, 12.558300 |  |
|  | D763 | *Stenothrophomonas indicatrix* soil isolate from sampling site 55.788800, 12.558300 |  |
|  | D764 | *Chryseobacterium* sp. soil isolate from sampling site 55.788800, 12.558300 |  |
|  | Sr | *Stenotrophomonas rhizophila* | **SPMX SynCom** | 6 |  |
|  | Pa | *Paenibacillus amylolyticus* |  |
|  | Mo | *Microbacterium oxydans* |  |
|  | Xr | *Xanthomonas retroflexus* |  |
|  | MWF001 | *Agrobacterium tumefaciens* | **MWF SynCom** | 7,8 |  |
|  | *Comamonas testosteroni* |  |
|  | *Microbacterium saperdae* |  |
|  | *Ochrobactrum anthropi* |  |
|  | UW85 | *Bacillus cereus* | **Thor SynCom** | 9 |  |
|  | UW101 | *Flavobacterium johnsoniae* |  |
|  | CI12 | *Pseudomonas koreensis* |  |
|  | XL380 | *Acinetobacter baumanni* | **Nanjing University SynCom** | 10 |  |
|  | XL97 | *Chryseobacterium rhizoplanae* |  |
|  | XL95 | *Enterobacter ludwigii* |  |
|  | XL123 | *Pantoea eucrina* |  |
|  | XL272 | *Pseudomonas stutzeri* |  |
|  | WLL | *Comamonas odontotermitis* |  |
|  | XL73 | *Burkholderia contaminans* |  |
|  |  | *Enterobacter cloacae* | **Kolter Lab SynCom** | 11 |  |
|  |  | *Stenotrophomonas maltophilia* |  |
|  |  | *Ochrobactrum pituitosum* |  |
|  |  | *Herbaspirillum frisingense* |  |
|  |  | *Pseudomonas putida* |  |
|  |  | *Curtobacterium pusillum* |  |
|  |  | *Chryseobacterium indologene* |  |
|  |  |  |  |  |  |

A diagram of a color chart

Description automatically generated

Fig S10**. Microtiter plate setup for the invasion test**. 200 µL of each SynCom were inoculated in the first row A. From that row, the SynCom member were 10-fold diluted by transferring 20 µL of culture to the next row containing 180 µL of medium. 6 dilution steps were made. Subsequently, 20 µL of *B. subtilis* the gfp-labelledvariants were added to each well to conform the co-culture. Rows G-H were dedicated to the two *Bacillus* strains and the individual members of each SynCom. Both *Bacillus* strains were cultured as a quadruplicate, and members were cultured as a quadruplicate when possible and a triplicate when not.

References for Supplementary materials

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