The plant host induces antibiotic production to select for most beneficial colonizers

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Supplementary information

Content:

Supporting Figures (S1-S9)

Supporting Table S1

Supporting Figures

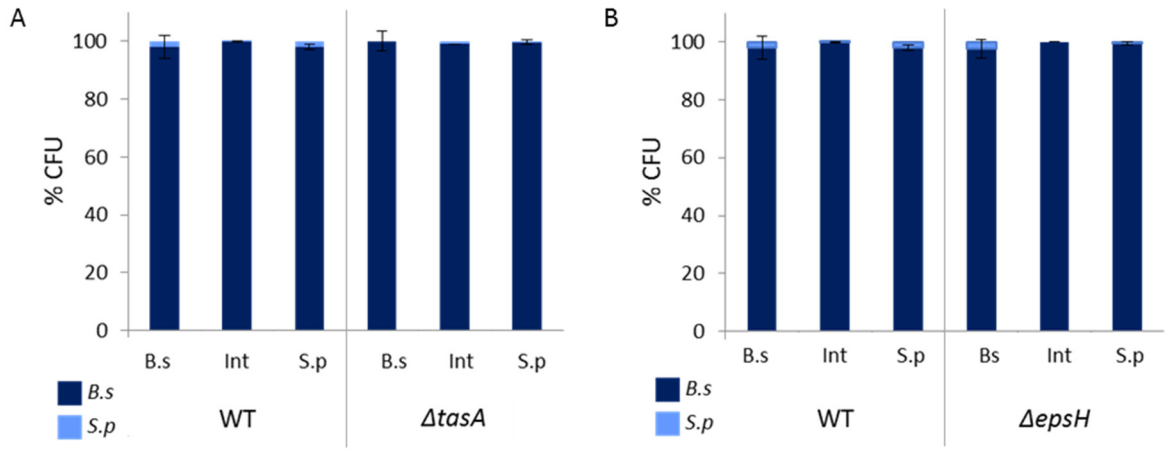

Figure S1: Extracellular matrix is not required to eliminate *S. plymuthica* cells.

Relative CFU counts at 48 – 96 hr after inoculation. Strains were inoculated at 0.5 cm (A) and 0.3 cm (B) distance to overcome the expansion defect of ECM mutants. The interacting colonies were divided into three areas as follows: *B.s* – the area of the *B. subtilis* colony most distant from the interaction area; *Int* – the area of direct interaction; *S.p* – the area of the *S. plymuthica* colony most distant from the interaction area. Once a direct contact was established, each section was separately harvested, sonicated and plated to determine the number of replicative cells of each species. The experiment was repeated at 24 hrs (no contact), 48 hrs (direct contact), and 72 hrs (covered) after inoculation. All experiments were performed at least 3 times, with at least three technical repeats, a representative experiment is shown.

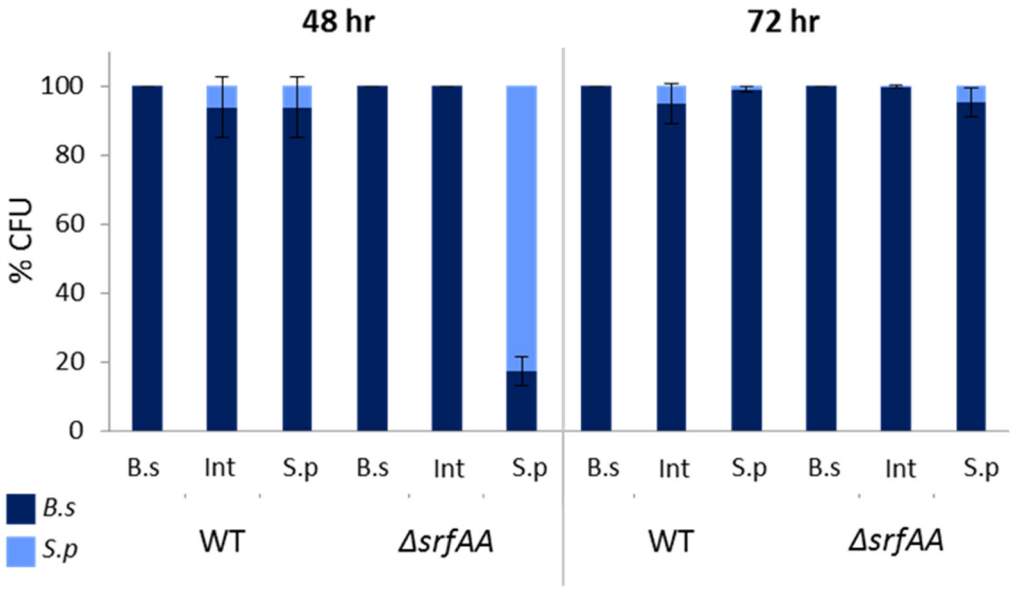

Figure S2: Surfactin has a minor role in the elimination of *S. plymuthica* cells. Relative CFU counts at 48 hrs and 72 hr after inoculation. Strains were inoculated at 0.3 cm distance to overcome the expansion defect of the surfactin mutant. The interacting colonies were divided into three areas as follows: *B.s* – the area of the *B. subtilis* colony most distant from the interaction area; *Int* – the area of direct interaction; *S.p* – the area of the *S. plymuthica* colony most distant from the interaction area. Once a direct contact was established, each section was separately harvested, sonicated and plated to determine the number of replicative cells of each species. The experiment was repeated at 24 hrs (no contact), 48 hrs (direct contact), and 72 hrs (covered) after inoculation. All experiments were performed at least 3 times, with at least three technical repeats, a representative experiment is shown.

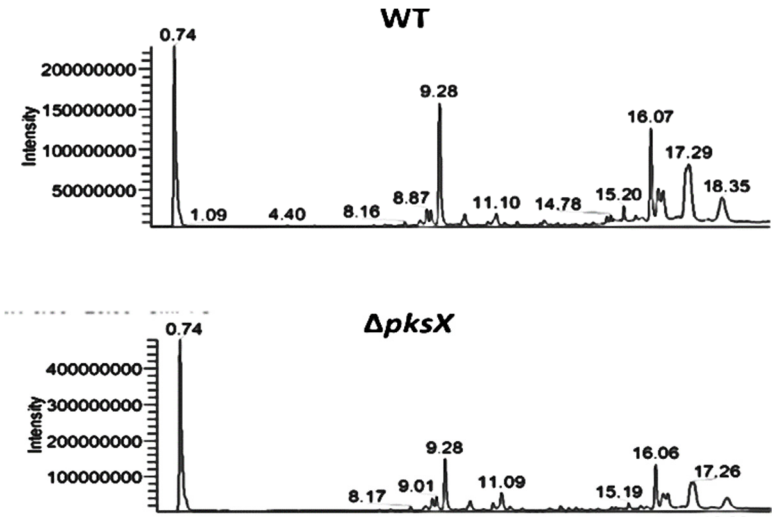

Figure S3. Full chromatogram of a wild type (right panel) and Δ*pksA-R* (left panel) biofilm colonies extracted with isopropanol obtained by liquid chromatography-MS.

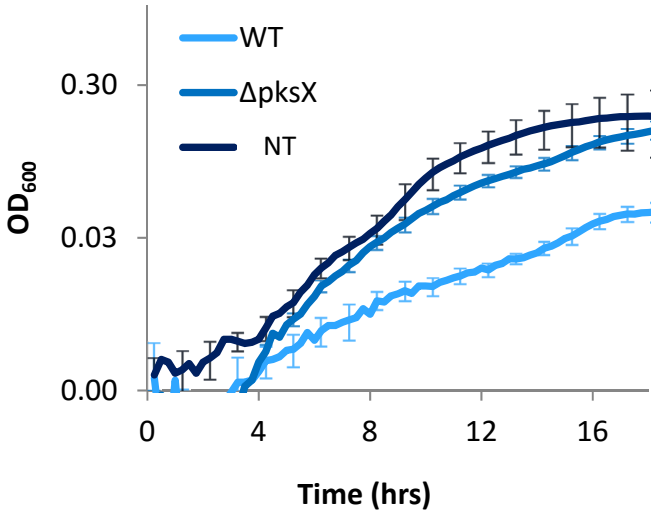

Figure S4. *pks* is required for the activity of *B. subtilis* colony extract versus *S. plymuthica.* Growth curves of *S. plymuthica* in liquid biofilm medium either untreated (NT) or supplemented with extraction of *B. subtilis* exudate. Active extracts were extracted directly from under a *B. subtilis* WT or Δ*pks* biofilms as indicated, grown on a solid biofilm medium (for details refer to Materials and Methods).

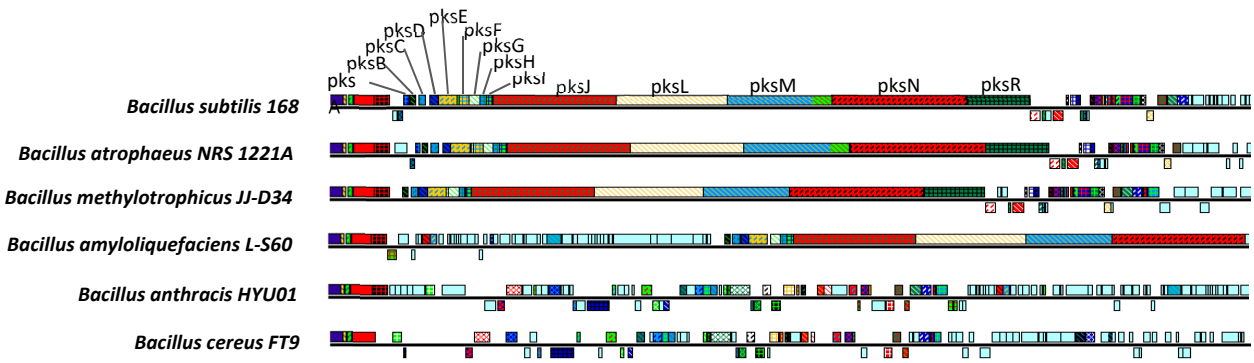

Figure S5 – *pks* operon is conserved among soil-dwelling *Bacillus* species.

Genome comparisons between *Bacillus subtilis 168, Bacillus atrophaeus NRS 1221A*, *Bacillus amyloliquefaciens L-S60* and *Bacillus methylotrophicus JJ-D34* reveals a conservation of the entire *pks* operon (*pksA-R*). *Bacillus anthracis HYU01* and *Bacillus cereus FT9* strains lacked the *pks* operon altogether. Orthologous genes were tagged by common colors across the different strains.

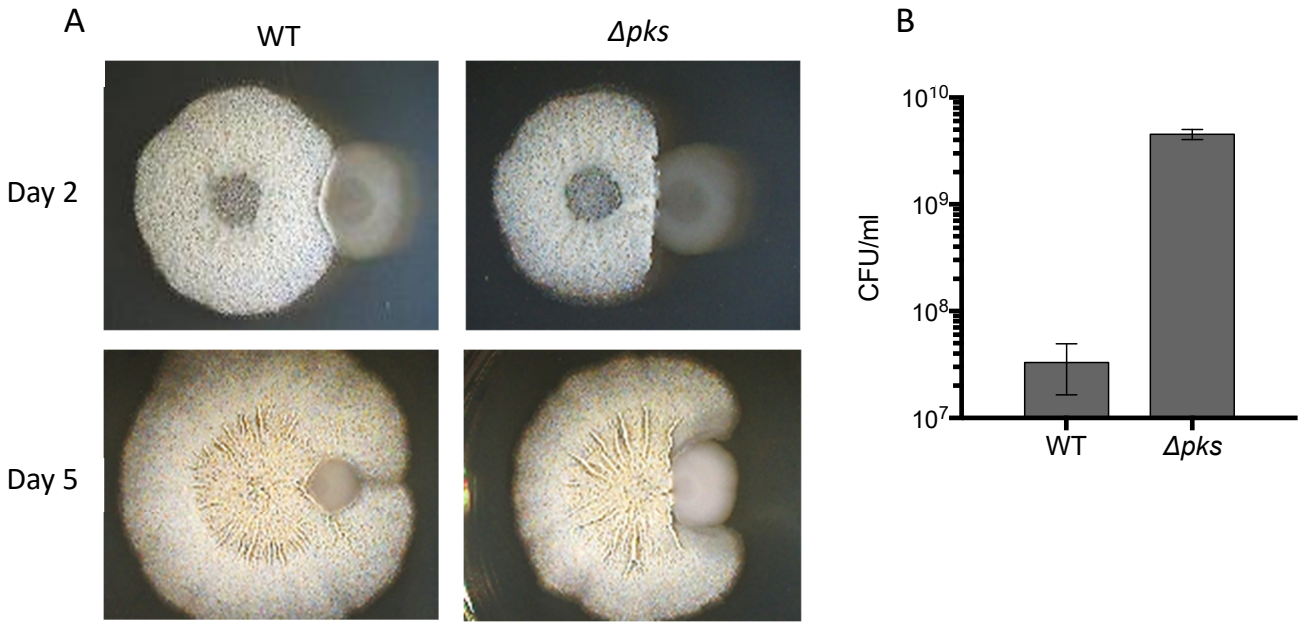

Figure S6: Polyketides synthesis is required for engulfment and outcompeting *Pseudomonas chlororaphis* during competition.

A. PKS-depended engulfment of *P. chlororaphis* by *B. subtilis*. *P. chlororaphis* (PC-449) biofilm inoculated next to WT *B. subtilis* or Δ*pks* strains at 30ºC for indicated times

B. The number of *P. chlororaphis* replicative cells at 72 hours after inoculated next to WT *B. subtilis* or Δ*pks* strains. Error bars represents ±S.D of 5 biological replicates.

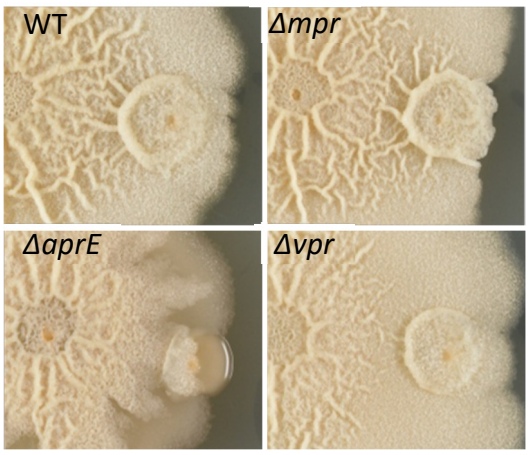

Figure S7: Extracellular protease AprE contributes to the elimination of *S. plymuthica.*

Indicated extracellular protease mutants were inoculated at a distance of 0.8 cm from *S. plymuthica.* In the absence of *aprE*, but not *mpr* nor *vpr*, a clear consumption defect was observed. Top-down images were taken 72 hrs after inoculation.

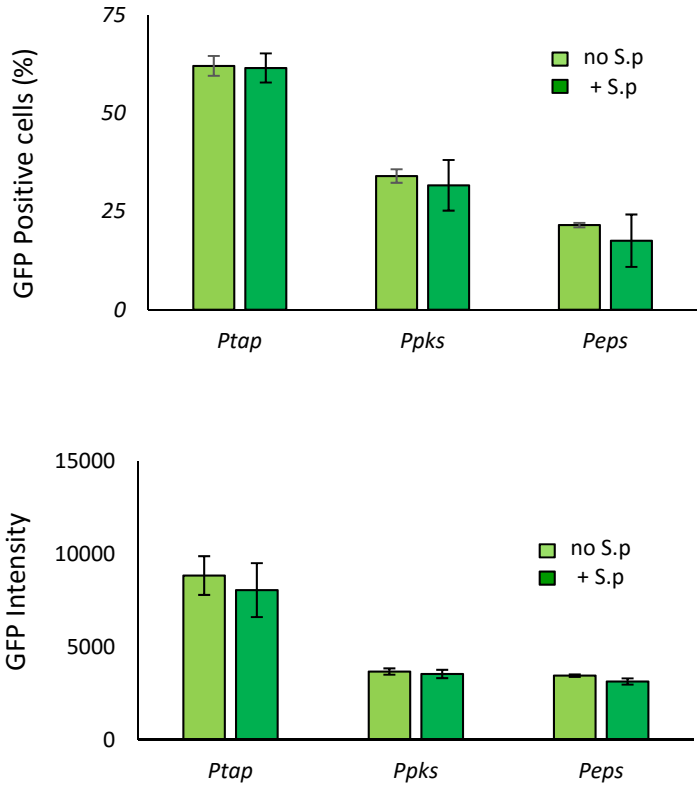

Figure S8: *S. plymuthica* does not induce the expression of *B. subtilis tasA*, *pks and eps*.

S. plymuthica was inoculated next to *B. subtilis* strains carrying two reporters: constitutively expressed mKate2, and GFP driven by the *tapA*, *pksC* or *eps* promoter (as indicated in the figure). After 48 hrs of incubation at 30°C, cells were harvested either from the area of direct interaction (+S.p) or a control area on the opposite side of the colony (no S.p). Cells were sonicated and analyzed by FACS. Only mKate-positive cells were chosen for analysis of GFP expression. Upper panel - percent of *B. subtilis* cells expressing GFP. Lower panel - mean GFP intensity of GFP-positive cells. The results presented are average of 6 repeats from three independent experiments. Bars represent standard deviation.

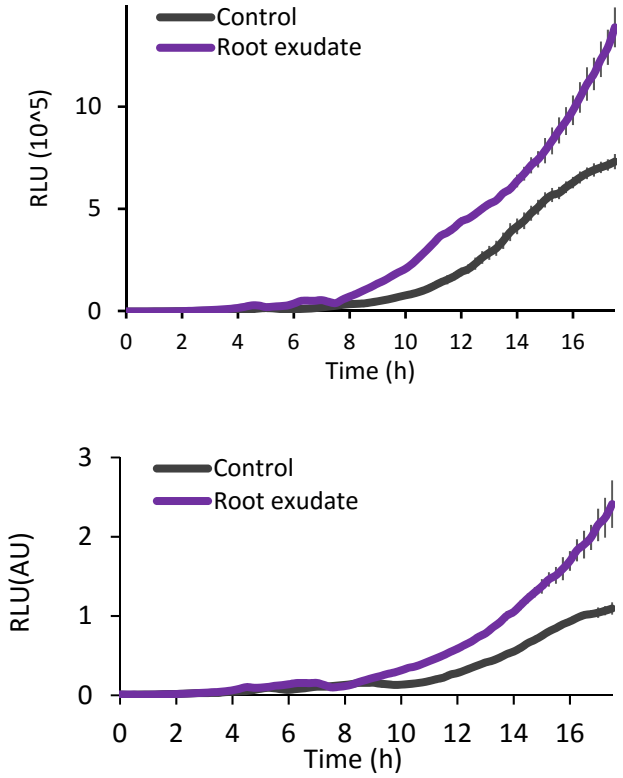

Figure S9: Root exudate induces the expression of *sdp* and *sinI*.

Growth-normalized RLU (in 10^5) of (A) P_{sdp}-lux strain and (B) P_{sinI}-lux treated with root exudate or plant growth medium. The results presented are average of 4 repeats from three independent experiments. Bars represent standard deviation.

Supporting Tables

Table S1: Separating killing from asymmetric expansion

Strain	Minimal distance to obtain direct contact (cm)	Killing time (days)
WT	0.8	2-3
Δpks(A-R)	0.8	-
Δpks(A-E)	0.8	-
ΔaprE	0.8	3-4
ΔsrfAA	0.3	2-4
ΔepsH	0.3	2-3
ΔtasA	0.5	2

Summarized are the killing versus expansion results for the indicated strains. Results were collected reproducibly over at six-twelve independent repeats performed with at-least duplicates. Both a truncated deletion of the *pks* operon (*ΔpksA-E*) and the deletion of the entire operon (*ΔpksA-R*) were incapable to eliminate *S. plymuthica*.