This PDF file includes:

**Supplementary Methods**

*In vitro* biocontrol assay

A standard co-inoculation technique was performed to determine the ability of isolates to inhibit growth of a fungal phytopathogen, *Botrytis cinerea* (strain Bcl16), and bacterial pathogen *Xanthomonas euvesicatoria* strain 85-10 (*Xcv*) [1, 2]. *Botrytis cinerea* was cultured on potato dextrose agar (PDA) at 22ºC for 5 days. A 5 mm diameter mycelial disc was cut from a 5-days-old *B. cinerea* colony and placed on one side of a dual media (PDA and LB media, 1:1) agar plates and incubated at 22ºC. After 2 days of inoculation, tested bacteria were streaked at opposite ends of the plate and cultured at 28°C for 5 days to examine antagonistic effects of these bacterial strains. The *B. cinerea* mycelia area ($cm^2$) in colony radius compared to *B. cinerea* colony radius in the inoculated agar control plates was calculated.

100 µL of *X. euvesicatoria* suspensions ($10^8$ cells $mL^{-1}$) was mixed with LB Agar (0.6% agar) in poured plates. After solidification, 1 µL of bacterial suspensions ($10^8$ cells $mL^{-1}$) were placed in the center of the agar plate and incubated at 28°C for 24 h. After incubation, inhibition halos were measured in terms of plate opacity (AU). Plates without bacteria served as control.

**Plant immunity assays**

Bacterial treatments

*S. lycopersicum*, cv. M82 seeds were sown after surface sterilization (with 1.5% NaOCl for five minutes, followed by three rinses with sterile water) in a tray containing potting mixture. After germination, a single tomato seedling (21-day-old) was transplanted to each pot (0.5 L, diameter = 10 cm) containing green quality soil mix, Tuff soil, Israel. Pots were kept in the greenhouse at 25±2°C, and 16-h photoperiod. Bacterial colonies of *B. pumilus* R2E, *B. megaterium* 4C, *R. pick*ettii R3C, *P. putida* IN68 and *B. subtilis* SB491 from a 24 h plate culture were washed twice in sterile distilled water, and then re-suspended in a 10 mM $MgCl_2$ solution. The cell suspension was adjusted to an optical density of $OD_{600}$=0.1 (approximately equal to $10^8$ CFU $mL^{-1}$) using a spectrophotometer (Tecan). Soil drenching of the plants was carried out by pouring 10 mL of bacterial suspension into each pot twice a week, for two weeks. Plants treated with sterile distilled water served as controls. Plants were used for pathogen assays and immunity assays.

Pathogen infection

*B. cinerea* (Bcl16) was cultured on PDA in petri dishes incubated at 22°C. Conidia were harvested from 14-day-old cultures and the suspension was then filtered through sterile cheesecloth. The concentration of conidia was determined using a haemocytometer under a light microscope, and adjusted to $10^6$ cells $mL^{-1}$ in solution (0.1% glucose and 0.1% $K_2HPO_4$). Tomato leaflets from bacteria (*B. pumilus* R2E, *B. megaterium* 4C, *R. pick*ettii R3C, *P. putida* IN68 and *B. subtilis* SB491) treated plants were inoculated with two droplets of 10 µL spore suspension. Controls consisted of leaves treated with above-mentioned solution without pathogen. The area of the necrotic lesions on infected leaf tissue was measured 5–10 days post-inoculation using ImageJ.

For *X. euvesicatoria* (*Xcv*) pathogenicity assays, culture was grown in LB medium containing 100 mg $L^{-1}$ of rifampicin and 300 mg $L^{-1}$ of streptomycin, overnight at 28°C [3]. Log phase bacterial cultures were harvested and re-suspended in 10 mM $MgCl_2$, at a final concentration of $10^4$ CFU $mL^{-1}$ ($OD_{600}$=0.0002). The fourth leaf of 4-week-old tomato plants was vacuum immersed with the bacterial suspensions. Three days after infiltration, three leaf discs of 0.9 cm diameter were sampled from the second left lateral leaflet from the infiltrated leaf of at least five plants, and ground in 1 mL of 10 mM $MgCl_2$. *Xcv* CFU were determined by plating and counting the resulting colonies [4]. Negative controls consisted of 10 mM $MgCl_2$ without pathogen inoculation.

*O. neolycopersici* was isolated from young leaves of 5–7 week old tomato plants grown in a commercial greenhouse in the winter of 2019 or 2020. Conidia of the pathogen were collected by rinsing infected leaves with sterile water. Following analysis under a light microscope using a hemocytometer, conidial suspensions were adjusted to $10^4$ $mL^{-1}$, and then sprayed onto 5–6-week old tomato plants (5 mL $plant^{-1}$), with a hand-held spray bottle. Plants were left to dry in an open greenhouse for up to 30 minutes. Suspensions were sprayed within 10–15 minutes of the initial conidia collection. Inoculated plants were kept in a humid growth chamber at 21°C.

Ethylene measurement

For ethylene production, leaf discs 0.9 cm in diameter were harvested from plants individually treated with bacteria, viz., *B. pumilus* R2E, *B. megaterium* 4C, *R. pick*ettii R3C, *P. putida* IN68, and *B. subtilis* SB491 , and average weight was measured for each plant. Discs were washed in water for 1-2 h. Every six discs were sealed in a 10

mL flask containing 1 mL assay medium (with or without 1 µg $mL^{-1}$ of Ethylene-Inducing Xylanase ; EIX) for 4 h at room temperature [5]. Ethylene production was measured by gas chromatography (Varian 3350, Varian, California, USA).

ROS measurement

Leaf discs of 0.5 cm in diameter were harvested from leaves 4-5 of 5 week old Mock and bacteria treated tomato plants, as indicated above. Discs were floated in a white 96-well plate (SPL Life Sciences, Korea) containing 250 µL distilled water for 4–6 h at room temperature. Further, water was removed and a ROS measurement reaction containing 1 mM flg-22 (flagellin peptide 22) or water was added. Light emission was measured for 30 minutes using a luminometer (Tecan Spark, Switzerland) [5] .

RNA preparation and qRT-PCR

RNA was isolated from ground leaf samples of plants individually treated with bacteria *viz.*, *B. pumilus* R2E, *B. megaterium* 4C, *R. pick*ettii R3C, *P. putida* IN68, and *B. subtilis* SB491, using Tri reagent (Sigma-Aldrich) as per the manufacturer's recommendations. RNA concentrations were quantified, and cDNA was then synthesized from 2 µg RNA in a 20 µL reaction, using both reverse transcriptase and oligo(dT) primers provided with the cDNA Synthesis kit (Promega, United States). RT-qPCR was performed according to the Power SYBR Green Master Mix protocol (Life Technologies, Thermo Fisher, United States), using a Rotor-Gene Q machine (Qiagen) detection system. Marker genes for JA, SA and ET signaling pathway: pathogenesis-related proteins (*SlPR1a*), Pto-interacting 5 (*SlPti-5*), 1-aminocyclopropane-1-carboxylate oxidase 1 (*SlACO-1*), Chitinase (*SlChi*), β-1, 3- glucanase (*Slb-Gluc*), and ethylene response factor-1 (*SlERF-1*), as well as the Pattern Recognition Receptors (PRRs) *LeEIX1* (Ethylene-inducing xylanase), and flagellin-sensing2 (*SlFLS2*), were characterized in previous studies (see supplementary Table 1) [6–8]. A geometric mean of the copy number of the three housekeeping genes, ribosomal protein *SlRPL8* (Solyc10g006580), *Slcyclophilin* (Solyc01g111170) and *SlActin* (Solyc03g078400) was used for normalization. All primer efficiencies were in the rage 0.98-1.03 (see supplementary Table 1). Relative expression was calculated using the copy number method for gene expression [9] .

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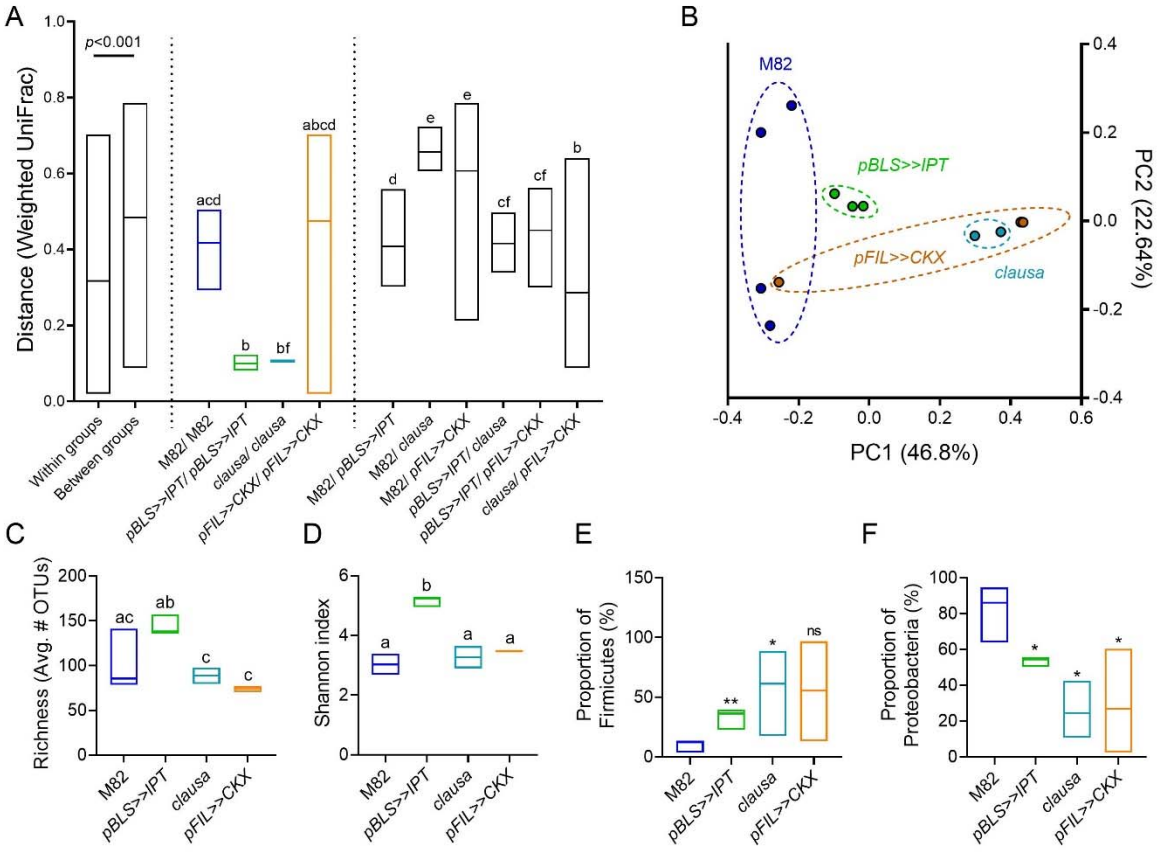


**Figure S1. Endogenous cytokinin supports Gram-positive bacteria in the pyllosphere-summer experiment.**

16S rRNA sequencing of the bacterial phyllosphere of randomly interspersed plants from each indicated genotype, grown in a net house in the summer of 2018, N=3 for each genotype.

**A** Weighted UniFrac beta diversity. Distance is significantly smaller within groups then between groups ($p$<0.001). **B** Principle coordinates analysis of distance between all individual samples in the weighted UniFrac beta diversity calculations. **C** Species richness- alpha diversity. **D** Shannon index. **E** Proportion of Firmicutes in the bacterial community of indicated genotypes. **F** Proportion of Proteobacteria in the bacterial community of indicated genotypes.

Floating bars encompass minimum to maximum values, line indicates mean. Different letters indicate statistically significant differences among samples (A,C,D), and asterisks indicate statically significant differences from M82 (E,F), in a two-tailed t-test with Welch's correction.

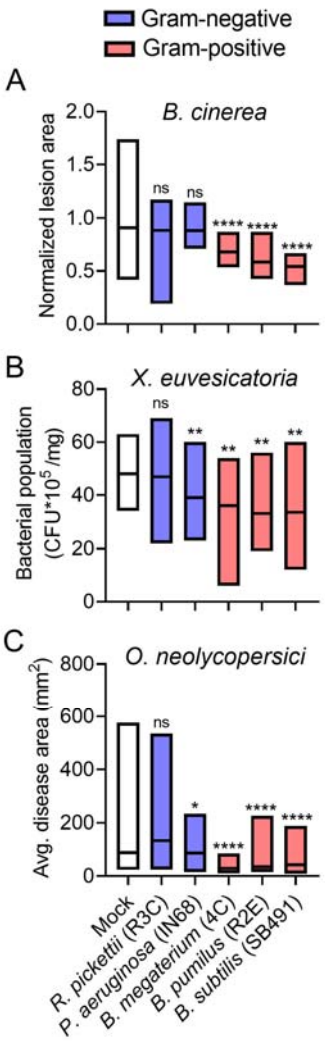


**Figure S2. Gram-positive bacilli isolated from the phyllosphere of high cytokinin genotypes promote disease resistance.**

*S. lycopersicum* cv. M82 plants were pre-treated with indicated bacteria ($OD_{600}$=0.1), and separately challenged with the indicated pathogens 3 days after bacterial treatment.

**A** Lesion area was measured 3 days after inoculation with *B. cinerea* ($10^6$ spores $mL^{-1}$).

**B** Bacterial growth (CFU) was measured 3 days after inoculation with *X. euvesicatoria pv. vesicatoria* ($10^5$ CFU $mL^{-1}$).

**C** Disease area was measured 7 days after inoculation with *O. neolycopersici* ($10^5$ spores $mL^{-1}$).

Floating bars encompass minimum to maximum values, line indicates mean. Asterisks indicate significance from Mock treatment in a one-way ANOVA with a Dunnett (A, C) or Tukey (B) post hoc test. **A** N=12, $p$<0.0001. **B** N=9, $p$<0.01. **C** N=24, $p$<0.02. (*$p$ value<0.05; **$p$ value<0.01; ****$p$ value<0.0001).

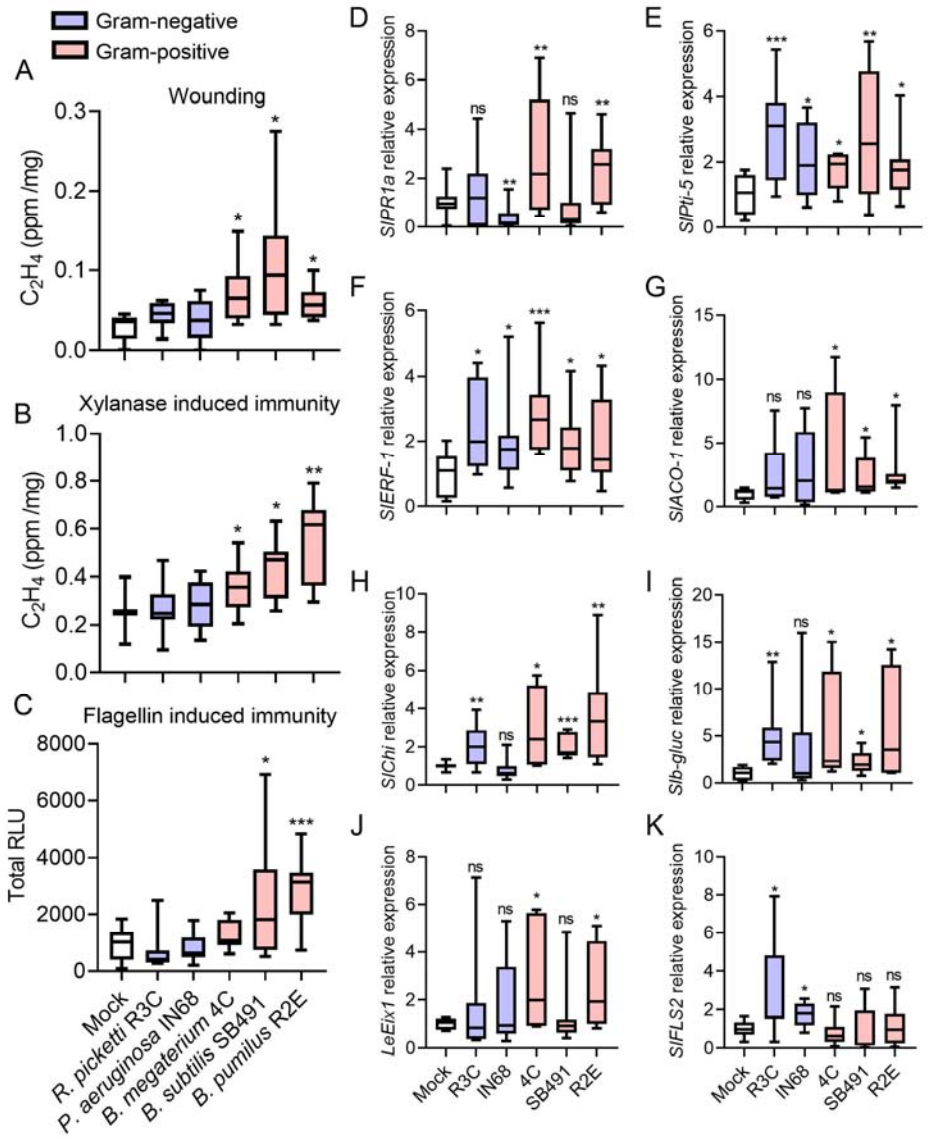


**Figure S3. Gram-positive phyllosphere bacilli isolates promote host immunity.**

*S. lycopersicum* cv. M82 plants were pre-treated with indicated bacteria ($OD_{600}$=0.1), and challenged with wounding or immunity elicitors 3 days after bacterial treatment. **A-B** Ethylene production was measured using gas chromatography. **C** ROS production was measured every 5 minutes for 90 minutes using the HRP luminol method in terms of total RLU (Relative Luminescence Units). **D-K** Gene expression was assayed by qRT-PCR**.**

Box plots indicate inner quartile ranges (box), outer quartile ranges (whiskers), median (line). **A** Asterisks indicate statistical significance from Mock treatment in an unpaired t-test, N=6, $p$<0.041. **B-C** Asterisks indicate significance from Mock treatment in a one-way ANOVA with a Dunnett post hoc test, **B** N=9, $p$<0.036. **C** N=10, $p$<0.0285. (*$p$ value<0.05; **$p$ value<0.01; ***$p$ value<0.001).

**D-K** Genes were normalized to a geometric mean of the expression of 3 normalizers: *SlActin* (Solyc03g078400), *SlCYP* (Solyc01g111170), and *SlRPL8* (Solyc10g006580). **D**- *SlPR1a*; **E**- *SlPti-5*; **F**- *SlERF-1*; **G**- *SlACO-1*; **H**- *SlChi*; **I**- *Slb-Gluc*; **J**- *LeEIX1*; **K**- *SlFLS2*. Asterisks indicate statistical significance from Mock treatment in an unpaired t-test, N=6, $p$<0.05. (*$p$ value<0.05; **$p$ value<0.01; ***$p$ value<0.001; ns- non significant).

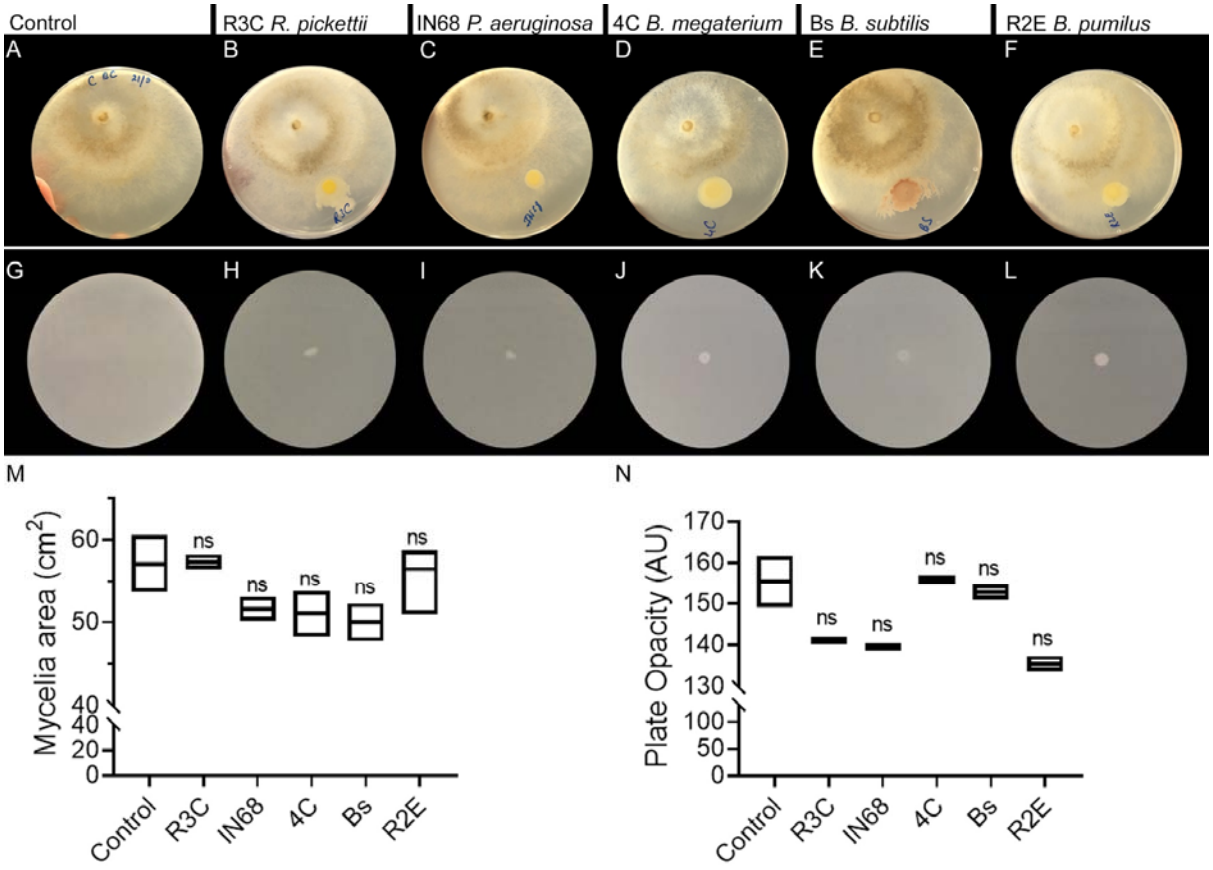


**Figure S4. No direct biocontrol activity of phyllosphere isolated disease resistance promoting microbes on *B. cinerea* or *X. euvesicatoria*.**

Antifungal and antibacterial activity against pathogens *B. cinerea* BcI16 and *X. euvesicatoria pv. vesicatoria* strain 85-10 of the selected bacterial isolates: *R. pickettii* (R3C) **B, H**; *P. aeruginosa* (IN68) **C, I**; *B. megaterium* (4C) **D, J**; *B. subtilis* (Bs, SB491) **E, K**; and *B. pumilus* (R2E) **F, L.**. Pathogens grown on plates without isolated bacteria **(A, G)** served as control.

**M, N** Graphs represent the results of 3 independent experiments ± SE. Floating bars depict minimum to maximum values, line indicates median. Results were analyzed for statistical significance using one way anova with a Bonferroni post hoc test, $p<0.05$ in all cases.

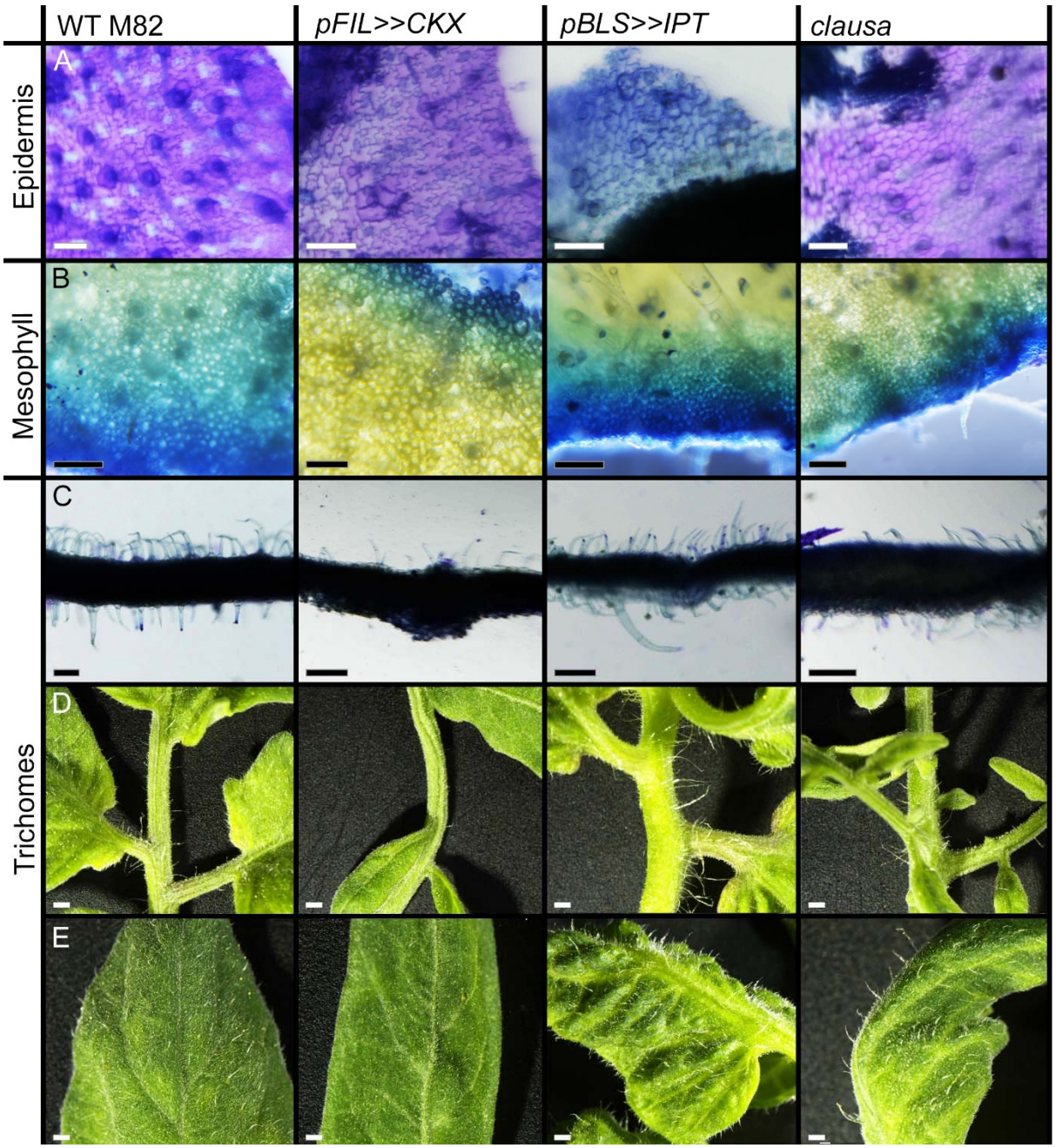


**Figure S5. Leaf morphology of different genotypes.**

**A** Adaxial leaf epidermal cells. **B** Mesophyll cells. **A, B** Leaf epidermal peels were stained for 5 minutes with 1% Toluidine Blue O in acetic acid. Bars= 100 µM. **C-E** Trichome density. **C** Leaf cross-sections, bars= 200 µm. **D** Petiolules, bars= 1000 µm. **E** Terminal leaflets**,** bars= 1000 µm.

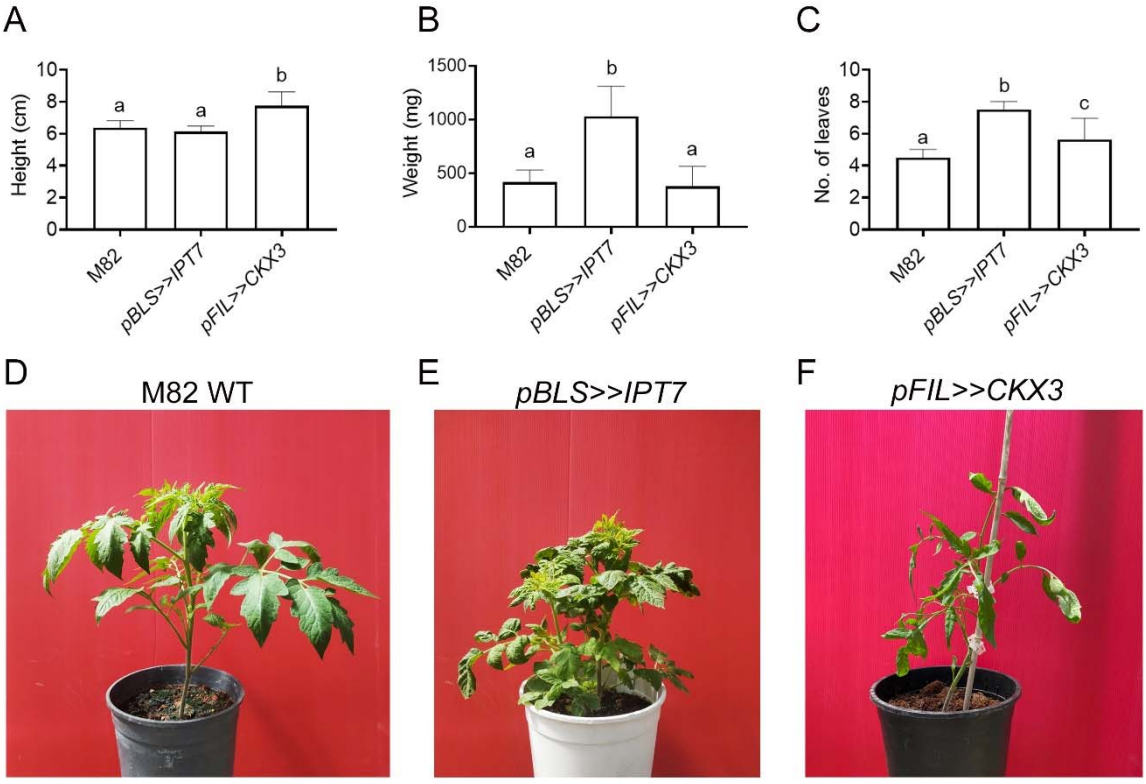


**Figure S6. Development of altered CK content genotypes.**

**A-C** Developmental parameters were assessed on 3 week old plants. Graphs display average ±SEM. **D-F** Representative images of each genotype at 5 weeks.

**A** Plant height. Different letters indicate statistically significant differences in a one way Anova with a Dunnett post hoc test, N=14, $p<0.0003$. **B** Plant weight. Different letters indicate statistically significant differences in a one way Anova with a Dunnett post hoc test, N=17, $p<0.0003$. **C** No. of leaves. Different letters indicate statistically significant differences in a one way Anova with a Dunnett post hoc test, N=17, $p<0.012$.

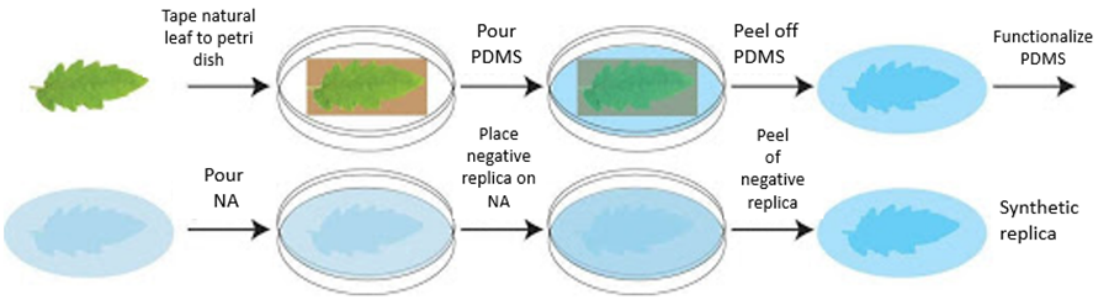


**Figure S7. Schematic of the process of leaf replica preparation.**

Natural leaves are replicated in PDMS. The "negative" PDMS imprint serves as a template to generate "positive" nutrient agar (NA) or PDMS leaf replicas. Procedure according to Kumari et al., 2020. Briefly, a leaf is taped to a petri dish. Liquid Polydimethylsiloxane (PDMS) is poured on the natural leaf, with vacuum application to ensure uniform and "deep" coverage of the microstructure. The polymer is cured overnight at room temperature. The leaf is then peeled off from the cured polymer, which is now a negative replica of the leaf.

To generate a positive PDMS replica which structurally simulates the natural leaf, the negative replica is functionalized to turn the polymer hydrophilic and prevent adherence of the two PDMS layers. The negative replica is then placed in a petri dish. Liquid polymer solution is once again poured onto the negative replica, vacuumed for 2 h, and cured at room temperature overnight. The negative replica is removed from the newly formed polymer layer, which represents the synthetic positive replica.

To generate agar plates containing an imprint, which structurally simulates the natural leaf, desired liquid media with 0.8% agar is poured into a petri dish, let to sit for 10 min, and when the media is cooled but not yet hardened, an autoclaved negative PDMS replica is placed on the petri dish. Once hardened, the PDMS negative is gently removed.

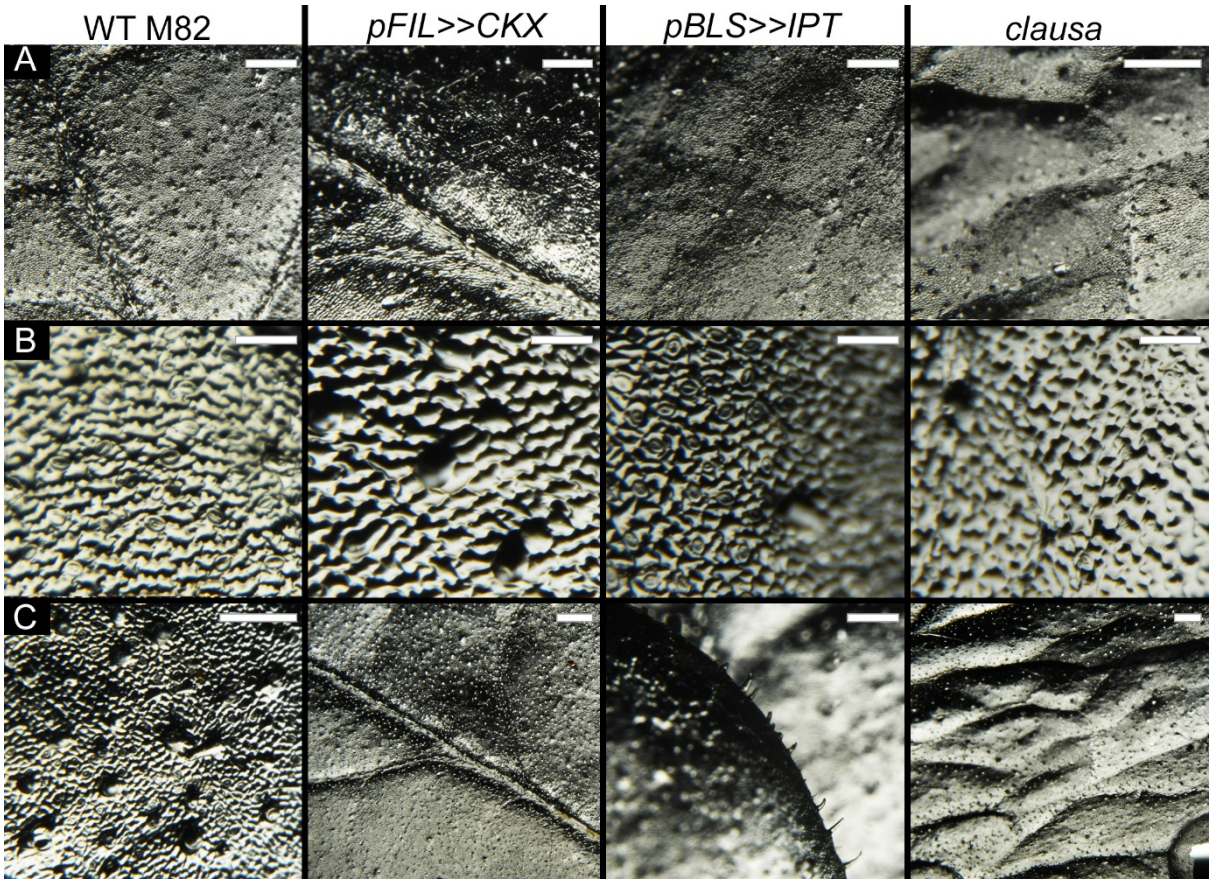


**Figure S8. Leaf structural features captured in PDMS replicas.**

Light microscopy images of positive PDMS replicas of leaves from the indicated genotypes. **A** Leaf adaxial topography including veins, bars= 500μM. **B** Recapitulation of leaf epidermal structure, including stomata, bars= 100 μM. **C** "Special" features are preserved in PDMS-placement of trichomes (M82), veins (*pFIL>>CKX*), trichomes (*pBLS>>IPT*), rugose topography (*clausa*). Bars= 500 μM, except in M82 where bar= 250 μM.

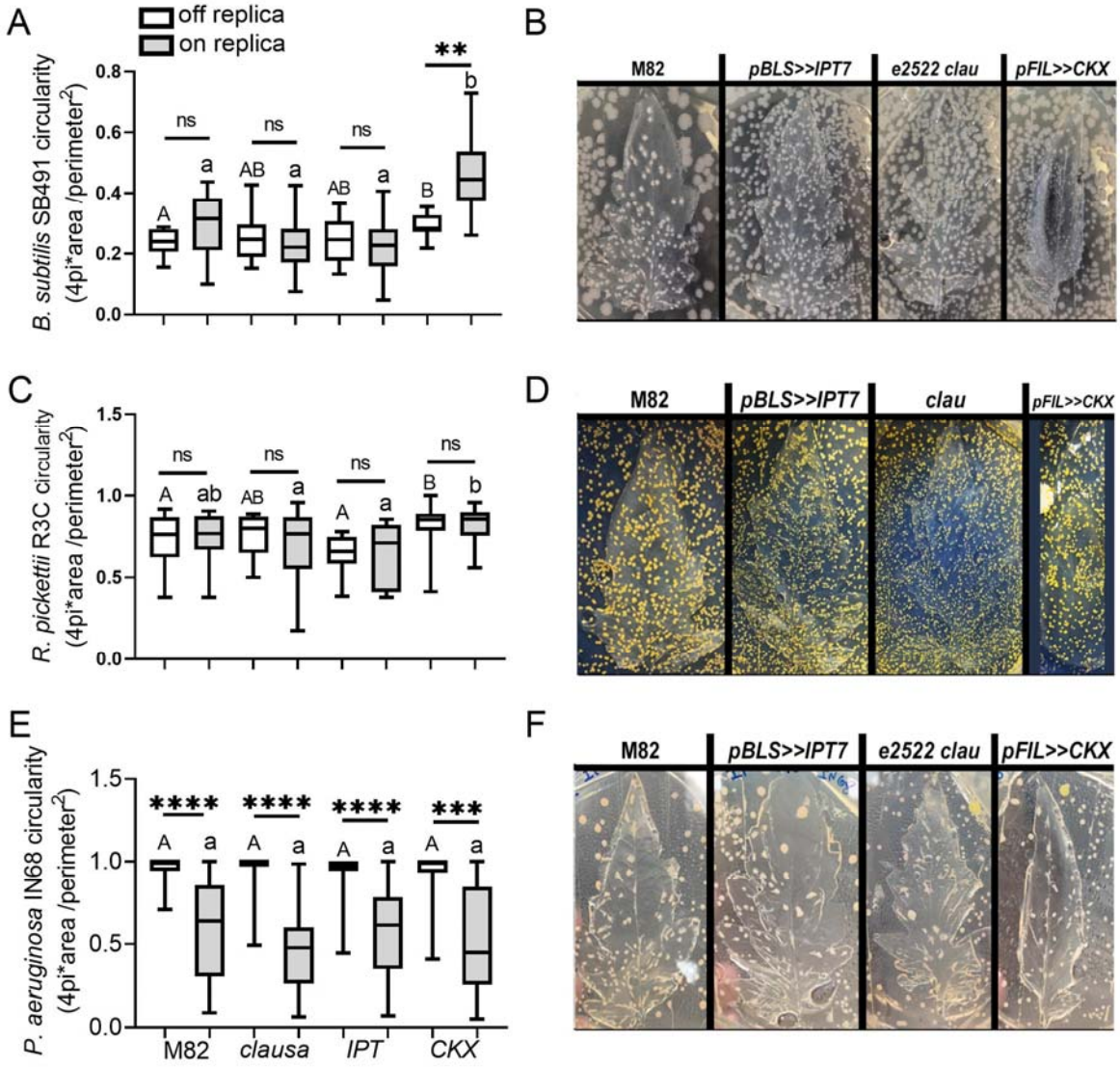


**Figure S9. Effect of synthetic leaf structures on colony circularity of additional bacterial isolates.**

Indicated bacteria ($OD_{600}$=0.01) were spray inoculated onto synthetic agar replicas of leaves of the indicated genotypes. Colony circularity of indicated bacteria was measured after 24 h using ImageJ.

**A, C, E**. Box plots are shown with inner quartile ranges (box), outer quartile ranges (whiskers), median (line), depicting results from 3 independent experiments. Asterisks denote significant differences in circularity on and off the replica derived from each genotype. Upper case letters denote differences in circularity on the structure-less agar ("off replica"). Lower case letters indicate statistically significant differences in circularity observed on the synthetic leaf replicas ("on replica") derived from different genotypes. Asterisks and different letters represent statistically significant differences found in a one-way ANOVA with a Dunnett post hoc test. **A** N>18, p<0.0084. **C** N>10, *p*<0.0003. **E** N>24, p<0.0001. (***p* value<0.01, ****p* value<0.001, *****p* value<0.0001, ns- non significant).

**B, D, F** Images depicting the colony morphology of *B. subtilis* SB491 (B), *R. pickettii* R3C (D), and *P. aeruginosa* IN68 (F) growing on the different genotype- derived synthetic leaf structures.

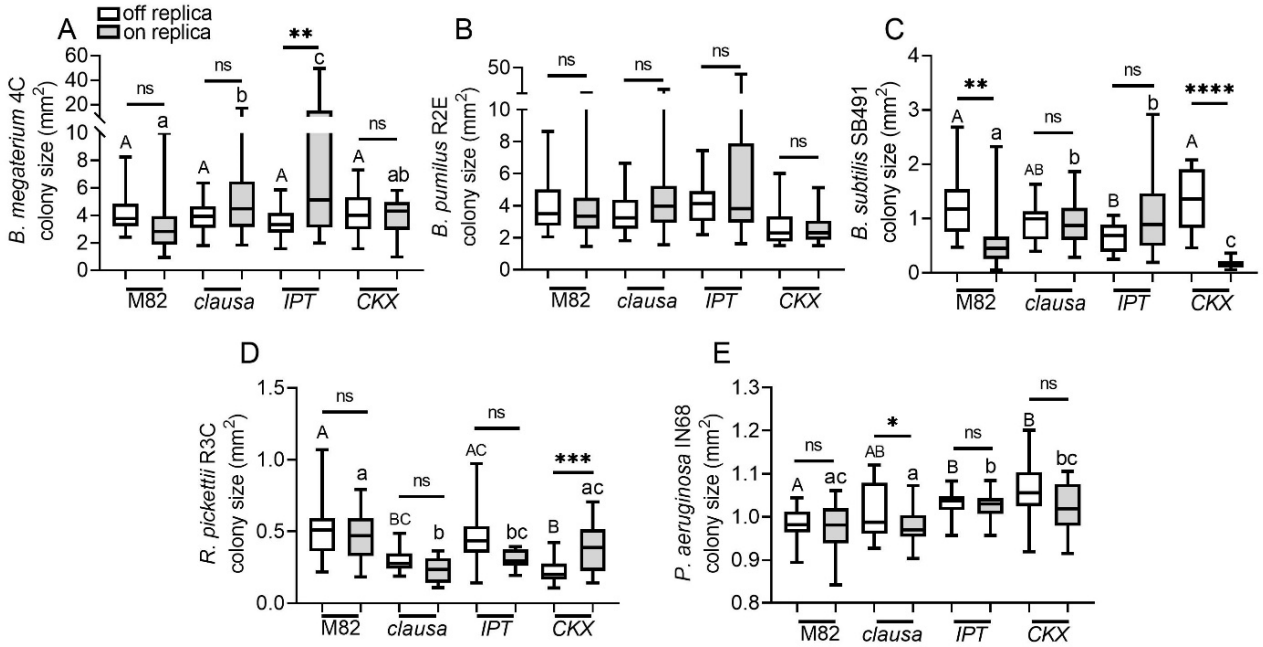


**Figure S10. Effect of synthetic leaf structures on colony size of bacteria.**

Indicated bacteria ($OD_{600}$=0.01) were spray inoculated onto agar replicas of leaves of the indicated genotypes. Colony size was measured after 24 h using ImageJ. Box plots are shown with inner quartile ranges (box), outer quartile ranges (whiskers), median (line), depicting results from 3 independent experiments. Asterisks denote significant differences in colony size on and off the replica derived from each genotype. Upper case letters denote differences in colony size on the structure-less agar ("off replica"). Lower case letters indicate statistically significant differences in colony size observed on the synthetic replicas ("on replica") derived from different genotypes.

**A** *B. megaterium* 4C. N>30, $p$<0.041. **B** *B. pumilus* R2E. N>20, no significant differences in colony size on or off the replica were observed. **C** *B. subtilis* SB491. N>18, $p$<0.022. **D** *R. pickettii* R3C. N>10, $p$<0.033. **E** *P. aeruginosa* IN68. N>24, p<0.05. Letters and asterisks indicate significance in a one-way ANOVA with a Dunnett post hoc test (*$p$ value<0.05; **$p$ value<0.01, ***$p$ value<0.001, ****$p$ value<0.0001, ns- non significant).

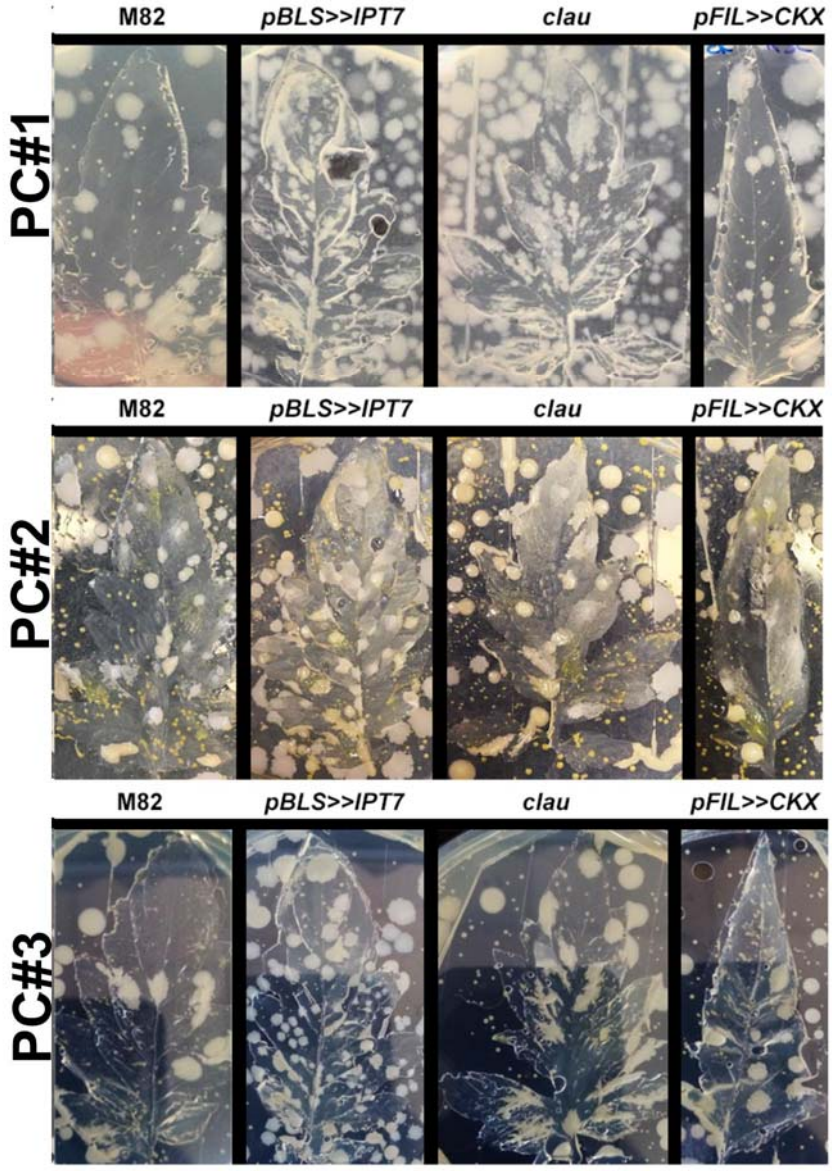


**Figure S11. Preference of Gram-positive bacteria to structures derived from cytokinin rich genotypes in a pairwise community context.**

Accompanying images to Figure 8.

PC#1: *B. subtilis* Bs SB491491 (large white dry colony) + *R. pickettii* R3C (small yellow colony).

PC#2: *B. pumilus* R2E (large white colony) + *R. pickettii* R3C (small yellow colony).

PC#3: *B. megaterium* 4C (large white colony) + *R. pickettii* R3C (small yellow colony).

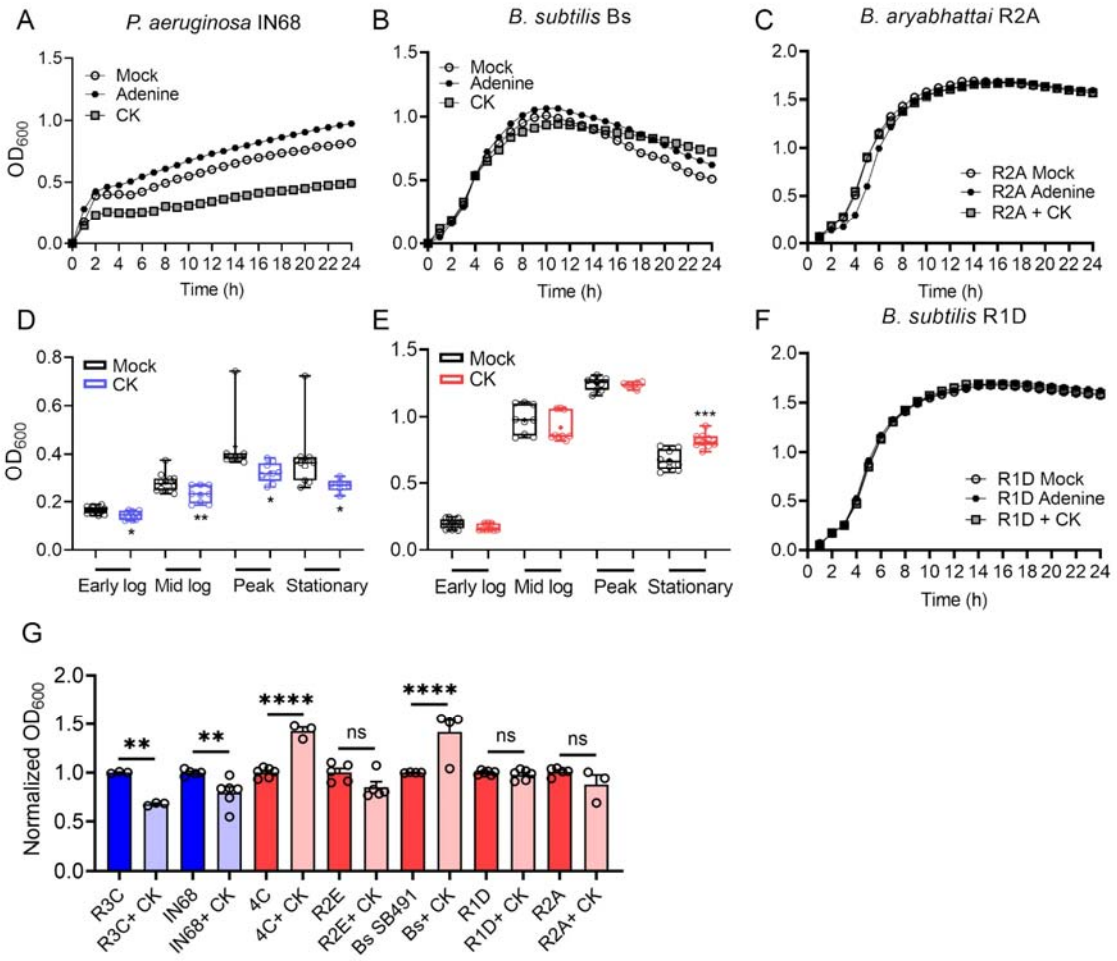


**Figure S12. Direct effect of cytokinin on phyllosphere derived microbes- IN68, Bs SB491, R1D, and R2A.**

Bacterial strains were grown in nutrient broth (**A-F**) or minimal media (**G**), with or without the addition of cytokinin (CK, 100 µM 6-Benzylaminopurine ) or adenine (100 µM) for 24 h. **A, B, C, F** growth curves; **D, E** analysis of growth with and without CK during log phase, peak, and stationary phase. **A, D** *P. aeruginosa* IN68*;* **B, E** *B. subtilis* (Bs, SB491); **C** *B. aryabhattai* R2A; **F** *B. subtilis* R1D. Graphs represent 3 independent experiments, N=9. **G** Normalized growth of all bacterial isolates (R3C, IN68, 4C, R2E, Bs SB491, R1D and R2A) with or without CK after 24 h in minimal media.

**D, E** Box plots are shown with inner quartile ranges (box), outer quartile ranges (whiskers), median (line), mean ("+"), depicting results from 3 independent experiments, all point shown. **G** graphs represent average ±SEM from 3-6 independent experiments, all point shown.

Asterisks indicate significance in an unpaired two-tailed t-test, **B** N=9, $p<0.05$; **D** N=9, $p<0.0002$; or **G** a one way anova with a Bonferroni post hoc test, N=3-6, $p<0.0092$ (*$p$ value <0.05; **$p$ value<0.01; ***$p$ value<0.001, ****$p$ value<0.0001).

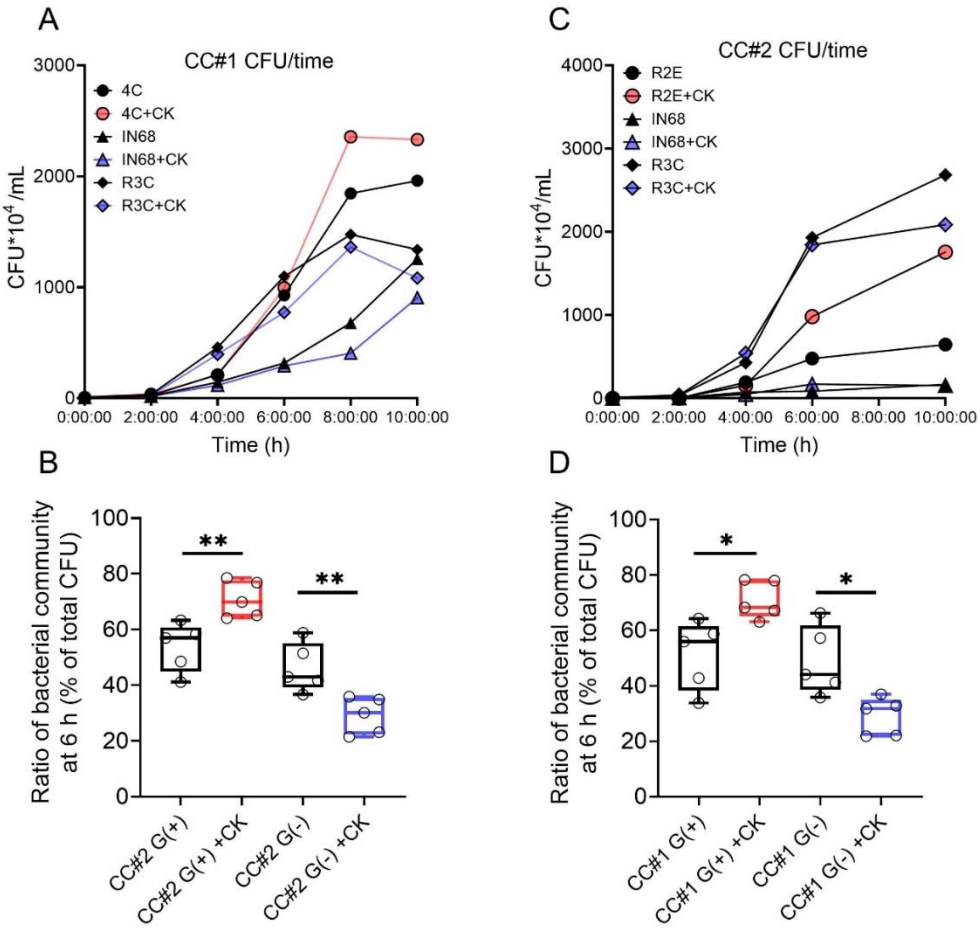


**Figure S13. The effect of cytokinin on phyllosphere derived microbes in a community context.**

Synthetic created communities ("CC") comprising 3 phyllosphere microbes each were generated by combining equal ratios of monocultures of the indicated bacteria. CCs were cultivated in nutrient broth, with or without the addition of 6-Benzylaminopurine (CK, 100 µM), until they reached the stationary phase. Experiment was repeated 5 times.

**A, C** CC#1: *B. megaterium* 4C + *P. aeruginosa* IN68 + *R. pickettii* R3C.

**B, D** CC#2: *B. pumilus* R2E + *P. aeruginosa* IN68 + *R. pickettii* R3C.

**A, B** Growth curves (average of 5 experiments), black symbols Mock, colored symbols + CK (blue: Gram-negative, red: Gram-positive).

**C, D** Percentage of Gram-positive (red) and Gram-negative (blue) bacteria out of the bacterial community after 10 h of cultivation.

Box plots are shown with inner quartile ranges (box), outer quartile ranges (whiskers), median (line), all points shown. Asterisks denote significant differences with and without CK in a one-way ANOVA with a Bonferroni post hoc test, N=5. **C** $p<0.03$**; D** $p<0.0059$, (*$p$ value<0.05, **$p$ value<0.01).

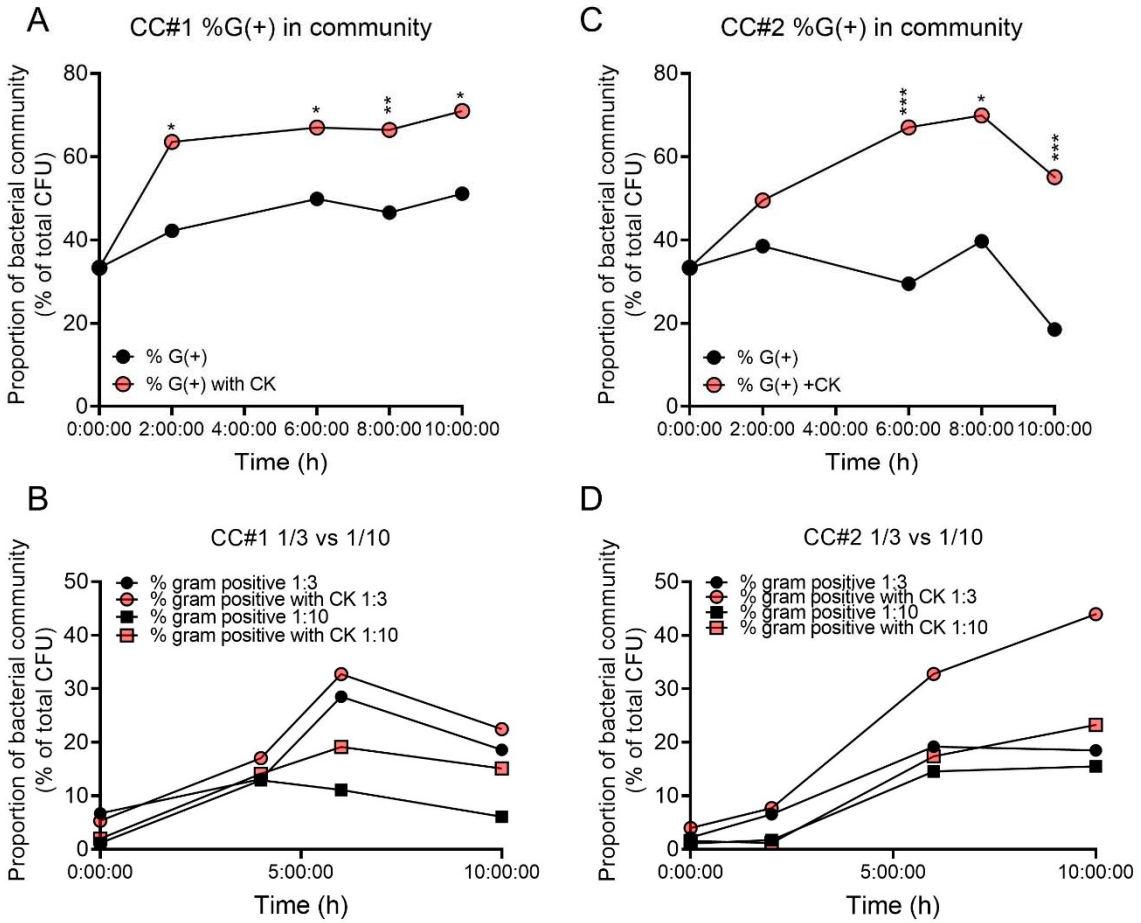


**Figure S14. The effect of CK on phyllosphere derived microbes in a community context at different starting *bacilli* ratios**.

Synthetic created communities ("CC") comprising 3 phyllosphere microbes each were generated by combining equal ratios of monocultures of the indicated bacteria (**A, C**), or starting ratios as indicated (1:3 or 1:10) (**B, D**). CCs were cultivated in nutrient broth, with or without the addition of 6-Benzylaminopurine (CK, 100 µM), until they reached the stationary phase. Experiment was repeated 5 times.

**A, B** CC#1: *B. megaterium* 4C + *P. aeruginosa* IN68 + *R. pickettii* R3C.

**C, D** CC#2: *B. pumilus* R2E + *P. aeruginosa* IN68 + *R. pickettii* R3C.

Growth curves (average of 3 experiments), Gram-positive bacterium only is plotted. Black symbols Mock, red symbols + CK. Growth of both bacilli is enhanced by CK irrespective of its starting ratio in the community. Asterisks denote significant differences with CK in multiple t-tests, N=5, $p<0.025$ (*$p$ value<0.05, **$p$ value<0.01, ***$p$ value<0.001).

**Supplementary Table S1** Primers used for qRT-PCR.

| Gene | Encoded protein | Accession No. | Primer pairs (5'-3') | Efficiency |
|---|---|---|---|---|
| ***PR1a*** | Pathogenesis related-1a | Solyc01g106620 | F: CTGGTGCTGTGAAGATGTGG<br>R: TGACCCTAGCACAACCAAGA | 1.01 |
| ***Pti 5*** | Pathogenesis-related genes transcriptional activator 5 | Solyc02g077370 | F: GACATGGTGCGAGAGTATGG<br>R: CTGAAACAGAGGCGTTCACT | 1.03 |
| ***ACO-1*** | 1-aminocyclopropane-1-carboxylate oxidase 1 | Solyc07g049530 | F: GGGCTTCTTTGAGTTGGTGA<br>R: GCTTGAACAGCCTCAAGTCC | 1.01 |
| ***ERF-1*** | Ethylene-responsive factor 1 | Solyc05g051200 | F: ATTAGGGATTCAACGCGTAA<br>R: AGAGACCAAGGACCCCTCAT | 0.98 |
| ***Chi*** | PR-3 (Chitinase) | Solyc10g055800 | F: TCGAACAGGAGGAGGATCTG R:<br>TCCAGGCTTTCTCGGACTAC | 1.02 |
| ***b-gluc*** | PR-2<br>(beta-1,3 glucanase) | Solyc01g060020 | F: TCGAACAGGAGGAGGATCTG<br>R: TCCAGGCTTTCTCGGACTAC | 1.02 |
| ***LeEIX1*** | LeEIX1 | Solyc07g008620 | F:TCAGACACGCTTCCAAGTTGGTTC<br>R: ATTGGTAGGGACTAGTGGCAAAGC | 0.98 |
| ***FLS2*** | FLS2 | Solyc02G070890 | F: GGGTTGGGGCAGTTATCCAA<br>R: GGTGGAATGGCACCTGAGAA | 1.02 |
| ***Act*** | Actin | Solyc03g078400 | F: TGGTCGGAATGGGACAGAAG<br>R: CTCAGTCAGGAGAACAGGGT | 1.03 |
| ***RPL8*** | Ribosomal protein L2 | Solyc10g006580 | F: TGGAGGGCGTACTGAGAAAC<br>R: TCATAGCAACACCACGAACC | 1.03 |
| ***CYP*** | Cyclophilin | Solyc01g111170 | F: TGAGTGGCTCAACGGAAAGC<br>R: CCAACAGCCTCTGCCTTCTTA | 0.99 |