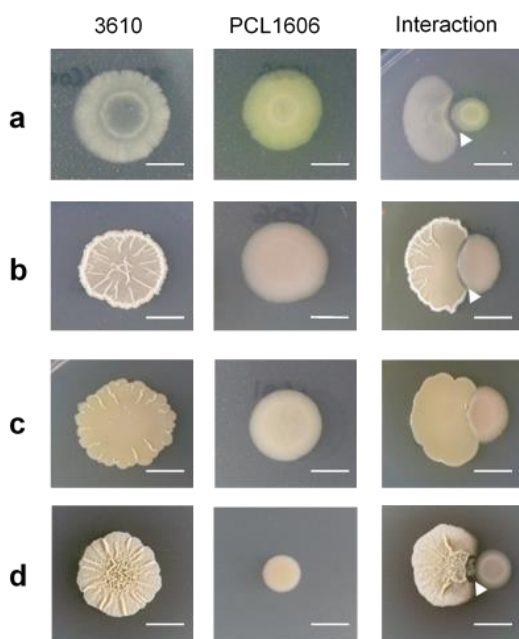


**The extracellular matrix protects *Bacillus subtilis* colonies from *Pseudomonas* invasion and modulates plant co-colonization**

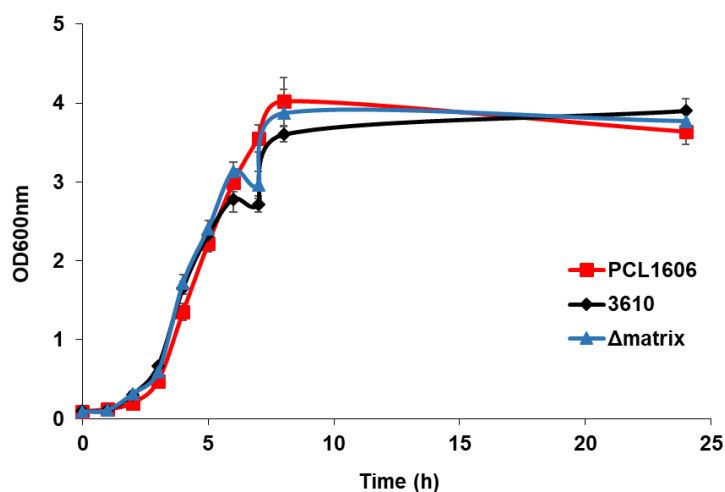
Molina-Santiago et al.,

## Supplementary material

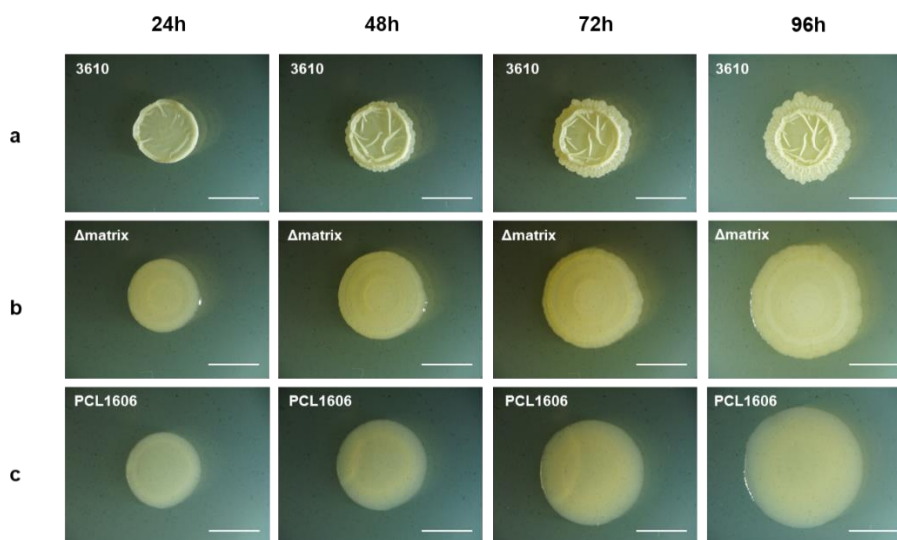
### Supplementary Figures



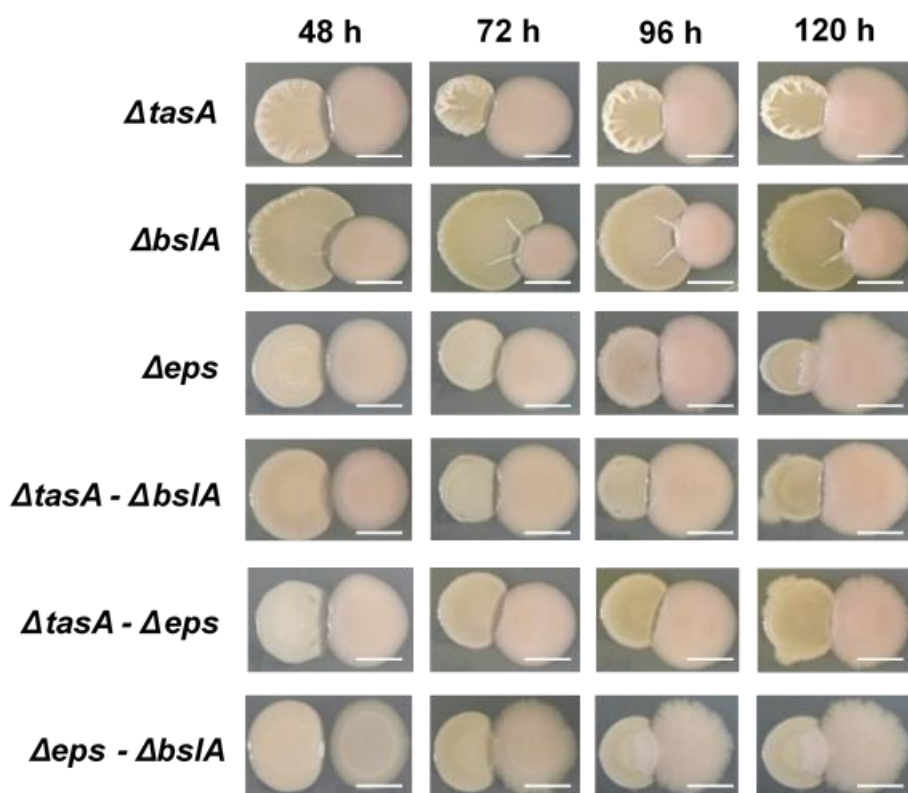
Suppl. Fig 1. Single colony growth and pairwise interactions between PCL1606 and 3610 in different culture mediums after 72 h of growth. (a) Growth in King's B medium; (b) growth in LB medium; (c) growth in TY medium; and (d) growth in Msgg medium. White arrows indicate the inhibition area of PCL1606 against 3610. Scale bar = 5mm.



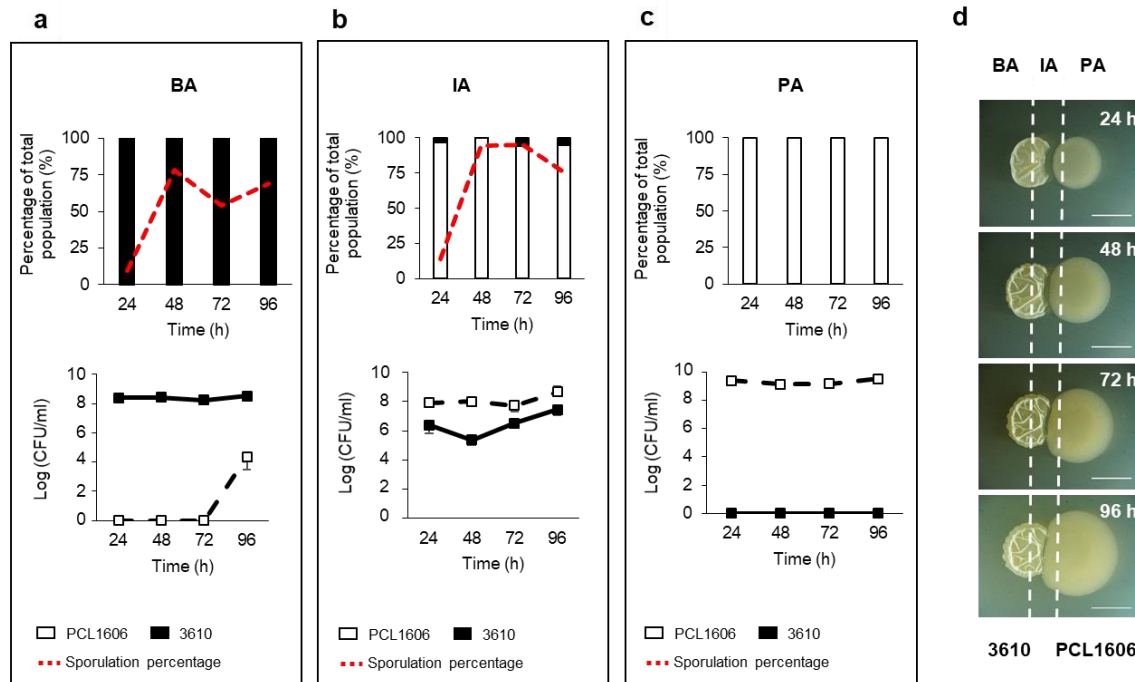
Suppl. Fig. 2. Growth curves of PCL1606 (red line), 3610 (black line) and Δmatrix (blue line) growing in LB medium. Average values of three biological replicates are shown, with error bars representing SD. Source data are provided as a Source Data file.



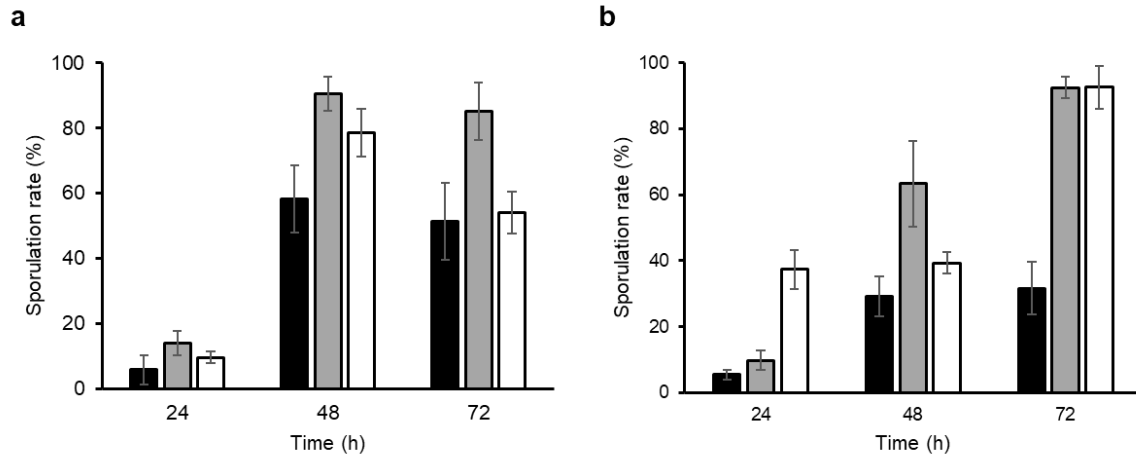
Suppl. Fig. 3. (a, b and c) Time-courses of *B. subtilis* (a) 3610, (b) Δmatrix and (c) *P. chlororaphis* PCL1606 colony morphologies at 24 h, 48 h, 72 h and 96 h when growing alone in LB medium. Scale bar = 5 mm.



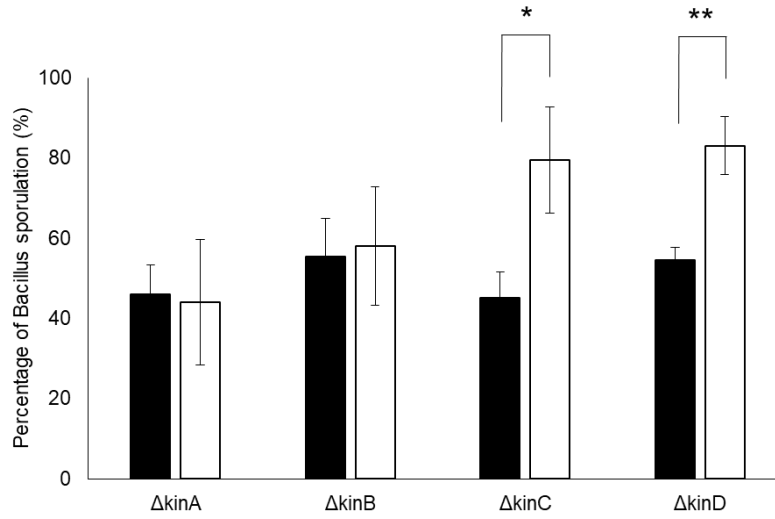
Suppl. Fig. 4. Time-courses of the interactions between PCL1606 (right side colonies) and *Bacillus* strains (left side colonies):  $\Delta$ TasA,  $\Delta$ BslA,  $\Delta$ Eps,  $\Delta$ TasA- $\Delta$ BslA,  $\Delta$ TasA- $\Delta$ Eps, and  $\Delta$ Eps- $\Delta$ BslA at 48 h, 72 h, 96 h and 120 h when growing as single-cultures in LB medium. Scale bar = 5 mm.



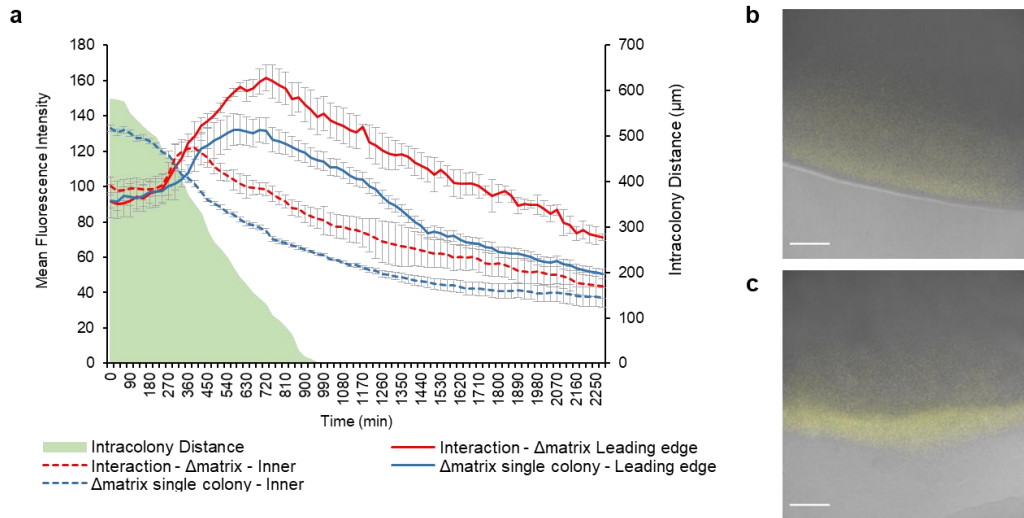
Suppl. Fig 5. a, b and c panels show (upper part) percentages and (bottom part) Log (CFU/ml) of PCL1606 (empty bars and squares) and 3610 (black bars and squares) in three different sections (BA [3610 area], IA [Intermediate area] and PA [PCL1606 area]) of the interaction at different time-points (24 h, 48 h, 72 h, 96 h). Sporulation rates are shown as red lines. (d) Scheme of the interactions and the sections: BA (3610 area), IA (Intermediate area) and PA (PCL1606 area). Scale bar = 5 mm. Cells from the three sections of the interaction were collected, sonicated and completely resuspended, diluted and plated to determine colony forming units (CFU), sporulation rates and percentages of each species over the total bacterial population in the interaction. Average values of three biological replicates are shown, with error bars representing SD. Source data are provided as a Source Data file.



Suppl. Fig 6. Sporulation percentages of (a) 3610 and (b)  $\Delta$ matrix growing in LB medium during 24 h, 48 h and 72 h of growth. Black bars indicate sporulation percentage of single colonies, grey bars indicate sporulation percentages of *B. subtilis* strains in the intermediate area (IA) after interaction with PCL1606, and white bars indicate sporulation percentages of *B. subtilis* strains in the Bacillus area (BA) after interaction with PCL1606 (n =3). Average values of three biological replicates are shown, with error bars representing SD. Source data are provided as a Source Data file.

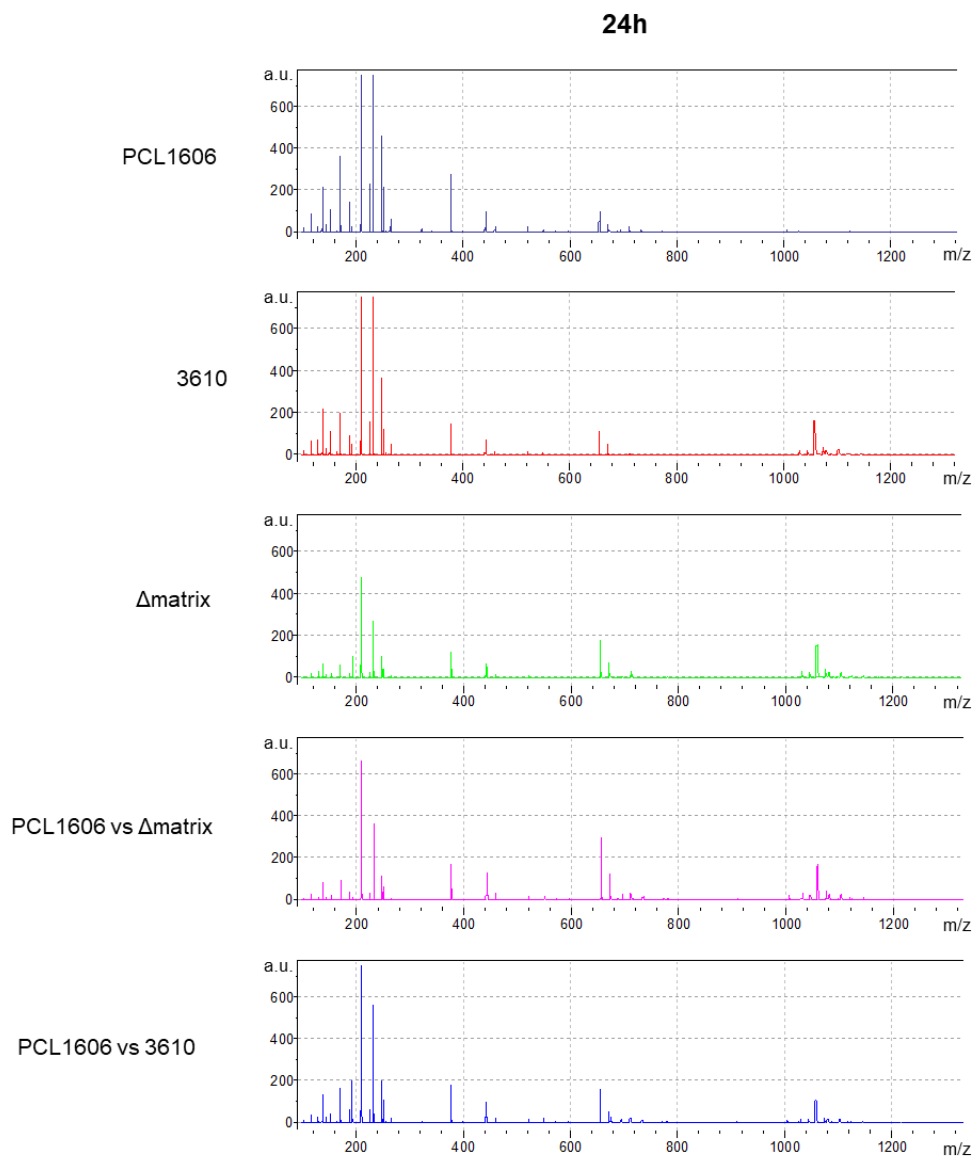


Suppl. Fig. 7. Percentage of sporulation in *B. subtilis* 3610 strains mutated in *kinA*, *kinB*, *kinC* and *kinD* histidine kinases when growing alone (black bars) and in the interaction with PCL1606 (empty bars) at 72 h of growth in LB medium. Average values of three biological replicates are shown, with error bars representing SD. \**P*-value < 0.05, \*\**P*-value < 0.01 (*t*-student). Source data are provided as a Source Data file.

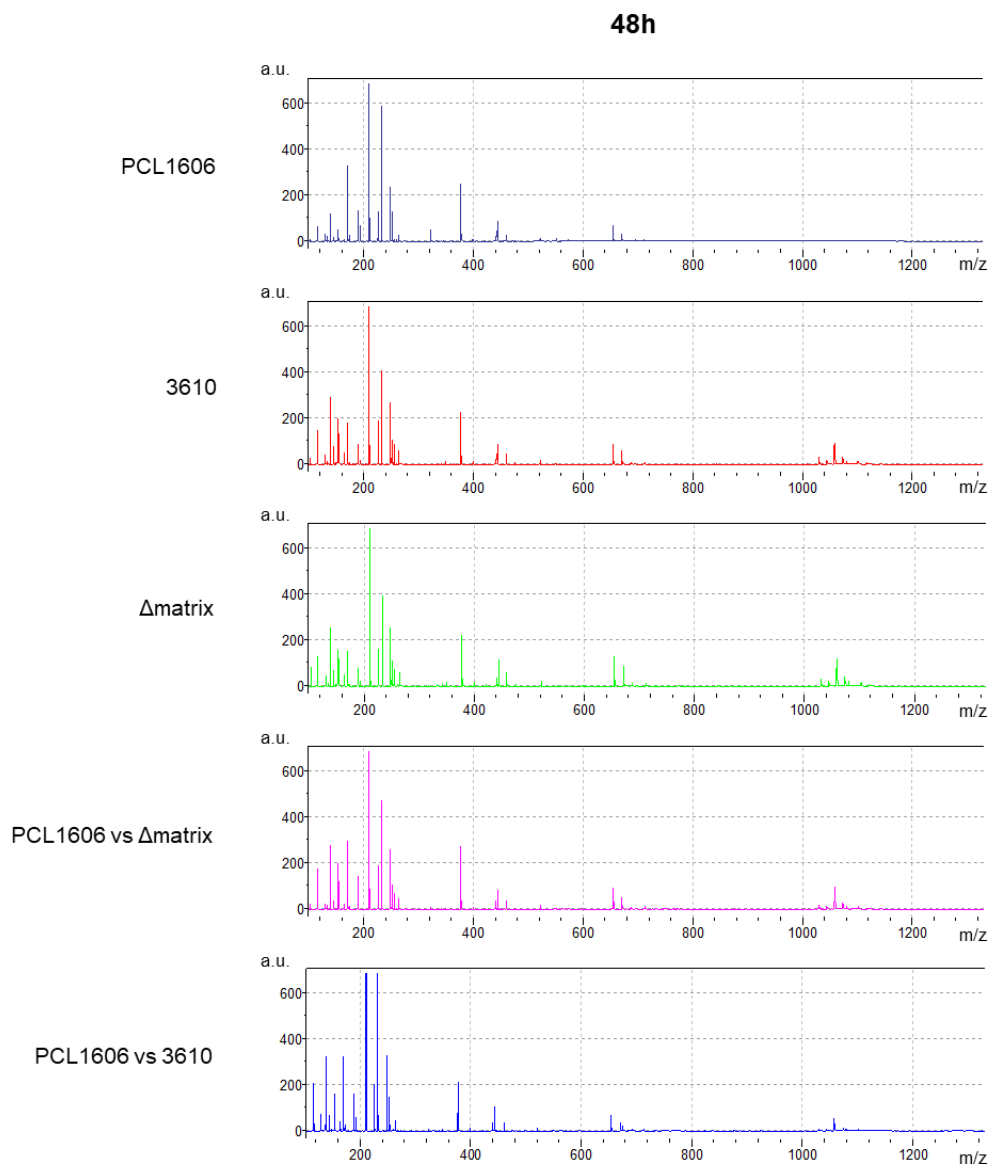


Suppl. Fig. 8. (a) Comparison of the mean of fluorescence intensity of the *sppB* promoter of a  $\Delta$ matrix single colony (blue lines) and of  $\Delta$ matrix in the interaction with PCL1606 (red lines). Dashed lines indicate sporulation in the inner of the colony while continuous lines indicate sporulation at the leading edge of the colony. Green area indicates the intracolony distance between  $\Delta$ matrix and PCL1606 during time. Average values of three biological replicates are shown, with error bars representing SD. (b) and (c) show the sporulation of *sppB* promoter in (b)  $\Delta$ matrix single colony and (c)  $\Delta$ matrix – PCL1606 interaction. Scale bar = 100  $\mu$ m. Source data are provided as a Source Data file.

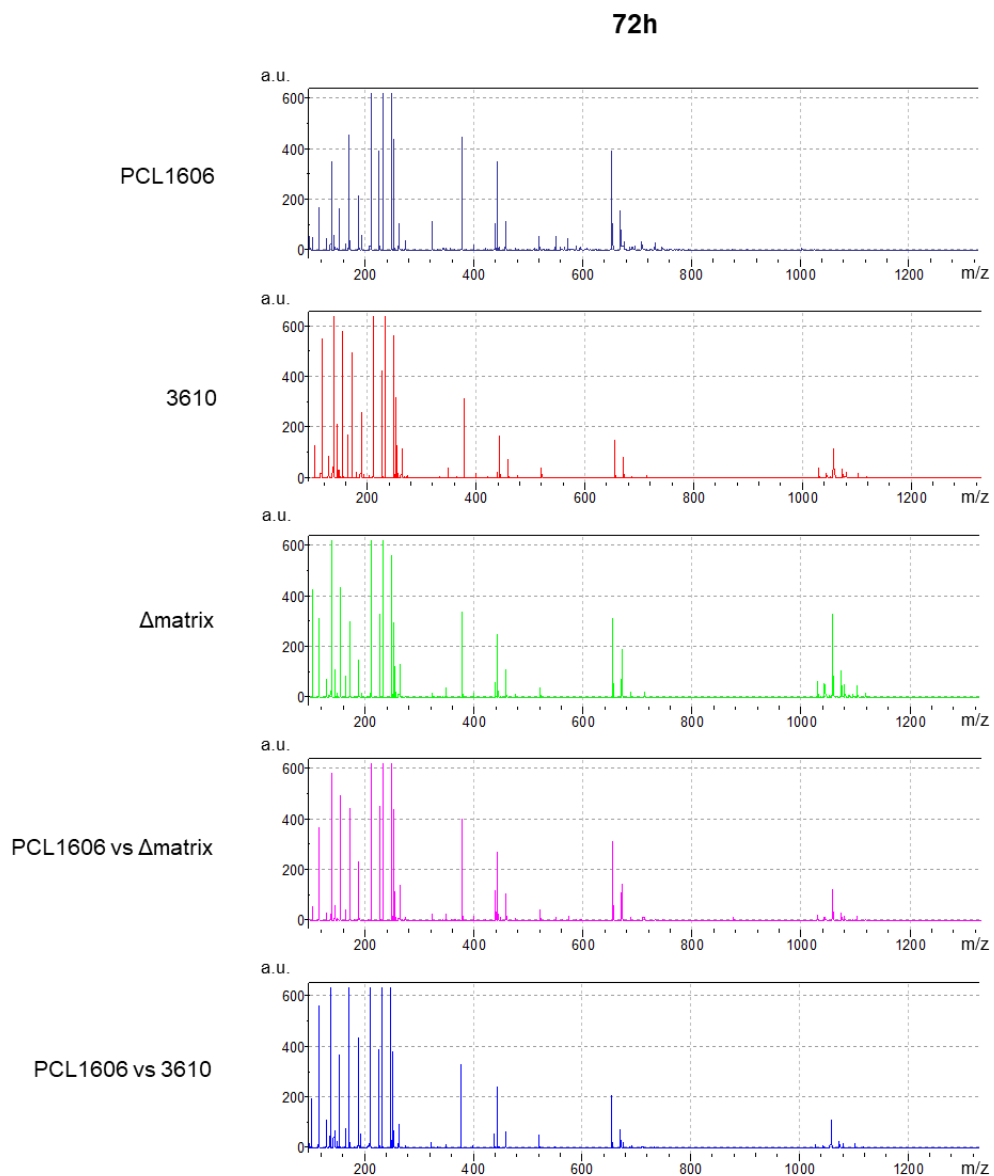




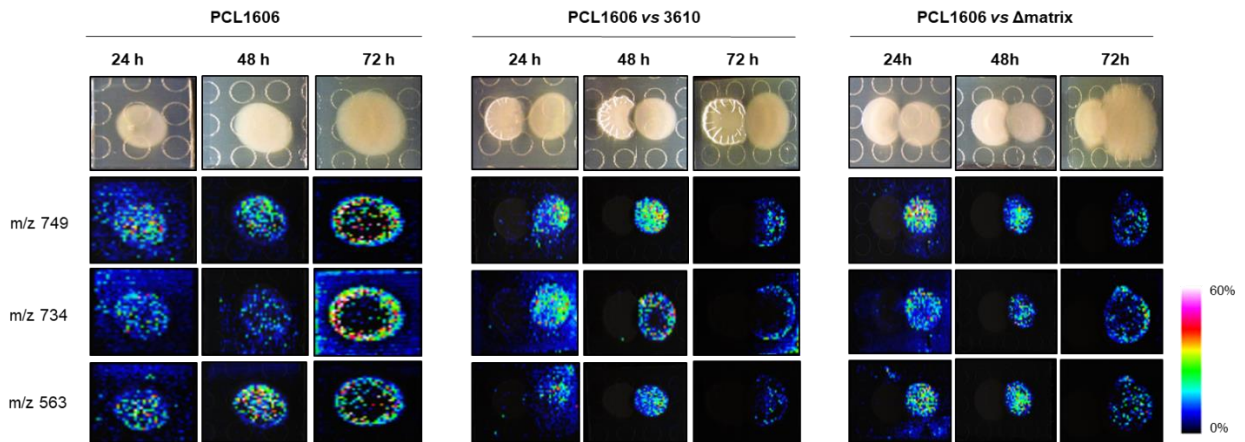
Suppl. Fig. 9. Average mass spectrum obtained by MALDI-TOF MSI of PCL1606, 3610 and  $\Delta$ matrix growing alone and in interactions after 24 h of culture in LB medium.



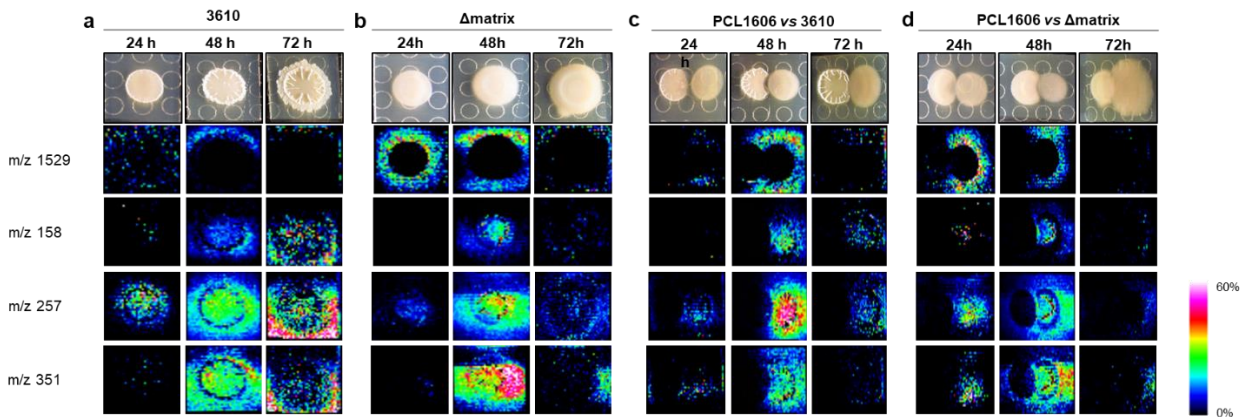
Suppl. Fig. 10. Average mass spectrum obtained by MALDI-TOF MSI of PCL1606, 3610 and  $\Delta$ matrix growing alone and in interactions after 48 h of culture in LB medium.



Suppl. Fig. 11. Average mass spectrum obtained by MALDI-TOF MSI of PCL1606, 3610 and  $\Delta$ matrix growing alone and in interactions after 72 h of culture in LB medium.

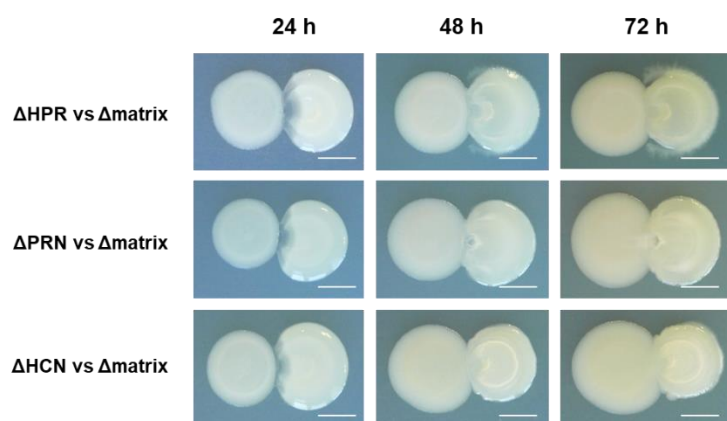


Suppl. Fig. 12. MALDI-TOF MSI heatmaps of selected molecules during time-lapse experiments (24 h, 48 h, 72 h) of PCL1606 growing alone and in interaction with *Bacillus* strains. As a representation of the similar metabolite distribution between samples, molecules with m/z of 749, 734 and 563 are shown.

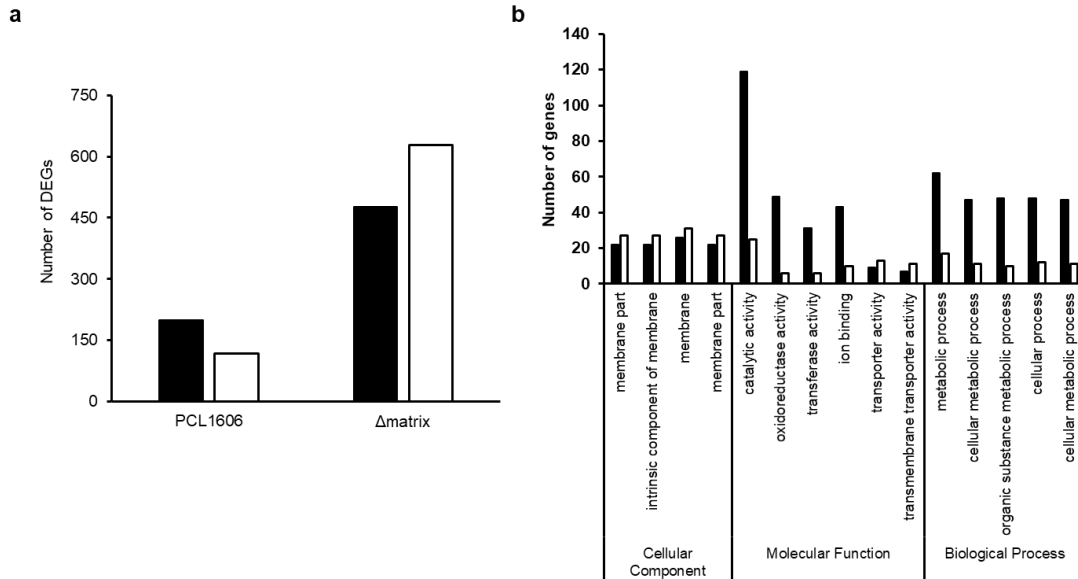


Suppl. Fig. 13. MALDI-TOF MSI heatmaps of selected molecules during time-lapse experiments (24 h, 48 h, 72 h) of (a) 3610 and (b)  $\Delta$ matrix growing alone and in (c) and (d) interaction with PCL1606. Molecules with similar distribution patterns are shown: m/z = 1529, 158, 257 and 351.

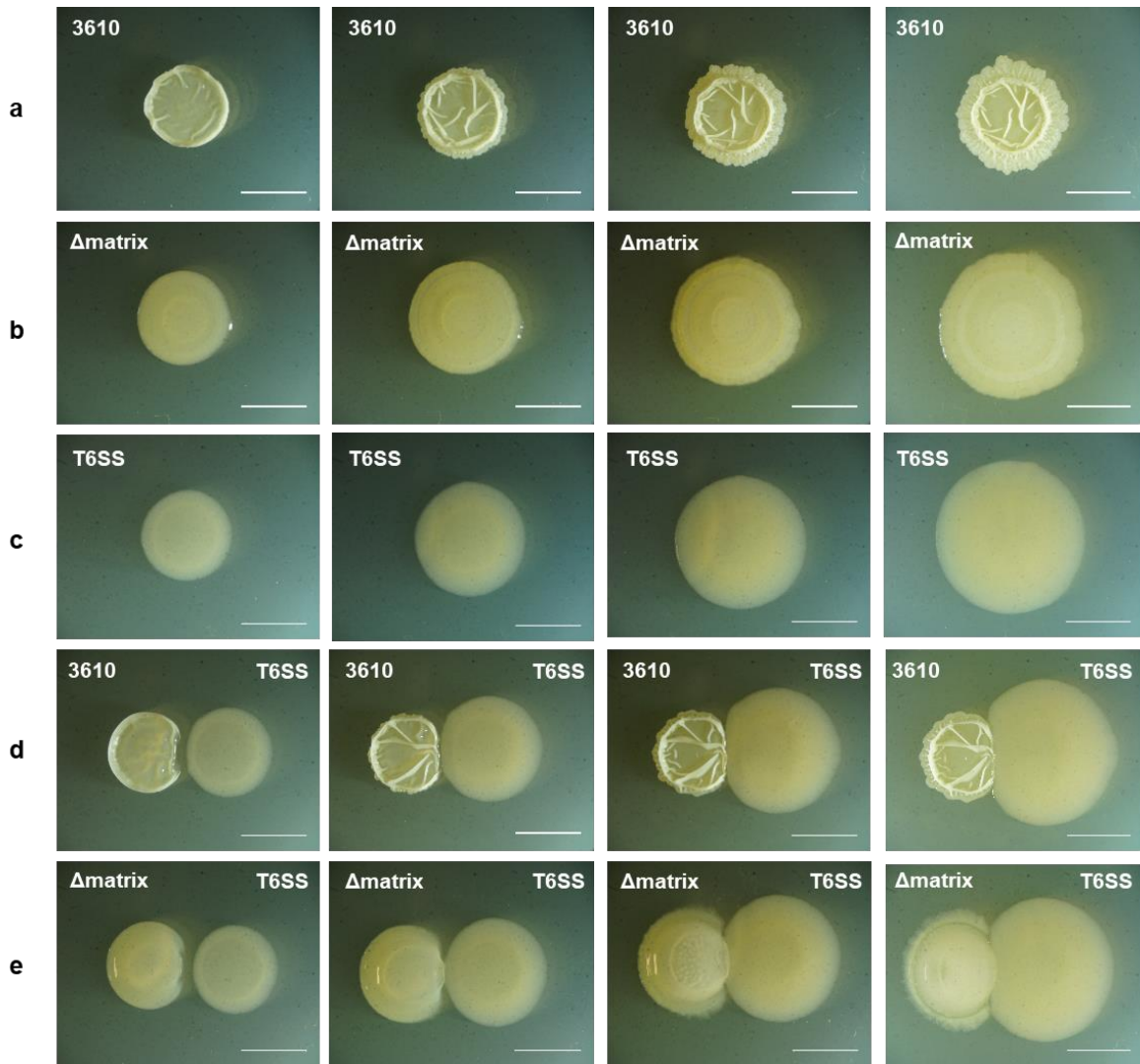




Suppl. Fig. 16. Time-course interaction experiments between PCL1606 mutants in secondary metabolites and  $\Delta\text{matrix}$ . Mutants of PCL1606 were unable to produce (a) HPR (2-hexyl-5-propyl-resorcinol), (b) PRN (pyrrolnitrin), and (c) HCN (hydrogen cyanide). Right colonies in the interaction are  $\Delta\text{matrix}$  while left colonies are PCL1606 mutants. Scale bar = 5 mm.

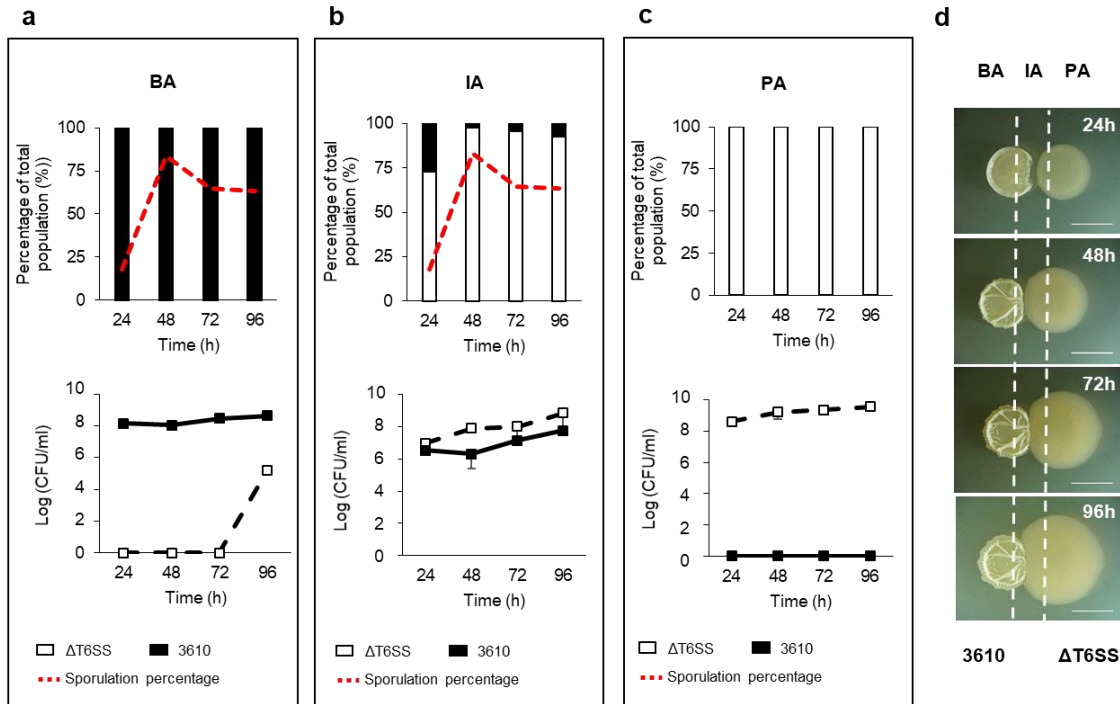


Suppl. Fig. 17. Gene ontology (GO) terms analysis of the differentially expressed genes during the interaction between PCL1606 and  $\Delta$ matrix. (a) Number of genes differentially expressed (DEGs) after 72 h of interaction of PCL1606 and  $\Delta$ matrix. Black bars indicate induced genes and empty bars indicate repressed genes. (b) GO terms differentially expressed in PCL1606. Black bars indicate induced GO terms while empty bars indicate repressed GO terms.

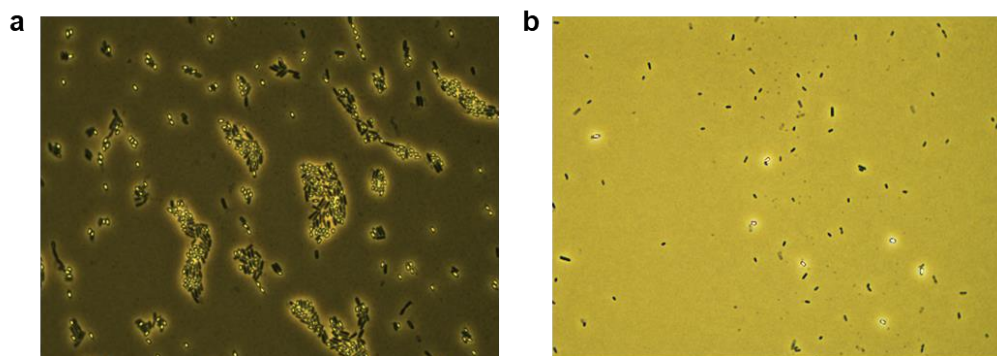


Suppl. Fig. 18. (a, b and c) Time-courses of *B. subtilis* (a) 3610, (b)  $\Delta$ matrix and (c)  $\Delta$ T6SS colony morphologies at 24 h, 48 h, 72 h and 96 h when growing alone in LB medium. (d and e) Time-courses of the interactions (d) 3610- $\Delta$ T6SS and (e)  $\Delta$ matrix- $\Delta$ T6SS at 24 h, 48 h, 72 h and 96 h growing in LB medium. Left colonies are *Bacillus* strains and right colonies are  $\Delta$ T6SS. Scale bar = 5 mm.

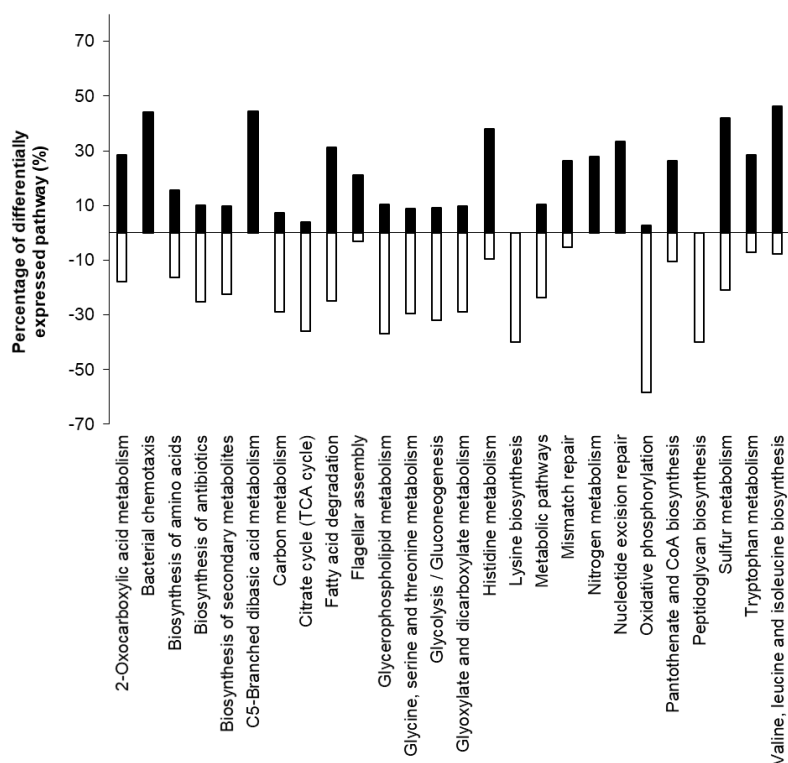




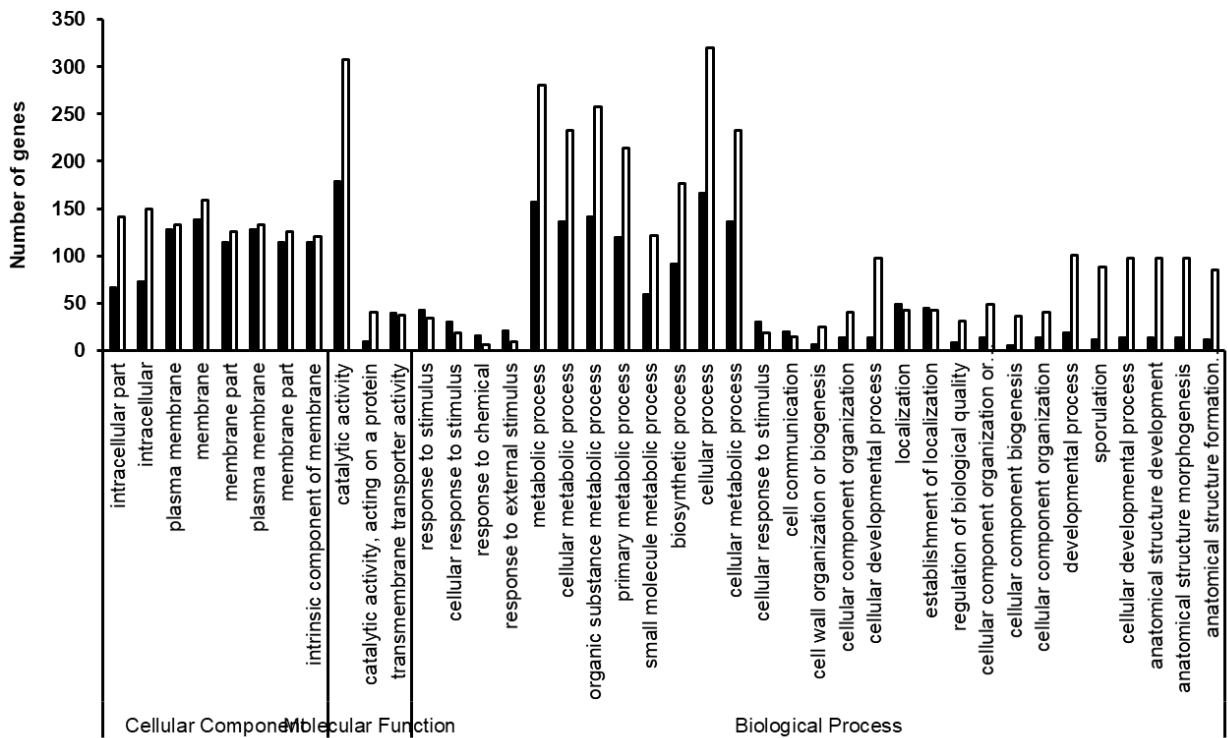
Suppl. Fig. 19. a, b and c panels show (upper part) percentages and (bottom part) Log (CFU/ml) of  $\Delta T6SS$  (empty bars and squares) and 3610 (black bars and squares) in three different sections (BA [3610 area], IA [Intermediate area] and PA [ $\Delta T6SS$  area]) of the interaction at different time-points (24 h, 48 h, 72 h, 96 h). Sporulation rates are shown as red lines. (d) Scheme of the interactions and the sections: BA (3610 area), IA (Intermediate area) and PA ( $\Delta T6SS$  area). Scale bar = 5 mm. Cells from the three sections of the interaction were collected, sonicated and completely resuspended, diluted and plated to determine colony forming units (CFU), sporulation rates and percentages of each species over the total bacterial population in the interaction. Average values of three biological replicates are shown, with error bars representing SD. Source data are provided as a Source Data file.



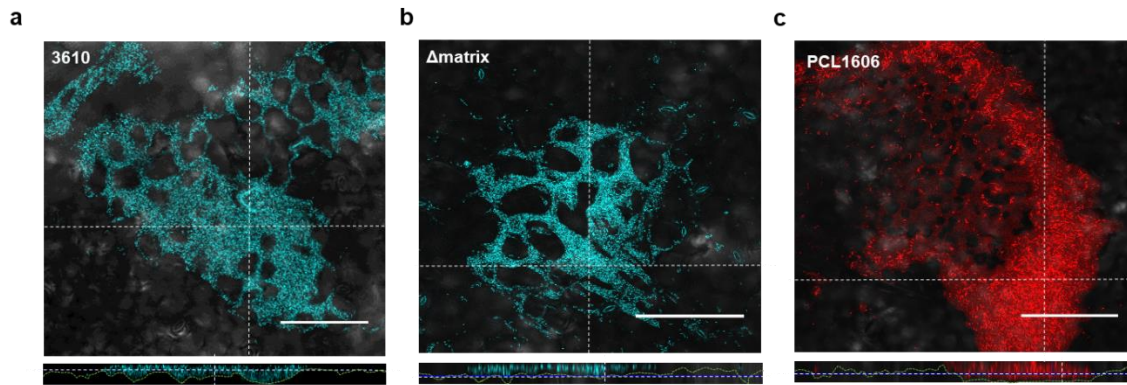
Suppl. Fig. 20. Microscopic images of the interaction area between PCL1606 and  $\Delta$ matrix at (a) 48 h and (b) 72 h of interaction. Spores can be observed as white brilliant spots. (a) shows the spores in the initial moments of the first contact between colonies while in (b) spores can be observed with an increase number of PCL1606 bacterial cells.



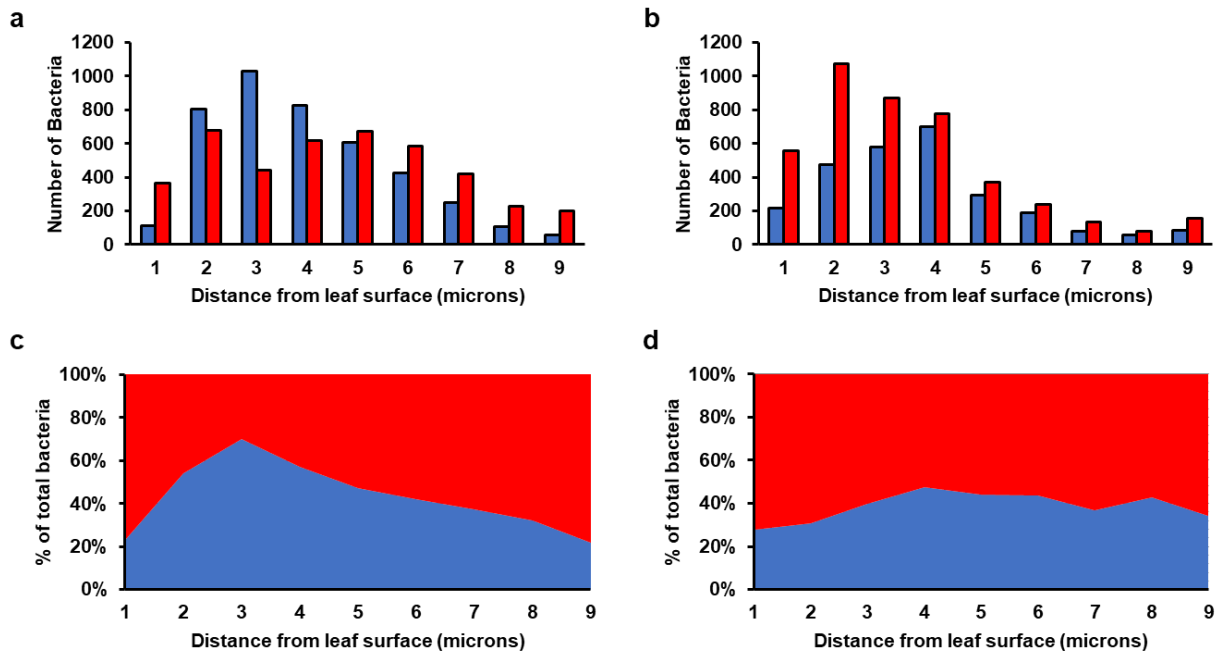
Suppl. Fig. 21. KEGG pathway analysis of the  $\Delta$ matrix genes induced (black bars) and repressed (empty bars) after 72 h of interaction with PCL1606 ( $P$ -value < 0.05).



Suppl. Fig. 22. GO terms differentially expressed in  $\Delta$ matrix during the interaction with PCL1606 (72 h). Black bars indicate induced GO terms while empty bars indicate repressed GO terms.



Suppl. Fig. 23. CLSM maximum projections and z axis slices at positions indicated by the discontinuous lines of the bacterial distribution of (a) 3610, (b)  $\Delta$ matrix and (c) PCL1606 after 9 days of inoculation. *Bacillus* strains were labeled with CFP and PCL1606 was labeled with DsRed. Scale bar = 100  $\mu$ m. Green dotted lines indicate the leaf surface.



Suppl. Fig. 24. Bacterial distribution relative to melon leaf surfaces after 9 days post-inoculation. *Bacillus* species are shown in blue whereas PCL1606 is shown in red. (a and b) Number of bacteria at different distances (1 to 9 microns) from the leaf surface when (a) 3610 and PCL1606, and (b)  $\Delta$ matrix and PCL1606 were co-inoculated. (c and d) Percentage of total bacteria at different distances from the leaf surfaces of interactions (c) 3610 – PCL1606 and (d)  $\Delta$ matrix – PCL1606. Source data are provided as a Source Data file.

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