Electronic Supplementary Information

Fluorescent detection of pyrene-stained *Bacillus subtilis* LPM1 rhizobacteria from colonized patterns of tomato roots

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I. Experimental procedures.

Chemicals. The following chemical products were obtained from Aldrich and used without further purification: 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt, propargyl bromide, N,N-diisopropylethylamine (DIPEA), 1-bromoundecane, 11-bromoundecanoic acid. All of the solvents such as CH_2Cl_2, $CHCl_3$, N-methylpyrrolidone, hexanes, methanol, were purchased from Aldrich, Baker or ASLO Reactivos. All solvents used for optical characterization were spectroscopic grade from Aldrich.

Synthesis of methyl 11-bromoundecanoate: A round bottomed flask was charged with 5g (18.85 mmol) of 11-bromoundecanoic acid, 30 mL of methanol and 0.40 mL (7.54 mmol) of sulfuric acid. The mixture is heated to 80°C for 4 h. After cooling, the solvent is removed under vacuum, and then a solution of $NaHCO_3$ is added, until a pH of 7 is reached. The organic phase is extracted with hexanes (3X), dried with $MgSO_4$, filtered and concentrated, to give a colorless liquid in 95 % yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm) 3.67 (s, 3H, CH_3), 3.41 (t, 2H, Br-CH_2), 2.31 (t, 2H, CH_2-α-CO), 1.85 (m, 2H, CH_2- β-Br), 1.62 (m, 2H, CH_2- β- CO), 1.42 (m, 4H, CH_2-γ-Br, CH_2-γ-CO), 1.29 (s, 8H, -CH_2).

General procedure for Williamson alkylations of 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt: A round bottomed flask was charged with 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (**M**), the alkylating agent and 30 mL of dry methanol. The mixture under nitrogen is heated to 70 °C and then DIPEA is added via syringe and let to react for 7 days. After cooling, the solvent is removed under vacuum, the product is dissolved in the minimum amount of methanol and precipitated in CH_2Cl_2 and then in acetone, filtrated and passed by flash silica gel chromatography.

Synthesis of sodium 8-((methyl 10-undecanoate)oxy)pyrene-1,3,6-trisulfonate: *Applying the general procedure for the Williamson alkylations of **M***: 600 mg (1.14 mmol) of **M**, 30 mL methanol, 2.05 g (7.32 mmol) of methyl 11-bromoundecanoate and 458 mg (0.62 mL, 3.55 mmol) of DIPEA. The crude product is precipitated in CH_2Cl_2 and then in acetone, filtrated and purified by flash chromatography (SiO_2, $CHCl_3$, and then $CHCl_3$:MEOH 1:1 v/v) to obtain a beige powder in 87 % yield. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 9.10 (d, *J* = 9.6 Hz, 1Hpyr), 9.03 (d, *J* = 9.9 Hz, 1Hpyr), 9.03 (s, 1Hpyr), 8.95 (d, *J* = 9.9 Hz, 1Hpyr), 8.38 (d, *J* = 9.6 Hz, 1Hpyr), 8.20 (s, 1Hpyr), 4.36 (t, 2H, CH_2-α-O), 3.56 (s, 3H, $COOCH_3$),

2.28 (t, 2H, CH_2-α-COO), 1.96 (m, 2H, CH_2-β-O), 1.59 (m, 2H, CH_2-β-COO), 1.51 (m, 2H, CH_2-γ-O), 1.40 (m, 2H, CH_2-γ-COO), 1.28 (bs, 8H, -CH_2-).

Synthesis of sodium 8-((10-carboxydecyl)oxy)pyrene-1,3,6-trisulfonate (M1): To a round bottom flask containing 850 mg (0.463 mmol) of sodium 8-((11-methoxy-11- oxoundecyl) oxy) pyrene-1,3,6-trisulfonate and 20 mL of methanol, were added 9.79 mL of NaOH-0.48 M solution (4 equiv., 188 mg de NaOH). The mixture was stirred at 40-50 °C for 2d. Once cool, the solution was acidified with HCl-1M, up to reach a pH of 2. The methanol is evaporated and to the syrup is added acetone, the formed precipitated is filtered off, dried overnight in an oven to obtain a pale yellow powder in 91 % yield. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 11.99 (bs, 1H, -COOH), 9.11 (d, *J* = 9.6, Hz, 1Hpyr), 9.02 (d, *J* = 9.7 Hz, 1Hpyr), 9.03 (s, 1H pyr), 8.94 (d, *J* = 9.9 Hz, 1Hpyr), 8.37 (d, *J* =9.6 Hz, 1Hpyr), 8.19 (s, 1Hpyr), 4.35 (t, 2H, CH_2-α-O), 2.19 (t, 2H, CH_2-α-COO), 1.96 (m, 2H, CH_2-β-O), 1.59 (m, 2H, CH_2-β-COO), 1.49 (m, 2H, CH_2-γ-O), 1.41 (m, 2H, CH_2-γ-COO), 1.28 (bs, 8H, -CH_2-).

Synthesis of sodium 8-(undecyloxy)pyrene-1,3,6-trisulfonate (M2): *Applying the general procedure for the Williamson alkylations of* ***M***: 150 mg (0.286 mmol) of **M**, 25 mL of methanol, 470 mg (2.0 mmol) of 1-bromoundecane and 115 mg (0.16 mL, 0.886 mmol) of DIPEA. The crude product is precipitated in CH_2Cl_2 and then in acetone, filtrated and purified by flash chromatography (SiO_2, $CHCl_3$, and then $CHCl_3$:MEOH 1:1 v/v) to obtain a beige powder in 85 % yield. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 9.11 (d, *J* = 9.6 Hz, 1Hpyr), 9.03 (d, *J* = 9.9 Hz, 1Hpyr), 9.02 (s, 1Hpyr), 8.94 (d, *J* = 9.9 Hz, 1Hpyr), 8.37 (d, *J* = 9.6 Hz, 1Hpyr), 8.19 (s, 1Hpyr), 4.35 (t, 2H, CH_2-α-O), 1.96 (m, 2H, CH_2-β-O), 1.59 (m, 2H, CH_2-γ-O), 1.42 (m, 2H, CH_2-β-CH_3), 1.25 (bs, 12H, -CH_2-), 0.84 (t, 3H, CH_3).

Synthesis of sodium 8-(prop-2-yn-1-yloxy)pyrene-1,3,6-trisulfonate (M3): *Applying the general procedure for the Williamson alkylations of* ***M***: 300 mg (0.572 mmol) of **M**, 35 mL of methanol, 176 mg (4.0 mmol) of 1-bromopropargyl and 229 mg (0.309 mL, 1.77 mmol) of DIPEA. The crude product is precipitated in CH_2Cl_2 and then in acetone, filtrated and purified by flash chromatography (SiO_2, $CHCl_3$, and then $CHCl_3$:MEOH 1:1 v/v) to obtain a pale brown powder in 72 % yield. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 9.14 (d, *J* = 9.6 Hz, 1Hpyr), 9.07 (d, *J* = 9.9 Hz, 1Hpyr), 9.06 (s, 2Hpyr), 8.99 (d, *J* = 9.9 Hz, 1Hpyr), 8.37 (d, *J* = 9.6 Hz, 1Hpyr), 8.39 (s, 1Hpyr), 5.20 (s, 2H, CH_2-≡), 3.65 (s, 1H, H-≡).

II. ^{1}H Nuclear Magnetic Resonance spectra

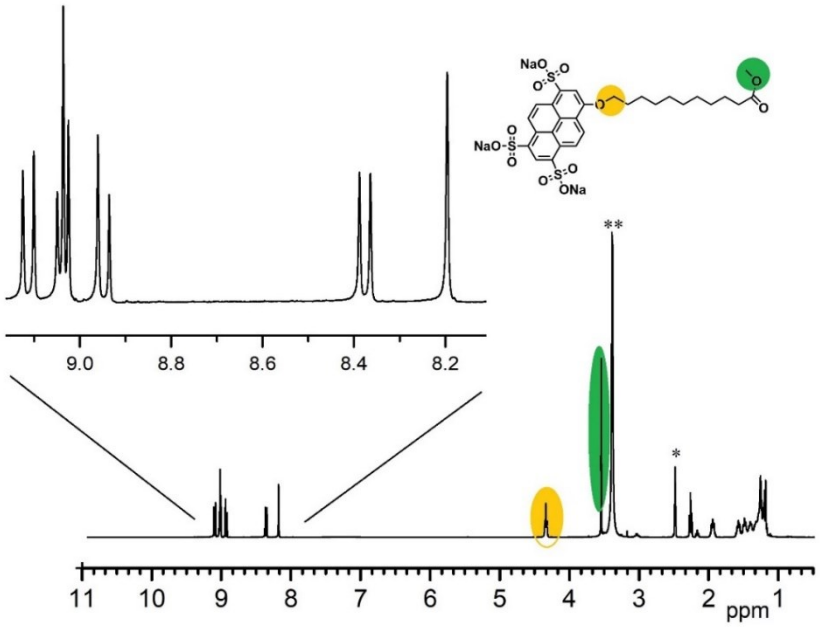

Figure S1. ^{1}H NMR spectra of sodium 8-((methyl 10-undecanoate)oxy)pyrene-1,3,6-trisulfonate in DMSO- *d*6. *Residual DMSO and **water.

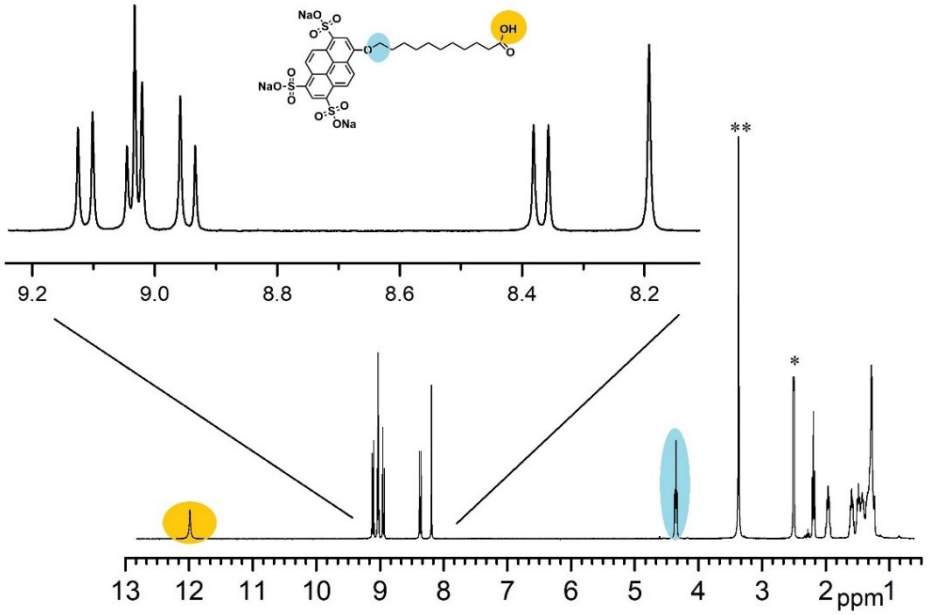

Figure S2. ^{1}H NMR spectra of sodium 8-((10-carboxydecyl)oxy)pyrene-1,3,6-trisulfonate (**M1**) in DMSO-*d*6. *Residual DMSO and **water.

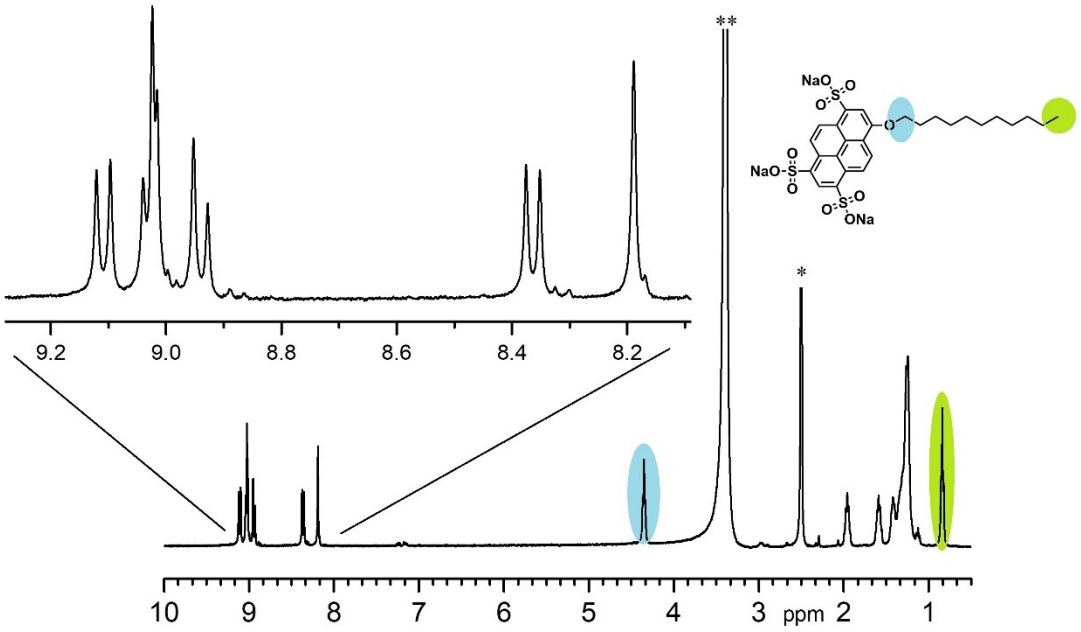

Figure S3. [1]H NMR spectra of sodium 8-(undecyloxy) pyrene-1,3,6-trisulfonate (**M2**) in DMSO-*d*6. *Residual DMSO and **water.

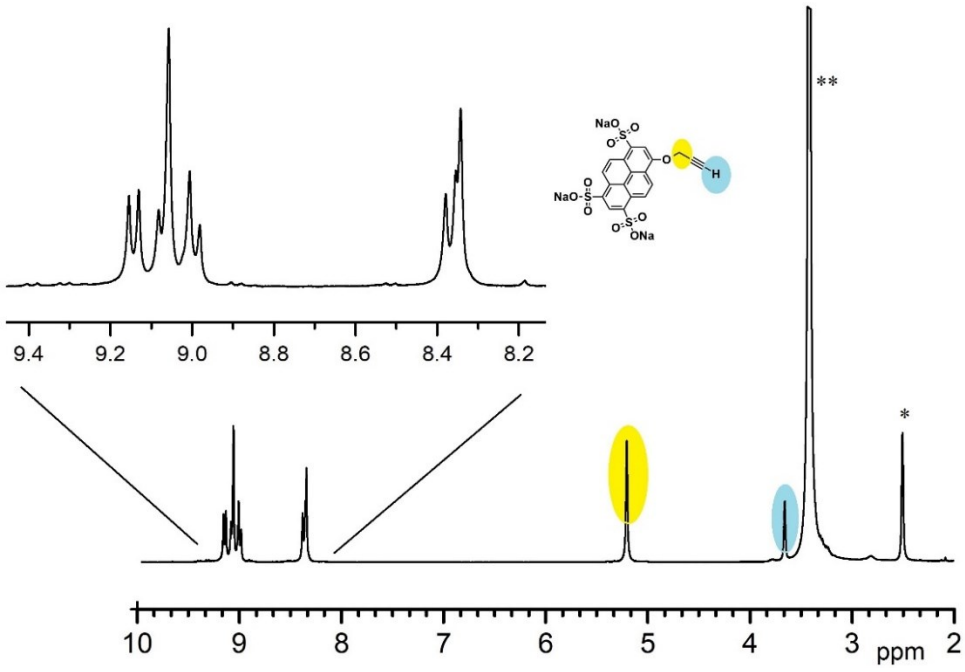

Figure S4. [1]H NMR spectra of sodium 8-(prop-2-yn-1-yloxy) pyrene-1,3,6-trisulfonate (**M3**) in DMSO-*d*6. *Residual DMSO and **water.

III. Chemotaxis assays

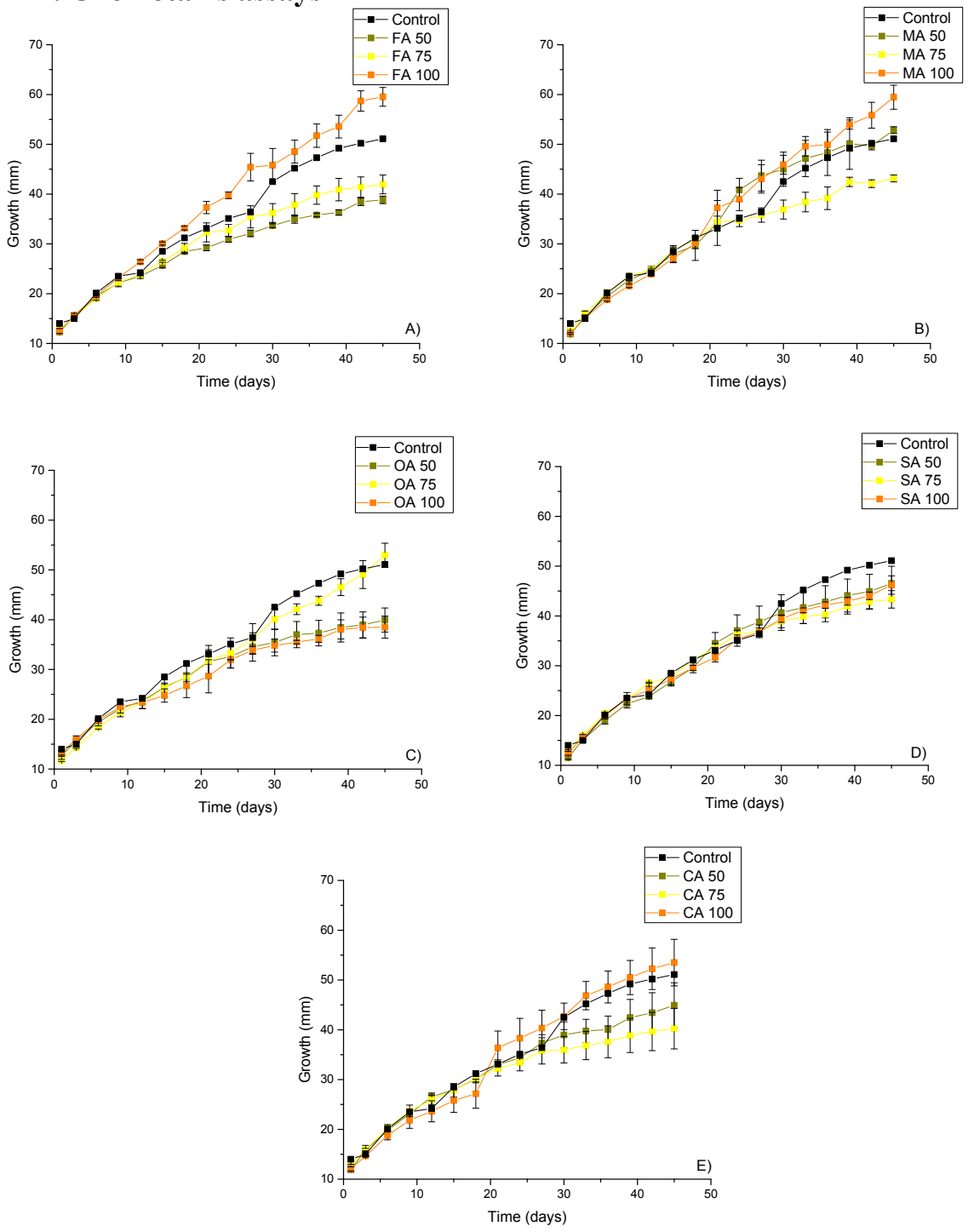

Figure S5. Chemotaxis assay of *Bacillus subtilis* LPM1 in plates with M9-medium supplemented with different organic acids at different concentrations (50, 75 and 100 µM): fumaric acid **(**FA), malic acid (MA), oxalic acid (OA), succinic acid (SA), citric acid (CA), and control water.

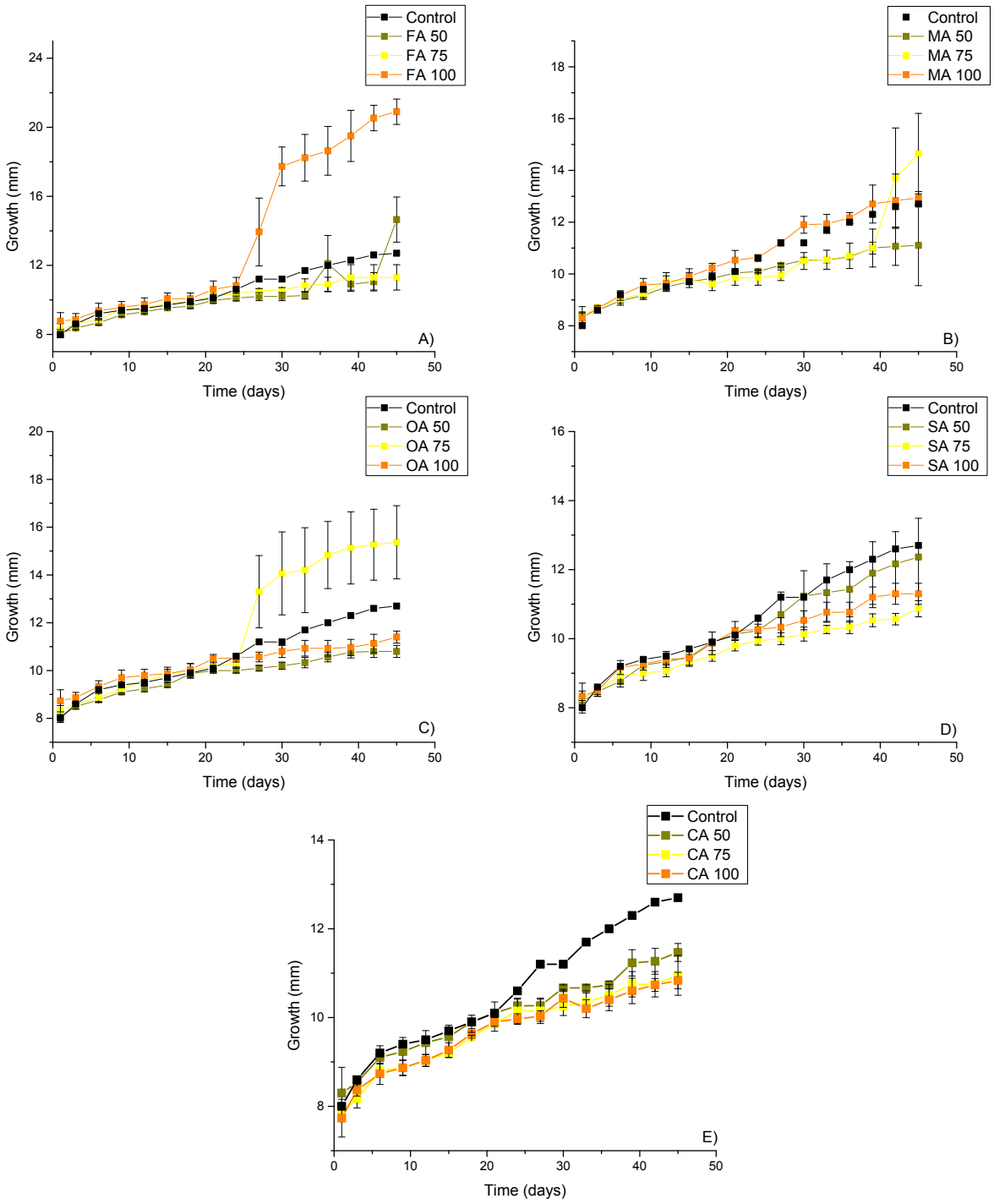

Figure S6. Chemotaxis assay of *Bacillus subtilis* PY79 in plates with M9-medium supplemented with different organic acids at different concentrations (50, 75 and 100 µM): fumaric acid **(**FA), malic acid (MA), oxalic acid (OA), succinic acid (SA), citric acid (CA), and control water.

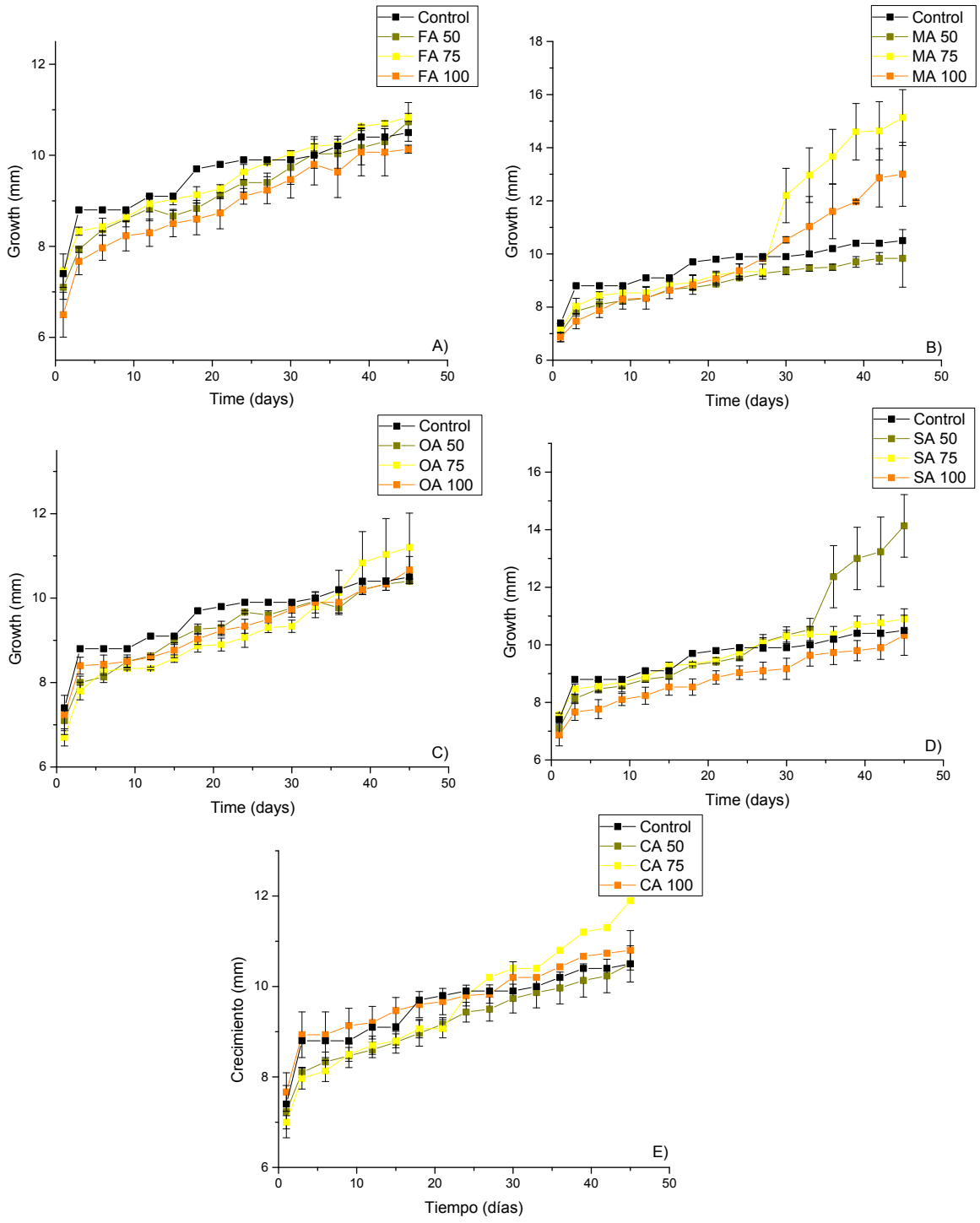

Figure S7. Chemotaxis assay of *Escherichia coli* DH5α in plates with M9-medium supplemented with different organic acids at different concentrations (50, 75 and 100 µM): fumaric acid **(**FA), malic acid (MA), oxalic acid (OA), succinic acid (SA), citric acid (CA), and control water.

Table S1. Chemotaxis assay of *Bacillus subtilis* PY79 in capillary tubes with M9-medium supplemented with different organic acids. Malic acid (**MA**), fumaric acid **(FA**), citric acid (**CA**), oxalic acid (**OA**), succinic acid (**SA**), and control (**C**).

	30 min	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
	[CFU mL^{-1}]	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**
MA	1.0×10^7	4.0×10^7	1.2×10^8	1.7×10^9	2.0×10^9	2.9×10^9
FA	1.0×10^7	1.0×10^7	6.0×10^7	2.1×10^8	1.1×10^9	1.7×10^9
CA	0	0	0	6.0×10^7	2.4×10^8	3.4×10^9
OA	1.0×10^7	1.0×10^7	1.0×10^7	8.0×10^7	5.5×10^8	2.0×10^9
SA	0	0	0	3.7×10^8	3.9×10^8	2.2×10^9
C	0	0	0	0	0	0

Table S2. Chemotaxis assays of *Escherichia coli* DH5α in capillary tubes with M9-medium supplemented with different organic acids. Malic acid (**MA**), fumaric acid **(FA**), citric acid (**CA**), oxalic acid (**OA**), succinic acid (**SA**), and control (**C**).

	30 min	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
	[CFU mL^{-1}]	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**	**[CFU mL^{-1}]**
MA	1.0×10^7	9.0×10^7	2.1×10^8	9.3×10^8	9.7×10^8	1.2×10^9
FA	2.0×10^7	8.0×10^7	9.0×10^7	1.0×10^8	1.6×10^8	2.6×10^8
CA	0	5.0×10^7	7.0×10^7	9.0×10^7	6.1×10^8	2.1×10^8
OA	0	7.0×10^7	8.0×10^7	1.7×10^8	8.3×10^8	1.7×10^9
SA	1.0×10^7	6.0×10^7	5.3×10^8	7.0×10^8	1.3×10^9	1.4×10^9
C	0	0	0	0	0	0

IV. Root colonization assays

Tomato seeds from three varieties (*Solanum lycopersicum* cv. Micro-Tom, *S. lycopersicum* cv. Floradade and *S. lycopersicum* cv. Wild) were germinated in Peat-moss for two weeks. Seedlings of three varieties of tomato were inoculated with *B. subtilis* LPM1 (approximately 10^8 CFU/mL) and were transplanted in 1.5 L pots containing peat-moss:perlite (70:30 v/v) as described in Experimental section. After 28 days, all of the roots were exposed in the M1 marker solution with agitation (150 rpm) at 30°C for 24 hours. Finally, roots were observed by LSCM with the same conditions described in the experimental section.

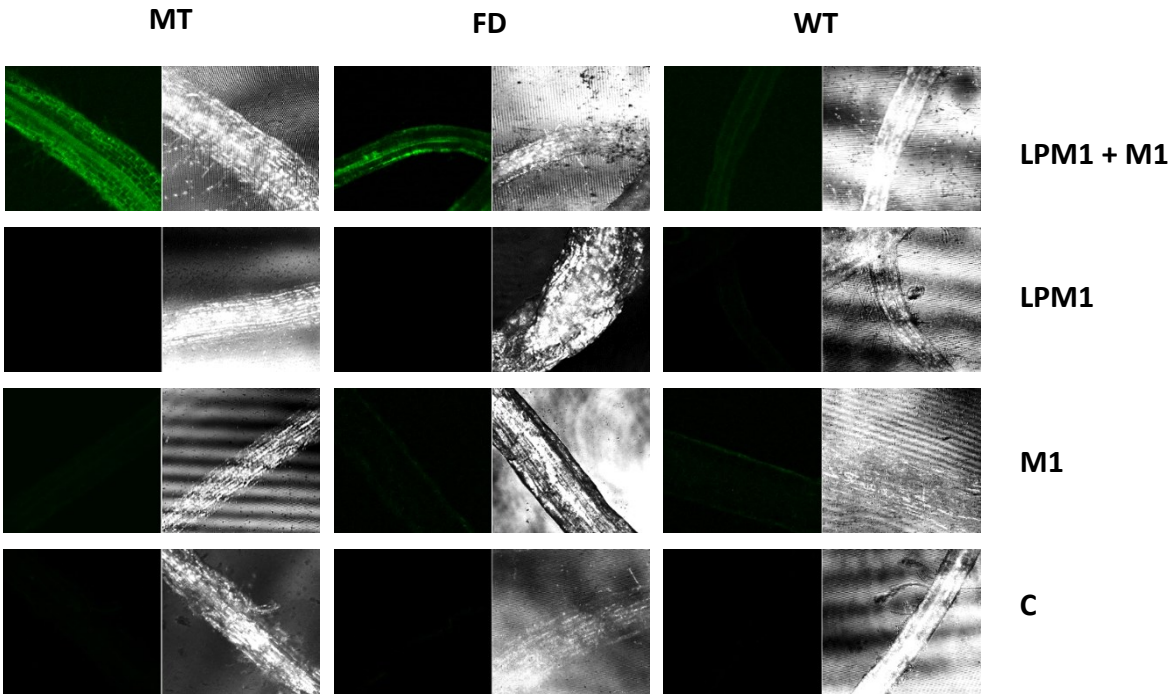

Figure S8. LSCM images of root colonisation patterns by *B. subtilis* LPM1 in three varieties of tomato plants: *Solanum lycopersicum* cv. Micro-Tom (**MT**), *S. lycopersicum* cv. Floradade (**FD**), and Wild *S. lycopersicum* cv. (**WT**). Roots treated with LPM1 strain and **M1** marker (**LPM1 + M1**), roots treated with LPM1strain (**LPM1**), roots treated with M1 marker (**M1**), and roots control water (**C**). Left images: fluorescence channel and right images: reflection channel.