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Novel form of collective movement by soil bacteria

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Supplementary Materials

Table 1: Strains used in this study

Name	Genotype ^a	Reference / Construction ^b
NCIB 3610	Wild type prototroph	Bacillus genetic stock centre
BAL834 (stocked as NRS1112)	JH642 <i>amyE::Phy-spac-gfp mut2</i> (cml)	(1)
BAL836 (stocked as NRS1113)	JH642 <i>amyE::Phy-spac-gfp mut2</i> (spc)	(2)
NRS1473	NCIB 3610 <i>sacA::Phy-spac-gfp mut2</i> (kan)	(3)
NRS2450	NCIB 3610 <i>epsA-O</i> (tet)	(4)
NRS5841	NCIB 3610 <i>comI amyE::Phy-spac-mKate2</i> (spc)	(5) (kindly provided by Prof Akos Kovacs)
NRS5852	NCIB 3610 <i>amyE::Phy-spac-mKate2</i> (spc)	SSP1 NRS5841-> NCIB 3610 Made by Margarita Kalamara (Stanley-Wall laboratory)
DS1677 (stocked as NRS2253)	NCIB 3610 Δ <i>hag</i>	(6) (kindly provided by Prof Daniel Kearns)
NRS6959	NCIB 3610 Δ <i>hag sacA::Phy-spac-gfp mut2</i> (kan)	SSP1 NRS1473-> DS1677
NRS3798	NCIB 3610 Δ <i>epsA-O</i> (tet) <i>amyE::Phy-spac-gfp mut2</i> (spc)	SSP1 BAL836 -> NRS2450 Made by Adam Ostrowski (Stanley-Wall laboratory)
NRS6958	NCIB 3610 Δ <i>srfAA</i> (kan)	(7)
NRS6963	NCIB 3610 Δ <i>srfAA</i> (kan) <i>amyE::Phy-spac-gfp mut2</i> (cml)	SSP1 BAL834 -> NRS6958
NRS5634	NCIB 3610 <i>comI</i> (Q12L) <i>sacA::Phy-spac-gfp mut2</i> (kan)	(8)
NRS7387	NCIB 3610 <i>comI</i> (Q12L) <i>sacA::Phy-spac-gfp mut2</i> (kan) Δ <i>comA</i> (spc)	pBL7 (1) -> NRS5634

^{a.} Antibiotic resistance cassettes are indicated as follows: *cml*, chloramphenicol resistance; *kan*, kanamycin resistance; *tet*, tetracycline resistance and *spc*, spectinomycin resistance.

^{b.} The direction of strain construction is indicated with phage (SPP1) or plasmid into the (→) recipient strain. A reference is provided if the strain or plasmid has previously been described and published.

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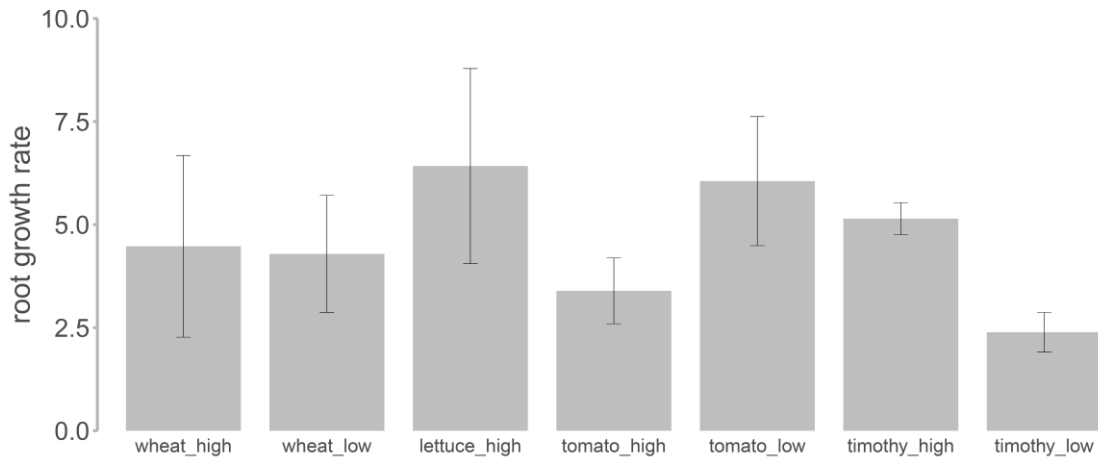


Fig. S2: Samples in flock analysis show not significant variation in root elongation between roots of different species or due to variation in soil liquid viscosity. Bargraphs show the average root elongation rates ($\mu\text{m min}^{-1}$) of different plant species (wheat, lettuce, tomato and timothy grass), grown in high or low viscosity soil solution. Error bars show $\pm\text{SD}$ and ANOVA analysis confirm no significant effect of viscosity on root elongation rate.

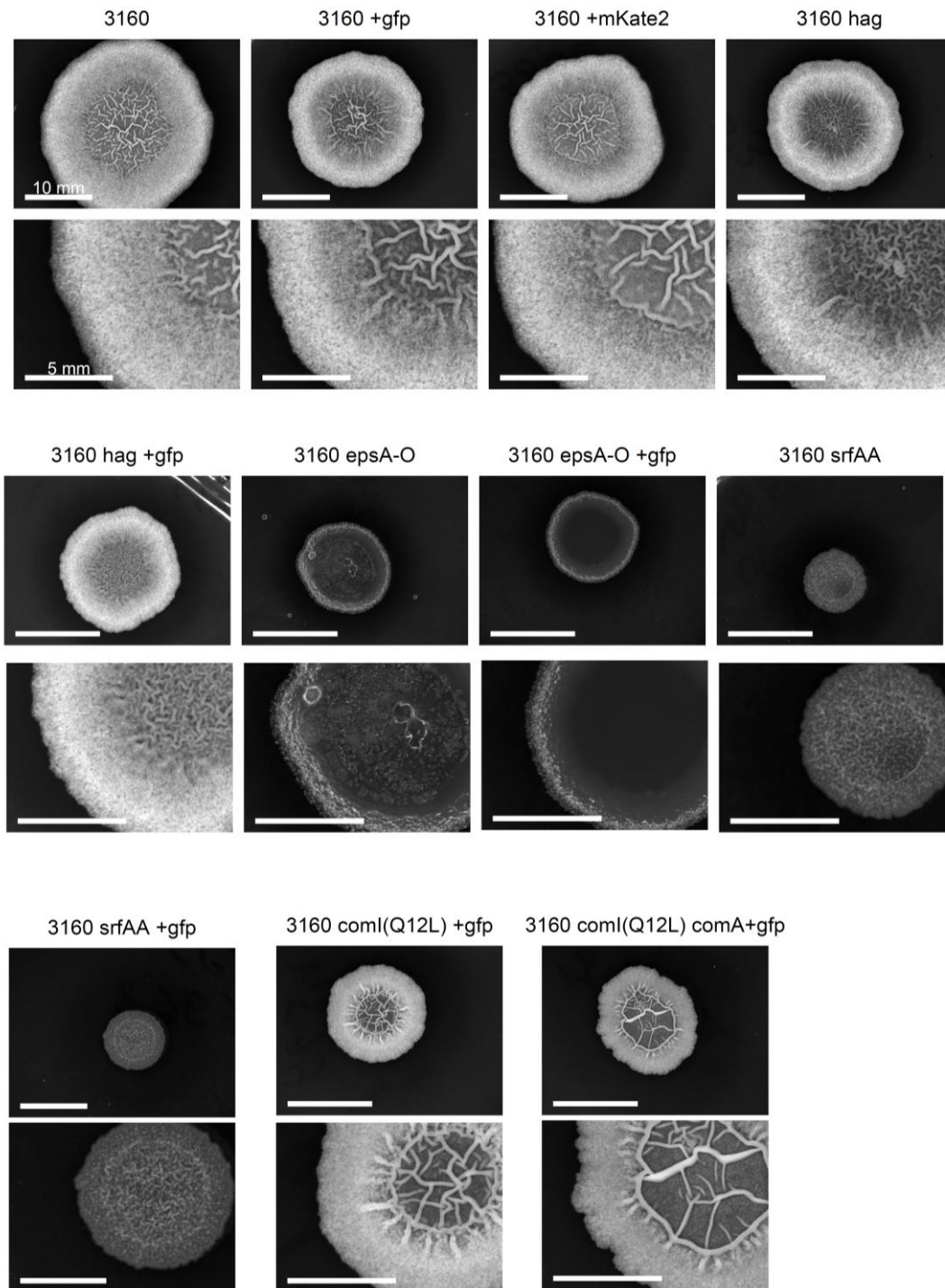


Fig. S3: Images of colony biofilms formed on MSgg agar for the different *B. subtilis* strains used in this study. Scale bar is 10 mm in the upper images and 5 mm the lower images. Images were taken after 48 hours incubation at 30°C.

Supplemental movie legends

Video S4. High quality time lapse of GFP labelled *B. subtilis* 3610 (NRS1473, green) exhibiting crowd movement around a lettuce root (*Lactuca sativa*, red) growing in sulphorhodamine B stained transparent soil (magenta). Images were captured in 60-minute intervals using a 10X objective. The time lapse covers a 24 hour period, approximately 24 hour post patch inoculation of the *B. subtilis*. The lettuce seedling is 3 days old.

Video S5. GFP labelled *B. subtilis* 3610 (NRS1473) exhibiting crowd movement around a tomato root (*Solanum lycopersicum*) growing in transparent soil saturated with a 78% percoll solution (6.6 mPa s^{-1}). Filamentous flocks show the defined, multiple branching morphology associated with higher viscosity solutions. Images were collected at 30-minute intervals using a 2X objective. Time lapse covers a 10 hour period.

Video S6. GFP labelled *B. subtilis* 3610 (NRS1473) exhibiting crowd movement around a tomato root (*Solanum lycopersicum*) growing in transparent soil saturated with a 39% percoll solution (2.2 mPa s^{-1}). Images were collected at 30 minute intervals using a 2X objective. Time lapse covers a 10 hour period.

Video S7. GFP labelled *B. subtilis* 3610 (NRS1473) exhibiting crowd movement around a tomato root (*Solanum lycopersicum*) growing in transparent soil saturated with water (1 mPa s^{-1}). Filamentous flocks show the persistent, diffuse and less branched morphology associated with non-viscous solutions. Images were collected at 30-minute intervals using a 2X objective. Time lapse covers a 10 hour period.

Video S8. GFP labelled WT-2 strain of *B. subtilis* 3610 (NRS5634) exhibiting filamentous, branched and highly co-ordinated crowd movement around a wheat root

(*Triticum aestivum* var. filon) growing in transparent soil. Images were collected at 30-minute intervals using a 1X objective. Time lapse covers a 10 hour period.

Video S9. GFP labelled surfactin (*surfAA*) mutant strain of *B. subtilis* 3610 (NRS6963) exhibiting filamentous, branched and co-ordinated crowd movement around a wheat root (*Triticum aestivum* var. filon) growing in transparent soil. Images were collected at 30-minute intervals using a 1X objective. Time lapse covers a 10 hour period.

Video S10. GFP labelled exopolysaccharide (*eps A-O*) mutant strain of *B. subtilis* 3610 (NRS3798) exhibiting crowd movement around a wheat root (*Triticum aestivum* var. filon) growing in transparent soil. Some loss of co-ordination and branching may be visible. Images were collected at 30-minute intervals using a 1X objective. Time lapse covers a 10 hour period.

Video S11. GFP labelled quorum sensing (*comA*) mutant strain of *B. subtilis* 3610 (NRS 7383) exhibiting crowd movement around a wheat root (*Triticum aestivum* var. filon) growing in transparent soil. Distinct loss of co-ordination and branching is visible. Images were collected at 30-minute intervals using a 1X objective. Time lapse covers a 10 hour period.

Video S12. GFP labelled flagellar (*hag*) mutant strain of *B. subtilis* 3610 (NRS6959) does not exhibit crowd movement around a wheat root (*Triticum aestivum* var. filon) growing in transparent soil. Images were collected at 30-minute intervals using a 1X objective. Time lapse covers a 10 hour period.

Video S13. Co-inoculation experiment with GFP labelled flagellar (*hag*) mutant strain of *B. subtilis* 3610 (NRS6959, green) and mKate labelled wild-type *B. subtilis* 3610 (NRS5852, magenta). The wild type exhibits crowd movement around the tomato root (*Solanum lycopersicum*, red) growing in transparent soil but the flagella mutant strain does not. Some groups of flagella mutant are visible on soil particles (top right). Images were collected at 30-minute intervals using a 2X objective. Time lapse covers a 10 hour period.