

Supplementary Material

1 Supplementary Figures and Tables

 Table S1. See attached Excel spreadsheet.

Table S2. Primers and plasmids used in this study.

Primers			
Name	Description	Sequence	Reference
PRK341F	Universal primer pair that amplifies	5'-CCTACGGGAGGCAGCAG-3'	
PRK806R	the V3-V4 region 16S rRNA gene.	5'-GGACTACHVGGGTWTCTAAT-3'	[1]
ermEKpnFwd	Used to amplify ermE* from the plasmid	CGA CTC GGT ACC AGC CCG ACC CGA GCA CGC	This study
ermENdeRev	PIJ110257	CGA ACC CAT ATG GGG CCT CCT GTT CT	This study

Plasmids			
Name	Description	Reference	
PIJ8660	Contains an optimised eGFP gene; aac ^R .	[2]	
PIJ10257	Used as a template from which to amplify ermEp*	[3]	
PIJ8660/erm	Contains an optimised eGFP gene under the control of the constitutive ermE* promotor; aac ^R .	This study	

Table S3. Characteristics of F2 Levington Compost.

Parameter	Measurement	SAC Rating	
рН	4.98	n/a	
Phosphorous	880.5 mg/kg	Excessively high	
Potassium	2508 mg/kg	Excessively high	
Magnesium	6021mg/kg	Excessively high	

Nitrate	5809.49 g/kg	n/a
Ammonium	192.18 g/kg	n/a
Organic matter	91.08 %	n/a

Table S4. Summary of stable isotope probing results displaying a list of genera that were identified as candidate autotrophs in either the rhizosphere or endosphere, as well as the genera (and their associated phyla) that were identified to be more than two-fold enriched in the heavy fractions (versus the light fractions) of samples from the rhizosphere or endosphere (or both, shown in red) suggesting they were metabolising labelled root exudates.

Autotrophic genera		Metabolisers of root exudates in the rhizosphere		Metabolisers of root exudates in the endosphere	
Rhizosphere	Endosphere	Phylum	Genus	Phylum	Genus
Bacteroides	Amaricoccus	Proteobacteria	Ensifer	Proteobacteria	Sphingobium
Bordetella	Bacteroides	Proteobacteria	Sideroxydans	Actinobacteria	Jatrophihabitans
Brevundimonas	Bordetella	Proteobacteria	Pseudomonas	Proteobacteria	Novosphingobium
Candidatus nitrotoga	Brevundimonas	Proteobacteria	Sinorhizobium	Proteobacteria	Methyloferula
Candidimonas	Candidatus nitrotoga	Proteobacteria	Telluria	Proteobacteria	Sinorhizobium
Delftia	Frateuria	Proteobacteria	Herbaspirillum	Proteobacteria	Shinella
Frateuria	Geobacter	Proteobacteria	Shinella	Proteobacteria	Pseudomonas
Hirschia	Heliobacterium	Proteobacteria	Limnobacter	Proteobacteria	Acidovorax
Methylibium	Hirschia	Proteobacteria	Massilia	Proteobacteria	Ensifer
Methylobacterium	Longilinea	Proteobacteria	Azohydromonas	Proteobacteria	Rhizobium
Nitrosospira	Luteimonas	Proteobacteria	Sphingopyxis	Proteobacteria	Agrobacterium
Paracoccus	Magnetospirillum	Proteobacteria	Chelatococcus	Planctomycetes	Blastopirellula
Prochlorococcus	Methylibium	Proteobacteria	Aquincola	Proteobacteria	Azospirillum
Sterolibacterium	Methylobacterium	Chloroflexi	Levilinea	Proteobacteria	Sphingopyxis
Woodsholea	Methyloversatilis	Proteobacteria	Altererythrobacter	Proteobacteria	Roseomonas
	Nannocystis	Proteobacteria	Hyphomicrobium	Proteobacteria	Lacibacterium
	Paracoccus	Proteobacteria	Methylocella	Proteobacteria	Pseudoxanthomonas
	Prosthecomicrobium	Proteobacteria	Pseudoxanthomonas	Proteobacteria	Rhodanobacter
	Rhodocista	Cyanobacteria	Chroococcidiopsis	Planctomycetes	Pirellula
	Thermoleophilum	Proteobacteria	Rhizobium	Proteobacteria	Telluria
	Woodsholea	Proteobacteria	Duganella	Proteobacteria	Massilia
		Proteobacteria	Asticcacaulis	Proteobacteria	Thermomonas
		Proteobacteria	Solimonas	Chloroflexi	Chloroflexus
		Proteobacteria	Thermomonas	Firmicutes	Chelativorans
		Proteobacteria	Acidovorax	Planctomycetes	Gemmata
		Planctomycetes	Pirellula	Proteobacteria	Aquincola
		Proteobacteria	Aetherobacter	Proteobacteria	Limimonas
		Firmicutes	Pelotomaculum		

 Table S5. Buffer and media recipes [see also 4]

Media	Component	g L ⁻¹ dH ₂ O
Soya Flour Mannitol (SFM) agar	Soy flour	20
	Mannitol	20
	Agar	20
	Maltose	4
Maltose-Yeast	Yeast Extract	4
(MYM) agar	Malt Extract	10
	Agar	18
ISP2 Agar	Yeast Extract	4
	Maltose	10
	D-glucose	4
	Agar	20
2xYT	Bacto-tryptone	16
	Yeast extract	10

	NaCl	5
	NaH2PO4.H2O	6.33 g
Silwett L-77 amended Phosphate Buffered Saline (PBS-S)	Na2HPO4.H2O	16.5 g
	Silwet L-77	200 µl added after autoclaving
	NH4SO4	1
	KH ₂ PO ₄	0.5
	MgSO4.7H2O	0.2
Minimal agarose media	FeSO ₄ .7H ₂ O	0.01
	Agarose	6
	Trace element solution	2 ml added after autoclaving
	Optional carbon source:	
	Mannitol or	5
	Maltose or	5

	Sucrose or	5
	Glucose or	10
	SA or	0.069
	Sodium citrate	1
Trace element solution	ZnCl ₂	0.04
	FeCl ₃ .6H ₂ O	0.2
	CuCl ₂ .2H ₂ O	0.01
	MnCl ₂ .4H ₂ O	0.01
	Na ₂ B ₄ O ₇ .10H ₂ O	0.01
	(NH4)6M07O24.4H2O	0.01
	Murashige and Skoog salts (Duchefa Biochemie, Harlem, Netherlands)	4.43
Murashige and Skoog (MSk) Agar	Sucrose	10
	Agar	8



Figure S1. *Streptomyces* isolates (as indicated) grown on minimal medium agarose plates containing either no carbon, 0.5 mM salicylic acid (SA), 3.875 mM sodium citrate or their preferred carbon source.



Figure S2. Salicylic acid chemoattractant assays. Streptomycete colonies were grown next to disks soaked in 0.1% DMSO (as a control for the SA solvent), 0.5 mM SA or 1 mM SA. Images were taken after 10 days of growth at 30°C. All assays were carried out on SFM agar media (Table S5). *Streptomyces* strains A) N2, B) N1, C) M3, D) *Streptomyces coelicolor* M145, E) M2, F) L2 and G) Actinovate were tested for their response to SA.



Figure S3. *Streptomyces* isolates growing on root exudates as a sole food source. Strains N1, L2, M3, M2 and N2 were isolated from surface washed *A. thaliana* roots. *Actinovate* is the *S. lydicus* WYEC106 cultured from the horticultural product Actinovate. *S. lydicus* 25470 was acquired from the American Type Culture Collection (see Table S1 in Additional File 1 for more details).



Figure S4. The relative abundance (%) of bacterial phyla in samples from the bulk soil, rhizosphere and root compartments of *Arabidopsis thaliana* grown in compost (n=3 replicate samples per compartment).



Figure S5. ¹³C DNA stable isotope probing with *A. thaliana* plants.



Figure S6. 16S rRNA gene copy number across buoyant density fractions of DNA isolated from root samples of *A. thaliana plants* incubated under either ${}^{12}CO_2$ (blue) or ${}^{13}CO_2$ (orange) conditions. Copy number is shown as a percentage of total copy number per sample. N=2 replicate measurements on the same fraction. Bars represent standard errors. Green and black dots represent the "light" and "heavy" fractions, respectively, that were pooled in each treatment and submitted for 16S rRNA gene amplicon sequencing.



Figure S7. The abundance of the eight bacterial genera metabolising the greatest quantities of labelled exudates in the endophytic compartment of *A. thaliana* (determined by ratios of abundance in 13CH to 12CH fractions). Their relative abundances (%) are shown across the bulk soil, rhizosphere and root compartments (light yellow to brown indicates increasing relative abundance). N = 3 replicate plants in individual pots. *Streptomyces* species were not metabolising root exudates, nor were they enriched in the endophytic compartment, but are shown for comparison. Clustering represents Bray-Curtis dissimilarities.

References

- 1. Yu, Y., et al., *Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction.* Biotechnol Bioeng, 2005. **89**(6): p. 670-9.
- 2. Sun, J., et al., *Green fluorescent protein as a reporter for spatial and temporal gene expression in Streptomyces coelicolor A3(2).* Microbiology, 1999. **145 (Pt 9)**: p. 2221-7.
- 3. Hong, H.J., et al., *The role of the novel Fem protein VanK in vancomycin resistance in Streptomyces coelicolor*. J Biol Chem, 2005. **280**(13): p. 13055-61.
- 4. ActinoBase. ActinoBase: an Actinomycete Community Wiki. Available from: <u>www.actinobase.org</u>.