Streptomyces endophytes promote the growth of *Arabidopsis thaliana*

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Abstract. *Streptomyces* bacteria are ubiquitous in soils and are well-known for producing secondary metabolites, including antimicrobials. Increasingly, they are being isolated from plant roots and several studies have shown they are specifically recruited to the rhizosphere and the endosphere of the model plant *Arabidopsis thaliana*. Here we test the hypothesis that *Streptomyces* bacteria have a beneficial effect on *A. thaliana* growth and could potentially be used as plant probiotics. To do this, we selectively isolated streptomycetes from surface washed *A. thaliana* roots and generated high quality genome sequences for five strains which we named L2, M2, M3, N1 and N2. Re-infection of *A. thaliana* plants with L2, M2 and M3 significantly increased plant biomass individually and in combination whereas N1 and N2 had a negative effect on plant growth, likely due to their production of polyene natural products which can bind to phytosterols and reduce plant growth. N2 exhibits broad spectrum antimicrobial activity and makes filipin-like polyenes, including 14-hydroxyisochainin which inhibits the Take-all fungus, *Gaeumannomyces graminis* var. *tritici*. N2 antifungal activity as a whole was upregulated ~2-fold in response to indole-3-acetic acid (IAA) suggesting a possible role during competition in the rhizosphere. Furthermore, coating wheat seeds with N2 spores protected wheat seedlings against Take-all disease. We conclude that at least some soil dwelling streptomycetes confer growth promoting benefits on *A. thaliana* while others might be exploited to protect crops against disease.

Importance. It is vital that we reduce our reliance on agrochemicals and there is increasing interest in using bacterial strains to promote plant growth and protect against disease. Our study follows up reports that *Arabidopsis thaliana* specifically recruits *Streptomyces* bacteria to its roots. In particular, we test the hypothesis that these bacteria can offer benefits to their *A. thaliana* hosts and that strains isolated from these plants might be used as probiotics. We isolated *Streptomyces* strains from surface washed *A. thaliana* roots and genome sequenced five phylogenetically distinct strains. Genome mining and bioassays indicated that all five strains have plant growth promoting properties, including production of IAA, siderophores and ACC deaminase activity. Three strains significantly increased *A. thaliana* growth *in vitro* and when applied in combination in soil. Another produces potent filipin-like antifungal metabolites and we used it as a seed coating to protect germinating wheat seeds against the fungal pathogen *Gaeumannomyces graminis* var. *tritici* (wheat Take-all fungus). We conclude that introducing an optimal combination of *Streptomyces* strains into the root microbiome can provide significant benefits to plants.

Introduction. The bacterial genus *Streptomyces* comprises more than 600 known species and they produce a diverse array of specialised metabolites that account for ~55% of the antibiotics currently used in human medicine (1). They are filamentous, spore-forming bacteria that are ubiquitous in soils where they play an important role in breaking down complex organic material (2, 3). Intriguingly, they only produce around 10% of their encoded secondary metabolites *in vitro* (2-4). Thus, understanding the role and regulation of their specialised metabolites in natural habitats is essential if we are to unlock the other 90% and discover new molecules (3). Increasingly, *Streptomyces* species are being recognised as important defensive symbionts of a wide range of invertebrate species including bees, beetles, digger wasps and ants (5-9). In addition to this, streptomycetes have also been shown to interact extensively with plant roots, inhabiting both the soil surrounding the plant root, called the rhizosphere, as well as the niche within and between root cells, referred to as the endophytic compartment (10, 11). It has even been suggested that their filamentous, hyphal growth and complex specialised metabolism may have evolved to facilitate interactions with plant roots, presumably to allow entry into root tissue and enable them to compete for food in the form of root exudates or more complex polymers that make up the plant cell wall (2).

Several recent studies have reported that streptomycetes are present, and sometimes enriched, in the endophytic compartment of the model plant *Arabidopsis thaliana* relative to the bulk soil (12-15) where they are attracted by plant metabolites in the root exudates such as salicylate and jasmonate (16, 17). They have also been isolated from the endosphere of many other plant species, including wheat, a crop of huge social and economic value (18-21). Due to their capacity to produce a large number of antimicrobial compounds and their ability to abundantly colonise plant roots, streptomycetes are gaining increasing interest from a biocontrol point of view (10, 11, 19, 22). A recent study demonstrated that certain strains can act as defensive mutualists of strawberry plants whereby they protect their host plant and pollinating bees against fungal infections (23). Many other isolates have been shown to protect important crops against infection and two strains have been developed into commercial biocontrol agents called Actinovate® and Mycostop® (10, 19, 22, 24). The ubiquity of streptomycetes in soil and their diverse specialised metabolism, combined with their ability to colonise plant roots, makes streptomycetes attractive for this purpose. Their spore-forming capabilities also makes them tolerant of many environmental pressures, allowing them to be applied as dried seed coatings which remain viable under various agricultural conditions.

The aim of this study was to test the hypothesis that plant-associated streptomycetes provide benefits to their host *A. thaliana* plant and that strains isolated from *A. thaliana* might confer benefits to important crop plants, such as wheat. We hypothesised that these strains may play defensive or plant growth promoting roles since this genus is consistently recruited to the plant root microbiome. To this end, we generated high quality genome sequences for five *Streptomyces* species which we isolated from the root microbiome of *A. thaliana* plants and which could recolonise *A. thaliana* roots when applied as seed coatings. All five strains, named L2, M2, M3, N1 and N2, encode a large number of secondary metabolite biosynthetic gene clusters (BGCs) and all strains inhibited at least one bacterial or fungal pathogen. Strain N2 demonstrated broad spectrum antifungal and antibacterial activity and was able to inhibit growth of the Take all fungus, an economically important pathogen of wheat, both *in vitro* and on germinating wheat seeds. The antifungal activity of N2 was increased 2-fold *in vitro* in response to indole-3-acetic acid (IAA) and purification of the antifungal molecules identified a number of filipin-like compounds. Curiously, N2 reduced *A. thaliana* growth *in vitro* (but not in soil) which may have been caused by filipins targeting sterols in the plant cell membranes at high concentrations. Strains L2, M2 and M3 all promoted *A. thaliana* growth *in vitro* and when applied in combination to seeds planted in soil, and they all have well characterised plant growth promoting (PGP) traits including the production of plant growth hormones, siderophores and ACC deaminase. We conclude that *A. thaliana* can acquire significant benefits from recruiting streptomycetes to their root microbiome which they likely attract through the deposition of root exudates and dead root material into the bulk soil. Additionally, mining plant-streptomycete interactions may yield novel biocontrol and plant-growth promoting agents that could be developed for future applications in agriculture.

Results

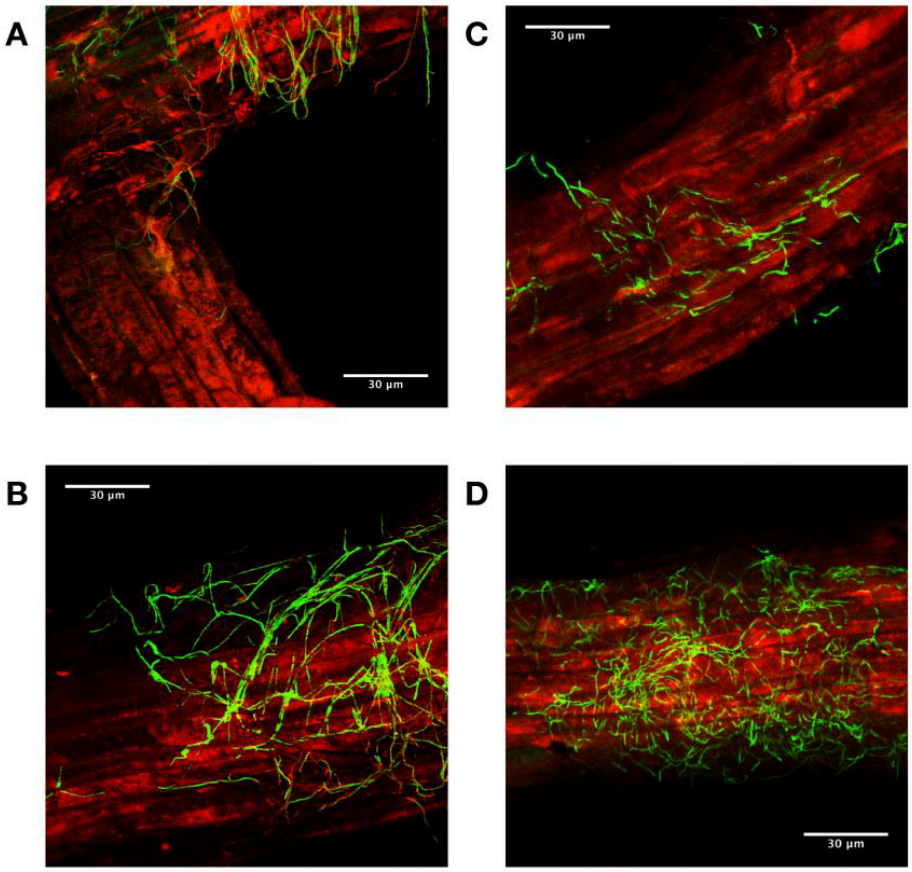
Isolation and genome analysis of *A. thaliana* root-associated *Streptomyces* strains. To culture bacteria from *A. thaliana* roots, the roots were washed and sonicated in sterile buffer (as described in 12) to remove soil particles. This process removed all bacteria apart from those there were tightly bound to the plant root surface (the rhizoplane) or were living within the plant root tissue as endophytes (12). The roots were then crushed in sterile 10% glycerol and serial dilutions were plated onto soya flour mannitol, starch casein and minimal salts agar. Colonies resembling streptomycetes were purified by restreaking and were then identified by colony PCR and 16S rRNA gene amplicon sequencing using the universal primers PRM341F

118 and MPRK806R (Table S1). Based on 16S rRNA sequencing, five phylogenetically distinct
119 strains (L2, M2, M3, N1 and N2) were then selected for genome sequencing. We used the
120 PacBio RSII platform (as described in 25) to generate high-quality genome sequences for the
121 five isolates, in addition to three strains of *Streptomyces lydicus*, which are known plant
122 endophytes (26, 27). One of these strains was isolated from the horticultural product
123 Actinovate® and the other two came from the ATCC culture collection (Table S1). All eight
124 linear genomes are within the size range typical for this genus and do not show any significant
125 reductions compared to the genomes of other sequenced *Streptomyces* species (Table 1). The
126 genomes of the *A. thaliana* associated strains L2, M2, M3, N1 and N2 were uploaded to the
127 automated multi-locus species tree (28) for phylogenetic classification. The highest average
128 nucleotide identity (ANI) values of strains L2, M2 and M3 to strains in the database are 88.3%,
129 94.7% and 91.1%, respectively; these are below the 95% threshold that is generally used to
130 assign strains to a known species (29, 30) so they could be new *Streptomyces* species. Strain
131 N1 has a 98.7% ANI to *Streptomyces albidoflavus* and strain N2 has a 97.6% ANI to
132 *Streptomyces griseofuscus*, suggesting they belong to these clades (Table 1).
133
134 **Table 1.** Genome features of root-associated *Streptomyces* strains sequenced for this study,
135 including their GenBank Accession numbers, their genome size in base pairs (bp) and the total
136 number of open reading frames (ORFs) per genome. Genomes were sequenced using the
137 PacBio RSII platform. Biosynthetic Gene Clusters (BGCs) were predicted using AntiSMASH
138 5.0 (31) and % ANI and closest relatives were determined using AutoMLST (28).

Strain	Accession no.	Size (bp)	ORFs	BGCs	Closest relative / ANI
L2	QBDT00000000	8,073,926	7079	29	*Streptomyces bungoensis* (88.3%)
M2	CP028834	8,718,751	8026	22	*Streptomyces* sp. HBG00200 (94.7%)
M3	QANR00000000	8,304,843	7561	25	*Streptomyces pratensis* (91.1%)
N1	QBDS00000000	7,207,104	6239	21	*Streptomyces albidoflavus* (98.7%)

N2	CP028719	8,428,700	7401	34	*Streptomyces griseofuscus* (97.6%)
Actinovate	RDTC00000000	9,139,876	7989	33	*S. lydicus*
ATCC25470	RDTD00000000	7,935,716	7084	25	*S. lydicus*
ATCC31975	RDTE00000000	9,244,118	8128	31	*S. lydicus*

***Streptomyces* bacteria colonize *A. thaliana* roots.** To investigate whether the sequenced *Streptomyces* strains can promote plant growth and fitness, we established root infection assays in which seeds were coated with a suspension of pre-germinated *Streptomyces* spores. Tagging the strains with eGFP and the apramycin resistance (*aac*) gene allowed visual confirmation of root infection using confocal microscopy (Fig. 1) and selective re-isolation of the strains on agar plates containing apramycin, confirming that they were able to re-colonise plant roots.



147 **Figure 1.** Confocal Laser Scanning Microscopy images of *A. thaliana* rhizoplane colonisation
148 by eGFP-tagged *Streptomyces* strains three days after inoculation. A and B show *A. thaliana*
149 roots (red) colonised by eGFP-tagged *Streptomyces coelicolor* M145 (green) which is a known
150 root endophyte and was used as a control (32). C and D show *A. thaliana* roots (red) colonised
151 by eGFP-tagged *Streptomyces* strain M3 which was isolated in this study (green).
152
153 ***Streptomyces* strains M2, M3 and L2 have growth-promoting effects in *A. thaliana.*** Next
154 we wanted to determine if any of the *Streptomyces* strains isolated from *A. thaliana* roots can
155 enhance plant growth, so we established root infection assays on agar plates, whereby
156 *Streptomyces* spores were inoculated directly onto the roots of young *A. thaliana* seedlings.
157 We tested all the genome-sequenced strains from this study and found that the inoculation of
158 different strains had a significant effect on the dry weights of plants grown on agar, compared
159 to a sterile control (Fig. 2, $F_{(8,135)}= 27.63$, $P < 0.001$ in an ANOVA test). Strains L2, M2 and
160 M3 significantly increased *A. thaliana* dry biomass under these conditions when compared to
161 sterile control plants (Fig. 2, $P < 0.05$ in Tukey's Honestly Significant Difference (HSD) tests).
162 In comparison, the three strains of *S. lydicus* had no effect on *A. thaliana* plant biomass *in vitro*
163 despite this species being previously noted to have plant-growth-promoting effects (26).
164 *Streptomyces* strains N1 and N2 significantly reduced the growth of *A. thaliana in vitro* relative
165 to control plants (Fig. 2; $P < 0.05$ in Tukey's HSD).
166

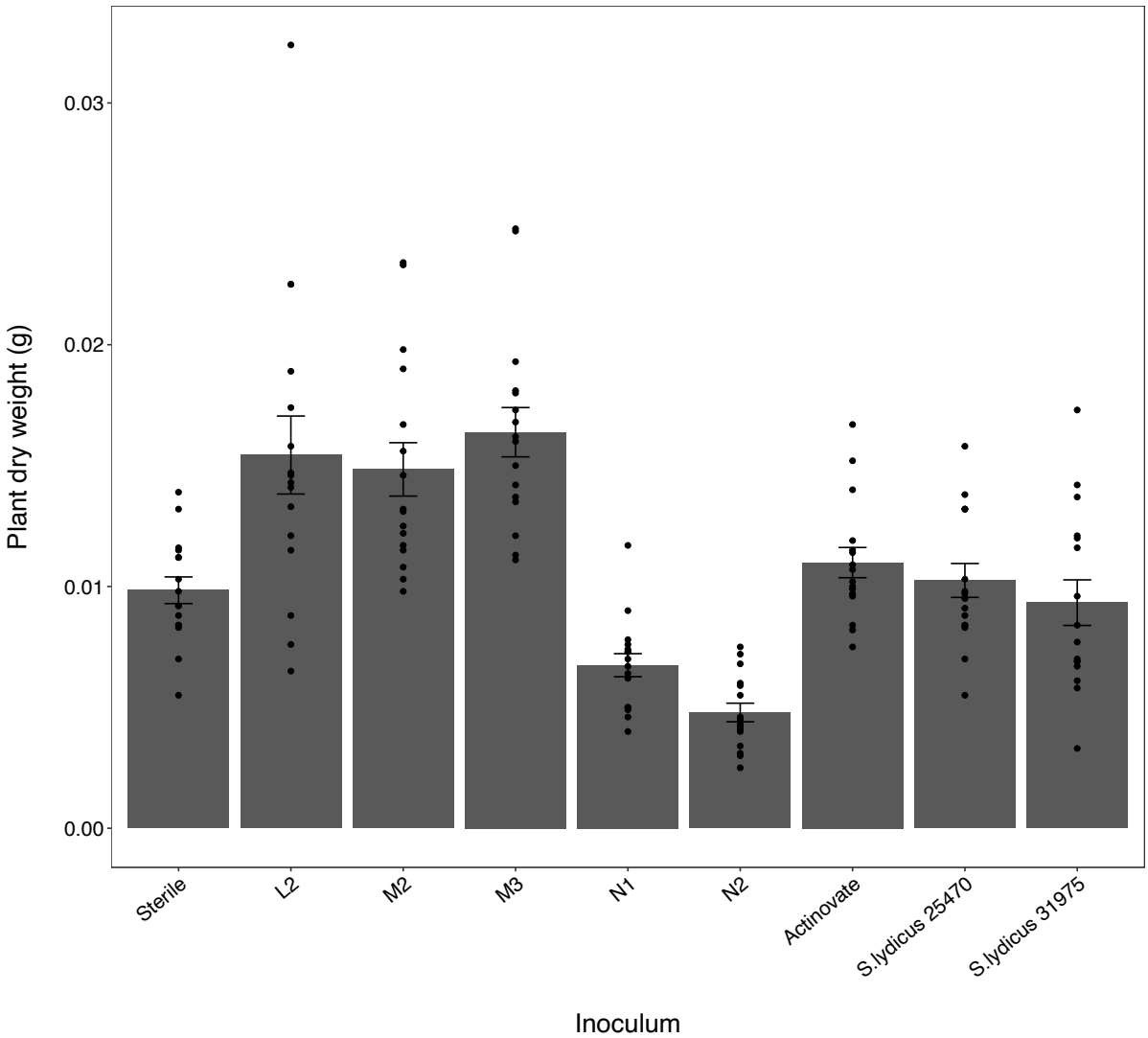
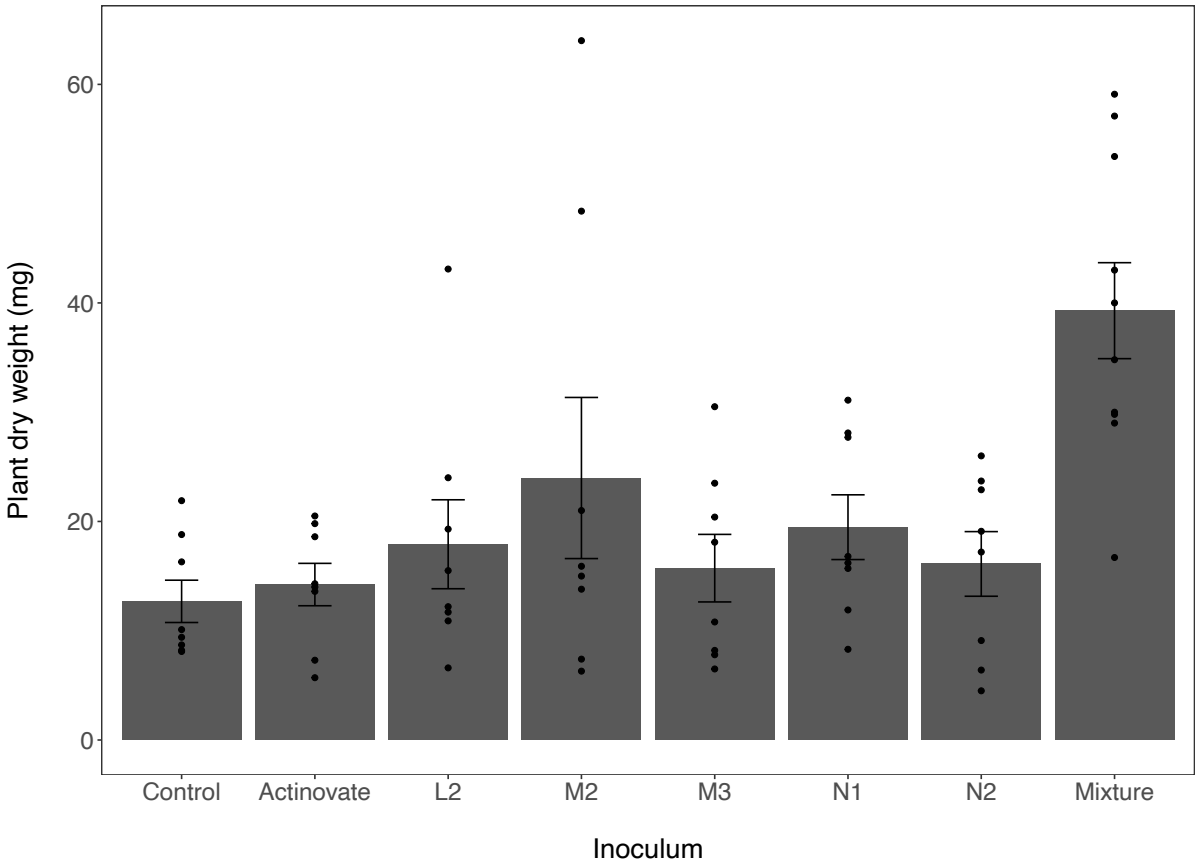


Figure 2. The average biomass of *Arabidopsis thaliana* plants growing on agar plates, following inoculation with sequenced *Streptomyces* isolates. Biomass (dry weight in grams) was measured 16 days after inoculation. Sterile plants were grown as a control. N=16 plants per treatment, represented by points. Error bars represent standard errors.

To test whether each of the new isolates could promote plant growth in soil, spores of each strain were applied individually to *A. thaliana* seeds planted in Levington's F2 seed and modular compost. Since, it is known that some strains can work synergistically to promote plant growth (33-35), a mixture of spores of L2, M2 and M3 (the strains that promoted plant growth *in vitro*) was also added to seeds. Strain inoculation had a significant influence on the dry weight of plants grown in compost (ANOVA test on log-transformed dry weight: $F_{(7,58)}$ = 3.9358, P = 0.001). However, interestingly none of the strains had an effect on plant growth when applied individually (Fig. 3, P >0.05 in Tukey HSD test), but the application of a spore

181 mixture of L2, M2 and M3 significantly increased plant dry weight from an average of 12.69
182 mg ± 1.94 (mean ± SE) for control plants, to 39.29 mg ± 4.39 (Fig. 3).
183



184
185 **Figure 3.** Total dry weight of *A. thaliana* plants grown in Levington's F2 compost from seeds
186 inoculated with spores of Actinovate, L2, M2, M3, N1 and N2 or a mixture of L2, M2 and M3
187 *Streptomyces* spores. Dry weight is shown in milligrams. Sterile seeds were grown as a control.
188 N=8 replicate plants per treatment (shown as points).
189
190 **Characterisation of plant growth promoting traits.** KEGG pathway analysis revealed that
191 the genomes of all our sequenced *Streptomyces* strains possess genes encoding proteins
192 involved in the biosynthesis of IAA, which can contribute to shoot and root growth (Table S2).
193 For example, all strains have genes encoding key proteins involved in the indole-3-acetamide
194 (IAM) pathway, whereby tryptophan is converted to IAM via a tryptophan 2-monooxygenase
195 enzyme (KEGG reaction R00679). IAM is then further converted to IAA through the action of
196 an amidase enzyme (KEGG reaction R03096). Several strains also possessed genes encoding
197 enzymes involved in the tryptamine (TAM) pathway. *In vitro* colorimetric assays using
198 Salkowski reagent (as described in 36) qualitatively confirmed the ability of all strains to make

199 IAA (Fig. S1). In addition to IAA, the genomes of all the *Streptomyces* isolates possess up to
200 two copies of genes encoding the enzyme aminocyclopropane-1-carboxylate (ACC)
201 deaminase. This cleaves ACC, which is the direct precursor for the plant phytohormone
202 ethylene, into ammonia and 2-oxobutanoate, Kegg reaction: R00997 (Table S2). Bacteria can
203 use the ammonium released in this reaction as a nitrogen source, and all the isolates are capable
204 of utilising ACC as a sole nitrogen source in minimal medium (Fig. S2). There is evidence that
205 the activity of the ACC deaminase enzyme can reduce plant damage and early-onset
206 senescence caused by excessive ethylene production under prolonged periods of plant stress,
207 by removing the substrate for ethylene biosynthesis (37, 38).
208
209 **Bioactivity of root-associated *Streptomyces* isolates.** *Streptomyces* bacteria are well-known
210 for their ability to produce a wide range of specialised metabolites which can have bioactivity
211 against bacteria, fungi, viruses, nematodes, insects and plants (1, 3, 39). We reasoned that our
212 strains likely make antimicrobial natural products and that strains N1 and N2 may encode
213 herbicidal compounds given that they have an adverse effect on *A. thaliana* growth *in vitro*
214 (Fig. 2). All eight sequenced genomes were submitted to the bacterial antiSMASH 5.0 portal
215 (31) which can predict BGCs for major types of specialised metabolite. This identified between
216 21 and 34 putative specialised metabolite BGCs in the sequenced genomes, which is within the
217 typical range for this genus (Table S1). This includes BGCs predicted to encode polyketide
218 synthases (PKS), non-ribosomal peptide synthases (NRPS), ribosomally encoded post
219 translationally modified peptides (RiPPs) and terpenes (Table S3). All five strains encode
220 multiple siderophore BGCs which are molecules that chelate metal ions, such as iron,
221 generating soluble complexes that can be taken up by plant roots and contribute to plant growth
222 (40). Siderophores also help microorganisms to compete in the soil, rhizosphere and
223 endosphere, with the added benefit that this may act to exclude plant pathogens that are also
224 competing for iron (41).
225 To test whether antimicrobial compounds could be produced *in vitro,* sequenced strains
226 were tested for their ability to inhibit a range of bacterial and fungal pathogens, including the
227 bacterial plant pathogen *Pseudomonas syringae* and the fungus *G. graminis* var. *tritici* which
228 is the causative agent of wheat Take-all, one of the most economically damaging diseases of
229 wheat worldwide (19). All eight strains exhibited antibacterial activity and N2 inhibited all of
230 the bacterial strains that were tested: *Bacillus subtilis*, methicillin resistant *Staphylococcus*
231 *aureus* (MRSA), *Escherichia coli* and *Pseudomonas syringae* (Fig. S3; Table S4). The N2
232 genome encodes a rich repertoire of BGCs including several putative antibacterial BGCs

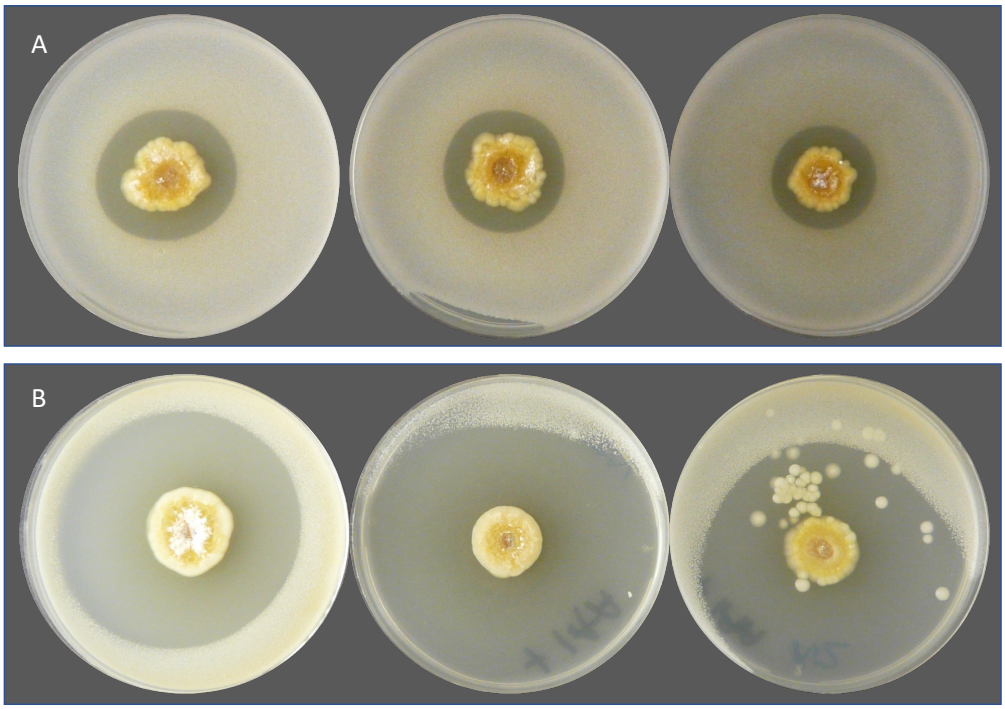
233 predicted to encode the proteins responsible for the biosynthesis of albusnodin, albaflavenone
234 and diisonitrile antibiotic SF2768-like antibiotics, the antiproliferative actinomycin D which is
235 used clinically as an anti-cancer therapeutic, in addition to a possible analogue of the
236 proteasome inhibitor cinnabaramide (Table S3). The actinomycins exhibit broad-spectrum
237 bioactivity by binding to DNA and inhibiting transcription; thus it could result in the inhibition
238 of all the pathogenic indicator strains (42). M2 is interesting because it inhibits *Pseudomonas*
239 *syringae* but none of the other bacteria that were tested (Table S4). It encodes a relatively
240 modest number of BGCs with one for a putative aquamycin-type antibiotic (Table S3).
241 However, this effect may be due to a siderophore because an antibacterial compound that
242 inhibits *Pseudomonas* would also be expected to inhibit *B. subtilis* and *E. coli* (Table S4). L2
243 encodes clusters for the biosynthesis of albaflavenone and thioviridamide-like molecules
244 (Table S3) and inhibits *B. subtilis in vitro* (Table S4). M3 inhibits *B. subtilis* and *P. syringae*
245 (Table S4) and encodes a type 3 PKS for the biosynthesis of alkylresorcinol-type phenolic
246 lipids (Table S4) which may have antibacterial activity. Finally, N1 inhibits *B. subtilis* (Table
247 S4) and encodes several BGCs encoding molecules with potential antibacterial activity
248 including a putative polycyclic tetramate macrolactam (PTM) antibiotic which are widely
249 distributed but often cryptic (43), and a desotamide like antibiotic (Table S3). It should be
250 noted that up to 90% of specialised metabolite BGCs are cryptic (i.e. not expressed under
251 laboratory conditions) in *Streptomyces* species, largely because we do not understand the
252 environmental or host-derived signals that activate their expression (3). In addition, there are
253 many known specialised metabolites for which the BGCs have not yet been identified so it is
254 likely that at least some of the known BGCs in these genomes are not expressed under the
255 growth conditions used in this study and/or that these strains encode antimicrobials whose
256 BGCs cannot be predicted by antiSMASH 5.0.

257 The strains L2, M2 and M3 do not exhibit antifungal activity against *C. albicans*, *L.*
258 *prolificans* or *G. graminis* var. *tritici in vitro* (Table S4), although it is possible that these strains
259 may encode cryptic antifungal compounds that are not expressed under the conditions used in
260 experiments. For example, M3 encodes a clavam-like cluster which has a similar structure to
261 those that encode the antifungal compound clavamycin (Table S3). In comparison, strains N1
262 and N2 both have type 1 (T1) PKS gene clusters which are predicted to encode the biosynthesis
263 of known polyene antifungal compounds (Table S3). The N1 T1PKS gene cluster is predicted
264 to encode candicidin and the N2 T1PKS gene cluster is predicted to encode filipin-type
265 antifungals (Table S4). Both of these metabolites inhibit fungal growth by binding to sterols in
266 their cell walls and it is known that filipins can also bind to phytosterols in plant cell membranes

267 and reduce the growth of plant roots at high concentrations (44-46). Thus, it is possible that N1
268 and N2 are making polyenes *in planta* and this is what caused the reduction in growth observed
269 during *in vitro* inoculation experiments (Fig. 2). These strains did not influence plant growth
270 in compost (Fig. 3) suggesting that the effect was diluted in a non-sterile system, or that the
271 polyenes were not expressed under these conditions. Despite the presence of antifungal-like
272 clusters in the other *A. thaliana* isolates, only N2 exhibited antifungal activity *in vitro* (Table
273 S4). In fact, N2 demonstrated potent and broad-spectrum antifungal activity as it inhibited all
274 three test strains; these were the human pathogenic fungal species *Candida albicans* and multi-
275 drug resistant *Lomentospora prolificans* as well as the plant pathogenic Take-all fungus
276 *Gaeumannomyces graminis* var *tritici* (Table S4).

277 Production of antifungal compounds likely gives N2 an advantage in the rhizosphere
278 and, consistent with this, N2 antifungal activity against *C. albicans* was shown to increase 2-
279 fold *in vitro* in the presence of indole-3-acetic acid (IAA), as judged by the size of the inhibition
280 zone (Fig. 4). IAA is the precursor to the plant phytohormone auxin which regulates processes
281 involved in growth and development (47). It is also made by many microbial species in the
282 rhizosphere, including the bacterial strains that were isolated in this study (Fig. S1 & Table
283 S2), and has been noted previously for its role in both intra- and inter-kingdom signalling (47-
284 49). Thus, N2 may be driven to produce antifungals in close proximity to plant roots when it
285 is in competition with other microbes.

286



287

288 **Figure 4:** (A) Three biological replicates of strain N2 (centre) growing on minimal medium
289 agar that has been overlaid with soft LB agar inoculated with *Candida albicans.* (B) is the same
290 but the minimal medium agar contains 0.1 mg ml^{-1} of indole-3-acetic acid. This experiment
291 was repeated 4 times (each time with 3 replicates), with consistent results.
292
293 **Purification and identification of antifungal compounds from *Streptomyces* strain N2**. To
294 extract the molecules with antifungal activity, strain N2 was plated on SFM agar (4 L, 120
295 plates) and grown for eight days at 30 °C before extraction with ethyl acetate. The crude extract
296 had an intense orange colour and HPLC analysis identified components with UV characteristics
297 typical of polyene metabolites. Comparison to an authentic commercial sample of the filipin
298 complex of *Streptomyces filipinensis* (Sigma Aldrich) confirmed the presence of several filipin
299 related molecules, although the two major components eluted earlier than these molecules (Fig.
300 S4). The two major components eluted very closely together and had *m/z* values consistent
301 with the previously reported molecules pentamycin (also known as fungichromin) and 14-
302 hydroxyisochainin (50-52). Despite a challenging elution profile sufficient separation was
303 accomplished by applying multiple purification steps, and the isolated material enabled
304 structural confirmation by 2D NMR (Figs S5-S16, Tables S5-S7). The extract also contained
305 a mixture of components with an absorption maximum at 444 nm and this mixture was red
306 after separation from the polyene fraction. LCMS analysis indicated that these components
307 were consistent with the known actinomycin congeners D, X_2 and $X_{0\beta}$ respectively (Fig. S4).
308 These are most likely encoded by BGC5 in the N2 genome (Table S3) (53). The filipin-like
309 compounds (Fig. 5) can all be assigned to the type 1 PKS BGC in region 1 (Table S3, Fig. S17)
310 (51, 54, 55).
311 Pentamycin is active against *Candida albicans* and *Trichomonas vaginalis* and is used
312 for the treatment of vaginal candidiasis, trichomoniasis, and some mixed infections (56-58). It
313 is identical in structure to filipin III apart from the presence of an additional secondary hydroxyl
314 group at C14 (Fig. 5) which is added by a cytochrome P450 monooxygenase encoded by a
315 gene located directly upstream of the first PKS gene (A1) in the *pentamycin* BGC as reported
316 recently (51); the same BGC architecture is observed in *Streptomyces* strain N2 (Fig. S17). 14-
317 hydroxyisochainin shares the same polyene core structure as pentamycin but carries an altered
318 side chain lacking two carbon atoms and is indicative of different length extender units being
319 utilized by the final module of the type I PKS during biosynthesis (Fig. 5). This observation is
320 unexpected as the co-production of pentamycin and 14-hydroxyisochainin has only been

observed as a result of precursor-directed biosynthesis (52). Thus, this example of co-production by *Streptomyces* strain N2 appears to be novel.

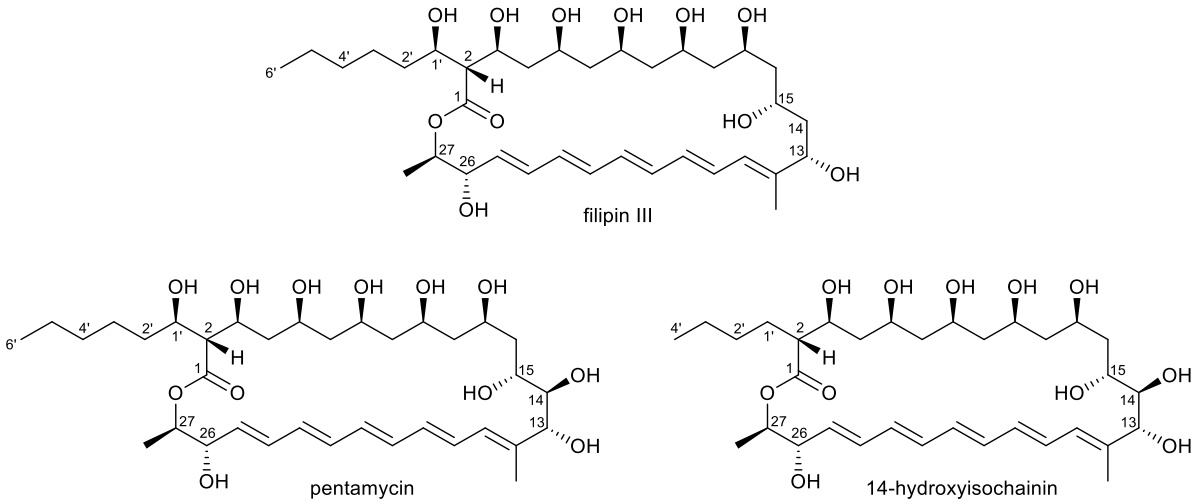
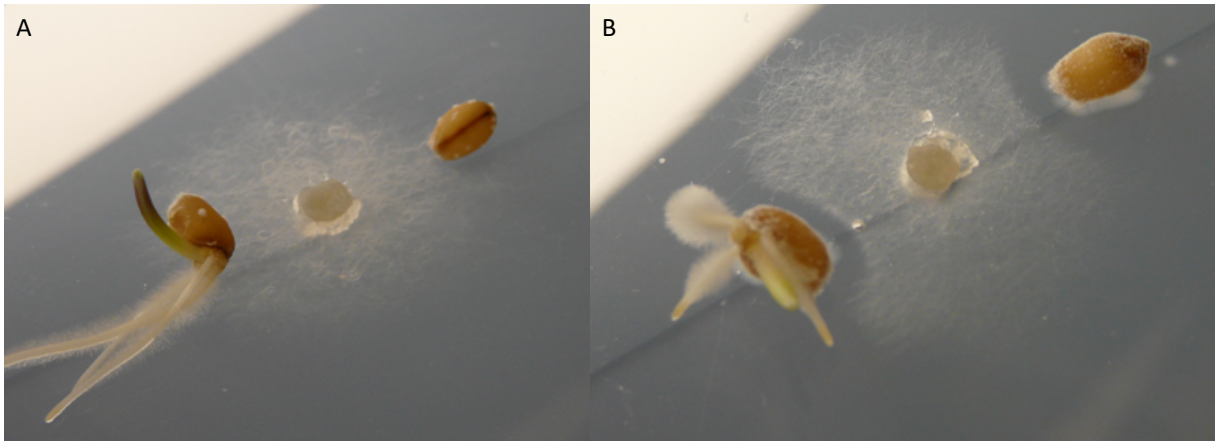


Figure 5. Structures of filipin III, pentamycin and 14-hydroxyisochainin.

Disc diffusion bioassays confirmed that pentamycin and 14-hydroxyisochainin inhibit the growth of *C. albicans* (Fig. S18), but only 14-hydroxyisochainin was able to inhibit *G. graminis* var. *tritici* (Fig. S19), suggesting that 14-hydroxyisochainin is responsible for the antifungal activity of N2 against the Take-all fungus, potentially in combination with lower quantities of other products of the filipin-like BGC. The complex consisting of actinomycin D, X_2 and $X_{0\beta}$, which were co-purified from N2 extracts (Fig. S4), did not have antifungal activity suggesting they are not responsible for N2 antifungal bioactivity *in vitro* (Fig. S18). Neither pentamycin, 14-hydroxyisochainin or the actinomycin complex inhibit the growth of *E. coli* K12 (Fig. S18) suggesting that a previously undescribed compound is responsible for the observed inhibition of *E. coli* and *P. syringae* by N2 *in vitro* (Fig. S3, Table S4).

***Streptomyces* strain N2 protects wheat against Take-all.** In order to test whether strain N2 has the potential to protect wheat plants against *G. graminis* var. *tritici,* we inoculated surface sterilised wheat seeds (*Triticum aestivum* var. Paragon) with N2 spores or left them sterile as a control. These seeds were germinated next to a central plug of Take-all fungus on potato glucose agar (PGA) plates. On the control plates, *G. graminis* grew outwards across the agar plate and over the sterile wheat seedlings, whereas the seeds that had been inoculated with N2 spores were resistant to *G. graminis* var. *tritici*, as indicated by a zone of inhibition around the germinating wheat seeds (Fig. 6). The most parsimonius explanation is that the N2 spores have

346 germinated and are producing 14-hydroxyisochainin and possibly other filipins, that inhibit the
347 Take-all fungus.
348



349

350 **Figure 6. Inhibition of *G. graminis* var. *tritici* in wheat seedlings.** Germinating wheat seeds
351 are either A) sterile or B) inoculated with a spore preparation of *Streptomyces* isolate N2,
352 growing next to a plug of *G. graminis* var. *tritici*, the Take-all fungus. *G. graminis* is prevented
353 from growing towards inoculated seeds, as demonstrated by the zone of inhibition.
354

355 To further test the potential of the *Streptomyces* strain N2 to act as a biocontrol strain against
356 Take-all *in vivo*, wheat seeds were soaked in N2 spores, allowed to dry, and then grown in
357 sterile vermiculite containing *G. graminis* var. *tritici* mycelia. After 3 weeks of growth at 25°C,
358 Take-all infection severity was scored on a scale of 0-8, using an infection scoring system as
359 follows: : 0=no infection; 1=maximum of one lesion per root; 2=more than one lesion per root;
360 3=many small and at least one large lesion per root; 4=many large lesions per root; 5=roots
361 completely brown; 6=roots completely brown plus infection in stem; 7=roots completely
362 brown, infection in stem and wilted yellow leaves; 8=entire plant brown and wilted (Fig. S20).
363 The wheat plants that had germinated and grown in the absence of Take-all (with or without
364 N2 spores) were healthy and infection free (Figs. S21 & S22). However, those seeds that had
365 grown from un-inoculated seeds in the presence of the Take-all fungus showed extensive and
366 severe levels of Take-all disease, with an average infection score of 7.24 ± 0.26 SE (Fig. S21).
367 Most of the plants in this treatment group exhibited infected roots, stems and leaves, which all
368 appeared senescent and brown (Fig. S22). However, there was a significant effect of plant
369 treatment on infection score (Kruskal-Wallis test $H_{DF=3}$= 83.41, P = <0.001). Plants that had
370 grown from seeds coated in N2 spores (N = 25), demonstrated a small, but significant, decrease
371 in average infection severity to 5.47 ± 0.59, compared to plants grown from uninoculated seeds

372 in the presence of Take-all (Fig. S22, Dunn's test between inoculated and sterile wheat grown
373 with *G. graminis*: *P*= 0.023). As plants were grown in a sterile system in this experiment, it is
374 likely that the streptomycete experienced low levels of nutrient availability compared to in a
375 natural soil environment. We hypothesise that greater levels of competition and nutrients may
376 fuel greater levels of antibiotic production by N2, and thus the strain could offer greater levels
377 of protection against host infection in a more natural soil-plant system.
378
379 **Discussion.** *Streptomyces* species have traditionally been described as free-living soil bacteria
380 but given that most bare soils are rapidly colonised by vegetation it is perhaps not surprising
381 that they are also effective at colonising the rhizosphere and endosphere of plants (10). It has
382 even been suggested that their filamentous growth may have evolved to facilitate plant root
383 colonisation since this trait evolved 50 million years after plants first colonised land, around
384 450mya (2, 10). Certainly, their ability to sporulate provides a useful mechanism for vertical
385 transmission across plant generations, via the soil. In this study we followed up reports that
386 *Streptomyces* bacteria are enriched in the *A. thaliana* rhizosphere and specifically recruited by
387 plant-produced compounds such as the plant hormones salicylate and jasmonate (12-17). We
388 aimed to isolate and characterise plant-associated streptomycetes from *A. thaliana* and test
389 whether they can be beneficial to their plant host. We generated high quality genome sequences
390 for five *A. thaliana* root-associated strains and three strains of the known endophyte *S. lydicus*.
391 We found that three of the five root-associated strains significantly increased the biomass of *A.*
392 *thaliana* plants when they were applied to seeds or roots, both *in vitro* and when applied in
393 combination in soil. Two others, N1 and N2, significantly decreased the biomass of *A. thaliana*
394 *in vitro*, most likely because they make polyenes that bind to sterols, including the phytosterols
395 found in plant cell walls (44-46), which probably had a negative effect on the plant in a sterile
396 system. However, this effect was removed in a compost system with neither strain influencing
397 plant growth. Our work suggests therefore, that while *Streptomyces* are consistently associated
398 with *A. thaliana* roots and enriched in the root microbiome compared to the surrounding bulk
399 soil, not all strains that are competitive in the rhizosphere and endosphere necessarily have a
400 beneficial effect on host fitness. This is important from an ecological and applied perspective,
401 the former because it helps us to better understand the microbial and host factors influencing
402 plant microbiome assembly and the latter because tipping the balance in the plant's favour, for
403 example by applying beneficial species as seed coatings or soil additives that can competitively
404 colonise roots, could improve crop yields (19, 59, 60). Such strains could be used as biological
405 growth promoters to replace the use of harmful pesticides and fertilisers which have negative

effects on the wider ecosystem and also contribute to climate change (10, 11, 19, 59, 60). As a proof of this concept we took strain N2, which makes the polyene antifungals including pentamycin, 14-hydroxyisochainin and filipin III, and coated seeds of spring bread wheat with its spores. N2 was able to protect germinating wheat seedlings against the Take-all fungus *in vitro* and significantly reduce Take-all disease progression in wheat plants grown in sterile vermiculite. Although we do not have an assay for Take-all disease in soil the protective effect may be even greater because nutrients in the form of organic matter could provide a more beneficial growing environment for the *Streptomyces* strain than sterile vermiculite (61) and the presence of a greater diversity of microbes may fuel antimicrobial production by this strain as a result of interference competition (62). It is also intriguing that IAA increased the antifungal activity of strain N2 since it provides compelling evidence that environmental signals can alter the expression of secondary metabolites, and probably as an extension also influence interspecies competition (47, 49). Although there is much future work to do to understand this phenomenon our results provide a system to begin to understand how secondary metabolites are regulated and used by microbes in nature and may also provide new tools for activating the 90% of BGCs that are silent in these bacteria. Plant-associated *Streptomyces* strains may yet provide us with a new generation of antimicrobials for the clinic and might also be harnessed to improve our food security. Understanding the ecology of these bacteria and their associated natural products will be crucial if we are to achieve these goals.

Materials and Methods

Isolation of root-associated *Streptomyces* strains. Buffers and media recipes are listed in Table S8. Strains, primers and plasmids are listed in Table S1. Wild-type *A. thaliana* Col-0 seeds were sterilised by washing in 70% (v/v) ethanol for 2 minutes, 20% (v/v) sodium hypochlorite for 2 minutes, then five times in sterile water. Individual seeds were sown into pots of sieved Levington's F2 seed and modular compost, placed at 4°C for 24 hours, then grown for 4 weeks under a 12-hour photoperiod (12 hours light/12 hours dark) at 22°C. Plants were taken aseptically from pots and their roots tapped firmly to remove as much adhering soil as possible. Root material was placed into sterile PBS-S buffer for 30 minutes on a shaking platform and before being transferred into fresh PBS-S and washed for 30 minutes. Any remaining soil particles were removed with sterile tweezers. Cleaned roots were then transferred to 25ml of fresh PBS-S and placed in a sonicating water bath for 20 minutes to

439 remove any residual material still attached to the root surface; this was to ensure that any
440 remaining bacteria were either present in the endophytic compartment or were very firmly
441 attached to the root surface ("the rhizoplane") (12). The roots were crushed in sterile 10% (v/v)
442 glycerol and serial dilutions were spread onto either soya flour mannitol (SFM) agar, starch
443 casein agar, or minimal salts medium agar containing sodium citrate. Plates were incubated at
444 30°C for up to 14 days. Colonies resembling streptomycetes were re-streaked onto SFM agar
445 and identified by 16S rRNA gene PCR amplification and sequencing with the universal primers
446 PRK341F and MPRK806R. Sequencing was carried out by Eurofins Genomics, Germany.
447 Three strains of *Streptomyces lydicus,* which are known to associate with plant roots, were also
448 used in experiments; *S. lydicus* WYEC108 was isolated from the commercial biocontrol
449 product Actinovate® and two more *S. lydicus* strains (ATCC25470 and ATCC31975) were
450 obtained from the American Type Culture Collection. *Streptomyces* strains were maintained
451 on SFM agar (N1, N2, M2, M3), Maltose/Yeast extract/Malt extract (MYM) agar with trace
452 elements (L2) or ISP2 agar (*S. lydicus* strains). Strains were spore stocked as described
453 previously (63).
454
455 **Genome sequencing and analysis** High quality genome sequences were obtained for
456 *Streptomyces* strains N1, N2, M2, M3, and L2, as well as the three known strains of
457 *Streptomyces lydicus* using PacBio RSII sequencing technology at the Earlham Institute,
458 Norwich, UK, as described previously (25). The automated Multi-Locus Species Tree
459 (autoMLST) server (28) was used to phylogenetically classify the *Streptomyces* strains L2, M2,
460 M3, N1 and N2. BGCs were predicted using antiSMASH 5.0 (31) and genomes were annotated
461 using RAST (64). Amino acid sequences were uploaded to the KEGG Automatic Annotation
462 Server (KAAS) for functional annotation of genes and metabolic pathway mapping (65).
463
464 **Generating eGFP-labelled *Streptomyces* strains.** Plasmid pIJ8660 containing a codon
465 optimised eGFP gene under the control of the constitutive *ermE** promoter and the *aac*
466 apramycin resistance marker (66) was conjugated into *Streptomyces* strains (63). Exconjugants
467 were selected and maintained on SFM agar plates (Table S8) containing 50 µg ml^{-1} apramycin.
468 For confocal microscopy, *A. thaliana* Col-0 seeds were germinated on MSk (Table S8) and
469 grown vertically at 22 °C for 9 days under a 12-hour photoperiod. Seedlings were then
470 transferred to MSk (1.5% agar, 0% sucrose, Table S5) and allowed to equilibrate for 24 hours
471 before being inoculated with 1µl of spore suspension (10^6 spores ml^{-1}) of the eGFP-tagged M3

472 or *Streptomyces coelicolor* M145 strains. As a known coloniser of plant roots, *S. coelicolor*
473 was used as a control (32). Inoculated seedlings were then left to grow for 3 days before being
474 washed in a 20% (v/v) solution of glycerol containing 1 μg ml^{-1} 276 SynaptoRed™ for 10
475 minutes. A 20 mm section of root (taken from the base of the petiole) was then mounted onto
476 a slide with 100 μl of the SynaptoRed™/glycerol solution. Samples were imaged using a Zeiss
477 LSM510 META laser-scanning confocal microscope with a PlanApochromat 63x (1.4 NA)
478 objective. Green fluorescent protein was excited at 488 nm and emission collected through a
479 527.5 ± 22.5 nm bp filter, and FM4-64 excited at 543 nm and emission collected through a
480 587.5 ± 27.5 nm bp filter.
481
482 **Plant growth promotion assays**. *A. thaliana* Col-0 seeds were sterilised and plated onto MSk
483 medium (1% (w/v) sucrose and 0.8% (w/v) agar, Table S8). These were then left at 4 °C in the
484 dark for 24 hours before being placed, vertically, under long day growth conditions (12 h light/
485 12 h dark) at 22°C for 10 days. Seedlings were then transferred to square agar plates containing
486 MSk agar (as above, Table S8) and allowed to equilibrate, vertically, overnight at 22 °C. 1 μl
487 of *Streptomyces* spores (10^6 ml^{-1}) from each of the sequenced isolates was added to the top of
488 the root system of each seedling and allowed to dry. 16 replicate seedlings were inoculated per
489 sequenced *Streptomyces* strain. 10% (v/v) glycerol was added to control seedlings. Plates were
490 grown vertically for 16 days, 12 h light/ 12 h dark at 22°C before measuring plant biomass (dry
491 weight). The biomass of plants with different inocula were compared via ANOVA and Tukey's
492 Honestly Significant Difference (HSD) tests using R 3.2.3 (67); biomass was log-transformed
493 during analyses to ensure normality of residuals. Strains were also tested for their ability to
494 promote *A. thaliana* growth in compost. Sterile *A. thaliana* Col-0 seeds were placed into a
495 solution of 2xYT (Table S8) containing 10^6 pregerminated spores ml^{-1} of each strain or no
496 spores as a control. Seeds were incubated in the spore solution for 2 hours, before being
497 transferred to pots containing sieved Levington's F2 seed and modular compost. An additional
498 3 ml of pre-germinated spores (or sterile 2xYT) was pipetted into the soil surrounding each
499 seed. The strains L2, M2 and M3 were also tested for their ability to promote plant growth in
500 combination; 10^3 spores ml^{-1} of each strain were mixed together and pregerminated in 2xYT
501 before being used as above. Pots were then placed at 4°C for 48 hours before being grown for
502 6 weeks under a photoperiod of 12 hours light/12 hours dark. There were 8 replicate pots per
503 treatment. After 6 weeks, the plants were removed from pots and cleaned by washing in PBS-
504 S (Table S8) and using tweezers to remove adhering soil particles. Plants were then dried in an

505 oven at 50°C enabling plant dry weight to be calculated. An ANOVA test and Tukey's HSD
506 tests were used (as above) to test for an effect of strain inoculation on plant dry weight. Dry
507 weights were log-transformed prior to analysis.
508
509 **Indole 3 Acetic Acid (IAA) production assays.** *Streptomyces* isolates were grown on
510 cellophane membranes covering YMD media supplemented with 5 mM tryptophan (Table S8).
511 After 7 days, cellophane membranes with bacterial biomass were removed and plates were
512 flooded with Salkowski reagent (as described in 36). A red colour indicates that IAA has
513 leached into the medium.
514
515 **1-Aminocyclopropane-1-carboxylic acid (ACC) degradation assays.** To test for the use of
516 ACC as a sole nitrogen source, *Streptomyces* strains were streaked onto Dworkin and Foster
517 medium (68) in which 0.2% (w/v) NH_4SO_4 or 0.051% (w/v) ACC was added as a sole nitrogen
518 source, or no nitrogen source as a control. Plates were incubated for 10 days at 30°C before
519 imaging.
520
521 **Antibiotic bioassays.** Spores (4 µl of 10^6 ml^{-1} solution) of individual *Streptomyces* isolates
522 were pipetted onto the centre of agar plates and incubated at 30°C for 7 days before adding the
523 pathogenic indicator strains (see Table S1). A clinical isolate of *Candida albicans* (gift from
524 Prof Neil Gow), *Bacillus subtilis* 168 (from Prof Nicola Stanley Wall), a clinical isolate of
525 Methicillin Resistant *Staphylococcus aureus* isolated from a patient at the Norfolk and Norwich
526 University Hospital UK (69), an *Escherichia coli* K12 lab strain and the plant pathogen
527 *Pseudomonas syringae* DC3000 (gift from Dr Jacob Malone) were grown overnight in 10 ml
528 Lysogeny Broth (LB) at 30°C, 250 rpm. These were sub-cultured 1 in 20 (v/v) for a further 4
529 hours at 30°C before being used to inoculate 100 ml of molten LB (0.5% agar), 3 ml of which
530 was used to overlay each agar plate containing a *Streptomyces* colony. Plates were incubated
531 for 48 hours at 30°C. Bioactivity was indicated by a clear halo around the *Streptomyces* colony.
532 For bioassays using the fungal strains *Lomentospora prolificans* or *Gaeumannomyces graminis*
533 var. *tritici* (Table S1), a plug of the fungus (grown for 14 days on potato glucose agar) was
534 placed at the edge of the agar plate, 2 cm away from the growing streptomycete colony. Plates
535 were incubated at 25°C for up to 14 days to assess inhibition of fungal growth. Bioassays were
536 carried out on a range of different media, including minimal medium supplemented with
537 Indole-3-Acetic Acid (IAA) (See Table S8 for media recipes).

538

539 **Purification and elucidation of filipin-like compounds from strain N2.** Spores of strain N2
540 were spread onto 120 plates (4 L) of SFM agar and grown for eight days at 30 °C. The resulting
541 agar was then sliced into small pieces and extracted with ethyl acetate (3 x 1.5 L). An analytical
542 sample was taken for analysis whereby the extract was filtered through gauze and the solvent
543 removed under reduced pressure yielding 9.2 g of crude material. This was split into two halves
544 and each treated identically: after dissolving in acetone (50 ml), loose normal phase silica gel
545 (~30 g, Sigma Aldrich) was added and the solvent removed under reduced pressure. The
546 impregnated silica gel was dry loaded onto a Biotage® SNAP Ultra cartridge (50 g, HP-Sphere
547 normal phase silica). The resulting sample was chromatographed using a Biotage flash
548 chromatography system to separate the polyene fraction (338 nm) and the actinomycin(s)
549 containing fraction (444 nm; hereafter referred to as the 'actinomycin complex') using the
550 following gradient with a flow rate of 100 ml min^{-1}: hold at 0% B for 1 CV; linear gradient 0-
551 50% B over 10 CV; 50-100% B over 0.5 CV; and hold at 100 % B for 3 CV; (mobile phase A,
552 chloroform; mobile phase B, methanol).

553 The polyene containing fractions were combined, the solvent removed under reduced
554 pressure and the residues split into two fractions. Each fraction (in 800 µL DMSO) was loaded
555 onto a Biotage® SNAP Ultra cartridge (12 g, C_{18}) and chromatography was achieved using the
556 following gradient at a flow rate of 12 ml min^{-1}: hold at 0% B for 5 CV; linear gradient 0-
557 55% B over 1 CV, 55-85% B over 10 CV; 85-100% B over 2 CV; and hold at 100% B for
558 1 CV; (mobile phase A, water; mobile phase B, methanol). The resulting fractions were
559 analysed using LCMS and combined to yield three polyene samples of 53 mg, 34 mg and 9 mg
560 after solvent was removed. LCMS spectra of these fractions and the original crude extract were
561 then uploaded onto the GNPS (Global Natural Products Social Molecular Networking
562 platform). The largest network containing the spectra of the filipin related compounds was
563 manually adapted and processed in Cytoscape 3.6.1. The second fraction contained the most
564 interesting compounds so only this sample was further purified by chromatography over a
565 Synergi Fusion 4 micron C_{18} 250 x 10 mm column (Phenomenex) using an Agilent 1100 series
566 HPLC system fitted with a fraction collector and eluting at a flow rate of 3.5 ml min^{-1} with the
567 following gradient. 0-2 min, 45% B; 2-5 min, 45-50% B; 5-10 min, 50% B; 10.0-10.1 min, 50-
568 45% B; 10.1-12.0 min, 45% B; (mobile phase A, 0.1% formic acid in water; mobile phase B,
569 acetonitrile). This yielded pentamycin (4.2 mg) and 14-hydroxyisochainin (2.3 mg). Both
570 structures were assigned using 2D NMR recorded on a Bruker Avance Neo 600 MHz
571 spectrometer equipped with a helium-cooled cryoprobe and dissolved in DMSO-d_6. Additional

572 1D-experiments were carried out in CD_3OD for 14-hydroxyisochainin in order to compare
573 directly with published data for this compound (Figs S10-16 and Tables S5-8) (52).
574 Disc-diffusion bioassays were used to test whether purified pentamycin, 14-
575 hydroxyisochainin and the actinomycin complex were active against the pathogenic strains *C.*
576 *albicans* and *E. coli* (Table S1). Both indicator strains were grown in 10 ml of LB broth (Table
577 S5) at 30°C and 200 rpm overnight. Cultures were then diluted 1 in 20 (v/v) into 10 ml of LB
578 and grown for a further 4 hours. The 10 ml sub-culture was added to 50 ml of soft LB (0.5%
579 agar) which was then poured into 10 cm square plates and allowed to set. Meanwhile, 6 mm
580 sterile filter paper discs (Whatman) were inoculated with 40 µl of each individual compound
581 (three technical replicates of each compound were tested). 40 µl of methanol was added to one
582 disc per plate as a solvent control and 40 µl of nystatin (5 mg ml^{-1}) or hygromycin (50 mg ml^{-}
583 ^{1}) were used as positive controls on *C. albicans* and *E. coli* plates, respectively. Once dry, discs
584 were placed onto plates 3 cm apart. These were then incubated at 30°C overnight. Inhibition
585 of the indicator strain was evidenced by a zone of clearing around the disc. Purified compounds
586 were also tested for their ability to inhibit the wheat take-all fungus *G. graminis* (Table S1).
587 For this, discs were placed onto PGA agar plates (Table S8) 2 cm away from an actively
588 growing plug of *G. graminis*. Plates were left to grow at room temperature for 5 days before
589 imaging. Three technical replicates were run for each purified compound (pentamycin, 14-
590 hydroxyisochainin and actinomycin).
591
592 **Wheat seedling bioassays with *Streptomyces* strain N2.** Seeds of *Triticum aestivum* (var.
593 Paragon, Table S1) were sterilized by placing them in 70% (v/v) ethanol for 2 minutes followed
594 by a wash in 3% (v/v) NaOCl for 10 minutes. Seeds were then rinsed five times in sterile dH_2O,
595 before placing them into a solution of pregerminated spores (10^7 spores ml^{-1}) of *Streptomyces*
596 strain N2 (Table S1). Spores were pregerminated in 2xYT (Table S8) at 50°C for 10 minutes.
597 Seeds were incubated in either the spore preparation or sterile 2xYT as a control, for 2 hours,
598 before being allowed to dry in a Petri dish under sterile conditions. Two wheat seeds were then
599 placed onto a 10 cm square plate of Msk agar (1.5% (w/v) agar, 0% (w/v) sucrose, Table S8),
600 on either side of a plug of the *G. graminis* fungus, which was placed in the centre of the agar
601 plate. Plugs were taken from an actively growing plate of *G. graminis* on PGA agar. Three
602 replicate plates each of N2-coated seeds and sterile control seeds were used in each experiment.
603 Plates were incubated for 5 days at room temperature after which inhibition of *G. graminis* was
604 indicated by a zone of clearing around the wheat seeds.

605 A sterile vermiculite system was used to investigate the ability of *Streptomyces* strain
606 N2 to protect older wheat seedlings against Take-all infection. 25 ml of sterile vermiculite was
607 placed into the bottom of a 50 ml Falcon tube. Five plugs of *G. graminis* actively growing on
608 PGA (Table S8), or uninoculated PGA plugs as a control, were placed on top of this layer,
609 before being covered with a further 10 ml of vermiculite. Five seeds of *T. aestivum* (soaked in
610 either N2 spores or uninoculated 2xYT, as described above) were then placed on top of this
611 vermiculite layer, before the addition of a further 10 ml of vermiculite. The Falcon tubes were
612 then sealed with parafilm and incubated at 25°C for 3 weeks, under a 12 hour light/ 12 hour
613 dark photoperiod. There were five replicates tubes, each containing five replicate seeds, of each
614 of the following combinations: PGA plugs with N2-coated seeds (wheat/*Streptomyces* control);
615 *G. graminis* plugs with N2-coated seeds (wheat/*Streptomyces*/fungus treatment); PGA plugs
616 with uninoculated seeds (wheat control); *G. graminis* plugs with uninoculated seeds
617 (wheat/fungus treatment). After three weeks of incubation, plants were taken from the Falcon
618 tubes and adhering vermiculite was removed from the roots. Take-all infection severity was
619 scored on a scale of 0-8, using an infection scoring system as follows: : 0=no infection;
620 1=maximum of one lesion per root; 2=more than one lesion per root; 3=many small and at least
621 one large lesion per root; 4=many large lesions per root; 5=roots completely brown; 6=roots
622 completely brown plus infection in stem; 7=roots completely brown, infection in stem and
623 wilted yellow leaves; 8=entire plant brown and wilted. Differences in infection score between
624 treatments were analysed in R 3.2.3 (67) using a Kruskal-Wallis test, coupled with a post-hoc
625 Dunn's multiple comparison test.
626

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References

1. Hutchings MI, Truman AW, Wilkinson B. 2019. Antibiotics: past, present and future. Curr Opin Microbiol 51:72-80.
2. Chater KF. 2016. Recent advances in understanding *Streptomyces*. F1000Res 5:2795.
3. van der Meij A, Worsley SF, Hutchings MI, van Wezel GP. 2017. Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiol Rev 41:392-416.
4. Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA. 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141-7.
5. Seipke RF, Kaltenpoth M, Hutchings MI. 2012. *Streptomyces* as symbionts: an emerging and widespread theme? FEMS Microbiol Rev 36:862-876.
6. Barke J, Seipke RF, Gruschow S, Heavens D, Drou N, Bibb MJ, Goss RJM, Yu DW, Hutchings MI. 2010. A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant *Acromyrmex octospinosus*. BMC Biol 8.
7. Kaltenpoth M. 2009. Actinobacteria as mutualists: general healthcare for insects? Trends Microbiol 17:529-535.
8. Kaltenpoth M, Goettler W, Dale C, Stubblefield JW, Herzner G, Roeser-Mueller K, Strohm E. 2006. '*Candidatus Streptomyces philanthi*', an endosymbiotic streptomycete in the antennae of *Philanthus* digger wasps. Int J Syst Evol Microbiol 56:1403-1411.
9. Hulcr J, Adams AS, Raffa K, Hofstetter RW, Klepzig KD, Currie CR. 2011. Presence and diversity of *Streptomyces* in *Dendroctonus* and sympatric bark beetle galleries across North America. Microb Ecol 61:759-68.
10. Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S. 2016. *Streptomyces* as a plant's best friend? FEMS Microbiology Ecology:fiw119.
11. Schrey SD, Tarkka MT. 2008. Friends and foes: streptomycetes as modulators of plant disease and symbiosis. Antonie Van Leeuwenhoek 94:11-19.
12. Bulgarelli D, Rott M, Schlaeppi K, van Themaat EVL, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E. 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488:91-95.
13. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Del Rio TG. 2012. Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86-90.
14. Bodenhausen N, Horton MW, Bergelson J. 2013. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. PloS one 8:e56329.
15. Schlaeppi K, Dombrowski N, Oter RG, Ver Loren van Themaat E, Schulze-Lefert P. 2014. Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. Proc Natl Acad Sci U S A 111:585-92.

684 16. Carvalhais LC, Dennis PG, Badri DV, Kidd BN, Vivanco JM, Schenk PM. 2015.
685 Linking jasmonic acid signaling, root exudates, and rhizosphere microbiomes. Mol
686 Plant Microbe Interact 28:1049-58.
687 17. Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M,
688 Malfatti S, Del Rio TG, Jones CD, Tringe SG. 2015. Salicylic acid modulates
689 colonization of the root microbiome by specific bacterial taxa. Science 349:860-864.
690 18. Coombs JT, Franco CM. 2003. Isolation and identification of actinobacteria from
691 surface-sterilized wheat roots. Appl Environ Microbiol 69:5603-8.
692 19. Newitt JT, Prudence SMM, Hutchings MI, Worsley SF. 2019. Biocontrol of cereal
693 crop diseases using streptomycetes. Pathogens 8.
694 20. Rey T, Dumas B. 2017. Plenty Is No Plague: *Streptomyces* Symbiosis with Crops.
695 Trends Plant Sci 22:30-37.
696 21. Edwards J, Johnson C, Santos-Medellin C, Lurie E, Podishetty NK, Bhatnagar S,
697 Eisen JA, Sundaresan V. 2015. Structure, variation, and assembly of the root-
698 associated microbiomes of rice. Proc Natl Acad Sci U S A 112:E911-20.
699 22. Law JW, Ser HL, Khan TM, Chuah LH, Pusparajah P, Chan KG, Goh BH, Lee LH.
700 2017. The potential of *Streptomyces* as biocontrol Agents against the Rice Blast
701 Fungus, *Magnaporthe oryzae* (*Pyricularia oryzae*). Front Microbiol 8:3.
702 23. Kim DR, Cho G, Jeon CW, Weller DM, Thomashow LS, Paulitz TC, Kwak YS.
703 2019. A mutualistic interaction between Streptomyces bacteria, strawberry plants and
704 pollinating bees. Nat Commun 10:4802.
705 24. LahdenperÄ ML, Simon E, Uoti J. 1991. Mycostop - a novel biofungicide based on
706 *Streptomyces* bacteria, p 258-263. *In* Beemster ABR, Bollen GJ, Gerlagh M, Ruissen
707 MA, Schippers B, Tempel A (ed), Developments in Agricultural and Managed Forest
708 Ecology, vol 23. Elsevier.
709 25. Holmes NA, Innocent TM, Heine D, Bassam MA, Worsley SF, Trottmann F, Patrick
710 EH, Yu DW, Murrell JC, Schiott M, Wilkinson B, Boomsma JJ, Hutchings MI. 2016.
711 Genome analysis of two *Pseudonocardia* phylotypes associated with *Acromyrmex*
712 leafcutter ants reveals their biosynthetic potential. Front Microbiol 7:2073.
713 26. Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF,
714 Morra M. 2002. Novel plant-microbe rhizosphere interaction involving *Streptomyces*
715 *lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl Environ Microbiol
716 68:2161-2171.
717 27. Yuan WM, Crawford DL. 1995. Characterization of *Streptomyces lydicus* WYEC108
718 as a potential biocontrol agent against fungal root and seed rots. Appl Environ
719 Microbiol 61:3119-3128.
720 28. Alanjary M, Steinke K, Ziemert N. 2019. AutoMLST: an automated web server for
721 generating multi-locus species trees highlighting natural product potential. Nucleic
722 Acids Res doi:10.1093/nar/gkz282.
723 29. Jain C, Rodriguez RL, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High
724 throughput ANI analysis of 90K prokaryotic genomes reveals clear species
725 boundaries. Nat Commun 9:5114.
726 30. Konstantinidis KT, Tiedje JM. 2005. Genomic insights that advance the species
727 definition for prokaryotes. Proc Nat Acad Sci USA 102:2567.
728 31. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T.
729 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline.
730 Nucleic Acids Res doi:10.1093/nar/gkz310.
731 32. Kumar J, Sharma VK, Singh DK, Mishra A, Gond SK, Verma SK, Kumar A,
732 Kharwar RN. 2016. Epigenetic Activation of Antibacterial Property of an Endophytic

Streptomyces coelicolor Strain AZRA 37 and Identification of the Induced Protein Using MALDI TOF MS/MS. PLoS One 11:e0147876.
33. Bradáčová K, Kandeler E, Berger N, Ludewig U, Neumann G. 2020. Microbial consortia inoculants stimulate early growth of maize depending on nitrogen and phosphorus supply. Plant, Soil and Environment 66:105-112.
34. Egamberdieva D, Jabborova D, Berg G. 2016. Synergistic interactions between Bradyrhizobium japonicum and the endophyte Stenotrophomonas rhizophila and their effects on growth, and nodulation of soybean under salt stress. Plant and Soil 405:35-45.
35. Jha CK, Saraf M. 2012. Evaluation of Multispecies Plant-Growth-Promoting Consortia for the Growth Promotion of Jatropha curcas L. Journal of Plant Growth Regulation 31:588-598.
36. Bric JM, Bostock RM, Silverstone SE. 1991. Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57:535-538.
37. Glick BR. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30-9.
38. Palaniyandi SA, Damodharan K, Yang SH, Suh JW. 2014. *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of 'Micro Tom' tomato plants. J Appl Microbiol 117:766-73.
39. Hopwood DA. 2007. *Streptomyces* in nature and medicine: the antibiotic makers. Oxford University Press, New York.
40. Scavino AF, Pedraza RO. 2013. The role of siderophores in plant growth-promoting bacteria, p 265-285, Bacteria in agrobiology: crop productivity. Springer.
41. Kloepper JW, Leong J, Teintze M, Schroth MN. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885-886.
42. Katz E. 1967. Actinomycin, p 276-341. *In* Gottlieb D, Shaw PD (ed), Biosynthesis doi:10.1007/978-3-662-38441-1_25. Springer Berlin Heidelberg, Berlin, Heidelberg.
43. Luo Y, Huang H, Liang J, Wang M, Lu L, Shao Z, Cobb RE, Zhao H. 2013. Activation and characterization of a cryptic polycyclic tetramate macrolactam biosynthetic gene cluster. Nat Commun 4:2894.
44. Bonneau L, Gerbeau-Pissot P, Thomas D, Der C, Lherminier J, Bourque S, Roche Y, Simon-Plas F. 2010. Plasma membrane sterol complexation, generated by filipin, triggers signaling responses in tobacco cells. Biochim Biophys Acta 1798:2150-9.
45. Milhaud J, Bolard J, Benveniste P, Hartmann MA. 1988. Interaction of the polyene antibiotic filipin with model and natural membranes containing plant sterols. Biochim Biophys Acta 943:315-25.
46. Moeller CH, Mudd JB. 1982. Localization of filipin-sterol complexes in the membranes of *Beta vulgaris* roots and *Spinacia oleracea* chloroplasts. Plant Physiol 70:1554-61.
47. Duca D, Lorv J, Patten CL, Rose D, Glick BR. 2014. Indole-3-acetic acid in plant-microbe interactions. Antonie Van Leeuwenhoek 106:85-125.
48. Patten CL, Blakney AJ, Coulson TJ. 2013. Activity, distribution and function of indole-3-acetic acid biosynthetic pathways in bacteria. Crit Rev Microbiol 39:395-415.
49. Daddaoua A, Matilla MA, Krell T, Chini A, Morel B. 2018. An auxin controls bacterial antibiotics production. Nucleic Acids Res 46:11229-11238.
50. Cope AC, Johnson HE. 1958. Fungichromin. Determination of the structure of the pentaene chromophore. J Am Chem Soc 80:1504-1506.

51. Zhou S, Song L, Masschelein J, Sumang FAM, Papa IA, Zulaybar TO, Custodio AB, Zabala D, Alcantara EP, de Los Santos ELC, Challis GL. 2019. Pentamycin biosynthesis in Philippine *Streptomyces* sp. S816: cytochrome P450-catalyzed installation of the C-14 hydroxyl group. ACS Chem Biol doi:10.1021/acschembio.9b00270.
52. Li Z, Rawlings BJ, Harrison PH, Vederas JC. 1989. Production of new polyene antibiotics by *Streptomyces cellulosae* after addition of ethyl (Z)-16-phenylhexadec-9-enoate. J Antibiot (Tokyo) 42:577-84.
53. Keller U, Lang M, Crnovcic I, Pfennig F, Schauwecker F. 2010. The actinomycin biosynthetic gene cluster of Streptomyces chrysomallus: a genetic hall of mirrors for synthesis of a molecule with mirror symmetry. J Bacteriol 192:2583-95.
54. Gao H, Grüschow S, Barke J, Seipke RF, Hill LM, Orivel J, Yu DW, Hutchings M, Goss RJM. 2014. Filipins: the first antifungal "weed killers" identified from bacteria isolated from the trap-ant. RSC Advances 4:57267-57270.
55. Payero TD, Vicente CM, Rumbero A, Barreales EG, Santos-Aberturas J, de Pedro A, Aparicio JF. 2015. Functional analysis of filipin tailoring genes from *Streptomyces filipinensis* reveals alternative routes in filipin III biosynthesis and yields bioactive derivatives. Microb Cell Fact 14:114.
56. Frey Tirri B, Bitzer J, Geudelin B, Drewe J. 2010. Safety, tolerability and pharmacokinetics of intravaginal pentamycin. Chemotherapy 56:190-6.
57. Kranzler M, Syrowatka M, Leitsch D, Winnips C, Walochnik J. 2015. Pentamycin shows high efficacy against *Trichomonas vaginalis*. Int J Antimicrob Agents 45:434-7.
58. Xiong ZQ, Zhang ZP, Li JH, Wei SJ, Tu GQ. 2012. Characterization of *Streptomyces padanus* JAU4234, a producer of actinomycin X(2), fungichromin, and a new polyene macrolide antibiotic. Appl Environ Microbiol 78:589-92.
59. Dessaux Y, Grandclement C, Faure D. 2016. Engineering the rhizosphere. Trends Plant Sci 21:266-278.
60. Ryan PR, Dessaux Y, Thomashow LS, Weller DM. 2009. Rhizosphere engineering and management for sustainable agriculture. Plant and Soil 321:363-383.
61. Hoitink HAJ, Boehm MJ. 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu Rev Phytopathol 37:427-446.
62. Scheuring I, Yu DW. 2012. How to assemble a beneficial microbiome in three easy steps. Ecol Lett 15:1300-1307.
63. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. 2000. Practical *Streptomyces* Genetics. John Innes Foundation, Norwich.
64. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75.
65. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35:W182-5.
66. Sun J, Kelemen GH, Fernandez-Abalos JM, Bibb MJ. 1999. Green fluorescent protein as a reporter for spatial and temporal gene expression in *Streptomyces coelicolor* A3(2). Microbiology 145 (Pt 9):2221-7.
67. R Core Team. 2017. R: A language and environment for statistical computing. Accessed

68. Dworkin M, Foster JW. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol 75:592-603.
69. Qin Z, Munnoch JT, Devine R, Holmes NA, Seipke RF, Wilkinson KA, Wilkinson B, Hutchings MI. 2017. Formicamycins, antibacterial polyketides produced by *Streptomyces formicae* isolated from African *Tetraponera* plant-ants. Chemical Science.