

1 Bacterium secretes chemical inhibitor that sensitizes competitor to
2 bacteriophage infection

3 Zhiyu Zang¹, Chengqian Zhang¹, Kyoung Jin Park¹, Daniel A. Schwartz², Ram Podicheti³, Jay T.
4 Lennon², Joseph P. Gerdt^{1*}

5 ¹Department of Chemistry, Indiana University, Bloomington, IN 47405, USA

6 ²Department of Biology, Indiana University, Bloomington, IN 47405, USA

7 ³Center for Genomics and Bioinformatics, Indiana University, Bloomington, IN 47405, USA

8 *Corresponding author: jpgerdt@iu.edu

9

10 **Abstract**

11 To overtake competitors, microbes produce and secrete secondary metabolites that kill
12 neighboring cells and sequester nutrients. This natural product-mediated competition likely
13 evolved in complex microbial communities that included viral pathogens. From this ecological
14 context, we hypothesized that microbes secrete metabolites that “weaponize” natural pathogens
15 (i.e., bacteriophages) to lyse their competitors. Indeed, we discovered a bacterial secondary
16 metabolite that sensitizes other bacteria to phage infection. We found that this metabolite provides
17 the producer (a *Streptomyces* sp.) with a fitness advantage over its competitor (*Bacillus subtilis*)
18 by promoting phage infection. The phage-promoting metabolite, coelichelin, sensitized *B. subtilis*
19 to a wide panel of lytic phages, and it did so by preventing the early stages of sporulation through
20 iron sequestration. Beyond coelichelin, other natural products may provide phage-mediated
21 competitive advantages to their producers—either by inhibiting sporulation or through yet-
22 unknown mechanisms.

23

24 Introduction

25 Competition is a common theme in microbial life.¹ Microbes frequently live in complex
26 communities with diverse bacterial, fungal, and protozoan species.² Given the finite resources and
27 space in their niches, microbes have evolved an arsenal of strategies that allow them to persist in
28 the face of competition.³ The secretion of secondary metabolites is one of the most common
29 methods by which bacteria and fungi compete with neighboring microbes.^{1, 3} For example,
30 antibiotics can kill or arrest the growth of competitors.⁴ Anti-adhesion molecules like
31 biosurfactants can impede invasion and colonization from potential competitors.⁵ Siderophores
32 that harvest the limited iron in the environment can also indirectly starve competitors and prevent
33 their rapid growth.⁶

34 To find new mechanisms of natural product-based microbial competition, we considered
35 environmental factors that microbes might leverage for a competitive advantage. Since microbes
36 naturally compete within complex populations that involve interactions with other predators and
37 pathogens, we asked if microbes secrete metabolites that sensitize their competitors to the
38 ubiquitous predators or pathogens around them. The major pathogens of bacteria are bacteriophage
39 viruses.⁷ These obligate parasites are strong agents of selection that can induce high rates of
40 mortality and have indirect effects on the competition between microbes and the flux of resources
41 in their environment.⁸ Bacteria have evolved myriad defenses that confer immunity to virus
42 infection.⁹ We hypothesize that microbes may inhibit these antiviral defenses of their competitors,
43 thereby sensitizing their competitors to phages to gain a relative fitness advantage. A similar form
44 of “weaponizing” phages has been reported in which the secretion of secondary metabolites by
45 one species induces lysogenic phages to become lytic and kill the host of another species.¹⁰⁻¹²
46 Otherwise, most cases report that secondary metabolites protect bacteria from phage infection (e.g.,
47 anthracyclines and aminoglycosides have exhibited antiviral effects).¹³ To identify cases where a
48 microbe sensitizes competing bacteria to phages, we screened soil bacteria for isolates that
49 improved the infectivity of bacteriophages on the model soil bacterium *Bacillus subtilis*.

50 We discovered that a *Streptomyces* sp. outcompetes *B. subtilis* in laboratory culture by
51 secreting a metabolite that sensitizes *B. subtilis* to phage predation. The metabolite is a common
52 siderophore named coelichelin, which elicited its effect via iron sequestration. We further found
53 that the improved phage predation was due to the ability of coelichelin to delay sporulation of *B.*

54 *subtilis*, which broadly sensitized *B. subtilis* to many lytic phages. This finding supports the
55 hypothesis that sporulation is a phage-defense strategy.¹⁴⁻¹⁶ Furthermore, given the abundance of
56 phages and iron-sequestering metabolites, this mechanism of metabolite-induced phage
57 sensitization may be a common approach to outcompete spore-forming bacteria.

58 **Results**

59 **Metabolites of *Streptomyces* sp. promote phage plaquing in *B. subtilis***

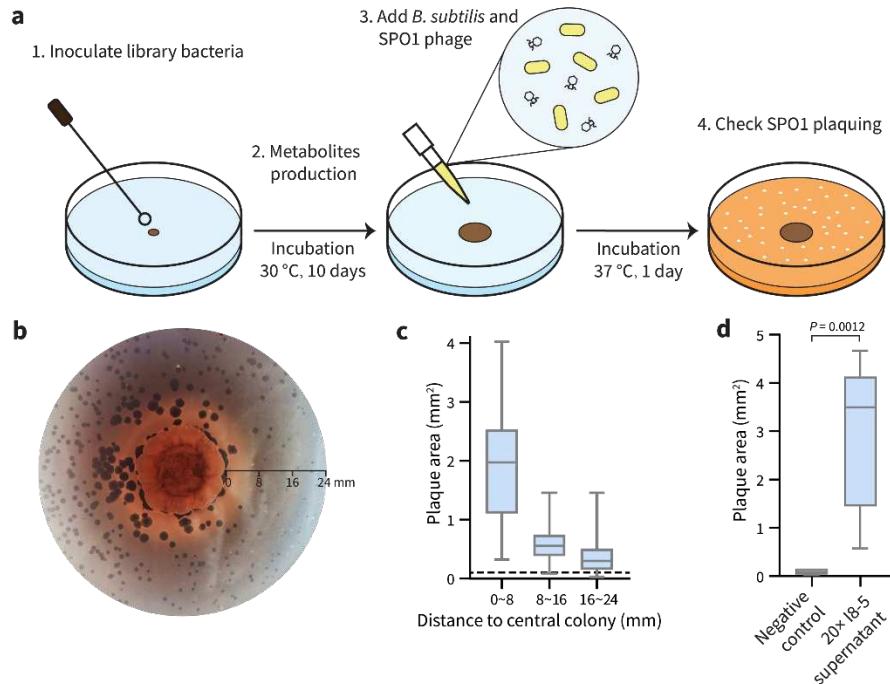
60 We performed a binary-interaction screen to discover bacteria that promote phage infections
61 in *B. subtilis*. Colonies of soil-isolated Actinobacteria were pre-grown on an agar plate to secrete
62 metabolites into their medium (Fig. 1a). Subsequently, *B. subtilis* and phage SPO1 were plated
63 around the mature Actinobacteria colonies. Without an adjacent Actinobacteria colony, the SPO1
64 plaque sizes were small, but we hypothesized that some adjacent colonies could sensitize the *B.*
65 *subtilis* to the phage and enlarge its plaques. In a small screen of 54 soil-derived Actinobacteria,
66 we found several that caused SPO1 to form larger plaques on *B. subtilis*. One of the clearest plaque-
67 enlarging isolates was *Streptomyces* sp. I8-5 (Fig. 1b,c). Notably, the plaque sizes were most
68 enlarged near the *Streptomyces* colony, and distant plaques were essentially the normal small size
69 (Fig. 1c). This distance dependence suggested the production of a diffusible plaque-promoting
70 substance—or possibly the depletion of a diffusible plaque inhibitory substance from the media.
71 To investigate whether the promoted plaquing was due to metabolites secreted from the I8-5
72 colony, we tested the activity of sterile-filtered conditioned medium of I8-5 culture. Concentrated
73 conditioned medium of I8-5 was spotted onto an agar plate containing *B. subtilis* and phage SPO1.
74 The conditioned medium increased the plaque sizes ~30-fold (Fig. 1d), suggesting that secreted
75 component(s) from I8-5 promote *Bacillus* phage infection.

76

77

78

79



80 **Figure 1. Binary-interaction screen identifies a *Streptomyces* sp. that promotes SPO1 phage**
81 **predation of *B. subtilis*.** (a) Scheme of the binary-interaction screen. (b) A mature colony of
82 *Streptomyces* sp. I8-5 (center) promoted SPO1 phage proliferation nearby (dark circles are
83 plaques), especially within a radius of 8 mm. (c) Quantification of plaque areas with increasing
84 distance from the *Streptomyces* sp. I8-5 colony. Data are represented as boxplots, showing the
85 median, interquartile ranges and minimum (bottom bar) and maximum (top bar). The dashed line
86 represents the average plaque area of SPO1 phages in the absence of the *Streptomyces* colony. At
87 least 72 plaques were measured for each condition. (d) The I8-5 supernatant was concentrated 20
88 times and tested for the ability to enlarge SPO1 plaques. Water was used as a negative control.
89 Data are represented as boxplots, showing the median, interquartile ranges and minimum (bottom
90 bar) and maximum (top bar). At least nine plaques were measured for each condition.

91

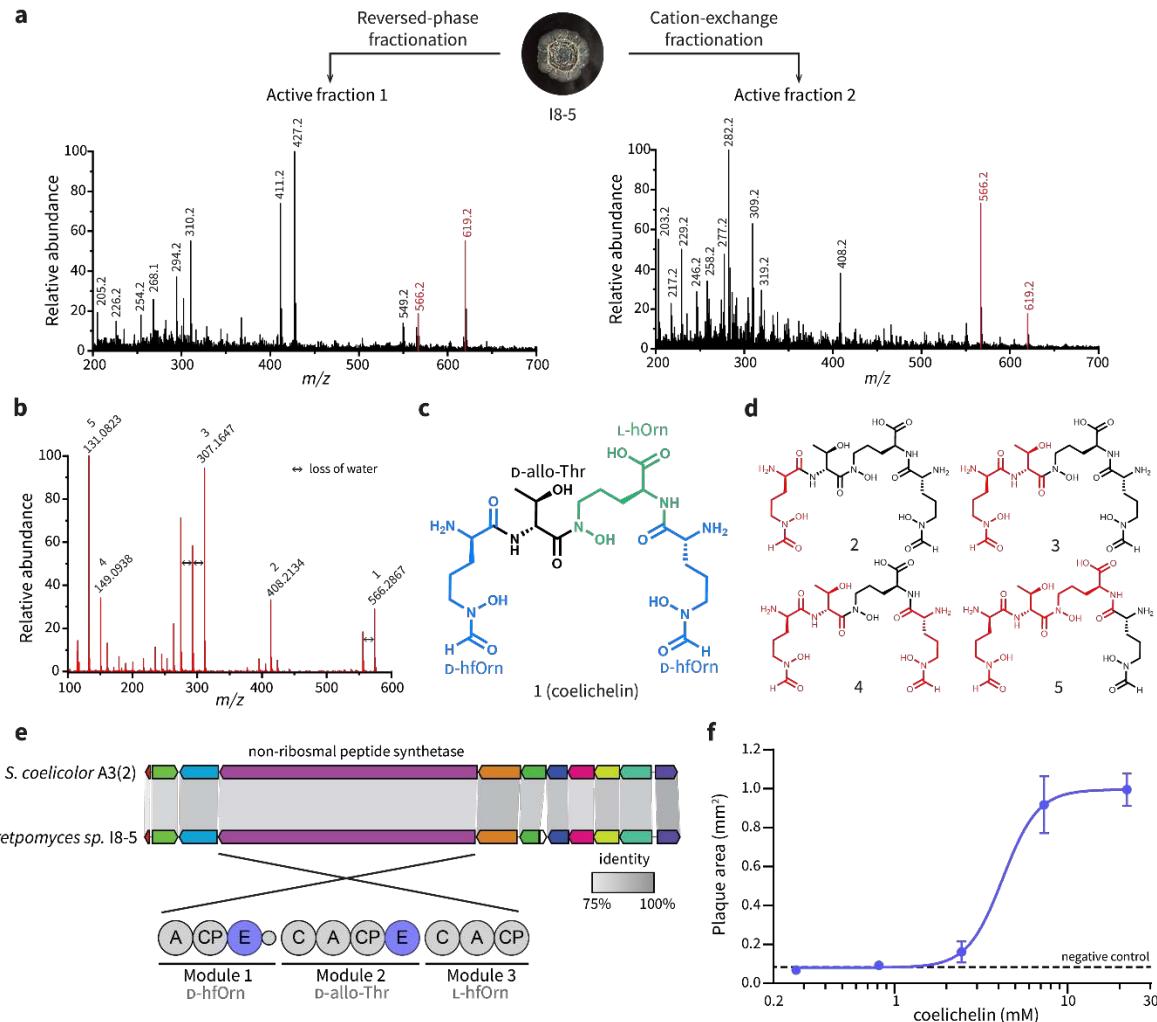
92 **Plaque-promoting metabolite is the siderophore coelichelin**

93 To identify the plaque-enlarging metabolite(s) made by *Streptomyces* sp. I8-5, the conditioned
94 medium of I8-5 culture was subjected to bioactivity-guided fractionation. Two active semi-pure
95 fractions were obtained from different purification strategies: one from reversed-phase
96 chromatography and the other from cation exchange chromatography (Fig. 2a). Since these are
97 relatively orthogonal separation methods, we suspected that few metabolites would be shared

98 between the active fractions. Therefore, we compared the composition of the two active fractions
99 using liquid chromatography-mass spectrometry (LC-MS), and we found that only two putative
100 metabolites were shared by the two fractions (Fig. 2a). High-resolution mass spectrometry of these
101 two metabolites revealed one with m/z 566.2867 and one with m/z 619.1946 (both were likely
102 $[\text{M}+\text{H}]^+$ adducts due to matching $[\text{M}-\text{H}]^-$ adducts observed by negative mode analysis [Extended
103 Data Fig. 1]). Tandem mass spectrometry (MS/MS) analysis of the 566.2867 peak (Fig. 2b)
104 revealed a fragmentation pattern that matched the known metabolite coelichelin (Fig. 2c,d).¹⁷ The
105 exact mass of the parent ion also matched the expected $[\text{M}+\text{H}]^+$ mass of coelichelin within 15 ppm
106 error. Because coelichelin is a siderophore with high affinity to iron,^{17, 18} the m/z 619.1946 peak
107 was attributed to the Fe-coelichelin complex $[\text{M}-2\text{H}+\text{Fe}]^+$ with 8 ppm error. To confirm the ability
108 of *Streptomyces* sp. I8-5 to produce coelichelin, we sequenced its genome and identified the
109 coelichelin biosynthetic gene cluster (BGC) (Fig. 2e). The coelichelin BGC in I8-5 has the same
110 organization as the reported one with high sequence identity (>75% for each gene, Fig. 2e).¹⁷
111 Consistent with the reported coelichelin non-ribosomal peptide synthetase (NRPS),¹⁷
112 epimerization domains were identified in the first and second module of the I8-5 coelichelin NRPS
113 (Fig. 2e). These domains strongly suggest that the absolute stereochemistry of the four amino acids
114 in our sample are the same as reported (Fig. 2c).¹⁷

115 To determine if coelichelin was the active component secreted by *Streptomyces* sp. I8-5, we
116 purified coelichelin from the *Streptomyces*-conditioned medium (Extended Data Fig. 2). NMR
117 analyses were performed on the gallium complex of the purified coelichelin to afford clean
118 spectroscopic data. These analyses confirmed the identity and purity of our isolated coelichelin
119 (Extended Data Fig. 3, Extended Data Table 1, and Supplementary Figures 1–4). As hypothesized,
120 the pure coelichelin enlarged plaques in a dose-dependent manner, confirming it is a phage-
121 promoting metabolite secreted by *Streptomyces* sp. I8-5 (Fig. 2f).

122



123 **Figure 2. Coelichelin is the active metabolite that promotes phage predation.** (a) Bioactivity-
124 guided fractionation and MS analysis identified only two putative metabolites present in both
125 active fractions purified from orthogonal separation techniques. Positive mode electrospray
126 ionization results are shown here, and matching negative mode peaks (m/z 564.2 and 617.2) are
127 shown in Extended Data Fig. 1. (b) MS/MS spectrum of the m/z 566.2867 peak, matching that of
128 the $[M+H]^+$ adduct of coelichelin.¹⁷ (c) Chemical structure of coelichelin, highlighting each amino
129 acid residue. (d) Key fragment losses of coelichelin in the MS/MS analysis, annotated with their
130 associated peak number from panel (b). Black atoms indicate the observed fragment ions, and the
131 neutral lost fragments are highlighted in red. (e) Comparison of the *Streptomyces* sp. I8-5
132 coelichelin biosynthetic gene cluster with the reported one from *S. coelicolor* A3(2). The percent
133 identity between each pair of genes is shown with shading (all were >75%). The modules of the
134 coelichelin non-ribosomal peptide synthetase are shown in detail below. The three modules are
135 responsible for installation of D- δ -N-formyl- δ -N-hydroxyornithine (D-hfOrn), D-allo-threonine (D-

136 allo-Thr), and L- δ -N-hydroxyornithine (L-hOrn), respectively. The adenylation domains (A),
137 thiolation and peptide carrier proteins (CP), condensation domains (C), and epimerization domains
138 (E) are shown. (f) Pure coelichelin enlarged phage plaques in a dose-dependent manner (EC₅₀=4.2
139 mM). Water was used as a negative control. Data are represented as the average \pm SEM from at
140 least seven individual plaques of each condition.

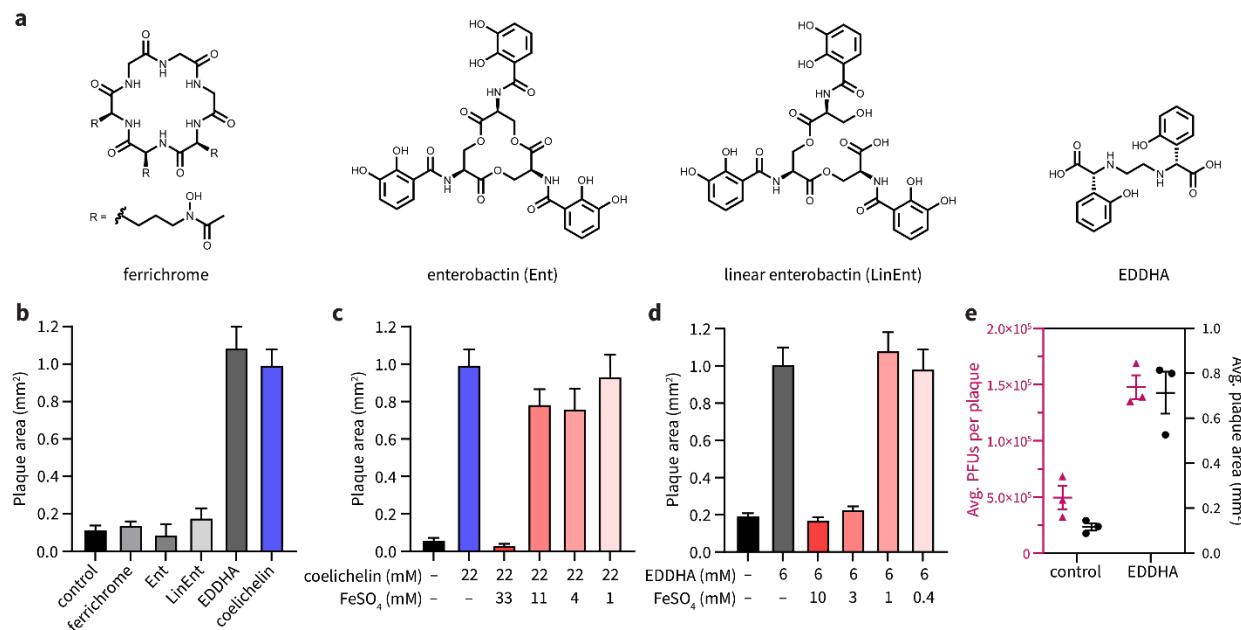
141

142 **Coelichelin promotes phage proliferation by iron sequestration**

143 Coelichelin is known to be a hydroxamate-type siderophore.¹⁷⁻²⁰ We hypothesized that other
144 siderophores would also enlarge plaques of SPO1. We tested three other common siderophores:
145 ferrichrome, enterobactin, and linear enterobactin (Fig. 3a). Surprisingly, none of these three
146 siderophores promoted phage plaquing (Fig. 3b). Previous work has demonstrated that *B. subtilis*
147 can import a range of xenosiderophores produced by other organisms (in addition to using its own
148 siderophore bacillibactin).²¹ This list of “pirated” siderophores notably includes all three that failed
149 to enlarge plaques: ferrichrome,²² enterobactin,^{23, 24} and linear enterobactin.²² Therefore we
150 hypothesized that only siderophores that cannot be imported and utilized by *B. subtilis* enlarge
151 plaques. The non-usable siderophores would sequester iron away from *B. subtilis* and, via an
152 unknown mechanism, improve phage replication on the iron-starved host.

153 It was previously unknown if coelichelin could sequester iron away from *B. subtilis* (or if *B.*
154 *subtilis* could instead use it as a xenosiderophore). Therefore, to test the iron sequestration
155 hypothesis, we utilized a synthetic iron chelator, ethylenediamine-*N,N'*-bis(2-hydroxyphenyl-
156 acetic acid) (EDDHA, Fig. 3a), which is known to sequester iron away from *B. subtilis*.²⁵ In line
157 with our hypothesis, EDDHA increased SPO1 plaque sizes to a similar level as coelichelin (Fig.
158 3b). Furthermore, if iron starvation is responsible for the improved plaquing, the plaque sizes
159 should decrease to their normal size when excess iron is co-administered with the siderophore. As
160 we expected, approximately equimolar concentrations of FeSO₄ quenched the plaque promotion
161 effect of both coelichelin and EDDHA. (Fig. 3c,d). We hypothesized that the enlarged plaques
162 were the result of increased phage proliferation within each plaque. To test this hypothesis, we
163 quantified the viable phages (plaque forming units [PFUs]) generated per plaque. Indeed, the larger
164 plaques afforded by iron limitation produced far more viable phages (Fig. 3e). Thus, we concluded

165 that *B. subtilis* does not use coelichelin as a xenosiderophore, and furthermore, the iron starvation
166 caused by this *Streptomyces* siderophore promotes the predation of *B. subtilis* by the SPO1 phage.



167 **Figure 3. Coelichelin promotes phage predation by sequestering iron. (a)** Chemical structures
168 of ferrichrome, enterobactin (Ent), linear enterobactin (LinEnt), and ethylenediamine-*N,N'*-bis(2-
169 hydroxyphenyl-acetic acid) (EDDHA). **(b)** Ferrichrome (20 mM), Ent (10 mM), LinEnt (20 mM),
170 and EDDHA (6 mM) were tested for the ability to increase SPO1 plaque areas. Water was used as
171 a negative control. Data are represented as the average \pm SEM from at least four individual plaques
172 of each condition. **(c)** Iron complementation antagonized the plaquing promotion effect of
173 coelichelin and **(d)** EDDHA. Water was used as a negative control. Data are represented as the
174 average \pm SEM from at least six individual plaques of each condition. **(e)** The average plaque
175 forming units (PFUs) per plaque and plaque area were measured with EDDHA (6 mM) or water
176 (control) treatment. At least 13 plaques were selected for each condition. Data are represented as
177 the average \pm SEM from three independent biological replicates. Symbols show the values of each
178 biological replicate.

179

180 Iron sequestration improves phage infection by inhibiting sporulation in *B. subtilis*

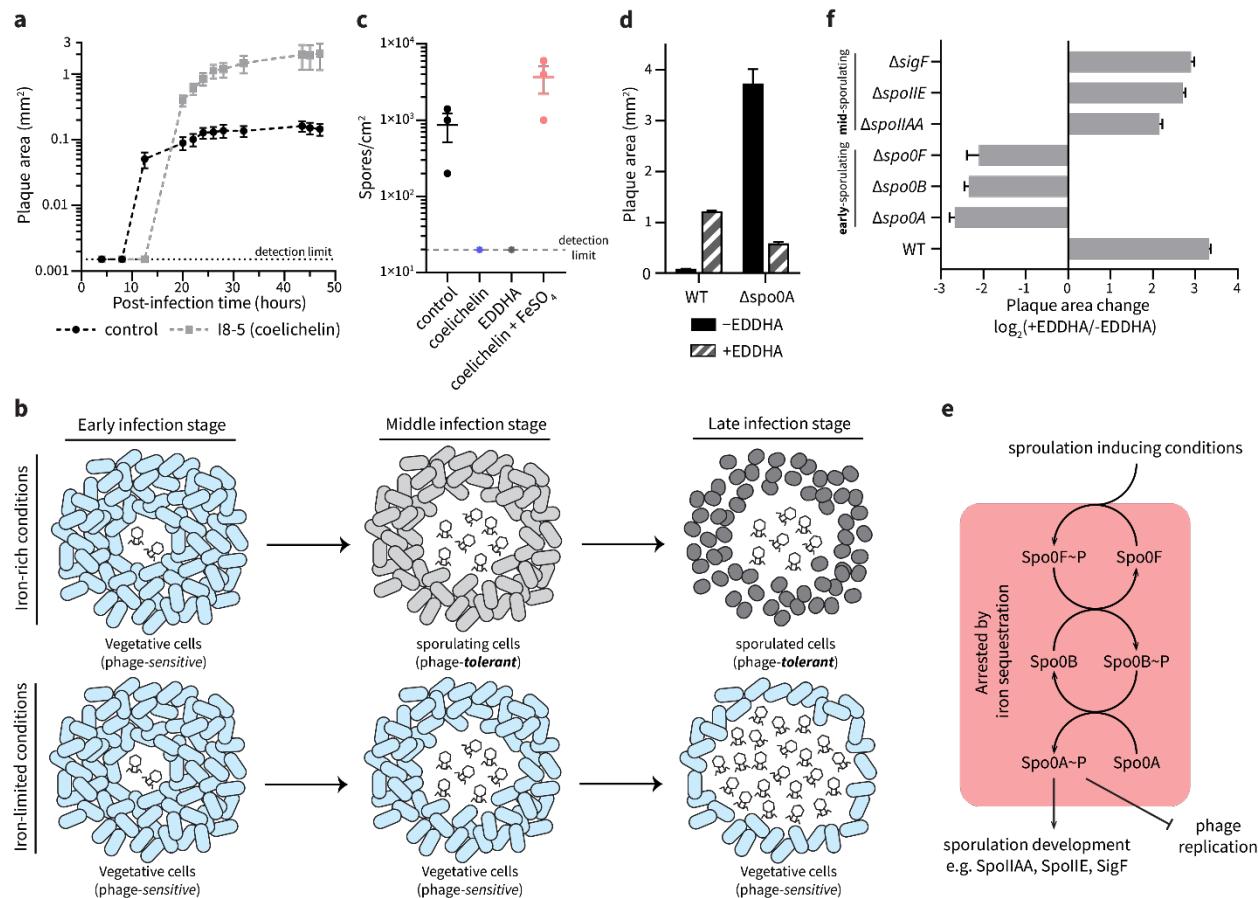
181 We next investigated the mechanism by which iron starvation promoted phage infection of *B.*
182 *subtilis*. Plaque size can be increased by many factors that either accelerate the rate of phage

183 replication or extend the period in which phages can replicate before the bacteria becomes
184 recalcitrant. For example, the rate of plaque expansion depends largely on the burst size (i.e., the
185 number of new phages released from each infected cell) and latent period (i.e., the time required
186 for phages to lyse the host cell and produce new progeny) of phage replication. Specifically, large
187 burst sizes and shorter latent periods maximize the phage reproduction rate, thus resulting in larger
188 plaques.²⁶ We considered whether iron starvation increased burst size and/or shortened the latent
189 period of phage replication. It has been reported that iron starvation actually has the opposite effect
190 in *Vibrio cholerae*: it reduces burst size and delays phage-mediated cell lysis.²⁷ In line with the *V.*
191 *cholerae* study, we observed that when *B. subtilis* grew next to *Streptomyces* sp. I8-5, the plaque
192 development of SPO1 was slower than plaque development alone, suggesting that iron
193 sequestration does not accelerate phage replication (Fig. 4a). However, we observed that the
194 plaque development process lasted longer in the presence of the *Streptomyces* colony (Fig. 4a),
195 which means that phages underwent more reproduction cycles, lysing more *B. subtilis* cells and
196 ultimately forming 10× larger plaque areas.

197 The extended period of phage infection led us to consider an alternative mechanism: iron
198 starvation may prevent host dormancy. Phage reproduction and plaque development requires
199 metabolically active host cells.²⁸ Under sub-optimal conditions, many bacteria enter dormant
200 stages with heavily reduced metabolic activity,²⁹ which is unfavorable for phage proliferation.
201 Therefore, dormancy can be considered a mechanism of phage defense. One form of dormancy in
202 *B. subtilis* is sporulation,³⁰ which has been shown to arrest phage infection by masking surface
203 receptors¹⁴ and inhibiting DNA replication and transcription.^{15, 16} Notably, sporulation in *B.*
204 *subtilis* relies on sufficient levels of intracellular iron.³¹ Therefore, we hypothesized that iron
205 starvation inhibits the sporulation process and maintains *B. subtilis* cells in their phage-susceptible
206 vegetative growth state. Consequently, delayed sporulation would promote phage proliferation,
207 leading to larger plaques (Fig. 4b). To test this hypothesis, we first determined if iron sequestration
208 inhibited sporulation of *B. subtilis* under our experimental conditions. Indeed, by quantifying heat-
209 treated spores,³² we determined that both coelichelin and EDDHA inhibited sporulation in *B.*
210 *subtilis* (Fig. 4c). This inhibitory effect was due to iron sequestration, as demonstrated by the
211 ability of iron supplementation to recover native levels of sporulation (Fig. 4c).

212 To determine if sporulation inhibition was the cause of increased phage proliferation, we
 213 employed a knockout mutant of *spo0A* in *B. subtilis*. Since Spo0A is a transcriptional regulator
 214 that initiates the sporulation process in *B. subtilis*, Δ *spo0A* mutants are incapable of sporulating
 215 and are “locked” into their vegetative growth state.³³ We predicted that this mutant would naturally
 216 form large plaques that are not further enlarged by iron limitation. Indeed, the SPO1 phage formed
 217 extremely large plaques on the Δ *spo0A* mutant (Fig. 4d). As expected, the mutant plaques were
 218 not enlarged by EDDHA-induced iron sequestration. In fact, the plaques were substantially smaller
 219 under EDDHA treatment, possibly due to the aforementioned slowing of phage proliferation under
 220 iron limitation.²⁷ Therefore, our results demonstrate that iron sequestration extends phage infection
 221 on *B. subtilis* by inhibiting its sporulation into dormant, phage-tolerant cells.

222



223 **Figure 4. Iron sequestration inhibits sporulation initiation in *B. subtilis*.** (a) The plaque size
 224 development of SPO1 phage on *B. subtilis* grown ~8 mm from to an I8-5 colony (coelichelin
 225 produced) or *B. subtilis* alone (control). Data are represented as the average \pm SEM from at least
 226 eight individual plaques of each condition. (b) Schematic model for iron sequestration-induced

227 promotion of phage infection: under iron-rich conditions, *B. subtilis* cells sporulate when nutrients
228 are limited. Once the sporulation process initiates in *B. subtilis* cells, phage proliferation is arrested
229 (top). However, when iron is limited, *B. subtilis* cells are unable to sporulate, allowing phages to
230 continue infecting vegetative *B. subtilis* cells (bottom). **(c)** The influence of iron starvation on *B.*
231 *subtilis* sporulation. The number of spores formed under treatment of water (control), coelichelin
232 (22 mM), EDDHA (6 mM), and coelichelin (22 mM) + FeSO₄ (33 mM). Iron starvation inhibited
233 sporulation. Data are represented as the average ± SEM from three independent biological
234 replicates. Circles show the values of each biological replicate. **(d)** The plaque-enlarging effect of
235 EDDHA (6 mM) was tested against *B. subtilis* WT and $\Delta spo0A$. Water was used as the –EDDHA
236 control. The $\Delta spo0A$ mutant naturally formed larger plaques that were not further increased by
237 iron sequestration. Data are represented as the average ± SEM from at least six individual plaques
238 of each condition. **(e)** Schematic representation of sporulation steps in *B. subtilis*. **(f)** The plaque
239 size ratio between EDDHA-treated and untreated samples of different mutants. Mutations in *spo0A*
240 and earlier genes eliminated the phage-promoting effects of iron sequestration. Data are
241 represented as the average ratio ± SEM calculated from at least four individual plaques of each
242 condition.

243

244 **Iron sequestration inhibits sporulation at an early stage**

245 Sporulation in *B. subtilis* is a multi-stage process involving initiation, development, and
246 maturation.³⁴ The process is transcriptionally initiated by Spo0A, which is activated by the kinase
247 Spo0B, which itself is activated by the kinase Spo0F in a phosphorelay (Fig. 4e).³⁵ The activated
248 Spo0A protein then functions as the master transcriptional regulator of the genes leading to the
249 formation of mature *B. subtilis* spores. As further confirmation of the necessity of activated Spo0A
250 protein for the small plaque phenotype in untreated *B. subtilis*, we tested $\Delta spo0F$ and $\Delta spo0B$
251 mutants. These, too, exhibited large plaques that did not enlarge upon iron sequestration (Fig. 4f).
252 Therefore, the phage inhibition effect occurs downstream of Spo0A activation, and iron
253 sequestration likely prevents the activation of Spo0A.

254 To determine if the iron-dependent phage inhibition phenotype requires the complete
255 maturation of spores, we tested mutants of three genes that are required for intermediate stages of
256 sporulation: *spoIIAA*, *spoIIE*, and *sigF*.³⁶ Unlike the early sporulation genes, these mutants plaqued

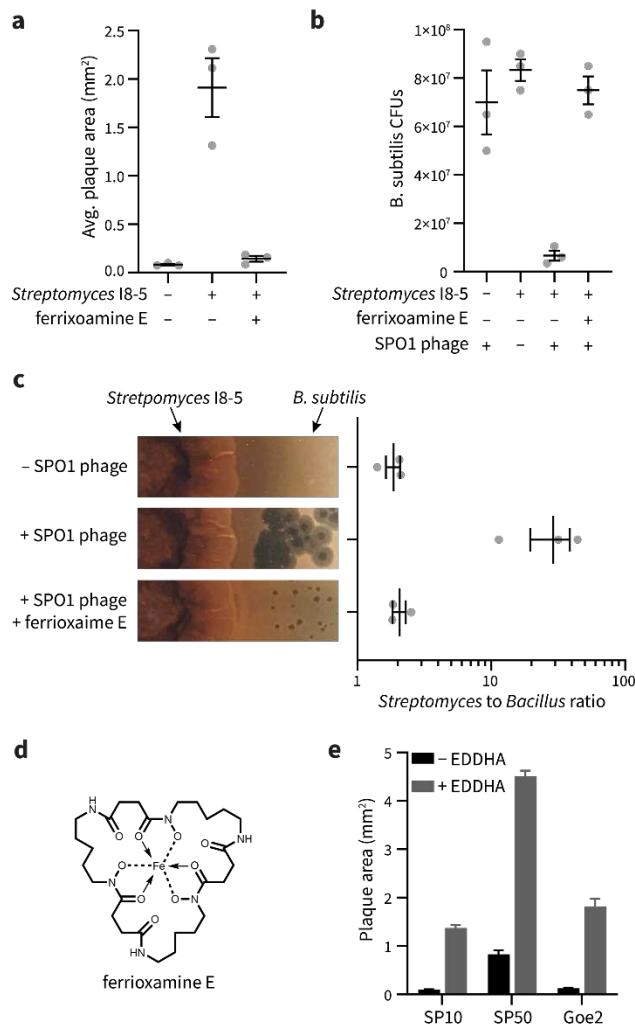
257 like wild-type: they formed small plaques under resource-replete conditions but large plaques
258 under iron starvation (Fig. 4f). Therefore, we propose that phage proliferation is arrested by the
259 earliest stages of sporulation and does not require the intermediate and final stages of spore
260 formation. Notably, prior work has shown that activated Spo0A can directly inhibit replication of
261 and transcription from phage phi29 DNA.^{15, 16} Therefore, two likely mechanisms may contribute
262 to the Spo0A-dependent plaque restriction: Spo0A may directly inhibit replication and
263 transcription of phage SPO1 genes, and/or Spo0A may activate early sporulation processes that
264 halt host metabolic processes that are essential for phage replication. These mechanisms are not
265 mutually exclusive and may synergize to fully arrest phage replication in our conditions. In either
266 case, iron sequestration prevents the activation of Spo0A, which frees the phage to continue
267 replicating and lysing the *B. subtilis* population.

268

269 **Siderophore production enables *Streptomyces* to outcompete *B. subtilis* in a phage-dependent**
270 **manner**

271 *B. subtilis* and *Streptomyces* are both soil bacteria and are likely to share habitats in nature.³⁷
272 Since coelichelin secreted by *Streptomyces* sp. I8-5 promoted phage infection on *B. subtilis* (Fig.
273 5a), we hypothesized that coelichelin offers *Streptomyces* a competitive advantage over *B. subtilis*
274 in the presence of *Bacillus* phages. Indeed, the combined action of SPO1 phages and a nearby
275 *Streptomyces* sp. I8-5 colony significantly decreased the *B. subtilis* population density relative to
276 phage treatment or *Streptomyces* treatment alone (Fig. 5b). Importantly, this effect allowed
277 *Streptomyces* to outcompete *B. subtilis* 15:1 under our growth conditions (Fig. 5c). To validate the
278 significance of coelichelin-induced iron sequestration for the decreased *B. subtilis* fitness, we
279 supplied the *B. subtilis* cells with excess bioavailable iron in the form of a xenosiderophore-iron
280 complex with ferrioxamine E (Fig. 5d). With ferrioxamine E as a supplemented iron source, the
281 phage promotion effect from *Streptomyces* was abolished as seen with normal size plaques (Fig.
282 5a), and the *B. subtilis* population increased to the same level as the no-*Streptomyces* and no-phage
283 controls (Fig. 5b). With the lost impact of coelichelin, *Streptomyces* lost its phage-induced
284 competitive advantage over *B. subtilis* (Fig. 5c). Therefore, secretion of coelichelin enables
285 *Streptomyces* sp. I8-5 to outcompete *B. subtilis* by facilitating phage predation on its competitor.

286 Finally, we asked if the facilitated phage predation was specific to only the SPO1 phage, or if
287 it could be generalized to a wide range of phages that would encounter mixed *B. subtilis* +
288 *Streptomyces* communities in nature. We tested three other *Bacillus* phages (SP10, SP50, and
289 Goe2),^{38, 39} and the virulence of all three was substantially increased by iron sequestration (Fig.
290 5e). Therefore, iron sequestration by competing microbes may broadly sensitize *B. subtilis* to a
291 variety of phages in nature.



292 **Figure 5. Coelichelin helps *Streptomyces* to outcompete *B. subtilis*.** (a) The average plaque areas
293 of SPO1 on *B. subtilis* alone, and *B. subtilis* neighboring *Streptomyces* I8-5 colony with or without
294 ferrioxamine E as excess iron source. At least eight plaques were selected for each replicate. Data
295 are represented as the average \pm SEM from three independent biological replicates. Circles show
296 the values of each biological replicate. (b) The colony forming units of *B. subtilis* measured in the
297 presence of phages, neighboring *Streptomyces* I8-5 colony with and without phages, and

298 neighboring *Streptomyces* I8-5 colony with ferrioxamine E as excess iron source in the presence
299 of phages. Data are represented as the average \pm SEM from three independent biological replicates.
300 Circles show the values of each biological replicate. **(c)** *Streptomyces* to *B. subtilis* ratio calculated
301 from colony forming units measured after 2 days of co-culture. Data are represented as the average
302 \pm SEM from three independent biological replicates. Circles show the values of each biological
303 replicate. **(d)** The chemical structure of ferrioxamine E. **(e)** The plaquing promotion effect of
304 EDDHA (6 mM) for phages SP10, SP50, and Goe2 on *B. subtilis*. Water was used as the –EDDHA
305 control. Data are represented as the average ratio \pm SEM calculated from at least four individual
306 plaques of each condition.

307 **Discussion**

308 We discovered that a bacterial strain gains a competitive advantage over neighboring bacteria
309 by producing a secondary metabolite that sensitizes its competitor to phage predation. In our
310 culture conditions, the metabolite/phage synergy switched the outcome of the bacterial
311 competition to strongly favor the metabolite producer. The secondary metabolite is the known
312 *Streptomyces*-produced siderophore coelichelin, which sequesters iron away from *B. subtilis* and
313 promotes phage infection by inhibiting sporulation. These findings reveal a new mechanism by
314 which siderophores can shape microbial competition through bacteria-phage ecology.

315 Siderophores are primarily believed to provide a means for the producing organism to acquire
316 scarce iron ions to supply their own metalloenzymes.^{6, 20} Beyond iron acquisition, siderophores
317 can benefit their producers in other ways that justify the maintenance of their biosynthetic genes.
318 Notably, siderophores can inhibit the growth of neighbors, allowing the producing microbe to
319 displace its competitors.^{6, 40} Our results also show that siderophores can block sporulation of
320 competing bacteria, which could be beneficial in fluctuating environments. For example, by
321 excluding spores of a competitor, the siderophore-producer's spores would revive without
322 competition when conditions become optimal for growth and reproduction.⁴¹⁻⁴³ Finally, our work
323 reveals a potential fourth benefit of siderophore production: microbes can sensitize competing
324 bacteria to lytic phages. Since iron sequestration sensitizes *B. subtilis* to several natural phages,
325 this phage-promoting effect may benefit the multitude of siderophore-producing bacteria and
326 fungi²⁰ that compete with *B. subtilis* and its endospore-forming relatives.

327 Beyond microbe-microbe competition, multicellular hosts also leverage iron sequestration to
328 stall the growth of pathogens by producing molecules like transferrin and lactoferrin.⁴⁴ Therefore,
329 host-induced iron sequestration may also sensitize certain endospore-forming bacteria to lytic
330 phages (either ones endogenously present or those administered therapeutically).

331 Although our studies focused on soil *Streptomyces* and the model soil bacterium *B. subtilis*,
332 endosporulation is a characteristic trait of many bacteria in the *Bacillota* (Firmicutes) phylum.⁴⁵
333 These endospore-forming bacteria are not only abundant in soil and aquatic sediments, but they
334 are also important members of host-associated microbiomes—including some common intestinal
335 pathogens and mutualistic taxa in humans.⁴⁶ Therefore, it is plausible that secondary metabolites
336 sensitize diverse *Bacillota* (Firmicutes) in varied environments to phage infection via sporulation

337 inhibition. In fact, secondary metabolites other than siderophores have also been shown to inhibit
338 sporulation. For example, a common signal molecule used for quorum sensing, autoinducer-2,
339 inhibits sporulation in *Bacillus velezensis*.³⁴ Furthermore, the bacterial macrocycle, fidaxomicin,
340 inhibits *Clostridioides difficile* sporulation.⁴⁷ Therefore, microbial siderophores, other microbial
341 secondary metabolites, and even host-produced molecules may sensitize competing bacteria to
342 phage infection in natural communities.

343 In conclusion, we found an example that a natural product, coelichelin, gives its producer an
344 advantage by sensitizing its competitors to phages. Despite a rich history of studies on the
345 “chemical warfare” waged between microbes via natural products, little emphasis has been placed
346 on how phage predation intersects with microbial secondary metabolites. This work reveals that
347 microbial natural products do not just directly inhibit the fitness of microbial competitors, but these
348 molecules can also sensitize competitors to lytic phages. Although the extent of this phenomenon
349 in nature is yet to be seen, it may shape the microbial ecology of both environmental and host-
350 associated ecosystems. Also, much like society has leveraged microbial competition to discover
351 life-saving antimicrobials, phage-promoting natural products may also prove useful one day as co-
352 administered adjuvants in phage-based interventions.

353

354 **Methods**

355 **Strains and growth conditions**

356 The strains and bacteriophages used in this study are listed in Table S1. All chemicals used in this
357 study are listed in Table S2. All primers used in this study are listed in Table S3. *B. subtilis* strains
358 were routinely grown in LB broth at 37 °C and 220 rpm. When appropriate, antibiotics were used
359 at the following concentrations: 7 µg/mL kanamycin and 1 µg/mL erythromycin. *Streptomyces*
360 strains were routinely grown in ISP2 media (4 g/L yeast extract, 10 g/L malt extract, and 4 g/L
361 dextrose) at 30 °C and 220 rpm.

362 **Bacteriophage lysate preparation**

363 To prepare the host culture, an overnight culture of *B. subtilis* RM125 WT was sub-cultured 1:100
364 into 4 mL LB + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 5 mM CaCl₂. The culture was incubated at 37 °C
365 and 220 rpm for 4 hours until the OD_{600nm} reached 0.2. About 1×10³ plaque forming units (PFUs)
366 of bacillus phage were added to the culture. The phage infected culture was incubated at 37 °C and
367 220 rpm until bacterial cells were lysed and the culture turned clear. The phage lysate was filtered
368 through a 0.2 µm polyethersulfone filter and stored at 4 °C.

369 **Binary-interaction screening**

370 To prepare plates with library bacteria, 5 µL of the frozen spore stock of each bacteria strain in our
371 library was suspended in 50 µL of ISP2 medium. Then 8 µL of the spore suspension was spotted
372 at the center of a ISP2 + 1.5% agar plate. The inoculated plates were incubated at 30 °C for 10
373 days to allow the library bacteria to grow and secrete their metabolites into the plate. To test the
374 influence of the metabolites on phage infectivity, an overnight culture of *B. subtilis* RM125 WT
375 was diluted 1:10 into 5 mL fresh LB broth and then ~1,000 PFUs of SPO1 phages were added into
376 the medium. The mixture of bacteria and phages was poured around the central colony formed by
377 the library bacteria. Bacteria and phages were allowed 10 mins to soak the plate, and then the
378 bacteria dilution was removed by pipet. The plate was dried under room temperature in biosafety
379 cabinet. The plate was incubated at 37 °C overnight and the plaques formed by SPO1 was
380 examined the next day.

381 **16S rRNA sequencing of I8-5**

382 A single colony of I8-5 was inoculated into 4 mL fresh ISP2 medium and incubated at 30 °C, 220
383 rpm for 4 days. The genomic DNA was extracted from 1 mL liquid culture using Promega Wizard
384 Genomic DNA Purification Kit (#1120). The 16S rRNA region of I8-5 genome was amplified by
385 PCR using 16S_F and 16S_R primers. Sanger sequencing result of its 16S rRNA with 16S_F
386 primer was available on NCBI (accession number: OR902106). The 16S rRNA sequence of I8-5
387 aligns well with species in *Streptomyces* genus. (Supplementary file 1)

388 **Coelichelin biosynthetic gene cluster identification in I8-5**

389 The library of the extracted genomic DNA was prepared by Illumina Nextera XT DNA Library
390 Prep Kit protocol (# FC-131-1096) and analyzed by Agilent D1000 ScreenTape. The libraries were
391 pooled and loaded on a NextSeq 1000/2000 P2 Reagents (100-cycles) v3 flow cell (#20046811)

392 configured to generate paired end reads. The demultiplexing of the reads was performed using
393 `bcl2fastq`, version 2.20.0. Reads were adapter trimmed and quality filtered using Trimmomatic⁴⁸
394 0.38 with the cutoff threshold for average base quality score set at 20 over a window of 3 bases
395 requiring a minimum trimmed read length of 20 bases (parameters: LEADING:20 TRAILING:20
396 SLIDINGWINDOW:3:20 MINLEN:20). The cleaned reads were assembled using SPAdes⁴⁹
397 version 3.15.4 with default parameters. The assembly was annotated using prokka⁵⁰ version 1.12
398 employing a sequence training set prepared from protein sequences obtained from 431 publicly
399 available *Streptomyces* assemblies (parameters: --minpid 70 --usegenus --hmmlist
400 TIGRFAM,CLUSTERS,Pfam,HAMAP). The coelichelin biosynthetic gene cluster was identified
401 by antiSMASH 7.0.⁵¹ The genomic sequences are available on NCBI (accession number:
402 JAYMFC000000000).

403 **Collection of I8-5 supernatant**

404 To revive the spores of I8-5, 5 μ L of the I8-5 frozen spore stock was streaked out on a ISP2 + 1.5%
405 agar plate. The plate was incubated at 30°C for 3 days until colonies formed. A colony was
406 inoculated into 4 mL fresh ISP2 medium and incubated at 30 °C, 220 rpm for 4 days. After the
407 incubation, 1 mL of the culture was added into 100 mL of ISP2 medium and grown for another 11
408 days to allow metabolite production. To harvest the metabolites in the supernatant, the bacteria
409 cells in the culture were pelleted at 4,820 \times g for 20 min and discarded. The supernatant was
410 lyophilized and stored at -20 °C until ready to use.

411 **Phage promotion activity test**

412 An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 mL fresh ISP2 + 0.1 mM
413 $MnCl_2$ + 5 mM $MgCl_2$, and poured onto an ISP2 + 0.1 mM $MnCl_2$ + 5 mM $MgCl_2$ + 1.5% agar
414 plate. Bacteria were allowed 10 min to attach to the plate, and then the unattached bacteria were
415 removed. The plate was dried under room temperature in biosafety cabinet. To test the phage
416 promotion effect, 2 μ L of compound was spotted on top of the bacterial lawn. After the compound
417 dried, incubated the plate at 37 °C for 1 h. Then 5 μ L of SPO1 phage lysate (~10 PFUs) was spotted
418 on top of the compound treated area. After the phage lysate dried, the plate was incubated at 37 °C
419 overnight and the plaques formed by SPO1 was examined the next day.

420 **Fractionation of I8-5 supernatant using reversed-phase chromatography**

421 The lyophilized supernatant was dissolved into a small amount of water as a concentrated sample.
422 The concentrated supernatant was further separated on a Phenomenex Synergi 4 μ m Hydro-RP 80
423 Å column (250 \times 10 mm) using an Agilent 1260 Infinity II HPLC system. The mobile phase A
424 was water + 0.01 % (v/v) formic acid and the mobile phase B was acetonitrile + 0.01 % (v/v)
425 formic acid. The flow rate was kept at 3 mL·min⁻¹ and the gradient was as follows: 0% B (0–5
426 min), increase to 20% B (5–6 min), 20% B for (6–11 min), increase to 80% B (11–31 min), increase
427 to 100% B (31–32 min), 100% B (32–37 min), decrease to 0% B (37–38 min), 0% B (38–43 min).
428 Eluted fractions were collected every 30 seconds and dried in vacuo. Each dried fraction was
429 redissolved into 2 μ L DMSO and spotted on a lawn of *B. subtilis* RM125 WT infected with ~1000
430 PFUs of SPO1 phages to test the phage promotion activity. The fraction eluting at 12.0~12.5 min
431 is active and labeled as “active fraction 1”. The composition of “active fraction 1” was analyzed

432 on a Phenomenex Synergi 4 μ m Hydro-RP 80 \AA column (250 \times 4.6 mm) using an Agilent 1260
433 Infinity II HPLC system coupled to a mass spectrometer Agilent InfinityLab LC/MSD XT. The
434 analysis was performed at a flow rate of 0.7 $\text{mL}\cdot\text{min}^{-1}$. The mobile phase and separation gradient
435 were the same as described above.

436 **Fractionation of I8-5 supernatant using cation-exchange and reversed-phase**
437 **chromatography**

438 The lyophilized supernatant was dissolved into 10 mL water and added 2% (v/v) formic acid to
439 adjust the pH to 2.02. The supernatant was loaded on a Waters Oasis MCX column (#186000255).
440 The column was then eluted with water + 2% (v/v) formic acid, methanol, and methanol + 5%
441 (v/v) ammonium hydroxide. The eluates were dried in vacuo and redissolved into water as a 100
442 mg/mL solution. 2 μ L of each redissolved fraction was spotted on a lawn of *B. subtilis* RM125
443 WT infected with \sim 1000 PFUs of SPO1 phages to test the phage promotion activity. The methanol
444 + 5% (v/v) ammonium hydroxide eluate was active and subjected to separation on a Phenomenex
445 Synergi 4 μ m Hydro-RP 80 \AA column (250 \times 10 mm) using an Agilent 1260 Infinity II HPLC
446 system. The mobile phase A was water + 0.1 % (v/v) formic acid and the mobile phase B was
447 acetonitrile + 0.1 % (v/v) formic acid. The flow rate was kept at 3 $\text{mL}\cdot\text{min}^{-1}$ and the gradient was
448 as follows: 10% B (0–10 min), increase to 50% B (10–30 min), increase to 100% B for (30–31
449 min), 100% B (31–38 min), decrease to 10% B (38–39 min), 10% B (39–44 min). Eluted fractions
450 were collected every 30 seconds and dried in vacuo. Each dried fraction was redissolved into 2 μ L
451 DMSO tested for phage promotion effect as described above. The fraction eluting at 3.5~4.0 min
452 is active and labeled as “active fraction 2”. The composition of “active fraction 2” was analyzed
453 on a Phenomenex Synergi 4 μ m Hydro-RP 80 \AA column (250 \times 4.6 mm) using an Agilent 1260
454 Infinity II HPLC system coupled to a mass spectrometer Agilent InfinityLab LC/MSD XT. The
455 analysis was performed at a flow rate of 0.7 $\text{mL}\cdot\text{min}^{-1}$. The mobile phase and separation gradient
456 were the same as described above.

457 **Tandem mass spectrometry (MS/MS) analysis of 566.2867 [M+H] $^+$**

458 High-resolution electrospray ionization (HR-ESI) mass spectra with collision-induced dissociation
459 (CID) MS/MS were obtained using a Waters Synapt G2S QTOF. Data-dependent acquisition was
460 employed to fragment the top three masses in each scan. The “active fraction 2” was separated on
461 a Phenomenex Synergi 4 μ m Hydro-RP 80 \AA column (250 \times 4.6 mm). The mobile phase A was
462 water + 0.1 % (v/v) formic acid and the mobile phase B was acetonitrile + 0.1 % (v/v) formic acid.
463 The flow rate was kept at 0.7 $\text{mL}\cdot\text{min}^{-1}$ and the gradient was as follows: 0% B (0–20 min), increase
464 to 20% B (20–21 min), increase to 40% B for (21–31 min), increase to 100% B (31–32 min), 100%
465 B (32–42 min), decrease to 0% B (42–43 min), 0% B (43–48 min). 566.2867 [M+H] $^+$ eluted at
466 17.2~18.2 min.

467 **Isolation of coelichelin from I8-5 supernatant**

468 The protocol was adapted from Challis et al.¹⁷ The lyophilized I8-5 supernatant (1.8533 g) was
469 dissolved in 5 mL of water. FeCl_3 was added to the supernatant (final concentration 40 mM) to
470 generate Fe-coelichelin complex. The reaction mixture was centrifuged at 16,000 \times g for 5 min and
471 the precipitates were discarded. The supernatant was separated on a Phenomenex Luna 10 μ m

472 Hydro-RP 100 Å column (250×21.2 mm) using an Agilent 1260 Infinity II HPLC system. The
473 mobile phase A was water + 10 mM NH₄HCO₃ (pH=8.01) and the mobile phase B was methanol.
474 The flow rate was kept at 10 mL·min⁻¹ and the gradient was as follows: 5% B (0–20 min), increase
475 to 90% B (20–21 min), 20% B for (21–31 min), decrease to 5% B (31–32 min), 5% B (32–42 min).
476 Fe-coelichelin eluted at 9.1~10.4 min and was collected by monitoring the absorbance at 435 nm.
477 The collected Fe-coelichelin was concentrated in vacuo, lyophilized, and obtained as an orange
478 solid.

479 The obtained Fe-coelichelin (45.9 mg) was dissolved into 74 mL water. The ferric iron was
480 removed from Fe-coelichelin complex by mixing the Fe-coelichelin solution with 74 mL 100 mM
481 8-hydroxyquinoline in methanol. The reaction was stirred for 30 min at room temperature. The Fe-
482 8-hydroxyquinoline complex was removed by extracting the aqueous phase using 50 mL
483 dichloromethane 3 times. The aqueous phase was concentrated in vacuo and separated on a
484 Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250×10 mm) using an Agilent 1260 Infinity
485 II HPLC system. The mobile phase A was water + 0.1 % (v/v) formic acid and the mobile phase
486 B was acetonitrile + 0.1 % (v/v) formic acid. The flow rate was kept at 3 mL·min⁻¹ and the gradient
487 was as follows: 0% B (0–10 min), increase to 5% B (10–11 min), 5% B for (11–21 min), increase
488 to 100% B (21–22 min), 100% B (22–32 min), decrease to 0% B (32–33 min), 0% B (33–43 min).
489 Apo-coelichelin eluted at 17.7~17.9 min and was collected by monitoring the absorbance at 210
490 nm. The collected apo-coelichelin was concentrated in vacuo, lyophilized, and obtained as a white
491 solid. The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data of apo-
492 coelichelin was obtained on a Thermo Scientific Finnigan LTQ Orbitrap XL Mass Spectrometer
493 equipped with a nano-electrospray ionization source operated in positive ionization mode. HR-
494 ESI-MS (positive-ion mode): *m/z* 566.2776 [M+H]⁺ (calcd for C₂₁H₄₀N₇O₁₁⁺: 566.2780)

495 **Coelichelin purity check by LC-MS**

496 The purified coelichelin was analyzed on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column
497 (250×4.6 mm) using an Agilent 1260 Infinity II HPLC system coupled to a mass spectrometer
498 Agilent InfinityLab LC/MSD XT. The mobile phase A was water + 0.1 % (v/v) formic acid and
499 the mobile phase B was acetonitrile + 0.1 % (v/v) formic acid. The flow rate was kept at 0.7
500 mL·min⁻¹ and the gradient was as follows: 0% B (0–10 min), increase to 5% B (10–30 min),
501 increase to 100% B for (30–31 min), 100% B (31–41 min), decrease to 0% B (41–42 min), 0% B
502 (42–52 min).

503 **Preparation of Ga-coelichelin**

504 Coelichelin (10 mg) was dissolved in 400 µL of water and reacted with 26.5 mg of Ga₂(SO₄)₃ in
505 400 µL of water. The reaction was performed at room temperature for 30 mins and subjected to
506 separation on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250×10 mm) using an Agilent
507 1260 Infinity II HPLC system. The mobile phase A was water + 0.1 % (v/v) formic acid and the
508 mobile phase B was acetonitrile + 0.1 % (v/v) formic acid. The flow rate was kept at 3 mL·min⁻¹
509 and the gradient was as follows: 0% B (0–10 min), increase to 100% B (10–11 min), 100% B for
510 (11–21 min, decrease to 0% B (21–22 min), 0% B (22–32 min). Ga-coelichelin eluted at 4.6~5.0
511 min and was collected by monitoring the absorbance at 210 nm. The collected Ga-coelichelin was

512 concentrated in vacuo, lyophilized, and obtained as a white solid. HR-ESI-MS (positive-ion mode):
513 m/z 632.1794 [M+H]⁺ (calcd for C₂₁H₃₇GaN₇O₁₁⁺: 632.1801). ¹H and TOCSY (mixing time of 60
514 ms) NMR spectra were obtained on a Varian 600 MHz Inova NMR spectrometer. ¹³C, DQF-COSY,
515 HSQC, and HMBC NMR spectra were obtained on a Bruker 500 MHz Avance Neo NMR
516 spectrometer.

517 **Iron complementation experiment**

518 An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 mL fresh ISP2 + 0.1 mM
519 MnCl₂ + 5 mM MgCl₂, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar
520 plate. Bacteria were allowed 10 mins to soak the plate, and then the bacteria dilution was removed
521 by pipet. The plate was dried under room temperature in a biosafety cabinet. 2 μ L of compound
522 was spotted as a small circle on top of the bacterial lawn. After the compound dried, 2 μ L of FeSO₄
523 aqueous solution was spotted on top of the compound-treated area. After the FeSO₄ solution dried,
524 the plate was incubated at 37 °C for 1 hour. Then 5 μ L of SPO1 phage lysate (~10 PFUs) was
525 spotted on top of the compound treated area. After the phage lysate dried, the plate was incubated
526 at 37°C overnight and the plaques formed by SPO1 were examined the next day.

527 **Quantification of phage reproduction from individual plaques**

528 An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 mL fresh ISP2 + 0.1 mM
529 MnCl₂ + 5 mM MgCl₂, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar
530 plate. Bacteria were allowed 10 mins to soak the plate, and then the bacteria dilution was removed
531 by pipet. The plate was dried under room temperature in biosafety cabinet. 2 μ L of 6 mM EDDHA
532 or water was spotted as a small circle on top of the bacterial lawn. After the compound dried, the
533 plate was incubated at 37 °C for 1 hour. Then 5 μ L of SPO1 phage lysate (10~40 PFUs) was
534 spotted on top of the compound treated area. After the phage lysate dried, the plate was incubated
535 at 37°C for 2 days. The number of plaques formed in each phage spot were enumerated and the
536 average plaque areas were measured for each phage spot. All the plaques in one phage spot were
537 pooled by carving out the agar with the plaques and resuspended in 5 ml phage buffer (10 mM
538 Tris, 10 mM MgSO₄, 4g/L NaCl, pH=7.5). The suspension was vortexed at the highest speed for
539 20 seconds to allow phages to fully detach from the agar. The PFUs of the pooled plaques were
540 quantified by the small drop plaque assay.⁵² For each individual phage spot, the average PFUs per
541 plaque was calculated using the following equation:

542
$$Avg. PFUs \text{ per plaque} = \frac{PFUs \text{ of pooled plaques}}{number \text{ of plaques}}$$

543 **Sporulation quantification**

544 An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 mL fresh ISP2 + 0.1 mM
545 MnCl₂ + 5 mM MgCl₂, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar
546 plate. Bacteria were allowed 10 mins to soak the plate, and then the bacteria dilution was removed
547 by pipet. The plate was dried under room temperature in biosafety cabinet. 2 μ L of compound or
548 water (control) was spotted as a small circle on top of the bacterial lawn. After the compound dried,
549 the plate was at 37 °C for 16 hours. Then ~1cm² area of bacteria was scraped off the plate and

550 resuspended in 200 μ L water. The cell suspension was heated at 85 °C for 15 mins to kill non-
551 sporulated cells. Then the spores in the heat-treated cell suspension were quantified by measuring
552 the colony forming units.

553 **Generation of sporulation mutants**

554 The gene knockout mutants in *B. subtilis* 168 were purchased from Bacillus Genomic Stock Center.
555 The mutation was then transferred to *B. subtilis* RM125 using SPP1-mediated generalized phage
556 transduction.^{53, 54} The SPP1 phage lysate was obtained from the *B. subtilis* 168 knockout donor
557 strain as described above and stored at 4 °C until ready to use. A single colony of the recipient *B.*
558 *subtilis* RM125 was inoculated into 10 mL LB + 10 mM CaCl₂. The recipient culture was
559 incubated at 37 °C and 220 rpm for 4 hours. For phage transduction, 950 μ L of the recipient culture
560 was mixed with 50 μ L of donor SPP1 lysate, and incubated at 37 °C for 10 mins to allow phage
561 adsorption. Then the infected culture was transferred into 9 mL of prewarmed LB + 20 mM sodium
562 citrate and incubated at 37 °C for another 10 mins. The cells were pelleted at 4000 \times g for 5 min
563 and plated onto an LB + 20 mM sodium citrate + 1.5% agar plate with appropriate antibiotics. The
564 plates were incubated at 37 °C overnight and the mutant colonies were re-streaked twice on LB +
565 20 mM sodium citrate + 1.5% agar plate with appropriate antibiotics to clean out phages. The
566 knockout mutation was validated by PCR with primers reported by Gross et al.⁵⁵ The sporulation
567 mutants were verified to not produce spores by the sporulation quantification experiment described
568 above.

569 ***Streptomyces* sp. I8-5 and *B. subtilis* competition**

570 To inoculate plates with *Streptomyces*, 5 μ L of the frozen spore stock of I8-5 was suspended in 50
571 μ L of ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂. Then 8 μ L of the spore suspension was spotted at
572 the center of an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar plate. The plates were incubated
573 at 30°C for 22 days to allow I8-5 colony to grow and secrete coelichelin into the plate. To inoculate
574 the *B. subtilis* next to *Streptomyces*, an overnight culture of *B. subtilis* RM125 WT was diluted
575 1:10 into 5 mL fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂, and poured around the I8-5 colony.
576 *B. subtilis* cells were allowed 10 mins to soak the plate, and then the bacteria dilution was removed
577 by pipet. The plate was dried under room temperature in biosafety cabinet. 2 μ L of water or
578 ferrioxamine E was spotted as a small circle on top of the *B. subtilis* lawn. After the spotted solution
579 dried, the plate was incubated at 37 °C for 1 hour. Then 5 μ L of SPO1 phage lysate (~10 PFUs)
580 was spotted on top of the compound treated area. After the phage lysate dried, the plate was
581 incubated at 37°C for two days. The average plaque area was measured for each phage spot. The
582 *B. subtilis* lawn and *Streptomyces* colony were carved out and resuspended in 5 ml LB broth and
583 5 ml ISP2 medium respectively. The cell suspensions were vortexed at the highest speed for 20
584 seconds to allow bacteria cells to fully detach from the agar. The colony forming units of *B. subtilis*
585 cell suspensions were quantified by plating serial dilutions of the cell suspension on LB + 1.5%
586 agar plates. The colony forming units of *Streptomyces* cell suspensions were quantified by plating
587 serial dilutions of the cell suspension on ISP2 + 10 ug/ml nalidixic acid + 1.5% agar plates.

588

589 **Acknowledgments**

590 We thank Andreea Măgălie (Georgia Institute of Technology) and Joshua Weitz (University of
591 Maryland) for helpful discussions. We thank the Bacillus Genomic Stock Center (Ohio State
592 University), the Félix d'Hérelle Reference Center for Bacterial Viruses (University of Laval), and
593 Robert Hertel (University of Goettingen) for providing bacteria and phages. We thank E. M. Nolan
594 (Massachusetts Institute of Technology) for providing enterobactin. The research was supported
595 by a research starter grant from the American Society of Pharmacognosy to J.P.G. and a National
596 Science Foundation CAREER award (IOS-2143636) to J.P.G. Research support was also provided
597 by the National Science Foundation (DEB-1934554 to J.T.L., D.A.S.; DBI-2022049 to J.T.L.), the
598 US Army Research Office (W911NF-22-1-0014, W911NF-22-S-0008 to J.T.L.), and the National
599 Aeronautics and Space Administration (80NSSC20K0618 to J.T.L.). Z.Z. was supported in part
600 by the John R. and Wendy L. Kindig Fellowship. K.J.P. and the Laboratory for Biological Mass
601 Spectrometry were supported by the Indiana University Precision Health Initiative. The 500 MHz
602 NMR and 600 MHz spectrometer of the Indiana University NMR facility were supported by NSF
603 grant CHE-1920026, and the Prodigy probe was purchased in part with support from the Indiana
604 Clinical and Translational Sciences Institute funded, in part, by NIH Award TL1TR002531.

605

606 **Author Contributions**

607 Conceptualization, Z.Z., J.P.G.; Methodology, Z.Z., D.S., J.P.G.; Investigation, Z.Z., C.Z., K.J.P.,
608 R.P.; Writing – Original Draft, Z.Z., J.P.G.; Writing – Review & Editing, Z.Z., C.Z., D.S., J.T.L.,
609 J.P.G.; Visualization, Z.Z., J.P.G.; Supervision, J.T.L., J.P.G.; Funding Acquisition, J.T.L., J.P.G.

610

611 **References**

612 1. Hibbing, M. E.; Fuqua, C.; Parsek, M. R.; Peterson, S. B. Bacterial Competition:
613 Surviving and Thriving in the Microbial Jungle. *Nature Reviews Microbiology* **2010**, *8* (1),
614 15–25.

615 2. Friedrich, M. W., Microbial Communities, Structure, and Function. In *Encyclopedia of*
616 *Geobiology*, Reitner, J.; Thiel, V., Eds. Springer Netherlands: Dordrecht, **2011**; p 592–595.

617 3. Ghoul, M.; Mitri, S. The Ecology and Evolution of Microbial Competition. *Trends in*
618 *Microbiology* **2016**, *24* (10), 833–845.

619 4. Westhoff, S.; Kloosterman, A. M.; Hoesel, S. F. A. v.; Wezel, G. P. v.; Rozen, D. E.
620 Competition Sensing Changes Antibiotic Production in *Streptomyces*. *mBio* **2021**, *12* (1),
621 10.1128/mBio.02729-20.

622 5. Valle, J.; Da Re, S.; Henry, N.; Fontaine, T.; Balestrino, D.; Latour-Lambert, P.; Ghigo,
623 J.-M. Broad-Spectrum Biofilm Inhibition by a Secreted Bacterial Polysaccharide. *Proceedings of*
624 *the National Academy of Sciences* **2006**, *103* (33), 12558–12563.

625 6. Kramer, J.; Özkaya, Ö.; Kümmerli, R. Bacterial Siderophores in Community and Host
626 Interactions. *Nature Reviews Microbiology* **2020**, *18* (3), 152–163.

627 7. Suttle, C. A. The Significance of Viruses to Mortality in Aquatic Microbial Communities.
628 *Microbial Ecology* **1994**, *28* (2), 237–243.

629 8. Koskella, B.; Meaden, S. Understanding Bacteriophage Specificity in Natural Microbial
630 Communities. *Viruses* **2013**, *5* (3), 806–823.

631 9. Hampton, H. G.; Watson, B. N. J.; Fineran, P. C. The Arms Race Between Bacteria and
632 Their Phage Foes. *Nature* **2020**, *577* (7790), 327–336.

633 10. Otsuji, N.; Sekiguchi, M.; Iijima, T.; Takagi, Y. Induction of Phage Formation in the
634 Lysogenic *Escherichia coli* K-12 by Mitomycin C. *Nature* **1959**, *184* (4692), 1079–1080.

635 11. Jancheva, M.; Böttcher, T. A Metabolite of *Pseudomonas* Triggers Prophage-Selective
636 Lysogenic to Lytic Conversion in *Staphylococcus aureus*. *Journal of the American Chemical
637 Society* **2021**, *143* (22), 8344–8351.

638 12. Silpe, J. E.; Wong, J. W. H.; Owen, S. V.; Baym, M.; Balskus, E. P. The Bacterial Toxin
639 Colibactin Triggers Prophage Induction. *Nature* **2022**, *603*, 315–320.

640 13. Hardy, A.; Kever, L.; Frunzke, J. Antiphage Small Molecules Produced by Bacteria –
641 Beyond Protein-Mediated Defenses. *Trends in Microbiology* **2023**, *31* (1), 92–106.

642 14. Schwartz, D. A.; Lehmkuhl, B. K.; Lennon, J. T. Phage-Encoded Sigma Factors Alter
643 Bacterial Dormancy. *mSphere* **2022**, *7* (4), e00297-22.

644 15. Castilla-Llorente, V.; Muñoz-Espín, D.; Villar, L.; Salas, M.; Meijer, W. J. J. Spo0A, the
645 Key Transcriptional Regulator for Entrance into Sporulation, is an Inhibitor of DNA Replication.
646 *The EMBO Journal* **2006**, *25* (16), 3890–3899.

647 16. Meijer, W. J.; Castilla-Llorente, V.; Villar, L.; Murray, H.; Errington, J.; Salas, M.
648 Molecular Basis for the Exploitation of Spore Formation as Survival Mechanism by Virulent
649 Phage φ29. *The EMBO Journal* **2005**, *24* (20), 3647–3657.

650 17. Lautru, S.; Deeth, R. J.; Bailey, L. M.; Challis, G. L. Discovery of a New Peptide Natural
651 Product by *Streptomyces coelicolor* Genome Mining. *Nature Chemical Biology* **2005**, *1* (5),
652 265–269.

653 18. Williams, J. C.; Sheldon, J. R.; Imlay, H. D.; Dutter, B. F.; Draelos, M. M.; Skaar, E. P.;
654 Sulikowski, G. A. Synthesis of the Siderophore Coelichelin and Its Utility as a Probe in the
655 Study of Bacterial Metal Sensing and Response. *Organic Letters* **2019**, *21* (3), 679–682.

656 19. Challis, G. L.; Ravel, J. Coelichelin, a New Peptide Siderophore Encoded by the
657 *Streptomyces coelicolor* Genome: Structure Prediction From the Sequence of its Non-Ribosomal
658 Peptide Synthetase. *FEMS Microbiology Letters* **2000**, *187* (2), 111–114.

659 20. Hider, R. C.; Kong, X. Chemistry and Biology of Siderophores. *Natural Product Reports*
660 **2010**, *27* (5), 637–657.

661 21. May, J. J.; Wendrich, T. M.; Marahiel, M. A. The dhb Operon of *Bacillus subtilis*
662 Encodes the Biosynthetic Template for the Catecholic Siderophore 2,3-Dihydroxybenzoate-
663 Glycine-Threonine Trimeric Ester Bacillibactin. *Journal of Biological Chemistry* **2001**, *276* (10),
664 7209–7217.

665 22. Schneider, R.; Hantke, K. Iron-hydroxamate Uptake Systems in *Bacillus subtilis*:
666 Identification of a Lipoprotein as Part of a Binding Protein-Dependent Transport System.
667 *Molecular Microbiology* **1993**, *8* (1), 111–121.

668 23. Abergel, R. J.; Zawadzka, A. M.; Hoette, T. M.; Raymond, K. N. Enzymatic Hydrolysis
669 of Trilactone Siderophores: Where Chiral Recognition Occurs in Enterobactin and Bacillibactin
670 Iron Transport. *Journal of the American Chemical Society* **2009**, *131* (35), 12682–12692.

671 24. Dertz, E. A.; Xu, J.; Stintzi, A.; Raymond, K. N. Bacillibactin-Mediated Iron Transport in
672 *Bacillus subtilis*. *Journal of the American Chemical Society* **2006**, *128* (1), 22–23.

673 25. Ollinger, J.; Song, K.-B.; Antelmann, H.; Hecker, M.; Helmann, J. D. Role of the Fur
674 Regulon in Iron Transport in *Bacillus subtilis*. *Journal of Bacteriology* **2006**, *188* (10),
675 3664–3673.

676 26. Gallet, R.; Kannoly, S.; Wang, I.-N. Effects of Bacteriophage Traits on Plaque
677 Formation. *BMC Microbiology* **2011**, *11* (1), 181.

678 27. Zang, Z.; Park, K. J.; Gerdt, J. P. A Metabolite Produced by Gut Microbes Represses
679 Phage Infections in *Vibrio cholerae*. *ACS Chemical Biology* **2022**, *17* (9), 2396–2403.

680 28. Woody, M. A.; Cliver, D. O. Effects of Temperature and Host Cell Growth Phase on
681 Replication of F-specific RNA Coliphage Q beta. *Applied and Environmental Microbiology*
682 **1995**, *61* (4), 1520–1526.

683 29. Rittershaus, Emily S. C.; Baek, S.-H.; Sassetti, Christopher M. The Normalcy of
684 Dormancy: Common Themes in Microbial Quiescence. *Cell Host & Microbe* **2013**, *13* (6),
685 643–651.

686 30. Sonenshein, A. L.; Roscoe, D. H. The Course of Phage Øe Infection in Sporulating Cells
687 of *Bacillus subtilis* Strain 3610. *Virology* **1969**, *39* (2), 265–276.

688 31. Grandchamp, G. M.; Caro, L.; Shank, E. A. Pirated Siderophores Promote Sporulation in
689 *Bacillus subtilis*. *Applied and Environmental Microbiology* **2017**, *83* (10), e03293-16.

690 32. Paidhungat, M.; Setlow, P. Role of Ger Proteins in Nutrient and Nonnutrient Triggering
691 of Spore Germination in *Bacillus subtilis*. *Journal of Bacteriology* **2000**, *182* (9), 2513–2519.

692 33. Molle, V.; Fujita, M.; Jensen, S. T.; Eichenberger, P.; González-Pastor, J. E.; Liu, J. S.;
693 Losick, R. The Spo0A Regulon of *Bacillus subtilis*. *Molecular Microbiology* **2003**, *50* (5),
694 1683–1701.

695 34. McKenney, P. T.; Driks, A.; Eichenberger, P. The *Bacillus subtilis* Endospore: Assembly
696 and Functions of the Multilayered Coat. *Nature Reviews Microbiology* **2013**, *11* (1), 33–44.

697 35. Hoch, J. A. Regulation of the Phosphorelay and the Initiation of Sporulation in *Bacillus*
698 *subtilis*. *Annual Review of Microbiology* **1993**, *47* (1), 441–465.

699 36. Tan, I. S.; Ramamurthi, K. S. Spore Formation in *Bacillus subtilis*. *Environmental*
700 *Microbiology Reports* **2014**, *6* (3), 212–225.

701 37. Straight, P. D.; Willey, J. M.; Kolter, R. Interactions between *Streptomyces coelicolor*
702 and *Bacillus subtilis*: Role of Surfactants in Raising Aerial Structures. *Journal of Bacteriology*
703 **2006**, 188 (13), 4918–4925.

704 38. Hemphill, H. E.; Whiteley, H. R. Bacteriophages of *Bacillus subtilis*. *Bacteriological*
705 *Reviews* **1975**, 39 (3), 257–315.

706 39. Willms, I. M.; Hoppert, M.; Hertel, R. Characterization of *Bacillus subtilis* Viruses
707 vB_BsuM-Goe2 and vB_BsuM-Goe3. *Viruses* **2017**, 9 (6), 146.

708 40. Niehus, R.; Picot, A.; Oliveira, N. M.; Mitri, S.; Foster, K. R. The Evolution of
709 Siderophore Production as a Competitive Trait. *Evolution* **2017**, 71 (6), 1443–1455.

710 41. Henriques, A. O.; Charles P. Moran, J. Structure, Assembly, and Function of the Spore
711 Surface Layers. *Annual Review of Microbiology* **2007**, 61 (1), 555–588.

712 42. Nicholson, W. L.; Munakata, N.; Horneck, G.; Melosh, H. J.; Setlow, P. Resistance of
713 *Bacillus* Endospores to Extreme Terrestrial and Extraterrestrial Environments. *Microbiology and*
714 *Molecular Biology Reviews* **2000**, 64 (3), 548–572.

715 43. Lennon, J. T.; Jones, S. E. Microbial Seed Banks: The Ecological and Evolutionary
716 Implications of Dormancy. *Nature Reviews Microbiology* **2011**, 9 (2), 119–130.

717 44. Golonka, R.; Yeoh, Beng S.; Vijay-Kumar, M. The Iron Tug-of-War between Bacterial
718 Siderophores and Innate Immunity. *Journal of Innate Immunity* **2019**, 11 (3), 249–262.

719 45. Khanna, K.; Lopez-Garrido, J.; Pogliano, K. Shaping an Endospore: Architectural
720 Transformations During *Bacillus subtilis* Sporulation. *Annual Review of Microbiology* **2020**, 74
721 (1), 361–386.

722 46. Schwartz, D. A.; Rodríguez-Ramos, J. A.; Shaffer, M.; Flynn, R. M.; Daly, R. A.;
723 Wrighton, K. C.; Lennon, J. T. Human-Gut Phages Harbor Sporulation Genes. *mBio* **2023**, 14
724 (3), e00182-23.

725 47. Chiu, C.-W.; Tsai, P.-J.; Lee, C.-C.; Ko, W.-C.; Hung, Y.-P. Inhibition of Spores to
726 Prevent the Recurrence of *Clostridioides difficile* Infection - A Possibility or an Improbability?
727 *Journal of Microbiology, Immunology and Infection* **2021**, 54 (6), 1011–1017.

728 48. Bolger, A. M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible Trimmer for Illumina
729 Sequence Data. *Bioinformatics* **2014**, 30 (15), 2114–2120.

730 49. Prjibelski, A.; Antipov, D.; Meleshko, D.; Lapidus, A.; Korobeynikov, A. Using SPAdes
731 De Novo Assembler. *Current Protocols in Bioinformatics* **2020**, 70 (1), e102.

732 50. Seemann, T. Prokka: Rapid Prokaryotic Genome Annotation. *Bioinformatics* **2014**, 30
733 (14), 2068–2069.

734 51. Blin, K.; Shaw, S.; Augustijn, H. E.; Reitz, Z. L.; Biermann, F.; Alanjary, M.; Fetter, A.;
735 Terlouw, B. R.; Metcalf, W. W.; Helfrich, E. J. N., et al. AntiSMASH 7.0: New and Improved
736 Predictions for Detection, Regulation, Chemical Structures and Visualisation. *Nucleic Acids*
737 *Research* **2023**, 51 (W1), W46–W50.

738 52. Mazzocco, A.; Waddell, T. E.; Lingohr, E.; Johnson, R. P., Enumeration of
739 Bacteriophages Using the Small Drop Plaque Assay System. In *Bacteriophages: Methods and*
740 *Protocols, Volume 1: Isolation, Characterization, and Interactions*, Clokie, M. R. J.; Kropinski,
741 A. M., Eds. Humana Press: Totowa, NJ, **2009**; p 81–85.

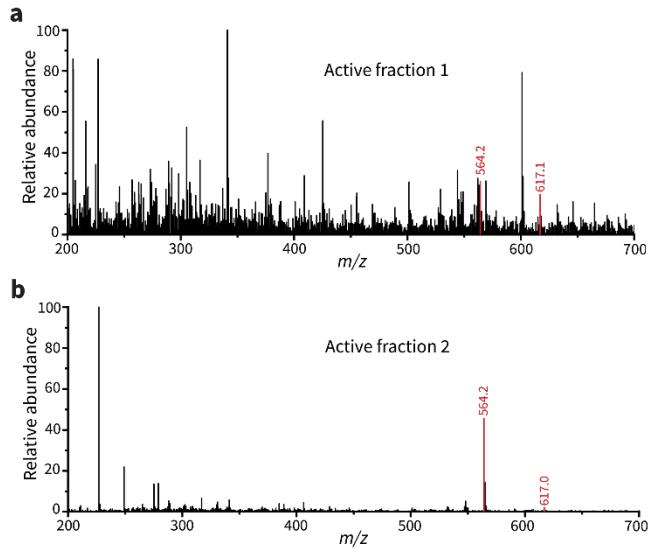
742 53. Yasbin, R. E.; Young, F. E. Transduction in *Bacillus subtilis* by Bacteriophage SPP1.
743 *Journal of Virology* **1974**, 14 (6), 1343–1348.

744 54. Konkol, M. A.; Blair, K. M.; Kearns, D. B. Plasmid-Encoded ComI Inhibits Competence
745 in the Ancestral 3610 Strain of *Bacillus subtilis*. *Journal of Bacteriology* **2013**, 195 (18),
746 4085–4093.

747 55. Koo, B.-M.; Kritikos, G.; Farelli, J. D.; Todor, H.; Tong, K.; Kimsey, H.; Wapinski, I.;
748 Galardini, M.; Cabal, A.; Peters, J. M., et al. Construction and Analysis of Two Genome-Scale
749 Deletion Libraries for *Bacillus subtilis*. *Cell Systems* **2017**, *4* (3), 291-305.e7.

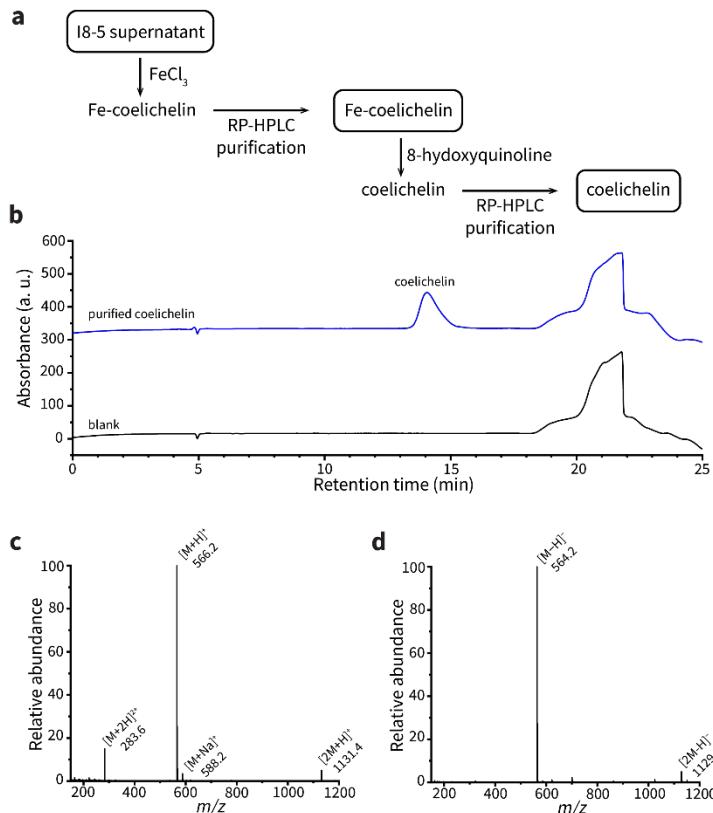
750

751



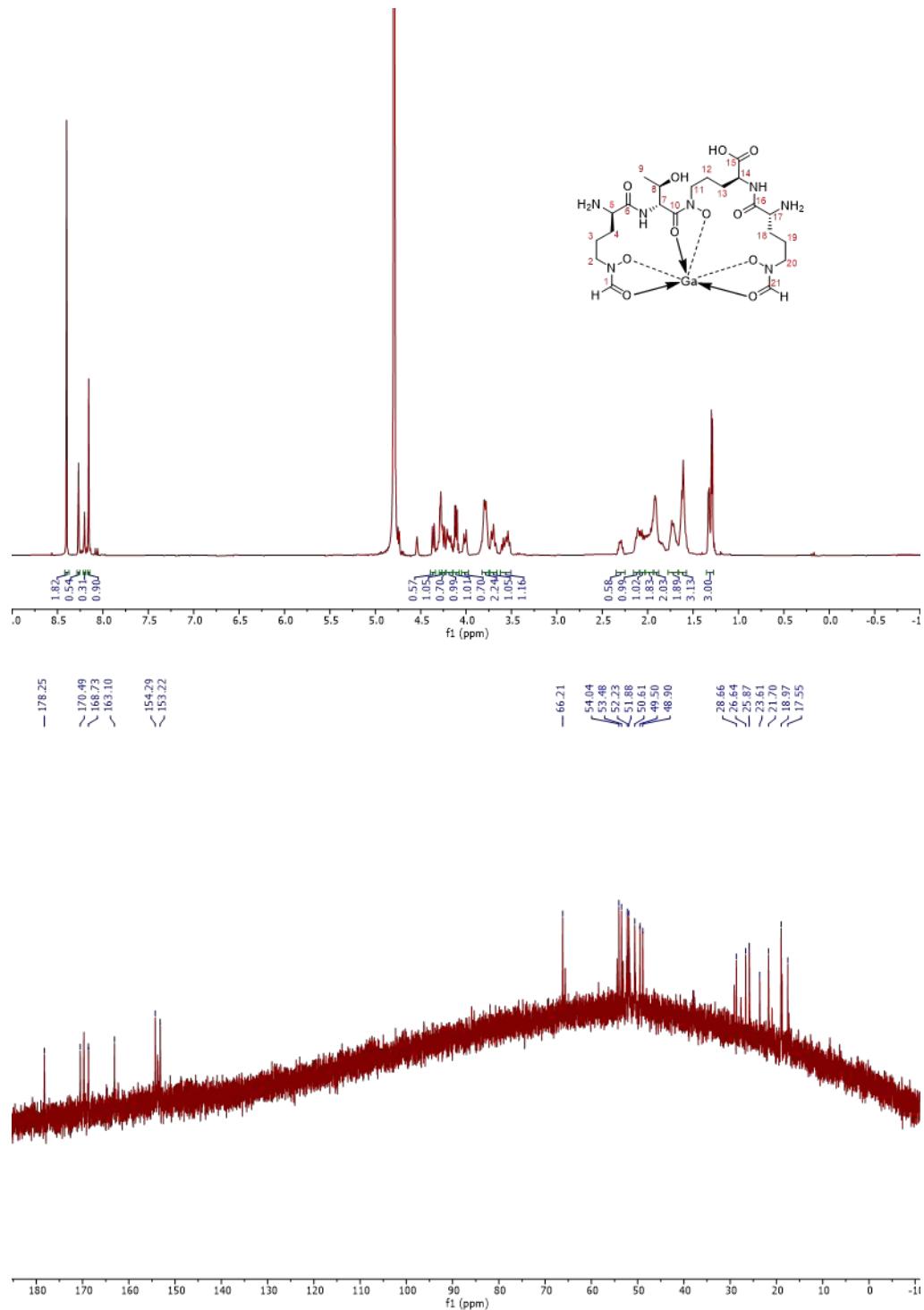
752 **Extended data Fig. 1 | The negative mode electrospray ionization MS spectra of active**
753 **fraction 1 (a) and active fraction 2 (b).** The shared peaks are highlighted red.

754



755 **Extended Data Fig. 2 | Coelichelin isolation from I8-5 supernatant. (a)** Isolation scheme.
756 **(b)** UV chromatogram at 210 nm. Water was used as the blank. **(c)** The averaged MS spectrum at
757 positive mode between retention time 13.5~14.8 min. **(d)** The averaged MS spectrum at negative
758 mode between retention time 13.5~14.8 min. M represents coelichelin.

759



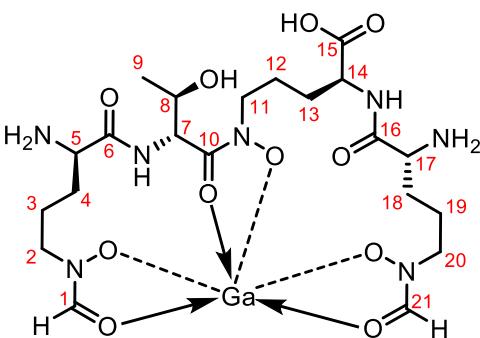
760 **Extended Data Fig. 3 | ^1H NMR spectrum (600 MHz, top) and ^{13}C NMR spectrum (125**
761 **MHz, bottom) of Ga-coelichelin in D_2O .**

762

763 **Extended Data Table 1 | Summary of ^1H (600 MHz) and ^{13}C (125 MHz) NMR spectral data**
 764 **of Ga-coelichelin in D_2O**

Position	δ_{C}	δ_{H} (mult., J in Hz)	Position	δ_{C}	δ_{H} (mult., J in Hz)
1	153.22	8.16 (1H, s)	12	21.70	1.91 (1H, m)
2	50.61	3.78 (1H, m) 3.56 (1H, m)	13	23.61	1.62 (1H, m) 1.98 (2H, m)
3	17.55	1.72 (1H, m) 1.62 (1H, m)	14	54.04	4.25 (1H, d, 12.2) -
4	25.87	2.12 (1H, m) 1.62 (1H, m)	15	178.25	-
5	51.88	4.28 (1H, m)	16	170.49	-
6	168.73	-	17	52.23	4.36 (1H, d, 11.0)
7	53.48	4.79 ^a (1H)	18	28.66	2.30 (1H, dt, 16.2, 9.0) 1.91 (1H, m)
8	66.21	4.11 (1H, dp, 9.2, 6.3)	19	26.64	2.06 (1H, m) 1.72 (1H, m)
9	18.97	1.29 (3H, d, 6.3)	20	48.90	4.01 (1H, dt, 14.8, 6.0) 3.78 (1H, m)
10	163.10	-	21	154.29	8.27 (1H, s)
11	49.50	4.19 (1H, m) 3.70 (1H, m)			

^aProton signal was masked by solvent residual



765

766