

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

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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.<sup>1</sup> Microbes frequently live in complex  
26 communities with diverse bacterial, fungal, and protozoan species.<sup>2</sup> 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.<sup>3</sup> The secretion of secondary metabolites is one of the most common  
29 methods by which bacteria and fungi compete with neighboring microbes.<sup>1, 3</sup> For example,  
30 antibiotics can kill or arrest the growth of competitors.<sup>4</sup> Anti-adhesion molecules like  
31 biosurfactants can impede invasion and colonization from potential competitors.<sup>5</sup> Siderophores  
32 that harvest the limited iron in the environment can also indirectly starve competitors and prevent  
33 their rapid growth.<sup>6</sup>

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.<sup>7</sup> 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.<sup>8</sup> Bacteria have evolved myriad defenses that confer immunity to virus  
42 infection.<sup>9</sup> 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.<sup>10-12</sup>  
46 Otherwise, most cases report that secondary metabolites protect bacteria from phage infection (e.g.,  
47 anthracyclines and aminoglycosides have exhibited antiviral effects).<sup>13</sup> 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.<sup>14-16</sup> 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***

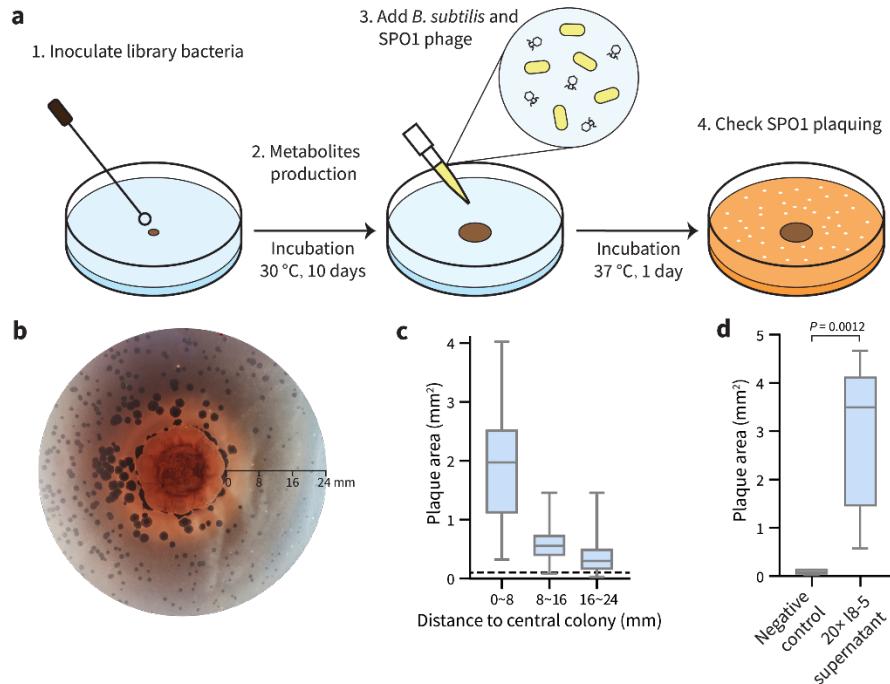
60 We performed a binary-interaction screen to discover bacteria that promote phage infections  
61 in *B. subtilis*. Colonies of soil-isolated Actinobacteria were pre-grown on an agar plate to secrete  
62 metabolites into their medium (Fig. 1a). Subsequently, *B. subtilis* and phage SPO1 were plated  
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.

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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.

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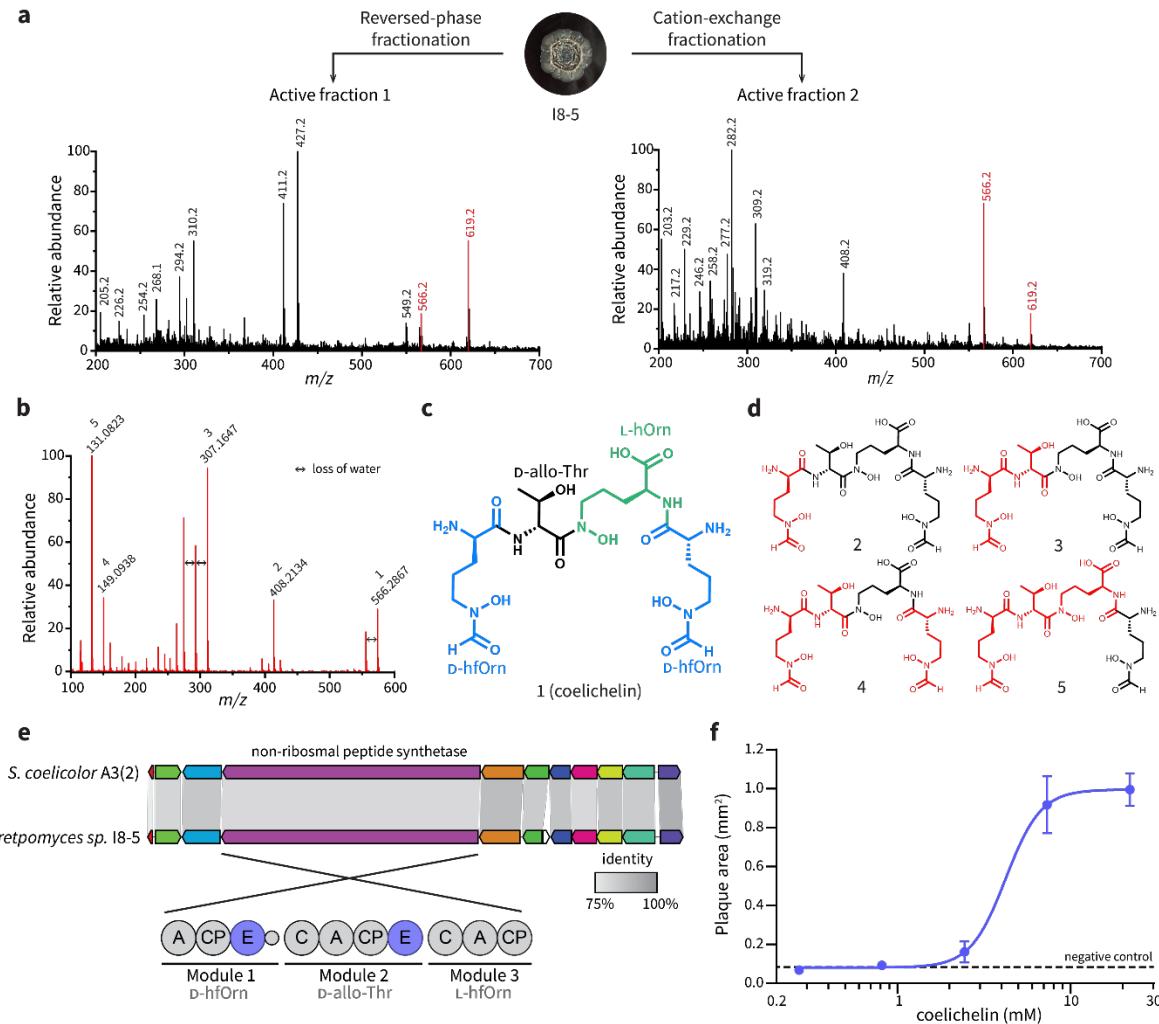
## 92 Plaque-promoting metabolite is the siderophore coelichelin

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).<sup>17</sup> 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,<sup>17, 18</sup> 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).<sup>17</sup>  
111 Consistent with the reported coelichelin non-ribosomal peptide synthetase (NRPS),<sup>17</sup>  
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).<sup>17</sup>

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.<sup>17</sup> (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- $\delta$ -N-formyl- $\delta$ -N-hydroxyornithine (D-hfOrn), D-allo-threonine (D-

136 allo-Thr), and L- $\delta$ -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 ( $EC_{50}=4.2$   
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.

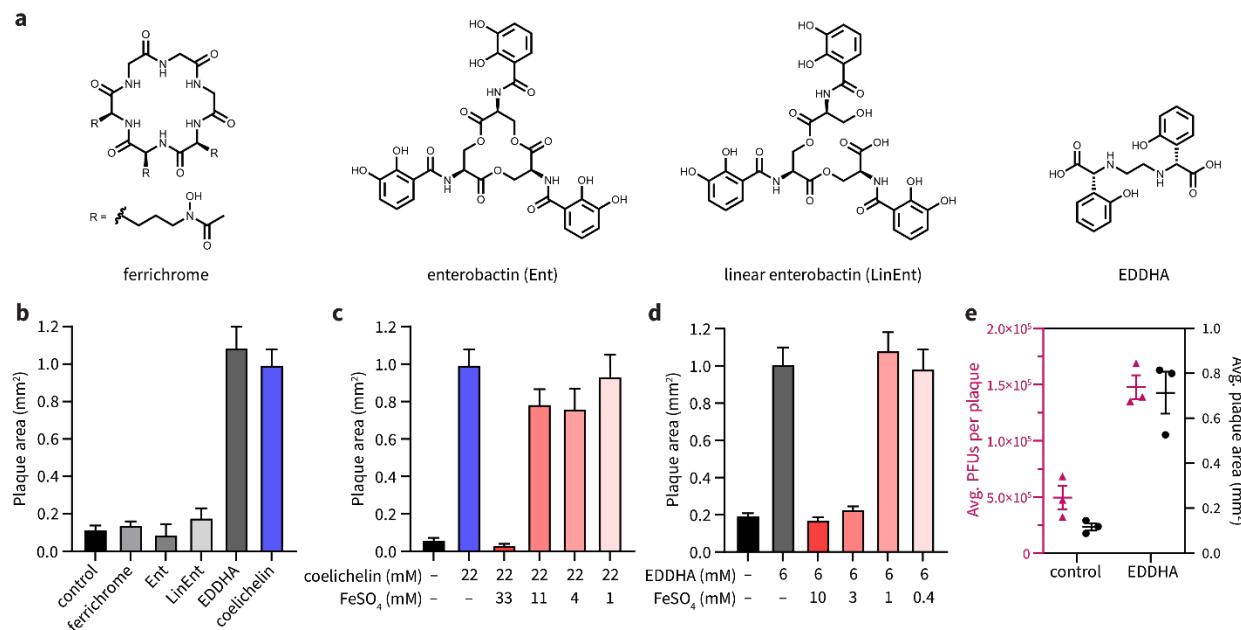
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## 142 **Coelichelin promotes phage proliferation by iron sequestration**

143 Coelichelin is known to be a hydroxamate-type siderophore.<sup>17-20</sup> 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).<sup>21</sup> This list of “pirated” siderophores notably includes all three that failed  
149 to enlarge plaques: ferrichrome,<sup>22</sup> enterobactin,<sup>23, 24</sup> and linear enterobactin.<sup>22</sup> 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*.<sup>25</sup> 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 FeSO<sub>4</sub> 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*

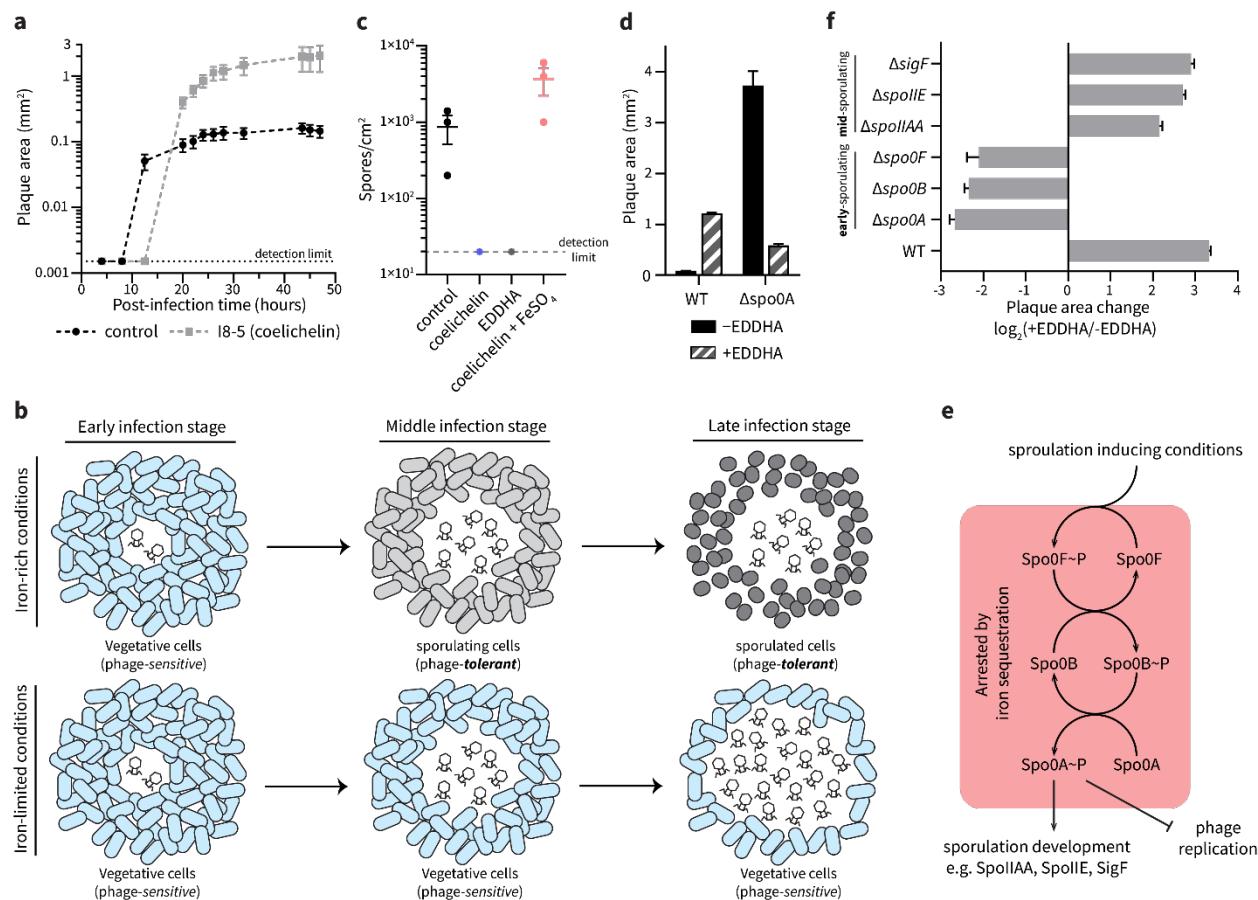
181 We next investigated the mechanism by which iron starvation promoted phage infection of *B.*  
182 *subtilis*. Plaque size can be increased by many factors that either accelerate the rate of phage

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.<sup>26</sup> 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.<sup>27</sup> 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.<sup>28</sup> Under sub-optimal conditions, many bacteria enter dormant  
200 stages with heavily reduced metabolic activity,<sup>29</sup> 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,<sup>30</sup> which has been shown to arrest phage infection by masking surface  
203 receptors<sup>14</sup> and inhibiting DNA replication and transcription.<sup>15, 16</sup> Notably, sporulation in *B.*  
204 *subtilis* relies on sufficient levels of intracellular iron.<sup>31</sup> 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,<sup>32</sup> 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*,  $\Delta$ *spo0A* mutants are incapable of sporulating  
 215 and are “locked” into their vegetative growth state.<sup>33</sup> 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  $\Delta$ *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.<sup>27</sup> 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) + FeSO<sub>4</sub> (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.<sup>34</sup> 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).<sup>35</sup> 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*.<sup>36</sup> Unlike the early sporulation genes, these mutants plaqued

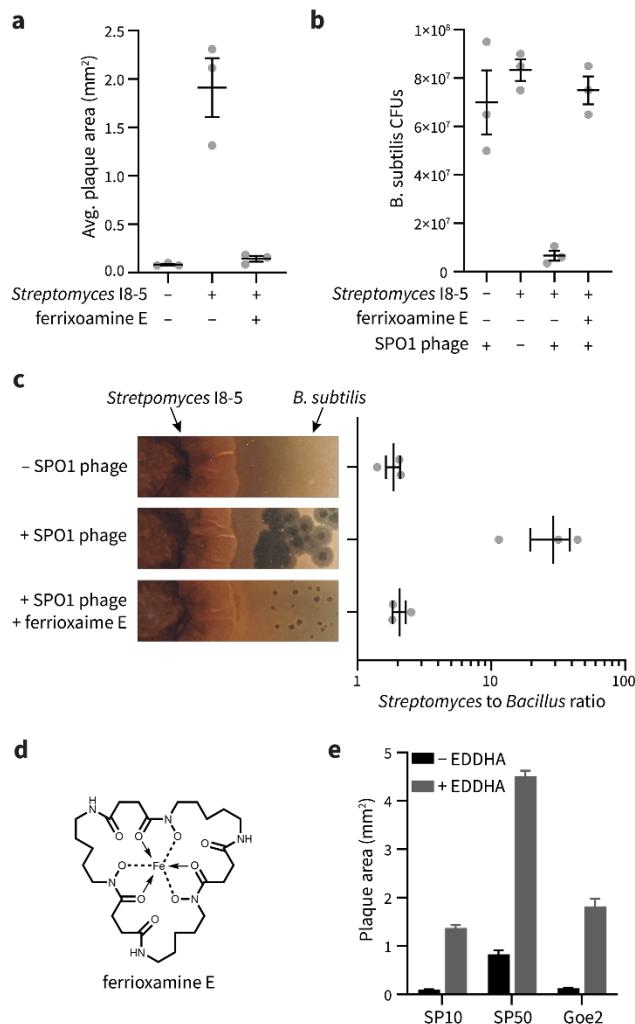
257 like wild-type: they formed small plaques under resource-replete conditions but large plaques  
258 under iron starvation (Fig. 4f). Therefore, we propose that phage proliferation is arrested by the  
259 earliest stages of sporulation and does not require the intermediate and final stages of spore  
260 formation. Notably, prior work has shown that activated Spo0A can directly inhibit replication of  
261 and transcription from phage phi29 DNA.<sup>15, 16</sup> 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.<sup>37</sup>  
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),<sup>38, 39</sup> 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.<sup>6, 20</sup> 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.<sup>6, 40</sup> 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.<sup>41-43</sup> 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 fungi<sup>20</sup> 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.<sup>44</sup> 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.<sup>45</sup>  
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.<sup>46</sup> 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*.<sup>34</sup> Furthermore, the bacterial macrocycle, fidaxomicin,  
340 inhibits *Clostridioides difficile* sporulation.<sup>47</sup> 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 MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub> + 5 mM CaCl<sub>2</sub>. The culture was incubated at 37 °C  
365 and 220 rpm for 4 hours until the OD<sub>600nm</sub> reached 0.2. About 1×10<sup>3</sup> 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 Trimmomatic<sup>48</sup>  
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 SPAdes<sup>49</sup>  
397 version 3.15.4 with default parameters. The assembly was annotated using prokka<sup>50</sup> 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.<sup>51</sup> 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  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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<sup>-1</sup> 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  $\mu$ 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  $\mu$ m Hydro-RP 80  $\text{\AA}$  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  $\mu$ 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  $\mu$ m Hydro-RP 80  $\text{\AA}$  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  $\mu$ 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  $\mu$ m Hydro-RP 80  $\text{\AA}$  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  $\mu$ m Hydro-RP 80  $\text{\AA}$  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.<sup>17</sup> The lyophilized I8-5 supernatant (1.8533 g) was  
469 dissolved in 5 mL of water.  $\text{FeCl}_3$  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  $\mu$ 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 NH<sub>4</sub>HCO<sub>3</sub> (pH=8.01) and the mobile phase B was methanol.  
474 The flow rate was kept at 10 mL·min<sup>-1</sup> 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<sup>-1</sup> 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]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>40</sub>N<sub>7</sub>O<sub>11</sub><sup>+</sup>: 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<sup>-1</sup> 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 Ga<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> 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<sup>-1</sup>  
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]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>37</sub>GaN<sub>7</sub>O<sub>11</sub><sup>+</sup>: 632.1801). <sup>1</sup>H and TOCSY (mixing time of 60  
514 ms) NMR spectra were obtained on a Varian 600 MHz Inova NMR spectrometer. <sup>13</sup>C, 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 MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub>, and poured onto an ISP2 + 0.1 mM MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub> + 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  $\mu$ L of compound  
522 was spotted as a small circle on top of the bacterial lawn. After the compound dried, 2  $\mu$ L of FeSO<sub>4</sub>  
523 aqueous solution was spotted on top of the compound-treated area. After the FeSO<sub>4</sub> solution dried,  
524 the plate was incubated at 37 °C for 1 hour. Then 5  $\mu$ 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 MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub>, and poured onto an ISP2 + 0.1 mM MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub> + 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  $\mu$ 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  $\mu$ 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 MgSO<sub>4</sub>, 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.<sup>52</sup> 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 MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub>, and poured onto an ISP2 + 0.1 mM MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub> + 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  $\mu$ 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 ~1cm<sup>2</sup> area of bacteria was scraped off the plate and

550 resuspended in 200  $\mu$ 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.<sup>53, 54</sup> 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 CaCl<sub>2</sub>. The recipient culture was  
559 incubated at 37 °C and 220 rpm for 4 hours. For phage transduction, 950  $\mu$ L of the recipient culture  
560 was mixed with 50  $\mu$ 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.<sup>55</sup> 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  $\mu$ L of the frozen spore stock of I8-5 was suspended in 50  
571  $\mu$ L of ISP2 + 0.1 mM MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub>. Then 8  $\mu$ L of the spore suspension was spotted at  
572 the center of an ISP2 + 0.1 mM MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub> + 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 MnCl<sub>2</sub> + 5 mM MgCl<sub>2</sub>, 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  $\mu$ 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  $\mu$ 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

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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.

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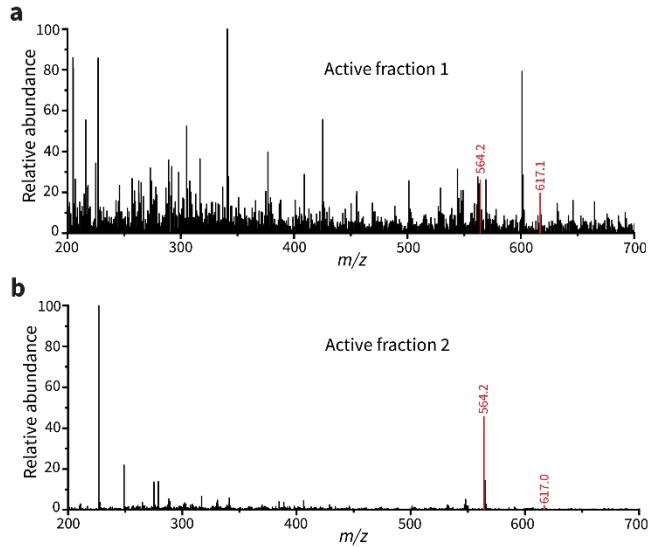
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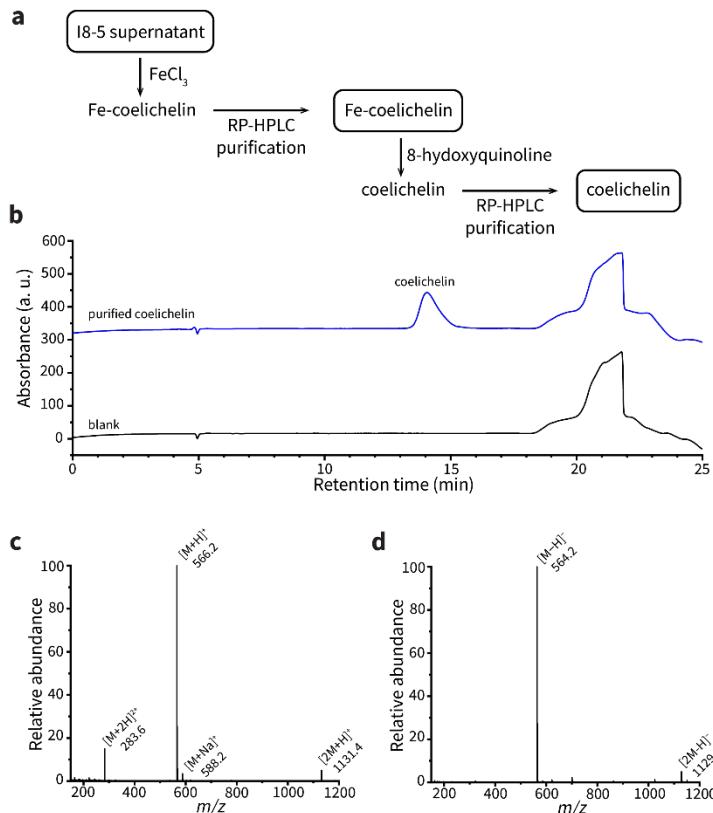
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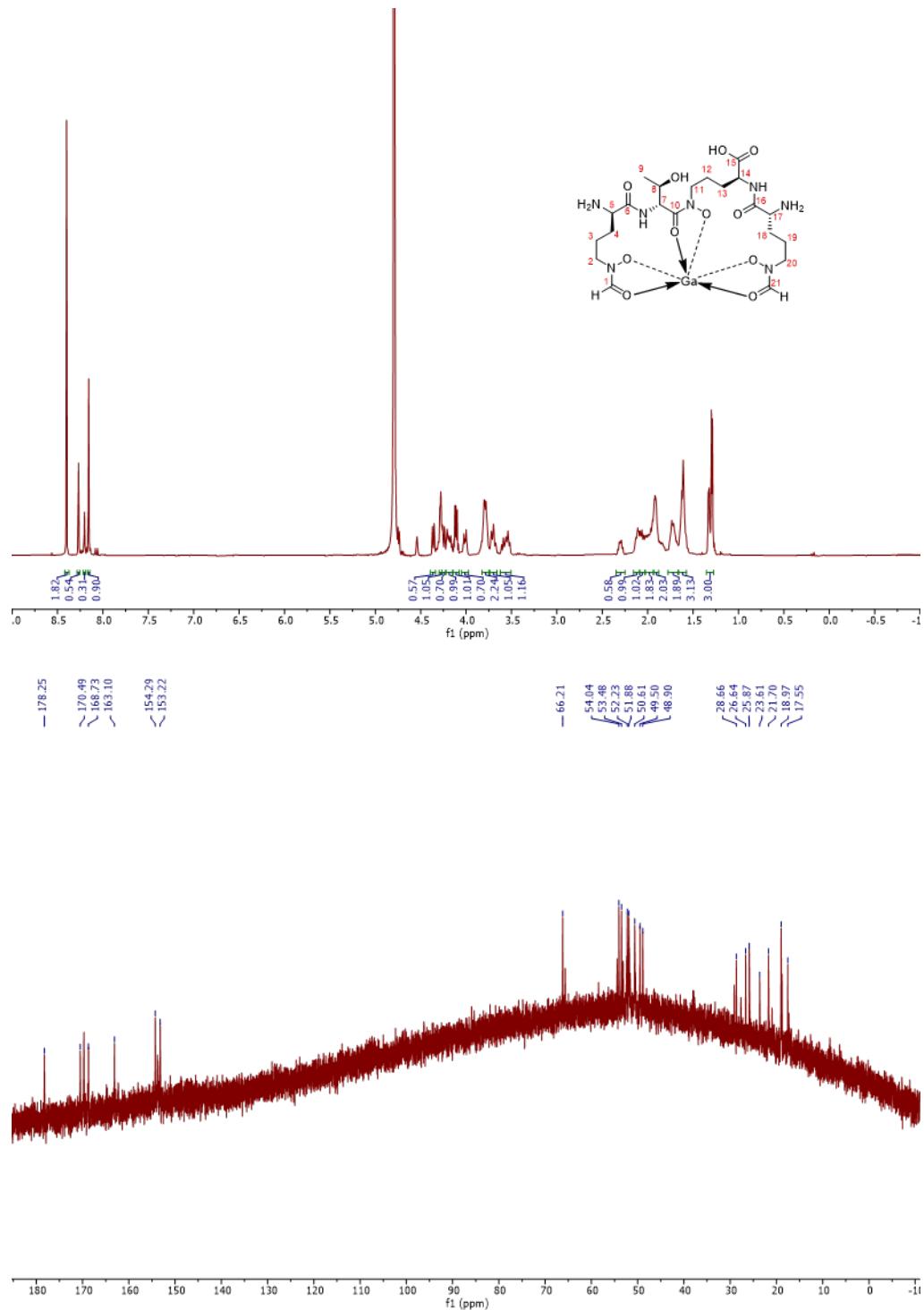
752      **Extended data Fig. 1 | The negative mode electrospray ionization MS spectra of active**  
753      **fraction 1 (a) and active fraction 2 (b).** The shared peaks are highlighted red.

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



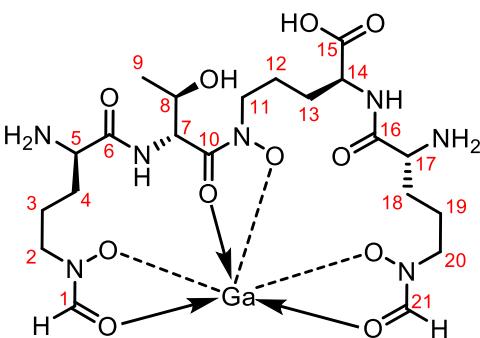
760      **Extended Data Fig. 3 |  $^1\text{H}$  NMR spectrum (600 MHz, top) and  $^{13}\text{C}$  NMR spectrum (125**  
761      **MHz, bottom) of Ga-coelichelin in  $\text{D}_2\text{O}$ .**

762

763 **Extended Data Table 1 | Summary of  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (125 MHz) NMR spectral data**  
 764 **of Ga-coelichelin in  $\text{D}_2\text{O}$**

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., J in Hz)	Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (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 <sup>a</sup> (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)			

<sup>a</sup>Proton signal was masked by solvent residual



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766