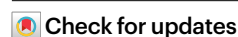


Streptomyces secretes a siderophore that sensitizes competitor bacteria to phage infection

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To overtake competitors, microbes produce and secrete secondary metabolites that kill neighbouring cells and sequester nutrients. This metabolite-mediated competition probably evolved in complex microbial communities in the presence of viral pathogens. We therefore hypothesized that microbes secrete natural products that make competitors sensitive to phage infection. We used a binary-interaction screen and chemical characterization to identify a secondary metabolite (coelichelin) produced by *Streptomyces* sp. that sensitizes its soil competitor *Bacillus subtilis* to phage infection in vitro. The siderophore coelichelin sensitized *B. subtilis* to a panel of lytic phages (SPO1, SP10, SP50, Goe2) via iron sequestration, which prevented the activation of *B. subtilis* Spo0A, the master regulator of the stationary phase and sporulation. Metabolomics analysis revealed that other bacterial natural products may also provide phage-mediated competitive advantages to their producers. Overall, this work reveals that synergy between natural products and phages can shape the outcomes of competition between microbes.

Competition is a common theme in microbial life¹. Given the finite resources and space in their niches, microbes have evolved an arsenal of strategies that allow them to persist in the face of competition with other microorganisms². The secretion of secondary metabolites is one of the most common methods by which bacteria and fungi compete with neighbouring microbes^{1,2}. For example, secreted metabolites frequently kill competitors³, prevent their attachment to surfaces⁴ and starve them of essential nutrients⁵.

To find new mechanisms of natural product-based microbial competition, we considered environmental factors that microbes might leverage for competitive advantage. Since microbes naturally compete within populations that interact with other predators and pathogens, we investigated whether microbes secrete metabolites that sensitize their competitors to the ubiquitous predators or pathogens around them. The major pathogens of bacteria are bacteriophage viruses (phages)⁶. These obligate parasites are strong agents of selection that can induce high rates of mortality, affecting the competition between microbes

and the flux of resources in their environment⁷. Bacteria have evolved myriad resistance mechanisms against phage infection⁸. Because bacteria have evolved to compete with each other within ecosystems that include phages, we hypothesize that microbes may deprive their competitors of phage resistance and sensitize competitors to phages to gain a relative fitness advantage. A similar form of ‘weaponizing’ phages has been reported in which the secretion of secondary metabolites by one bacterial species induces lysogenic phages to become lytic and kill the host of another species^{9–11}. Otherwise, most cases report that secondary metabolites protect bacteria from phages¹². To identify instances where a microbe sensitizes competing bacteria to phages, we screened soil bacteria for isolates that improved the infectivity of bacteriophages on the model soil bacterium *Bacillus subtilis*.

We discovered that a *Streptomyces* sp. outcompetes *B. subtilis* in laboratory culture by secreting a metabolite that sensitizes *B. subtilis* to phage infection. The metabolite is a common siderophore named coelichelin, which elicited its effect via iron sequestration. We further

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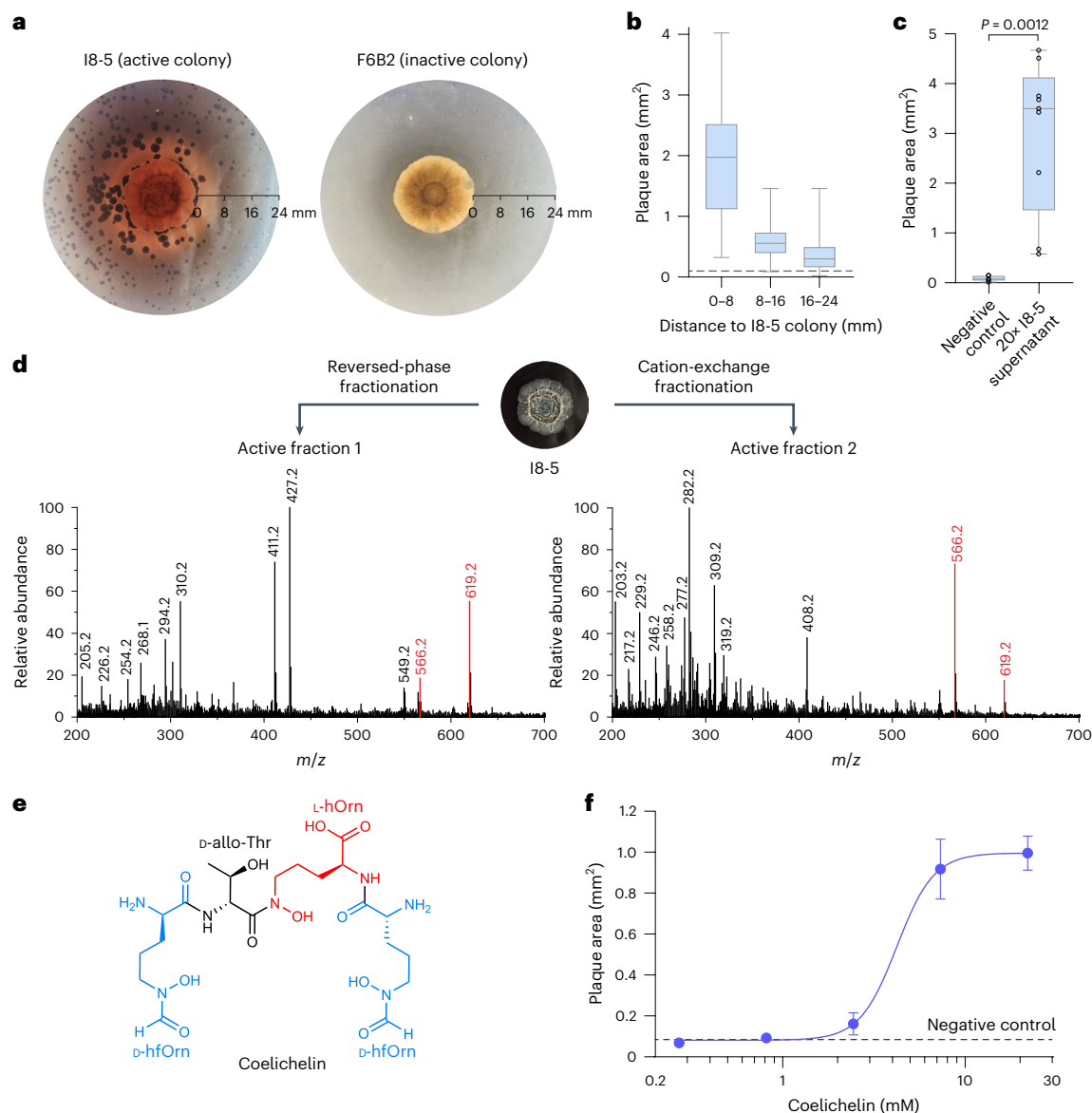


Fig. 1 | *Streptomyces* sp. produces metabolite that promotes SPO1 phage predation of *B. subtilis*. **a**, A mature colony of *Streptomyces* sp. 18-5 (centre of the plate) promoted SPO1 phage proliferation nearby (dark circles are plaques), especially within a radius of 8 mm (left). However, plaques remained small near a colony of an inactive *Streptomyces* sp. F6B2 (right). **b**, Quantification of plaque areas with increasing distance from the *Streptomyces* sp. 18-5 colony. Data are presented as boxplots, showing the median, interquartile ranges, minimum (bottom bar) and maximum (top bar). The dashed line represents the average plaque area of SPO1 phages in the absence of the *Streptomyces* colony. At least 72 plaques were measured for each condition. **c**, The 18-5 supernatant was concentrated 20 times and tested (2 μ l) for the ability to enlarge SPO1 plaques. Water was used as a

negative control. Data are presented as boxplots, showing the median, interquartile ranges, minimum (bottom bar) and maximum (top bar). Symbols represent individual plaque areas. At least five plaques were measured for each distance range. **d**, Bioactivity-guided fractionation and MS analysis identified two putative metabolites present in both active fractions purified from orthogonal separation techniques. Positive-mode electrospray ionization results are shown here, and matching negative-mode peaks (m/z 564.2 and 617.2) are shown in Extended Data Fig. 1b. **e**, Chemical structure of coelichelin, highlighting each amino acid residue. **f**, Purified coelichelin enlarged phage plaques in a dose-dependent manner (EC_{50} = 4.2 mM, 2 μ l). Water was used as a negative control. Data are presented as the average \pm s.e.m. of at least seven individual plaques for each condition.

found that the improved phage infection was due to the ability of coelichelin to delay the stationary phase transition of *B. subtilis*. This delay broadly sensitized *B. subtilis* to many lytic phages. Moreover, our data suggest that other bacterial metabolites beyond siderophores promote phage infectivity independently of iron. Therefore, microbes may leverage many mechanisms to sensitize neighbouring bacteria to phages, affording a phage-dependent competitive advantage.

Results

Streptomyces metabolites promote *B. subtilis* phage infection

We performed a binary-interaction screen to identify bacteria that promote phage infections in *B. subtilis*. Colonies of soil-isolated Actinomycetia

were pre-grown on an agar plate to secrete metabolites into the medium (Extended Data Fig. 1a). Subsequently, *B. subtilis* and phage SPO1 were plated around the mature Actinomycetia colonies. Without an adjacent Actinomycetia colony, the SPO1 plaque sizes were small (0.11 ± 0.05 mm²). In a screen of 97 soil-derived bacteria, we found several that caused SPO1 to form larger plaques on *B. subtilis* (Supplementary Figs. 1–5). One of the clearest plaque-enlarging isolates was *Streptomyces* sp. 18-5 (Fig. 1a,b). Notably, the plaque sizes were most enlarged (1.96 ± 0.21 mm²) near the *Streptomyces* colony, and distant plaques were essentially the normal small size (Fig. 1b). This distance dependence suggested the production of a diffusible substance that promoted plaque expansion, or possibly the depletion of a diffusible plaque inhibitory substance from the media.

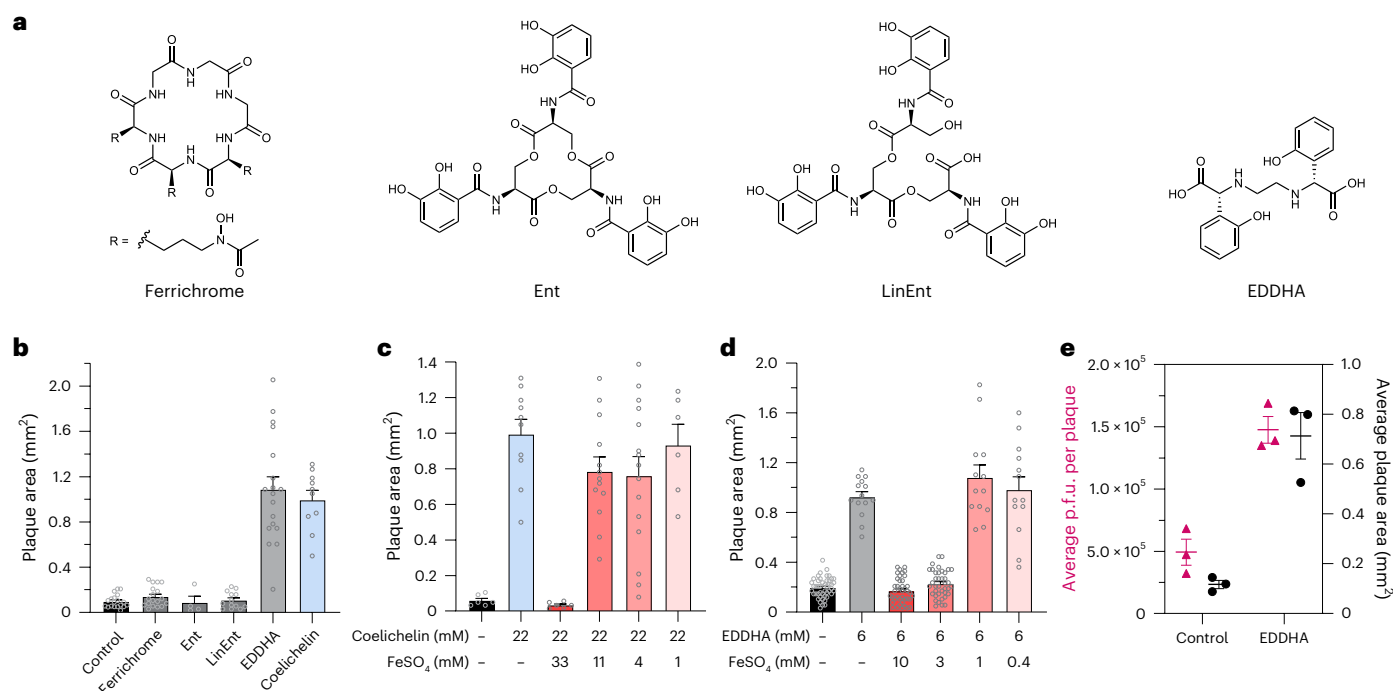


Fig. 2 | Coelichelin promotes phage predation by sequestering iron.

a, Chemical structures of ferrichrome, enterobactin (Ent), linear enterobactin (LinEnt) and ethylenediamine-*N,N'*-bis(2-hydroxyphenyl)-acetic acid (EDDHA). **b**, Ferrichrome (20 mM), Ent (10 mM), LinEnt (20 mM) and EDDHA (6 mM) were tested (2 μ l) for the ability to increase SPO1 plaque areas. Water was used as a negative control. Data are presented as the average \pm s.e.m. of at least four individual plaques for each condition. Symbols represent individual plaque areas. **c,d**, Iron complementation antagonized the plaquing promotion effect

of coelichelin (**c**) and EDDHA (**d**). Water was used as a negative control. Data are presented as the average \pm s.e.m. of at least 6 (**c**) or 14 (**d**) individual plaques for each condition. Symbols represent individual plaque areas. **e**, The average p.f.u. per plaque (magenta triangles, left axis) and plaque area (black circles, right axis) were measured with EDDHA (6 mM, 2 μ l) or water (control) treatment. Data are presented as the average \pm s.e.m. of three independent biological replicates. Symbols show the values of each biological replicate. At least 13 plaques were selected for each replicate.

To investigate whether the promoted plaquing was due to metabolites secreted from the I8-5 colony, we tested the activity of sterile-filtered conditioned medium of I8-5 culture on SPO1 plaquing. Adding conditioned medium increased the plaque sizes ~30-fold (Fig. 1c), suggesting that secreted component(s) from I8-5 promote SPO1 infection on *B. subtilis*.

Phage-promoting metabolite is the siderophore coelichelin

To identify the phage-promoting metabolite(s) made by *Streptomyces* sp. I8-5, the conditioned medium of I8-5 culture was subjected to bioactivity-guided fractionation. Two active semi-pure fractions were obtained from different purification strategies: one from reversed-phase chromatography and the other from cation-exchange chromatography (Fig. 1d). Since these are relatively orthogonal separation methods, we suspected that few metabolites would be shared between the active fractions. Indeed, by liquid chromatography-mass spectrometry (LC-MS), we found that only two putative metabolites were shared by the two fractions (Fig. 1d). High-resolution mass spectrometry of these two metabolites revealed one with m/z 566.2783 and one with m/z 619.1885 (both were probably $[M+H]^+$ adducts due to matching $[M-H]^-$ adducts observed by negative-mode analysis; Extended Data Fig. 1b). The accurate mass and the tandem mass spectrometry (MS/MS) fragmentation pattern of the m/z 566.2783 species matched the known metabolite coelichelin¹³ (Fig. 1e and Extended Data Fig. 1c). Because coelichelin is a siderophore with high affinity to iron^{13,14}, the m/z 619.1885 species was attributed to the Fe-coelichelin complex $[M-2H+Fe]^+$ with a matching MS/MS fragmentation pattern (Extended Data Fig. 1d). To confirm the ability of *Streptomyces* sp. I8-5 to produce coelichelin, we sequenced its genome and identified the coelichelin biosynthetic gene cluster (BGC) (Extended Data Fig. 1e). The coelichelin BGC in I8-5 has the same organization as the reported

one with high sequence identity¹³ (>75% for each gene; Extended Data Fig. 1e). Consistent with the reported coelichelin non-ribosomal peptide synthetase (NRPS)¹³, epimerization domains were identified in the first and second module of the I8-5 coelichelin NRPS (Extended Data Fig. 1e). These domains suggest that the absolute stereochemistry of our analyte is the same as reported¹³ (Fig. 1e).

To determine whether coelichelin was the active component secreted by *Streptomyces* sp. I8-5, we purified coelichelin from the *Streptomyces*-conditioned medium (Extended Data Fig. 2). Nuclear magnetic resonance (NMR) analyses confirmed the identity and purity of our isolated coelichelin (Supplementary Figs. 6–10 and Supplementary Table 1). As hypothesized, the purified coelichelin enlarged plaques in a dose-dependent manner, confirming that it is a phage-promoting metabolite secreted by *Streptomyces* sp. I8-5 (Fig. 1f).

Coelichelin promotes phage proliferation by iron sequestration

Since coelichelin is a hydroxamate-type siderophore^{13–16}, we hypothesized that other siderophores would also enlarge plaques of SPO1. We tested three other common siderophores: ferrichrome, enterobactin and linear enterobactin (Fig. 2a). Surprisingly, none of these siderophores increased plaque size (Fig. 2b). Previous work has demonstrated that *B. subtilis* can import a range of xenosiderophores produced by other organisms (in addition to using its own siderophore bacillibactin)¹⁷. This list of ‘pirated’ siderophores notably includes all three that failed to enlarge plaques^{18–20}. Therefore, we hypothesized that only siderophores that cannot be imported and utilized by *B. subtilis* enlarge plaques. The non-usable siderophores would sequester iron away from *B. subtilis* and, via an unknown mechanism, improve phage replication on the iron-starved host.

It was previously unknown whether coelichelin could sequester iron away from *B. subtilis* (or whether *B. subtilis* could instead use it as a xenosiderophore). Therefore, to test the iron sequestration hypothesis, we utilized a synthetic iron chelator, EDDHA (Fig. 2a), which is known to sequester iron away from *B. subtilis*²¹. In line with our hypothesis, EDDHA increased SPO1 plaque sizes to a similar level as coelichelin (Fig. 2b). Furthermore, if iron starvation was responsible for the improved plaquing, the plaque sizes should decrease to their normal size when excess iron is co-administered with the siderophore. As expected, approximately equimolar concentrations of FeSO₄ quenched the plaque-enlarging effect of both coelichelin and EDDHA (Fig. 2c,d). We hypothesized that the enlarged plaques were the result of increased phage proliferation within each plaque. To test this hypothesis, we quantified the viable phages (plaque-forming units (p.f.u.)) generated per plaque. Indeed, the larger plaques afforded by iron limitation produced more phages (Fig. 2e). Thus, we concluded that *B. subtilis* does not use coelichelin as a xenosiderophore. Furthermore, the iron starvation caused by this *Streptomyces* siderophore promotes the predation of *B. subtilis* by SPO1 phages.

Phage infection is promoted by inhibition of SpoOA activation

We next investigated the mechanism by which iron starvation promoted phage infection in *B. subtilis*. Plaque size can be increased by many factors that either accelerate the rate of phage replication or extend the period in which phages can replicate before the bacteria becomes recalcitrant. For example, the rate of plaque expansion depends largely on the burst size (that is, the number of new phages released from each infected cell) and latent period (that is, the time required for phages to lyse the host cell and produce new progeny) of phage replication. Specifically, large burst sizes and shorter latent periods maximize the phage reproduction rate, thus resulting in larger plaques²². We considered whether iron starvation increased burst size and/or shortened the latent period of phage replication. It has been reported that iron starvation actually has the opposite effect in *Vibrio cholerae*: it reduces burst size and delays phage-mediated cell lysis²³. In line with the *V. cholerae* study, we observed that when *B. subtilis* grew next to *Streptomyces* sp. I8-5, the plaque development of SPO1 was slower than plaque development alone, suggesting that iron sequestration does not accelerate phage replication (Fig. 3a). However, we observed that the plaque development process lasted longer in the presence of the *Streptomyces* colony, leading to larger plaques (Fig. 3a). This longer plaque development (in combination with higher p.f.u. production in larger plaques; Fig. 2e) indicated that phages underwent more reproduction cycles, lysing more *B. subtilis* cells and ultimately forming 10× larger plaque areas.

The extended period of phage infection led us to consider an alternative mechanism: iron starvation may interfere with host dormancy. Reproduction and plaque development by many phages is optimal on metabolically active host cells^{24–27}. Under suboptimal conditions, many bacteria enter dormant stages with heavily reduced metabolic activity²⁸, which is typically unfavourable for phage proliferation. Therefore, dormancy can be considered a mechanism of phage resistance. In *B. subtilis*, nutrient starvation and other stresses lead to the activation of the transcriptional master regulator SpoOA, which triggers a transition from vegetative exponential growth to stationary phase and eventually, sporulation²⁹. To test the hypothesis that SpoOA activation could repress phage infection, we examined plaque formation on *B. subtilis* strains encoding IPTG-inducible sad67-D56N, which is a mutant form of SpoOA 'locked' in its active state³⁰. When the active SpoOA (sad67-D56N) was expressed under IPTG induction, the plaque formation of SPO1 was restricted (Fig. 3b). Therefore, transition into stationary phase and/or sporulation prevents plaque enlargement in *B. subtilis* (Fig. 3c).

Since two SpoOA-activated stationary phase phenotypes (biofilm formation and sporulation) in *B. subtilis* rely on sufficient levels

of intracellular iron^{31,32}, we asked whether iron sequestration could prevent SpoOA-induced transition into stationary phase and maintain *B. subtilis* in the phage-susceptible vegetative growth state (Fig. 3c). To test this hypothesis, we first determined whether iron sequestration prevented the activation of SpoOA under our experimental conditions by quantifying the formation of *B. subtilis* spores. We found that both coelichelin and EDDHA inhibited sporulation (SpoOA activation) in *B. subtilis* (Fig. 3d). This inhibitory effect was due to iron sequestration, as demonstrated by the ability of iron supplementation to recover native levels of sporulation (Fig. 3d).

To further determine whether the cause of increased phage proliferation was inhibition of SpoOA activation, we employed a knockout mutant of *B. subtilis* *spoOA*, which is incapable of entering stationary phase and thus 'locked' in its vegetative growth state^{33,34}. We predicted that this mutant would naturally form large plaques that are not further enlarged by iron limitation. Indeed, the SPO1 phage formed extremely large plaques on the $\Delta spoOA$ mutant (Fig. 3e). As expected, the mutant plaques were not enlarged by EDDHA-induced iron sequestration. In fact, the plaques were substantially smaller under EDDHA treatment, possibly due to impeded phage proliferation under iron limitation, similar to what we have observed with *V. cholerae*²³. A nearly identical phenotype was observed for the mutants $\Delta spoOF$ and $\Delta spoOB$, which are part of the phosphorelay that activates SpoOA³⁵ (Fig. 3e,f), providing more evidence that iron sequestration enlarges plaques by inhibiting the activation of SpoOA (Fig. 3f). Therefore, our results demonstrate that iron sequestration extends phage infection on *B. subtilis* by inhibiting the activation of SpoOA, the master regulator of the stationary phase and sporulation.

Plaque enlargement is due to multiple SpoOA-regulated pathways

Sporulation has been shown to restrict plaque development³⁶ by masking surface receptors³⁷. Therefore, we hypothesized that our observed plaque enlargement (by inhibiting SpoOA activation) was due to inhibited sporulation. To test this hypothesis, we examined three sporulation genes regulated by SpoOA (Extended Data Fig. 3a): *spolI*, *sigF* and *spolE* (the earliest known sporulation-specific regulator³⁸). If inhibiting sporulation was the mechanism of plaque enlargement, we would expect these mutants to phenocopy the $\Delta spoOA$ mutant (large plaques that are not enlarged by iron limitation). However, the plaques in these mutants were as small as those in the wild type (WT) and were significantly enlarged by iron sequestration (Extended Data Fig. 3b). Therefore, sporulation is 'not' essential to the observed SpoOA-induced plaque restriction under our experimental conditions.

Before *B. subtilis* commits to sporulation, SpoOA activates many other pathways in *B. subtilis* during the transition from exponential growth to the stationary phase²⁹ (Fig. 3f). These pathways include decreasing motility, increasing biofilm formation, increasing competence, increasing cannibalism, decreasing translation and amino acid metabolism, and decreasing nucleotide metabolism^{29,34}. All of these responses (perhaps except for increased competence) could intuitively inhibit phage reproduction and decrease plaque sizes^{24–27,39}. Most of these pathways were regulated by two parallel pathways of repression mediated by SinI/SinR and AbrB⁴⁰ (Extended Data Fig. 3a). To test whether SinI/SinR or AbrB were important for plaque restriction, we investigated three mutants: $\Delta sinI$, $\Delta spoOA \Delta sinR$ and $\Delta spoOA \Delta abrB$ (Extended Data Fig. 3b). We observed that the $\Delta sinI$ mutant was able to generate slightly larger plaques, and iron sequestration marginally enlarged plaques. We also found that deletion of either *abrB* or *sinR* from the $\Delta spoOA$ background each partially (but incompletely) abrogated the enlarged-plaque phenotype of $\Delta spoOA$. Therefore, both SinI/SinR and AbrB appear to be partially responsible for plaque restriction by SpoOA activation. To further dissect which pathways regulated by SinI/SinR and AbrB were important for the plaque restriction, we examined mutants of each pathway.

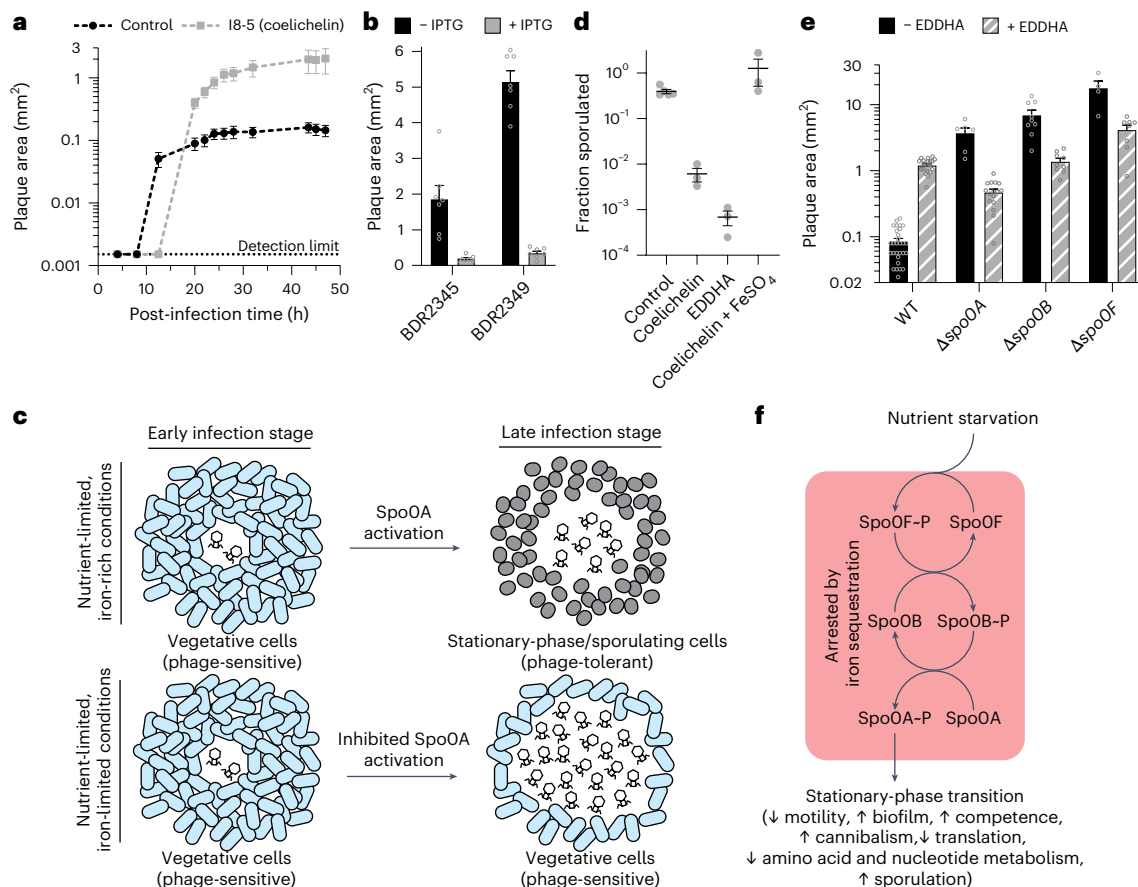


Fig. 3 | Iron sequestration inhibits SpoOA activation in *B. subtilis*. **a**, Plaque size development of SPO1 phage on *B. subtilis* grown ~8 mm away from an I8-5 colony (coelichelin producer) or *B. subtilis* alone (control). Data are presented as the average \pm s.e.m. of at least eight individual plaques for each condition. **b**, IPTG-induced expression of SpoOA (sad67-D56N) restricted phage infection in *B. subtilis* strains BDR2345 and BDR2349. Data are presented as the average \pm s.e.m. of at least seven individual plaques for each condition. Symbols represent individual plaque areas. **c**, Schematic model for iron sequestration-induced promotion of phage infection: under iron-rich conditions, *B. subtilis* cells enter stationary phase and eventually sporulate when nutrients are limited. Once the stationary-phase transition occurs in *B. subtilis* cells, phage proliferation is diminished (top). However, when iron is limited, the transition into stationary phase (and eventually sporulation) is delayed, allowing phages to continue

infecting vegetative *B. subtilis* cells (bottom). **d**, Influence of iron starvation on *B. subtilis* sporulation. The fraction of *B. subtilis* cells that sporulated under treatment (2 μ l) of water (control), coelichelin (22 mM), EDDHA (6 mM) and coelichelin (22 mM) + FeSO₄ (33 mM). Iron starvation inhibited sporulation. Data are presented as the average \pm s.e.m. of three independent biological replicates. Circles show the values of each biological replicate. **e**, The plaque-enlarging effect of EDDHA (6 mM, 2 μ l) was tested against *B. subtilis* WT, Δ spoOA, Δ spoOB and Δ spoOF. Water was used as the -EDDHA control. The Δ spoOA, Δ spoOB and Δ spoOF mutants naturally formed larger plaques that were not further increased by iron sequestration. Data are presented as the average \pm s.e.m. of at least four individual plaques for each condition. Symbols represent individual plaque areas. **f**, Schematic representation of the phosphorelay that activates SpoOA and downstream pathways in *B. subtilis*.

First, we investigated motility and biofilm formation. Activation of SpoOA switches *B. subtilis* from a motile cell to a sessile biofilm through parallel regulation by SinI/SinR and AbrB⁴⁰ (Extended Data Fig. 3a). Since motile cells may disperse phages more rapidly and biofilms may inhibit the diffusion of phages, this switch could constrict plaque sizes³⁹. Inhibition of these processes would thus enlarge plaques. We note that our model strain of *B. subtilis* is a 168 derivative, which is already deficient in motility and biofilm formation⁴¹. Nonetheless, we examined multiple mutants in motility (Δ sigD, Δ hag, Δ motA) and biofilm formation (Δ epsE, Δ tasA, Δ yuaB). All of the mutants showed the same phenotype as the WT strain (Extended Data Fig. 3b). Therefore, no individual biofilm or motility gene was necessary for the SpoOA-induced plaque restriction. However, a combination of them may still be required.

Then we investigated competence and cannibalism in *B. subtilis*, which are regulated by the AbrB pathway (Extended Data Fig. 3a). We first examined ComK, the key regulatory protein for competence development in *B. subtilis*⁴². However, inhibition of competence (Δ comK; Extended Data Fig. 3b) exhibited the same phenotype as WT,

suggesting that competence is not important for SpoOA-induced plaque restriction. We next examined the cannibalism pathway that delays the commitment of the *B. subtilis* population to sporulation. Due to the heterogeneity of SpoOA activation in the *B. subtilis* population, the two cannibalism toxins (Skf and Sdp) expressed by cells with activated SpoOA (SpoOA-P) kill neighbouring cells with unphosphorylated SpoOA^{43,44}. Since vegetative cells with unphosphorylated SpoOA are the ideal host for phage propagation, we hypothesized that the production of Skf and Sdp toxins could maintain a population of *B. subtilis* solely with activated SpoOA, which would restrict plaque development. To test this hypothesis, we generated a double knock-out mutant Δ skfA Δ sdpC that cannot produce these toxins. Indeed, the Δ skfA Δ sdpC mutant partially reproduced the phenotype of the Δ spoOA Δ abrB mutant (Extended Data Fig. 3b), suggesting that cannibalism may play a role in SpoOA/AbrB-activated plaque restriction.

From these analyses of several mutants, no single SpoOA-regulated phenotype was sufficient to completely explain the importance of SpoOA activation for plaque restriction. It is possible that multiple pathways may act synergistically to restrict plaque size. In addition,

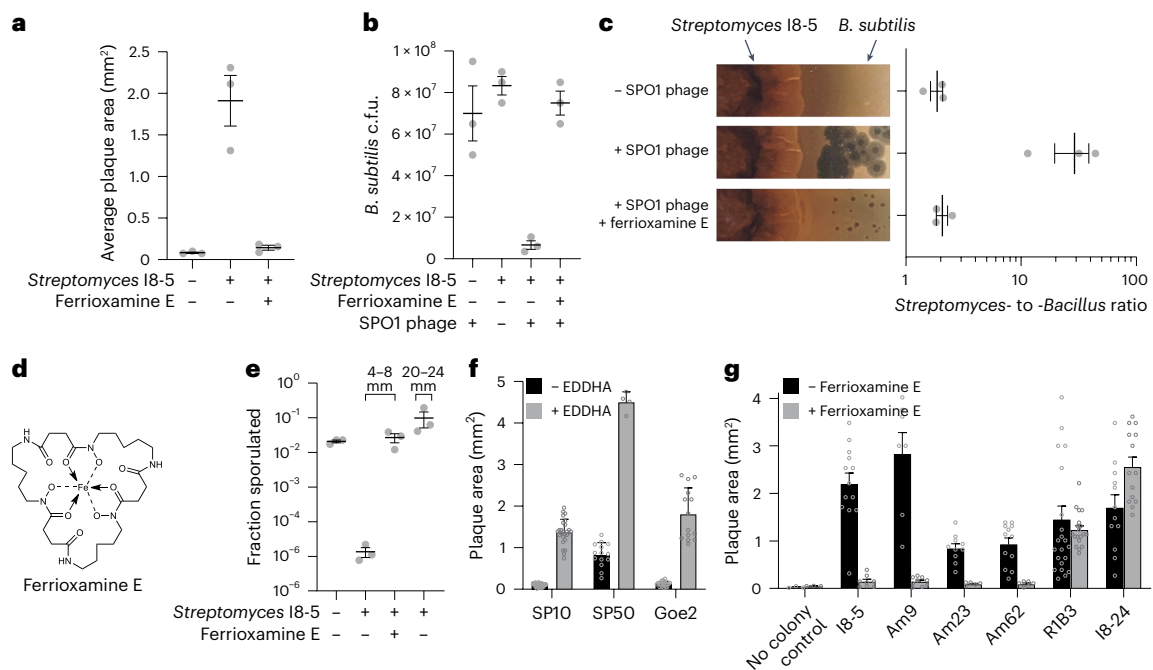


Fig. 4 | Phage-promoting metabolites help producers to outcompete

B. subtilis. **a**, The average plaque areas of SPO1 on *B. subtilis* alone and *B. subtilis* neighbouring *Streptomyces* I8-5 colony with or without ferrioxamine E (20 mM, 2 µl) as an excess iron source. Data are presented as the average ± s.e.m. of three independent biological replicates. Circles show the values of each biological replicate. At least eight plaques were selected for each replicate. **b**, Colony-forming units (c.f.u.) of *B. subtilis* measured when infected by phages, neighbouring *Streptomyces* I8-5 colony, and in a combination of phage and I8-5 with or without ferrioxamine E as excess iron source. Data are presented as the average ± s.e.m. of three independent biological replicates. Circles show the values of each biological replicate. **c**, *Streptomyces* to *B. subtilis* ratio calculated from c.f.u. measured after 2 days of co-culture. Data are presented as the average ± s.e.m. of three independent biological replicates. Circles show the values of each biological replicate. **d**, The chemical structure of ferrioxamine E. **e**, The impact of the *Streptomyces* I8-5 colony on *B. subtilis* sporulation

(an indicator of Spo0A activation). The distance of *B. subtilis* to the *Streptomyces* colony is indicated at the top. Data are presented as the average ± s.e.m. of three independent biological replicates. Circles show the values of each biological replicate. **f**, The plaque-enlarging effect of EDDHA (6 mM, 2 µl) for phages SP10, SP50 and Goe2 on *B. subtilis*. Water was used as the -EDDHA control. Data are presented as the average ratio ± s.e.m. calculated from at least four individual plaques for each condition. Symbols represent individual plaque areas. **g**, The plaque-enlarging effect of five other soil Actinomycetia. *B. subtilis* SPO1 plaque size was measured 4–8 mm away from the Actinomycetia. If addition of ferrioxamine E to the *B. subtilis*/SPO1 overcame the plaque enlargement, it would suggest that the phage metabolites produced by the Actinomycetia were siderophores. Data are presented as the average ratio ± s.e.m. calculated from at least three individual plaques for each condition. Symbols represent individual plaque areas.

the general decrease in ribosome number and amino acid and nucleotide metabolism in Spo0A-activated cells³⁴ may slow the progress of phage replication.

In conclusion, our analysis of plaquing in mutant *B. subtilis* strains suggests that multiple Spo0A-regulated pathways may redundantly restrict phage replication and decrease plaque sizes. Iron sequestration inhibits the activation of Spo0A, releasing these restrictions to allow continued phage propagation and enlarged plaques in *B. subtilis*.

Streptomyces outcompetes *B. subtilis* in a coelichelin- and phage-dependent manner

B. subtilis and *Streptomyces* are both soil bacteria and are likely to share habitats in nature⁴⁵. Since coelichelin secreted by *Streptomyces* sp. I8-5 promoted phage infection on *B. subtilis* (Fig. 4a), we hypothesized that coelichelin offers *Streptomyces* a competitive advantage over *B. subtilis* in the presence of *Bacillus* phages. Indeed, the combined action of SPO1 phages and a nearby *Streptomyces* sp. I8-5 colony significantly decreased the *B. subtilis* population density relative to phage treatment or *Streptomyces* treatment alone (Fig. 4b). Importantly, this effect allowed *Streptomyces* to outcompete *B. subtilis* 15:1 under our growth conditions (Fig. 4c).

To validate the importance of coelichelin-induced iron sequestration for the decreased *B. subtilis* fitness, we supplied the *B. subtilis* cells with excess bioavailable iron in the form of a xenosiderophore-iron complex, ferrioxamine E (Fig. 4d). With ferrioxamine E as a

supplemented iron source, the phage promotion effect from *Streptomyces* was abolished and *Streptomyces* lost its phage-induced competitive advantage over *B. subtilis* (Fig. 4a–c and Extended Data Fig. 4). We note that this experiment does not conclusively show the necessity of coelichelin per se. A second iron-sequestering metabolite could conceivably play a complementary role. Nonetheless, this experiment demonstrates that secretion of iron-sequestering metabolites enables *Streptomyces* sp. I8-5 to outcompete *B. subtilis* by facilitating phage predation on its competitor.

Furthermore, we hypothesized earlier that iron sequestration promoted phage infection by inhibiting the activation of Spo0A. Using sporulation as a proxy for Spo0A activation, we verified that the I8-5 colony inhibited sporulation of nearby *B. subtilis* (Fig. 4e). Just as the *Streptomyces* colony's impact on increased phage killing was quenched by bioavailable iron (+ferrioxamine E), so too was the colony's ability to inhibit sporulation (that is, inhibit Spo0A activation) (Fig. 4e). We also observed matching distance dependence from the *Streptomyces* colony for both plaque enlargement (Fig. 1b) and inhibition of sporulation (that is, inhibition of Spo0A activation) (Fig. 4e). This observed correlation between increased plaque sizes, increased relative fitness of *Streptomyces* and decreased *B. subtilis* sporulation (that is, decreased Spo0A activation) supports our model that siderophores from *Streptomyces* sp. I8-5 can confer a competitive advantage to their producer by sensitizing neighbouring *B. subtilis* to phage infection via inhibiting Spo0A activation.

Diverse competitors sensitize *B. subtilis* to multiple phages

Finally, we asked how widespread this metabolite-induced phage sensitization could be. We aimed to determine whether the effect was limited to the SPO1 phage and the *Streptomyces* sp. I8-5, or instead, whether a range of phages exhibit enlarged plaques in response to a range of microbial metabolites. We tested three other *Bacillus* phages (SP10, SP50 and Goe2)^{46,47}, and found that the virulence of all three was substantially increased by iron sequestration (Fig. 4f). Then we examined five of the other unique strains (Am9, Am23, Am62, R1B3, I8-24; Supplementary Figs. 1–5) in our library that also enlarged plaque sizes of SPO1 on *B. subtilis* in our initial screen (Fig. 4g). In three of the five strains, the effect was quenched by the addition of bioavailable iron, suggesting the same mechanism—secretion of iron-chelating metabolites (Fig. 4g). Coelichelin itself was not detected in the supernatant of the Am23 culture (Extended Data Fig. 5), suggesting that other siderophores may also be capable of enlarging plaques. Notably, the phage-promoting effects of R1B3 and I8-24 were independent of iron. This discovery suggests that beyond siderophores, other metabolites can also sensitize competitors to phage infections. Therefore, secondary metabolites produced by many competing microbes may broadly sensitize *B. subtilis* to a variety of phages in nature.

Discussion

We discovered that a bacterial strain gains competitive advantage over neighbouring bacteria by producing a secondary metabolite that sensitizes its competitor to phage predation. In our culture conditions, the metabolite–phage synergy switched the outcome of the bacterial competition to strongly favour the metabolite producer. Our discovery adds to the small but growing number of microbial natural products that influence bacteriophage infection, either by inhibiting phage proliferation¹², promoting phage proliferation⁴⁸, or triggering the lytic phase of lysogenic phages^{9–11}. We isolated the active metabolite, the known siderophore coelichelin. We further posited that this metabolite sequestered iron away from *B. subtilis* and promoted phage infection by inhibiting SpoOA activation. This finding reveals a new mechanism by which siderophores can shape microbial competition through bacteria–phage ecology.

Siderophores are primarily believed to enable their producer to acquire essential iron ions^{5,16}. Beyond iron acquisition, siderophores can also benefit their producers by starving competing microbes of iron^{5,49}. Our results further show that siderophores can block the stationary phase transition and sporulation of competing bacteria, which could be beneficial for the siderophore producer in fluctuating environments. For example, by excluding spores of a competitor, the siderophore-producer's spores would revive without competition when conditions become optimal for germination^{50–52}. Finally, our work reveals a potential fourth benefit of siderophore production: microbes can sensitize competing bacteria to lytic phages. This phage-promoting effect may benefit the multitude of siderophore-producing bacteria and fungi¹⁶ that compete with *B. subtilis* and its relatives.

Although our studies focused on soil *Streptomyces* and the model soil bacterium *B. subtilis*, SpoOA-regulated dormancy behaviours are characteristic of many bacteria in the Bacillota (Firmicutes) phylum⁵³. These bacteria are not only abundant in soil and aquatic sediments, but they are also important members of host-associated microbiomes, including some common intestinal pathogens and mutualistic taxa in humans⁵⁴. Therefore, it is plausible that secondary metabolites sensitize diverse Bacillota (Firmicutes) in varied environments to phage infection by inhibiting the stationary phase transition. In fact, secondary metabolites other than siderophores have also been shown to inhibit SpoOA activation or expression in Bacillota. For example, a common signal molecule used for quorum sensing, autoinducer-2, inhibits SpoOA activation in *Bacillus velezensis*⁵⁵. Furthermore, the bacterial macrocycle fidaxomicin inhibits *spoOA* gene expression in *Clostridioides difficile*⁵⁶. Beyond microbe–microbe competition,

multicellular hosts may also sensitize resident bacteria to lytic phages by producing iron-sequestering proteins⁵⁷ or other molecules that may inhibit SpoOA. Therefore, microbial siderophores, other microbial secondary metabolites and even host-produced molecules may sensitize competing bacteria to phage infection in natural communities.

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

Methods

Strains and growth conditions

The strains and bacteriophages used in this study are listed in Supplementary Tables 2 and 3. All chemicals used are listed in Supplementary Table 4. All primers used are listed in Supplementary Table 5. *B. subtilis* strains were routinely grown in LB broth at 37 °C and 220 r.p.m. When appropriate, antibiotics were used at the following concentrations: 7 µg ml^{−1} kanamycin and 1 µg ml^{−1} erythromycin. *Streptomyces* strains were routinely grown in ISP2 media (4 g l^{−1} yeast extract, 10 g l^{−1} malt extract and 4 g l^{−1} dextrose) at 30 °C and 220 r.p.m.

Bacteriophage lysate preparation

To prepare the host culture, an overnight culture of *B. subtilis* RM125 WT was subcultured 1:100 into 4 ml LB + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 5 mM CaCl₂. The culture was incubated at 37 °C and 220 r.p.m. for 4 h until the optical density (OD)_{600nm} reached 0.2. About 1 × 10³ plaque-forming units (p.f.u.) of *Bacillus* phage were added to the culture. The phage-infected culture was incubated at 37 °C and 220 r.p.m. until bacterial cells were lysed and the culture turned clear. The phage lysate was filtered through a 0.2-µm polyethersulfone filter and stored at 4 °C.

Binary-interaction screening

To prepare plates with library bacteria, 5 µl of the frozen spore stock of each bacterial strain in our library was suspended in 50 µl of ISP2 medium. Then 8 µl of the spore suspension was spotted at the centre of an ISP2 + 1.5% agar plate. The inoculated plates were incubated at 30 °C for 10 days to allow the library bacteria to grow and secrete their metabolites into the plate. To test the influence of the metabolites on phage infectivity, an overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 ml fresh LB broth and ~1,000 p.f.u. of SPO1 phages was then added into the medium. The mixture of bacteria and phages was poured around the central colony formed by the library bacteria. Bacteria and phages were allowed 10 min to soak the plate and the mixture was then removed using a pipette. The plate was dried under room temperature in a biosafety cabinet and then incubated at 37 °C overnight. The size of plaques formed by SPO1 was quantified using Fiji⁵⁸ the next day.

16S ribosomal RNA sequencing of Actinomycetia

A single colony of Actinomycetia was inoculated into 4 ml fresh ISP2 medium and incubated at 30 °C and 220 r.p.m. for 4 days. The genomic DNA was extracted from 1 ml liquid mycelia culture using Promega Wizard Genomic DNA Purification kit (1120). The 16S ribosomal RNA (rRNA) region of the bacterial genome was amplified by PCR using

16S_F and 16S_R primers. Sanger sequencing results of 16S rRNA are available on NCBI (18-5: GenBank [OR902106](#); Am9: GenBank [PQ178887](#); Am23: GenBank [PQ178944](#); Am62: GenBank [PQ178965](#); R1B3: GenBank [PQ178995](#); 18-24: GenBank [PQ179041](#)).

Coelichelin biosynthetic gene cluster identification in 18-5

The library of the extracted genomic DNA was prepared using the Illumina Nextera XT DNA Library Prep Kit protocol (FC-131-1096) and analysed using Agilent D1000 ScreenTape. The libraries were pooled and loaded on a NextSeq 1000/2000 P2 Reagents (100-cycles) v3 flow cell (20046811) configured to generate paired-end reads. The demultiplexing of the reads was performed using bcl2fastq v.2.20.0. Reads were adapter trimmed and quality filtered using Trimmomatic (0.38)⁵⁹ with the cut-off threshold for average base quality score set at 20 over a window of 3 bases requiring a minimum trimmed read length of 20 bases (parameters: LEADING:20 TRAILING:20 SLIDING-WINDOW:3:20 MINLEN:20). The cleaned reads were assembled using SPAdes (v.3.15.4)⁶⁰ with default parameters. The assembly was annotated using prokka (v.1.12)⁶¹, employing a sequence training set prepared from protein sequences obtained from 431 publicly available *Streptomyces* assemblies (parameters: --minpid 70 --usegenus --hmm TIGRFAM, CLUSTERS, Pfam, HAMAP). The coelichelin biosynthetic gene cluster was identified using antiSMASH (7.0)⁶². The genomic sequences are available on NCBI (accession number: JAYMFC000000000).

Collection of 18-5 supernatant

To revive the spores of 18-5, 5 µl of the 18-5 frozen spore stock was streaked out on an ISP2 + 1.5% agar plate. The plate was incubated at 30 °C for 3 days until colonies formed. A single colony was inoculated into 4 ml fresh ISP2 medium and incubated at 30 °C and 220 r.p.m. for 4 days. After the incubation, 1 ml of the culture was added into 1 l of ISP2 medium and grown for another 11 days to allow metabolite production. To collect the metabolites in the supernatant, the bacteria cells in the culture were pelleted at 4,820 × g for 20 min and the pellet was discarded. The supernatant was lyophilised and stored at -20 °C until ready to use.

Test of phage promotion activity of compounds/supernatant concentrate

An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 ml fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar plate. Bacteria were allowed 10 min to attach to the plate and the unattached bacteria were then removed. The plate was dried under room temperature in a biosafety cabinet. To test the phage promotion effect, 2 µl of compound/supernatant concentrate was spotted on top of the bacterial lawn. After the compound dried, the plate was incubated at 37 °C for 1 h. Then 5 µl of SPO1 phage lysate (~10 p.f.u.) was spotted on top of the compound-treated area. After the phage lysate dried, the plate was incubated at 37 °C and the size of plaques formed by SPO1 was quantified using Fiji⁵⁸ after 2 days.

Fractionation of 18-5 supernatant using reversed-phase chromatography

The lyophilised supernatant was dissolved into a small amount of water as a concentrated sample. The concentrated supernatant was further separated on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250 × 10 mm) using an Agilent 1260 Infinity II HPLC system. Mobile phase A was water + 0.01% (v/v) formic acid and mobile phase B was acetonitrile + 0.01% (v/v) formic acid. The flow rate was kept at 3 ml min⁻¹ and the gradient was as follows: 0% B (0–5 min), increase to 20% B (5–6 min), 20% B for (6–11 min), increase to 80% B (11–31 min), increase to 100% B (31–32 min), 100% B (32–37 min), decrease to 0% B (37–38 min), 0% B (38–43 min). Eluted fractions were collected every 30 s and dried in vacuo. Each dried fraction was redissolved into 2 µl

DMSO and spotted on a lawn of *B. subtilis* RM125 WT infected by SPO1 phages to test the phage promotion activity. The fraction eluting at 12.0–12.5 min was active and labelled as 'active fraction 1'. The composition of 'active fraction 1' was analysed on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250 × 4.6 mm) using an Agilent 1260 Infinity II HPLC system coupled to an Agilent InfinityLab LC/MSD XT mass spectrometer. The analysis was performed at a flow rate of 0.7 ml min⁻¹. The mobile phase and separation gradient were the same as described above.

Fractionation of 18-5 supernatant using cation-exchange and reversed-phase chromatography

The lyophilised supernatant was dissolved into 10 ml 2% (v/v) formic acid/water (pH 2.02). The supernatant was loaded on a Waters Oasis MCX column (186000255). The column was then eluted with water + 2% (v/v) formic acid, methanol and methanol + 5% (v/v) ammonium hydroxide. The eluates were dried in vacuo and redissolved into water as a 100 mg ml⁻¹ solution. Of each redissolved fraction, 2 µl was spotted on a lawn of *B. subtilis* RM125 WT infected by SPO1 phages to test the phage promotion activity. The methanol + 5% (v/v) ammonium hydroxide eluate was active and subjected to separation on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250 × 10 mm) using an Agilent 1260 Infinity II HPLC system. Mobile phase A was water + 0.1% (v/v) formic acid and mobile phase B was acetonitrile + 0.1% (v/v) formic acid. The flow rate was kept at 3 ml min⁻¹ and the gradient was as follows: 10% B (0–10 min), increase to 50% B (10–30 min), increase to 100% B (30–31 min), 100% B (31–38 min), decrease to 10% B (38–39 min), 10% B (39–44 min). Eluted fractions were collected every 30 s and dried in vacuo. Each dried fraction was redissolved into 2 µl DMSO and tested for phage promotion effect as described above. The fraction eluting at 3.5–4.0 min was active and labelled as 'active fraction 2'. The composition of 'active fraction 2' was analysed on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250 × 4.6 mm) using an Agilent 1260 Infinity II HPLC system coupled to an Agilent InfinityLab LC/MSD XT mass spectrometer. The analysis was performed at a flow rate of 0.7 ml min⁻¹. The mobile phase and separation gradient were the same as described above.

LC-MS/MS analysis of *m/z* 566.2783 and *m/z* 619.1885 species

The 'active fraction 2' was separated on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250 × 4.6 mm) using ACQUITY UPLC I-Class PLUS System. Mobile phase A was water + 0.1% (v/v) formic acid and mobile phase B was acetonitrile + 0.1% (v/v) formic acid. The flow rate was kept at 0.7 ml min⁻¹ and the gradient was as follows: 0% B (0–20 min), increase to 20% B (20–21 min), increase to 40% B (21–31 min), increase to 100% B (31–32 min), 100% B (32–42 min), decrease to 0% B (42–43 min), 0% B (43–48 min). *m/z* 566.2783 species eluted at 17.2–18.2 min and *m/z* 619.1885 species eluted at 26.0–26.1 min. High-resolution electrospray ionization (HR-ESI) mass spectra with collision-induced dissociation (CID) MS/MS were obtained using a Waters Synapt G2S QTOF. Data-dependent acquisition was employed to fragment the top three masses in each scan. The data were analysed using MassLynx 4.1 software.

Isolation of coelichelin from 18-5 supernatant

The protocol was adapted from ref. 13. The lyophilised 18-5 supernatant (1.8533 g) was dissolved in 5 ml of water. FeCl₃ was added to the supernatant (final concentration 40 mM) to generate an Fe-coelichelin complex. The reaction mixture was centrifuged at 16,000 × g for 5 min and the precipitates were discarded. The supernatant was separated on a Phenomenex Luna 10 µm Hydro-RP 100 Å column (250 × 21.2 mm) using an Agilent 1260 Infinity II HPLC system. Mobile phase A was water + 10 mM NH₄HCO₃ (pH 8.01) and mobile phase B was methanol. The flow rate was kept at 10 ml min⁻¹ and the gradient was as follows: 5% B (0–20 min), increase to 90% B (20–21 min), 90% B (21–31 min),

decrease to 5% B (31–32 min), 5% B (32–42 min). Fe–coelichelin eluted at 9.1–10.4 min and was collected by monitoring the absorbance at 435 nm. The collected Fe–coelichelin was concentrated in vacuo, lyophilised and obtained as an orange solid.

The obtained Fe–coelichelin (45.9 mg) was dissolved into 74 ml of water. The ferric iron was removed from the Fe–coelichelin complex by mixing the Fe–coelichelin solution with 74 ml of 100 mM 8-hydroxyquinoline in methanol. The reaction was stirred for 30 min at room temperature. The Fe–8-hydroxyquinoline complex was removed by extracting the aqueous phase using 50 ml dichloromethane three times. The aqueous phase was concentrated in vacuo and separated on a Phenomenex Synergi 4 μ m Hydro-RP 80 Å column (250 \times 10 mm) using an Agilent 1260 Infinity II HPLC system. Mobile phase A was water + 0.1% (v/v) formic acid and mobile phase B was acetonitrile + 0.1% (v/v) formic acid. The flow rate was kept at 3 ml min⁻¹ and the gradient was as follows: 0% B (0–10 min), increase to 5% B (10–11 min), 5% B (11–21 min), increase to 100% B (21–22 min), 100% B (22–32 min), decrease to 0% B (32–33 min), 0% B (33–43 min). Apo-coelichelin eluted at 17.7–17.9 min and was collected by monitoring the absorbance at 210 nm. The collected apo-coelichelin was concentrated in vacuo, lyophilised and obtained as a white solid. The HR-ESI mass spectrometry data of apo-coelichelin was obtained on a Thermo Scientific Finnigan LTQ Orbitrap XL mass spectrometer equipped with a nano-electrospray ionization source operated in positive ionization mode. The data were analysed using Xcalibur 4.0 software. HR-ESI-MS (positive-ion mode): m/z 566.2776 [M + H]⁺ (calculated for C₂₁H₄₀N₇O₁₁⁺: 566.2780).

Coelichelin purity check by LC–MS

The purified coelichelin was analysed on a Phenomenex Synergi 4 μ m Hydro-RP 80 Å column (250 \times 4.6 mm) using an Agilent 1260 Infinity II HPLC system coupled to an Agilent InfinityLab LC/MSD XT mass spectrometer. Mobile phase A was water + 0.1% (v/v) formic acid and mobile phase B was acetonitrile + 0.1% (v/v) formic acid. The flow rate was kept at 0.7 ml min⁻¹ and the gradient was as follows: 0% B (0–10 min), increase to 5% B (10–30 min), increase to 100% B (30–31 min), 100% B (31–41 min), decrease to 0% B (41–42 min), 0% B (42–52 min).

Preparation of Ga–coelichelin

Coelichelin (10 mg) was dissolved in 400 μ l of water, followed by the addition of 26.5 mg of Ga₂(SO₄)₃ in 400 μ l of water. The reaction was performed at room temperature for 30 min and subjected to separation on a Phenomenex Synergi 4 μ m Hydro-RP 80 Å column (250 \times 10 mm) using an Agilent 1260 Infinity II HPLC system. Mobile phase A was water + 0.1% (v/v) formic acid and mobile phase B was acetonitrile + 0.1% (v/v) formic acid. The flow rate was kept at 3 ml min⁻¹ and the gradient was as follows: 0% B (0–10 min), increase to 100% B (10–11 min), 100% B (11–21 min), decrease to 0% B (21–22 min), 0% B (22–32 min). Ga–coelichelin eluted at 4.6–5.0 min and was collected by monitoring the absorbance at 210 nm. The collected Ga–coelichelin was concentrated in vacuo, lyophilised and obtained as a white solid. HR-ESI-MS (positive-ion mode): m/z 632.1794 [M + H]⁺ (calculated for C₂₁H₃₇GaN₇O₁₁⁺: 632.1801). ¹H and TOCSY (mixing time of 60 ms) NMR spectra were obtained on a Varian 600 MHz Inova NMR spectrometer using Varian/Agilent VnmrJ and Linux workstations. ¹³C, DQF-COSY, HSQC and HMBC NMR spectra were obtained on a Bruker 500 MHz Avance Neo NMR spectrometer using Bruker IconNMR 5.1.9 software. All spectra were analysed using MestReNova 14.2.0-26256 software.

Iron complementation experiment

An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 ml fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar plate. Bacteria were allowed 10 min to soak the plate and the unattached bacteria were then removed. The plate was dried under room temperature in a biosafety cabinet. A volume of 2 μ l of compound was spotted as a small circle

on top of the bacterial lawn. After the compound dried, 2 μ l of FeSO₄ aqueous solution was spotted on top of the compound-treated area. After the FeSO₄ solution dried, the plate was incubated at 37 °C for 1 h. Then 5 μ l of SPO1 phage lysate (~10 p.f.u.) was spotted on top of the compound-treated area. After the phage lysate dried, the plate was incubated at 37 °C and the size of plaques formed by SPO1 was quantified using Fiji⁵⁸ after 2 days.

Quantification of phage reproduction from individual plaques

An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 ml fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar plate. Bacteria were allowed 10 min to attach to the plate and the unattached bacteria were then removed. The plate was dried under room temperature in a biosafety cabinet. EDDHA (2 μ l, 6 mM) or water was spotted as a small circle on top of the bacterial lawn. After the compound dried, the plate was incubated at 37 °C for 1 h. Then 5 μ l of SPO1 phage lysate (10–40 p.f.u.) was spotted on top of the compound-treated area. After the phage lysate dried, the plate was incubated at 37 °C for 2 days. The number of plaques formed in each phage spot was enumerated and the average plaque area for each phage spot was quantified using Fiji⁵⁸. All the plaques in one phage spot were pooled by carving out the agar containing the plaques and resuspending in 5 ml phage buffer (10 mM Tris, 10 mM MgSO₄, 4 g l⁻¹ NaCl, pH 7.5). The suspension was vortexed at the highest speed for 20 s to allow phages to fully detach from the agar. The p.f.u. of the pooled plaques were quantified using the small drop plaque assay⁶³. For each individual phage spot, the average p.f.u. per plaque was calculated using the following equation:

$$\text{Avg.p.f.u. per plaque} = \frac{\text{p.f.u. of pooled plaques}}{\text{number of plaques}}$$

Sporulation quantification

B. subtilis spores were quantified by determining the number of colony-forming units (c.f.u.) after heat-treating the population to kill vegetative cells⁶⁴. An overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 ml fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar plate. Bacteria were allowed 10 min to attach to the plate and the unattached bacteria were then removed. The plate was dried under room temperature in a biosafety cabinet. A volume of 2 μ l of compound or water (control) was spotted as a small circle on top of the bacterial lawn. After the compound dried, the plate was incubated at 37 °C for 16 h. Then ~1 cm² area of bacteria was scraped off the plate and resuspended in 200 μ l water. The cell suspension was heated at 85 °C for 15 min to kill non-sporulated cells. Then the spores in the heat-treated cell suspension were quantified by measuring the c.f.u.

Generation of sporulation mutants

The gene knockout donor in *B. subtilis* 168 were purchased from the Bacillus Genomic Stock Center. The mutation was then transferred to *B. subtilis* RM125 using SPP1-mediated generalized phage transduction^{65,66}. Briefly, the SPP1 phage lysate was obtained from the *B. subtilis* 168 knockout donor strain as described above and stored at 4 °C until ready to use. A single colony of the recipient *B. subtilis* RM125 was inoculated into 10 ml LB + 10 mM CaCl₂. The recipient culture was incubated at 37 °C and 220 r.p.m. for 4 h. For phage transduction, 950 μ l of the recipient culture was mixed with 50 μ l of donor SPP1 lysate and incubated at 37 °C for 10 min to allow phage adsorption. Then the 1 ml infected culture was transferred into 9 ml of prewarmed LB + 20 mM sodium citrate and incubated at 37 °C for another 10 min. The cells were pelleted at 4,000 $\times g$ for 5 min and plated onto an LB + 20 mM sodium citrate + 1.5% agar plate with appropriate antibiotics. The plates were incubated at 37 °C overnight and the mutant colonies were restreaked twice on LB + 20 mM sodium citrate + 1.5% agar plate with appropriate

antibiotics to clean out phages. The knockout mutation was validated using PCR with primers reported in ref. 67. The sporulation mutants were verified to not produce spores using the sporulation quantification experiment described above.

Spo0A (sad67-D56N) restricts phage infection

An overnight culture of *B. subtilis* (BDR2345/BDR2349) was diluted 1:10 into 5 ml fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1 mM IPTG, and poured onto an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1 mM IPTG + 1.5% agar plate. Bacteria were allowed 10 min to attach to the plate and the unattached bacteria were then removed. The plate was dried under room temperature in a biosafety cabinet and then incubated at 37 °C for 1 h. After incubation, 5 µl of SPO1 phage lysate (~10 p.f.u.) was spotted on top of the compound-treated area. After the phage lysate dried, the plate was incubated at 37 °C and the size of plaques formed by SPO1 was quantified using Fiji⁸⁸ after 2 days.

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

To inoculate plates with *Streptomyces*, 5 µl of the frozen spore stock of I8-5 was suspended in 50 µl of ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂. Then 8 µl of the spore suspension was spotted at the centre of an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar plate. The plates were incubated at 30 °C for 16 days to allow the I8-5 colony to grow and secrete coelichelin into the plate. To inoculate the *B. subtilis* next to *Streptomyces*, an overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 ml fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂, and poured around the I8-5 colony. *B. subtilis* cells were allowed 10 min to attach to the plate and the unattached bacteria were then removed. The plate was dried under room temperature in a biosafety cabinet. Water (2 µl) or ferrioxamine E (20 mM solution in 2 µl water) was spotted as a small circle on top of the *B. subtilis* lawn. After the spotted solution dried, the plate was incubated at 37 °C for 1 h. Then 5 µl of SPO1 phage lysate (~10 p.f.u.) was spotted on top of the compound-treated area. After the phage lysate dried, the plate was incubated at 37 °C for 2 days. The average plaque area for each phage spot was quantified using Fiji⁸⁸. The *B. subtilis* lawn and *Streptomyces* colony were carved out and resuspended in 5 ml LB broth and 5 ml ISP2 medium, respectively. The cell suspensions were vortexed at the highest speed for 20 s to allow bacterial cells to fully detach from the agar. The c.f.u. of *B. subtilis* cell suspensions were quantified by plating serial dilutions of the cell suspension on LB + 1.5% agar plates. The c.f.u. of *Streptomyces* cell suspensions were quantified by plating serial dilutions of the cell suspension on ISP2 + 10 µg ml⁻¹ nalidixic acid + 1.5% agar plates.

Test of phage promotion activity of Actinomycetia

To prepare plates with Actinomycetia, 5 µl of the frozen spore stock of each bacterial strain in our library was suspended in 50 µl of ISP2 medium. Then 8 µl of the spore suspension was spotted at the centre of an ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂ + 1.5% agar plate. The inoculated plates were incubated at 30 °C for 10 days to allow Actinomycetia to grow and secrete their metabolites into the plate. To test the influence of the metabolites on phage infectivity, an overnight culture of *B. subtilis* RM125 WT was diluted 1:10 into 5 ml fresh ISP2 + 0.1 mM MnCl₂ + 5 mM MgCl₂, and poured around the Actinomycetia colony. *B. subtilis* were allowed 10 min to attach to the plate and the unattached bacteria were then removed. The plate was dried under room temperature in a biosafety cabinet and then incubated at 37 °C for 1 h. After incubation, 5 µl of SPO1 phage lysate (~10 p.f.u.) was spotted on top of the *B. subtilis* lawn. After the phage lysate dried, the plate was incubated at 37 °C and the size of plaques formed by SPO1 was quantified using Fiji⁸⁸ after 2 days.

Detection of coelichelin production by Actinomycetia

To revive the spores of Actinomycetia, 5 µl of the Actinomycetia frozen spore stock was streaked out on an ISP2 + 1.5% agar plate. The plate was

incubated at 30 °C for 3 days until colonies formed. A colony was inoculated into 4 ml fresh ISP2 medium and incubated at 30 °C and 220 r.p.m. for 4 days. After the incubation, 1 ml of the culture was added into 100 ml of ISP2 medium + 0.1 mM MnCl₂ + 5 mM MgCl₂ and incubated at 30 °C and 220 r.p.m. To monitor the coelichelin level in the growth media, 1 ml of culture was removed and centrifuged at 16,000 × g for 5 min. Then the supernatant was collected and filtered through a 0.2 µm polyethersulfone filter. Of the supernatant, 10 µl was analysed on a Phenomenex Synergi 4 µm Hydro-RP 80 Å column (250 × 4.6 mm) using an Agilent 1260 Infinity II HPLC system coupled to an Agilent InfinityLab LC/MSD XT mass spectrometer. Mobile phase A was water + 0.1% (v/v) formic acid and mobile phase B was acetonitrile + 0.1% (v/v) formic acid. The flow rate was kept at 0.7 ml min⁻¹ and the gradient was as follows: 0% B (0–20 min), increase to 20% B (20–21 min), increase to 40% B (21–31 min), increase to 100% B (31–32 min), 100% B (32–42 min), decrease to 0% B (42–43 min), 0% B (43–48 min).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The genome sequence of strain I8-5 is available on NCBI (accession number [JAYMFC000000000](https://doi.org/10.1038/s41564-024-01910-8)). The 16S sequences of the other plaque-enlarging bacteria are available on NCBI (I8-5: GenBank [OR902106](https://doi.org/10.1038/s41564-024-01910-8); Am9: GenBank [PQ178887](https://doi.org/10.1038/s41564-024-01910-8); Am23: GenBank [PQ178944](https://doi.org/10.1038/s41564-024-01910-8); Am62: GenBank [PQ178965](https://doi.org/10.1038/s41564-024-01910-8); R1B3: GenBank [PQ178995](https://doi.org/10.1038/s41564-024-01910-8); I8-24: GenBank [PQ179041](https://doi.org/10.1038/s41564-024-01910-8)). Source data for plaque measurements are available on figshare at <https://doi.org/10.6084/m9.figshare.27269481> (ref. 68). Any further requests for data should be addressed to the corresponding author (jggerdt@iu.edu).

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Author contributions

Z.Z. and J.P.G. conceptualized the project. Z.Z., D.A.S. and J.P.G. developed the methodology. Z.Z., C.Z., K.J.P. and R.P. conducted investigations. Z.Z. and J.P.G. wrote the original draft of the paper. Z.Z., C.Z., D.A.S., J.T.L. and J.P.G. reviewed and edited the paper. Z.Z. and J.P.G. performed visualization. J.T.L. and J.P.G. supervised the project. J.T.L. and J.P.G. acquired funding.

Competing interests

The authors declare no competing interests.

Additional information

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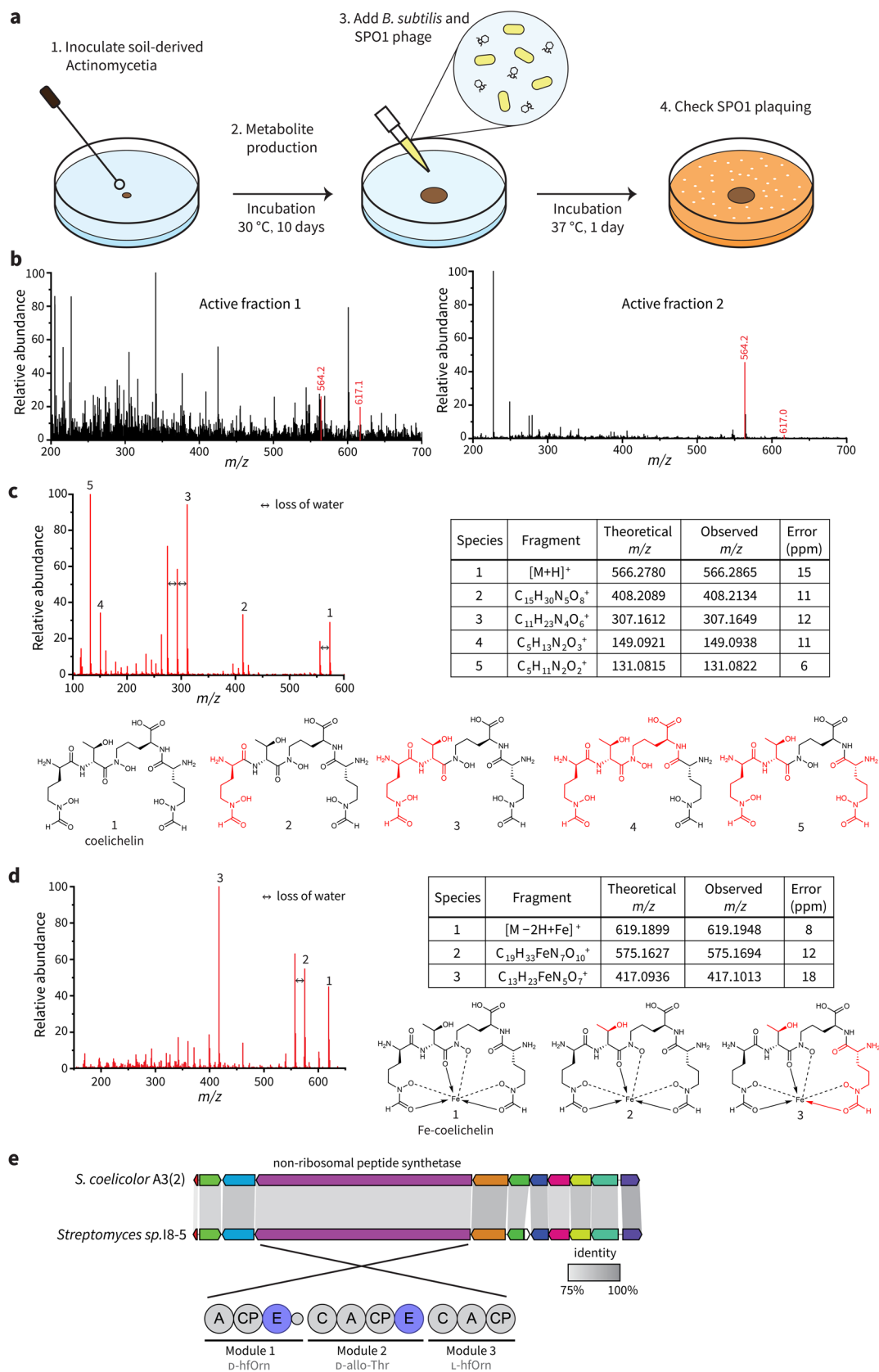
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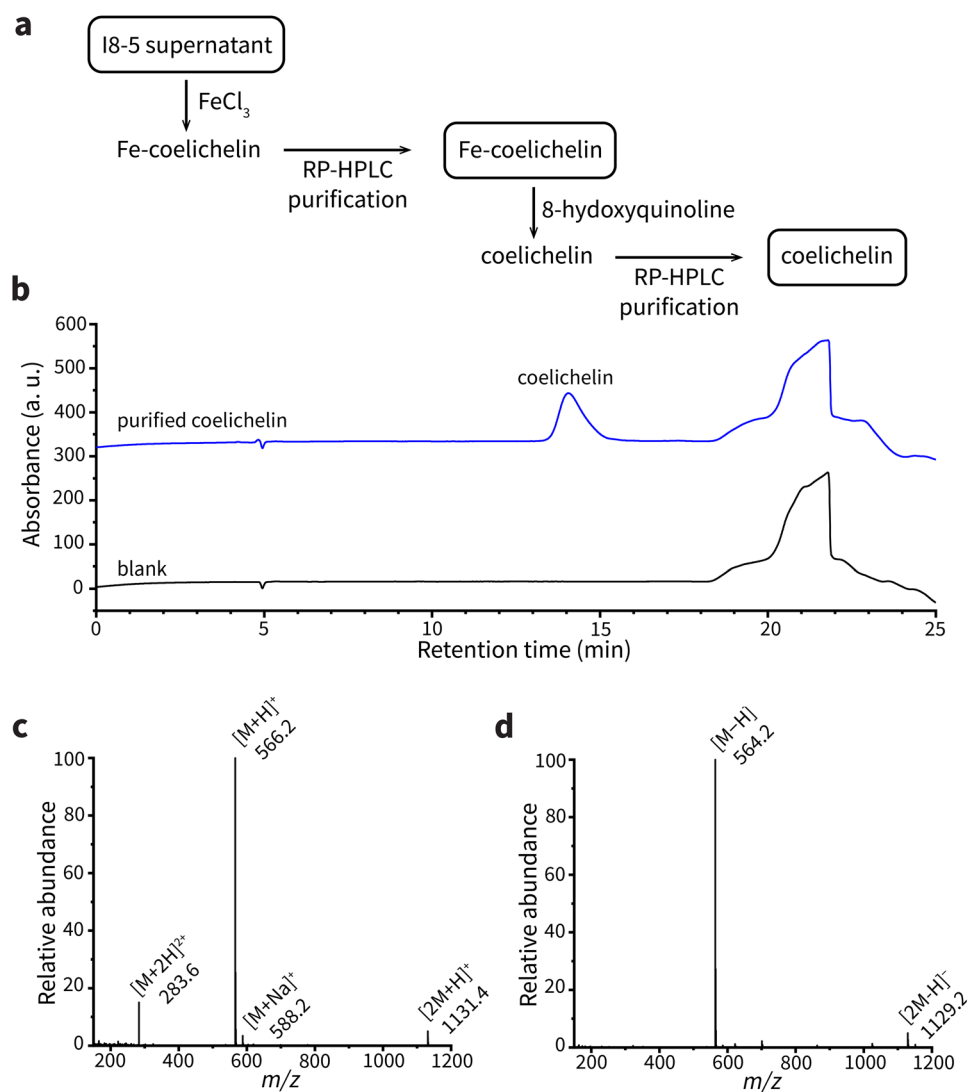
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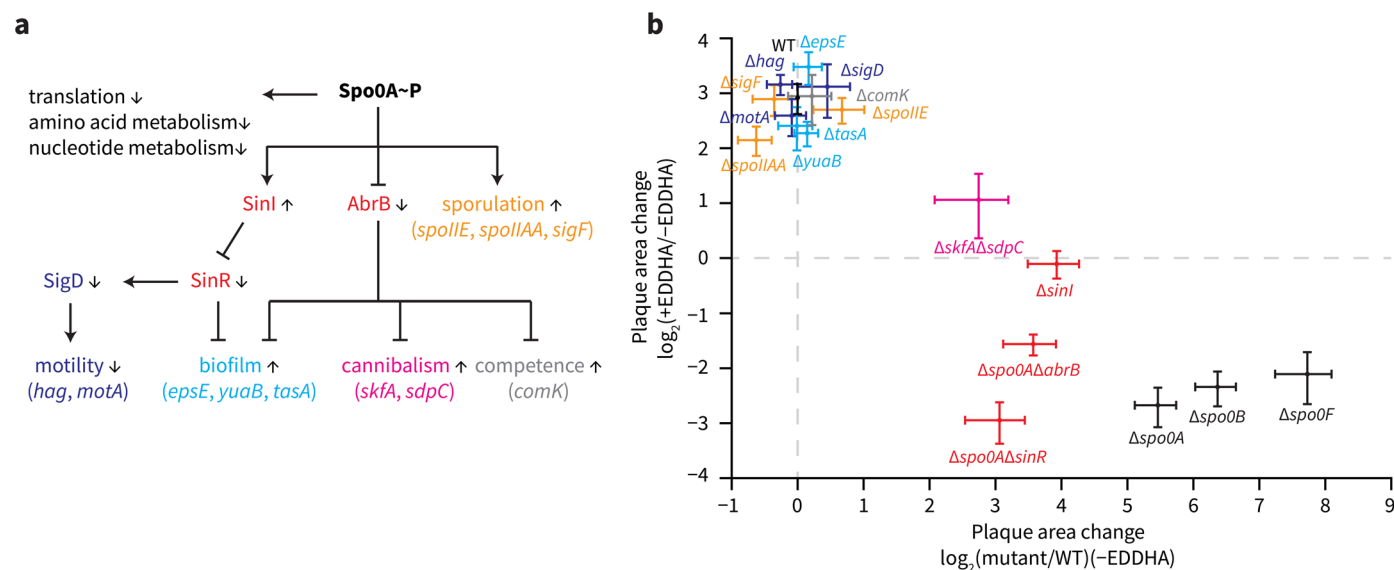


Extended Data Fig. 1 | Coelichelin is the active metabolite that promotes phage predation. (a) Scheme of the binary-interaction screen. (b) Negative mode electrospray ionization MS spectra of active fraction 1 (left) and active fraction 2 (right). The shared peaks are highlighted red. (c) MS/MS spectrum of the m/z 566.2783 species. Key fragments are annotated with their associated peak, and their losses are highlighted in red. (d) MS/MS spectrum of the m/z 619.1885 species. Key fragments are annotated with their associated peak, and their losses are highlighted in red. (e) Comparison of the *Streptomyces* sp. I8-5

coelichelin biosynthetic gene cluster with the reported one from *S. coelicolor* A3(2). The percent identity between each pair of genes is shown with shading (all were >75%). The modules of the coelichelin non-ribosomal peptide synthetase are shown in the lower region of the panel. The three modules are responsible for installation of D- δ -N-formyl-D-N-hydroxyornithine (D-hfOrn), D-allo-threonine (D-allo-Thr), and L- δ -N-hydroxyornithine (L-hOrn), respectively. The adenylation domains (A), thiolation and peptide carrier proteins (CP), condensation domains (C), and epimerization domains (E) are shown.



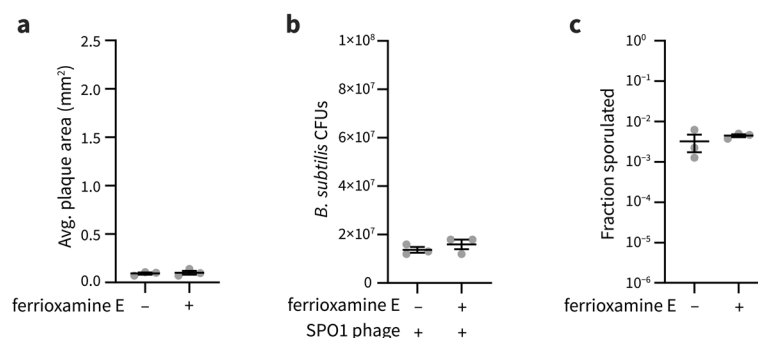
Extended Data Fig. 2 | Coelichelin isolation from I8-5 supernatant. (a) Isolation scheme. (b) UV chromatogram at 210 nm. Water was used as the blank. (c) The averaged MS spectrum at positive mode between retention time 13.5 - 14.8 min. (d) The averaged MS spectrum at negative mode between retention time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



Extended Data Fig. 3 | Multiple pathways regulated by Spo0A are important for the plaque enlargement phenotype caused by iron sequestration.

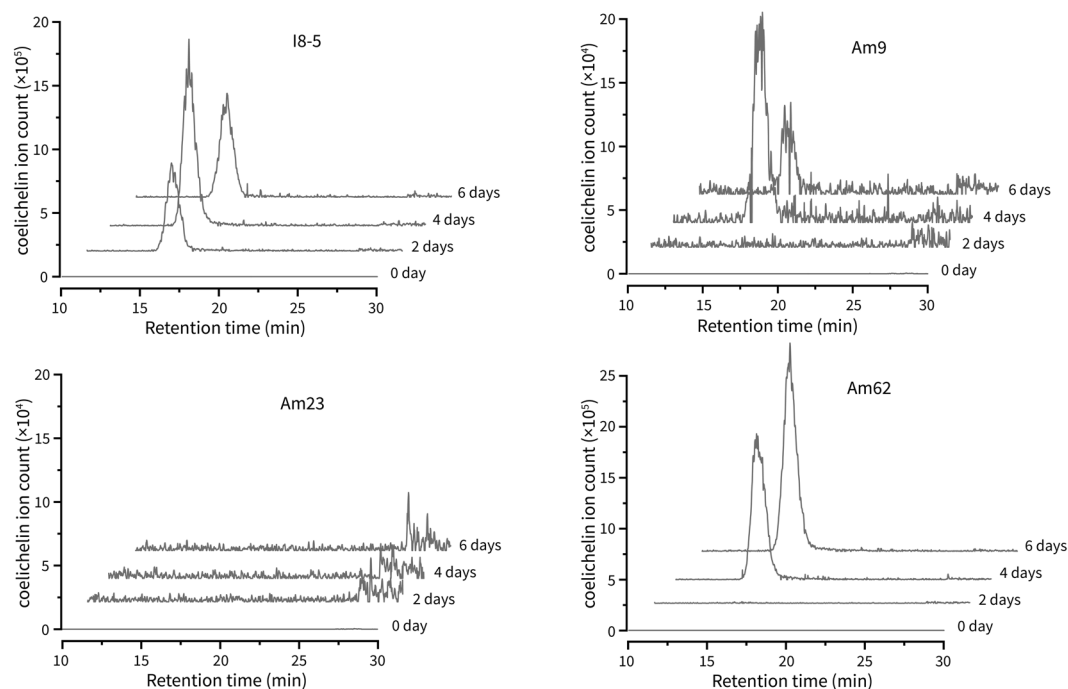
(a) Pathways regulated by Spo0A. (b) The x-axis shows the plaque size ratio between mutant and wild type (WT) under iron-rich conditions (– EDDHA).

The y-axis shows the plaque size ratio between iron-limited (6 mM EDDHA treated [2 μL]) and iron-rich conditions (– EDDHA) of different mutants. Water was used as the – EDDHA control. Data are represented as the average ratio \pm SEM calculated from at least four individual plaques of each condition.



Extended Data Fig. 4 | Ferrioxamine E alone has no substantial effect on plaque size, *B. subtilis* growth, and Spo0A activation. (a) The average plaque areas of SPO1 on *B. subtilis* were measured when treated with or without ferrioxamine E (2 μ l of 20 mM) as an excess iron source. Data are represented as the average \pm SEM from three independent biological replicates. Circles show the values of each biological replicate and at least 21 plaques were selected for each replicate. (b) The colony forming units of *B. subtilis* were measured when infected by SPO1

phages, treated with or without ferrioxamine E (2 μ l of 20 mM) as an excess iron source. Data are represented as the average \pm SEM from three independent biological replicates. Circles show the values of each biological replicate. (c) The impact of ferrioxamine E (2 μ l of 20 mM) on *B. subtilis* sporulation (an indicator of Spo0A activation). Data are represented as the average \pm SEM from three independent biological replicates. Circles show the values of each biological replicate.



Extended Data Fig. 5 | Coelichelin is not ubiquitously produced by all plaque-enlarging bacteria. The conditioned media resulting from the fermentation of 4 plaque-enlarging bacteria (collected at different time points) were subjected to LC-MS analysis. The extracted ion chromatogram of coelichelin is shown here.

No coelichelin was detected in the conditioned medium of Am23, suggesting that it does not produce coelichelin but instead an unknown phage-promoting siderophore.

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Data analysis	FIJI (ImageJ2) 2.9.0/1.53t was used to measure plaque sizes, GraphPad Prism 10 was used to plot results, MestreNova 14.2.0 was used to analyze NMR data. LCMS data was analyzed with Agilent Openlab CDS ChemStation C.01.10 (low resolution) and MassLynx 4.1 (high resolution).

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Am9: GenBank PQ178887; Am23: GenBank PQ178944; Am62: GenBank PQ178965; R1B3: GenBank PQ178995; l8-24: GenBank PQ179041). Raw image, NMR, and LCMS data are available on Figshare (doi: 10.6084/m9.figshare.27269481).

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<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.