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ABSTRACT

One of the most important ways that bacteria compete for resources and space is by producing antibiotics that inhibit competitors. Because antibiotic production is costly, the biosynthetic gene clusters coordinating their synthesis are under strict regulatory control and often require "elicitors" to induce expression, including cues from competing strains. Although these cues are common, they are not produced by all competitors and so the phenotypes causing induction remain unknown. By studying interactions between 24 antibiotic-producing *Streptomyces* we show that inhibition between competitors is common and occurs more frequently if strains are closely related. Next, we show that antibiotic production is more likely to be induced by cues from strains that are closely related or that share biosynthetic gene clusters. Unexpectedly, antibiotic production is less likely to be induced by antagonistic competitors, indicating that cell damage is not a general cue for induction. In addition to induction, antibiotic production often decreased in the presence of a competitor, although this response was not associated with genetic relatedness or overlap in biosynthetic gene clusters. Finally, we show that resource limitation increases the probability that antibiotic production declines. Our results clarify that social cues and resource availability are crucial determinants of interference competition in *Streptomyces*.

SIGNIFICANCE STATEMENT

Bacteria secrete antibiotics to inhibit their competitors, but the presence of competitors can also determine whether or not these toxins are produced. Here, we study the role of the competitive environment on antibiotic production in *Streptomyces*, a bacterial group renowned for their production of clinically useful antibiotics. We show that *Streptomyces* are more likely to induce antibiotic production when grown with closely related competitors or

bacteria that share secondary metabolite biosynthetic gene clusters, but not necessarily when they are threatened by competitor's toxins. In addition, *Streptomyces* often reduce their output of antibiotics when grown with competitors, especially under nutrient limitation. Our findings highlight the importance of the social and resource environment in the regulation of antibiotic production in these medicinally important bacteria.

INTRODUCTION

Bacteria live in diverse communities where they compete with other microbes for resources and space. Competition between different species can be regulated by the differential uptake and use of specific nutrients. It can also be driven by secreted toxins, like antibiotics or bacteriocins, that kill or inhibit competitors. Antibiotics and bacteriocins can allow producing strains to invade established habitats or repel invasion by other strains (1, 2). However, these compounds are expected to be metabolically expensive to make and so should only be produced against genuine threats from competitors. This idea, called "competition sensing", argues that microbes should upregulate toxin production when they sense competitors through cell damage or nutrient limitation (3). Bacteria can also sense competitors by detecting secreted signals that are used to regulate toxin production and thereby predict imminent danger (3). Consistent with this, many microbes change their production of secondary metabolites in response to cues from other strains when grown in co-culture (4, 5). However, these responses are not universal and it remains unclear if they can be predicted based on the identity or phenotype of their competitors and the cues they produce. Accordingly, at present we are unable to predict why some competitors alter toxin production while others do not.

We set out to address this question in the context of bacteria from the prolific antibiotic-producing family *Streptomycetaceae* (6). These filamentous, spore-forming bacteria are renowned for their production of secondary metabolites, including many clinically useful antibiotics, anti-helminthic agents and anti-cancer drugs (7). Antibiotic production in streptomycetes is associated with the developmental stage of the colony and typically coincides with the onset of sporulation (8, 9). We refer to this type of autonomous production as "constitutive" because it occurs in the absence of influence from other

species. In addition, the presence of other strains in co-culture can alter antibiotic production by increasing or reducing antibiotic output (4, 5, 10). These changes are caused by different cues that indicate the presence of competitors. These can include nutrient stress, if competitors have overlapping resource requirements, or cues that cause cellular damage or predict immediate danger, e.g. antibiotics or quorum-dependent regulators of antibiotic production, like gamma-butyrolactones (3, 11–13). We hypothesize that these competitive cues are more likely to be produced by strains with similar primary and secondary metabolism due to shared resource requirements or mechanisms of antibiotic regulation (14). More specifically, because these traits are phylogenetically conserved (15–18), we predict that *Streptomyces* will be more likely to respond to cues from closely related species.

To examine cues that regulate antibiotic production in streptomycetes, we studied antagonistic interactions between 24 different strains across a broad phylogenetic range in two nutrient environments. First, in each nutrient environment, we tested all possible pairwise interactions between these strains (24 x 24 = 576) by growing them as colonies and then testing if they could inhibit the growth of all other strains by inoculating these on top of the focal colony (Fig. 1). Next, we tested if growth in co-culture with a second strain altered the inhibitory behaviors we recorded during pairwise interactions. These three-way interactions (a total of 24 x 24 x 24 = 13,824 unique interactions in each nutrient environment) allowed us to compare the inhibitory capacity of strains during solitary growth, reflecting constitutive expression, to their behavior after interacting with a competitor during co-culture (Fig. 1). These approaches allowed us to directly test if altered antibiotic production during growth in co-culture could be predicted as a function of the identity of the competitor.

RESULTS

Constitutive antagonism

We first measured constitutive antibiotic production by growing each strain on a defined minimal medium and then testing if it could inhibit an overlay of each target strain (Fig. 1). These results formed the baseline against which we examined facultative responses. These assays revealed that approximately half of all possible pairwise interactions were inhibitory (47.7%) (Fig. 2A). We next identified the biosynthetic gene clusters in the complete genomes of these strains using the bioinformatics tool antiSMASH (19). This revealed considerable variability in the number of secondary metabolite biosynthetic gene clusters (BGCs) encoded within each genome (mean = 34 +/- 1.85 (SE), range = 22 to 64), suggesting broad diversity in inhibitory capacities (Fig. S1).

The antagonistic behavior of each strain against the 24 possible targets generated a unique fingerprint of inhibition, which we designate the inhibition phenotype. As anticipated, we found a significant correlation between inhibition phenotype and phylogeny (Fig. 2B) (Mantel test, $P < 0.001$, r = 0.27), suggesting that closely related strains inhibit the same targets. We then tested if this was due to the possibility that related strains produce similar antagonistic compounds. This idea is supported by a significant correlation between inhibition phenotype and biosynthetic gene cluster (BGC) similarity (Mantel test, $P < 0.001$, r = 0.43) (Fig. 2C).

Consistent with the idea that closely related strains are more likely competitors, strains showed a stronger tendency to inhibit closely related targets (logistic regression, $P < 0.001$, McFadden $R^2 = 0.02$, N = 536). As BGCs often also provide resistance against the product they encode, we expected that strains with a high degree of BGC similarity would

not inhibit each other. Indeed, strains were most likely to inhibit targets that are closely related but have dissimilar BGCs (logistic regression, $P_{\text{phylogenetic distance}} < 0.001$, $P_{\text{BGC distance}} = 0.064$, McFadden $R^2 = 0.02$, N = 536) (Fig. 2D). In contrast to results from a study that examined inhibitory interactions between phylogenetically diverse bacteria (20), we found no association between the probability of inhibition and the metabolic overlap between strains, assessed using BiOLOG plates (Fig. S2).

Altered inhibition during co-culture

These results show that streptomycetes constitutively produce antibiotics that are directed at closely related strains. However, constitutive antibiotic production does not account for facultative changes that are caused by cues from other strains. We measured facultative responses by inoculating each strain next to a competitor and then assessing if it could inhibit the growth of the different target strains, as above. By this approach, we could directly compare differences in the inhibitory capacity of each strain in the presence and absence of each competitor (Fig. 1).

The results of these assays confirm that facultative responses are extremely widespread. The inhibition phenotype was affected by a competitor in approximately half of the unique focal-competitor interactions (48%), meaning that the focal strain showed a change in antagonism against at least one target strain in the presence of a given competitor (Fig. 3A). There was considerable variability in the responsiveness of strains to competitors; whereas some strains responded to none of the competitors, others responded to nearly all of them (Fig. 3B).

Facultative responses fall into two categories: induction and suppression (Fig. 1), and both are common. Induction occurs when the presence of a competitor causes a strain to

inhibit a target strain that it didn't inhibit during constitutive assays. Suppression constitutes the opposite scenario, where growth next to a competitor suppresses antibiotic production. We observed induction in 33% of all tested co-cultures and suppression in 45%. On average a strain was induced by 7.4 +/- 1.5 (SE) competitors and suppressed by 9.6 +/- 1.7 (SE) competitors, with considerable variability in both values (induced: 0-20, suppressed: 0 -22) (Fig. 3B). Notably, in many cases, a given strain was both induced and suppressed by the same competitor against different targets. Accordingly, the dots in Figure 3A represent the net influence of these two types of changes, in some cases leading to no net change in the number of inhibited strains, even though the inhibition phenotype of the strain is different.

Competition sensing predicts that bacteria will change their behavior in response to antagonistic competitors that they detect by sensing cell damage (3). Although we found that induction was significantly related to the competitor being antagonistic (logistic regression, $P < 0.001$, McFadden $R^2 = 0.06$, N = 354), the direction of this result was counter to our expectations (Fig. 3C). Unexpectedly, antibiotic production was more likely to be induced by competitors that did not inhibit the producer strain (probability of induction 0.41 vs 0.22), suggesting that cell damage was not a strong cue for antibiotic induction. Other ways that cells could sense competitors is by detecting compounds they produce, such as antibiotics and quorum sensing signals, or through nutrient stress due to resource competition. Since both primary and secondary metabolism are correlated with phylogeny, we examined if induction was correlated with phylogenetic distance. As predicted, strains are more frequently induced by a closely related competitor (logistic regression, $P < 0.001$, McFadden $R^2 = 0.02$, N = 487) (Fig. 3D). To examine if this effect was driven by the production of similar secondary metabolites, we tested if differences in induction could be explained by BGC similarity. Indeed, strains are more likely induced by competitors with

which they share more BGC clusters (logistic regression, $P < 0.001$, McFadden $R^2 = 0.04$, N = 487) (Fig. 3E).

In addition to induction, we found that antibiotic production was also commonly suppressed in the presence of competitors. Although this strategy can be beneficial by preventing a competitor from producing a potentially harmful secondary metabolite, it could also benefit the suppressed strain by allowing it to redirect energy towards other functions. However, we found no relationship between suppression and the competitor's ability to inhibit the focal strain (logistic regression, $P = 0.83$, McFadden $R^2 = 0.025$, N = 473). Suppression was also not associated with phylogenetic or BGC distance.

Effect of resource stress on inhibition

To address the role of nutrient limitation on antibiotic production, we tested whether constitutive or facultative inhibition changed if the carbon source concentration was reduced by 10-fold (Fig. 4A). Constitutive inhibition was only marginally affected by these conditions, with 49.2% vs 47.7% of all pairwise interactions being inhibitory on low versus high resource medium. And only few pairwise interactions (6.7%) showed antagonism on one resource concentration but not on the other, indicating that a 10-fold change in carbon source concentration has a minimal effect on constitutive inhibition (McNemar's $X^2 = 0$, df = 1, $P = 1$). Likewise, we found a strong correlation between the inhibition phenotypes of the strains at both resource concentrations (Mantel test, r = 0.93, $P < 0.001$), with phylogenetic and BGC distance both significantly correlated with inhibition phenotype (Mantel test, $P < 0.001$, r = 0.30 and $P < 0.001$, r = 0.39 respectively) (Fig. 4B and C). As at the higher glycerol concentration, strains are more likely to inhibit closely related targets with dissimilar BGCs

(logistic regression, $P_{\text{phylogenetic distance}} < 0.001$, $P_{\text{BGC distance}} = 0.023$, McFadden $R^2 = 0.02$, N = 526) (Fig. 4D).

We then tested whether strains reacted differently to a competitor under resource stress (Fig. 5A and B). Competition sensing predicts that cells counter-attack when they sense competition through nutrient stress. As expected, streptomycetes responded differently to the presence of a competitor under varying resource conditions (McNemar's $X^2 = 5.43$, df = 1, $P < 0.05$), with a change in inhibition phenotype in 56.1% versus 49.1% in low versus high resource conditions, respectively (Fig. 5C). However, counter to expectations, we found that the incidence of induction declined at lower resource levels (30.2% vs 33.0%) (Fig. 5C). Just as at the higher resource level, strains were more likely to be induced by competitors that did not inhibit them (Fig. 5D) (Logistic regression, $P < 0.001$, McFadden $R^2 = 0.12$, N = 419). In contrast to the higher resource conditions, neither phylogenetic nor BGC similarity was associated with induction in the low resource environment.

Suppression dramatically increased in the low resource environment from 45.0% to 59.1% (Fig. 5C). Despite this increase, suppression was still not associated with any of the factors that we tested, suggesting that antibiotic suppression may be a general reaction to resource stress in streptomycetes.

DISCUSSION

Microbial populations in soil are highly diverse even at small spatial scales, suggesting that competitive and social interactions between neighboring cells are common as different species vie for space and resources (21). One of the ways that species compete is by secreting toxins like antibiotics or bacteriocins. Typically, the production of these compounds has been studied without consideration of this social context; however, both

theory and experiments have shown that this perspective is limited because it neglects crucial factors that induce or suppress toxins and also fails to identify toxins whose production is dependent on competitive interactions (4, 5, 22–25). In this context, the aims of our work were twofold: first to characterize the role of social interactions on antibiotic production in common soil microbes of the *Streptomycetaceae*, and second to identify factors that were predictive of competition-mediated responses.

By comparing antibiotic production in the absence and presence of another species in co-culture, termed constitutive and facultative production, respectively, we found that production was induced in more than 1/3 of co-cultures, confirming and considerably expanding results from earlier studies that facultative antibiotic production is widespread in these bacteria (4, 5). We also found that induction was strongly predicted by phylogeny, as anticipated if closely related strains are likely competitors. However, unexpectedly, a strain was no more likely to be induced during co-culture if it was grown with a competitor that inhibited it versus one that did not. In other words, while co-culture frequently altered antibiotic production, this was not evidently driven by cellular damage caused by the second strain, as specifically predicted by the "competition sensing" hypothesis (3). Instead, our results suggest that cells are more likely to induce antibiotic production in response to cues that are strongly correlated with phylogeny, rather than direct harm itself. For example, strains that share BGCs are more likely to induce each other, which suggests two possible sources for cues. First, antibiotic intermediates or antibiotics themselves, can serve as inducers of antibiotic production or resistance (13). These responses can prevent autotoxicity or killing by neighboring clonemates and also act as regulators of the expression of their own biosynthetic gene cluster (24, 26). Second, related strains that share one or more BGCs may be more likely to utilize similar secreted factors that induce antibiotic

production, e.g. the quorum-dependent gamma-butyrolactone signals. *Streptomyces* contain multiple receptors for cognate and non-cognate gamma-butyrolactones, thereby allowing them to detect these signals as a precursor of the antibiotics another strain might produce (12, 27, 28). Similar eavesdropping of quorum-dependent signals has been observed for bacteriocins in *Streptococcus pneumoniae*, which leads to cross-induction of strain-specific antimicrobials (29). Testing this idea in *Streptomyces* using chemically synthesized signals and reporter strains remains an important objective for future work.

When we repeated our assays at 1/10 the glycerol concentration, constitutive expression was only marginally changed; however, these lower resource concentrations led to slightly reduced induction rates and a marked increase in suppression. Moreover, the associations between induction and phylogeny and BGC distance disappeared. These results indicate that antibiotic regulation integrates information about the competitive environment as well as environmental resource availability. This is unsurprising, as links between nutrient sensing or carbon catabolite repression and antibiotic production in streptomycetes are well established (30, 31). Competition may exacerbate nutrient stress overall, leading to a general suppressive response that doesn't depend on the particulars of the competitor. By this view, suppression is best considered a response to nutrient stress, rather than the result of a specific action by the second strain. More generally, this result indicates that further work will need to consider responses other than antibiotic production when examining the behavior of cells in co-culture. For example, strains may respond to nutrient stress from competitors by redirecting energy used for antagonism towards functions that help them to avoid competition, e.g. hyphal growth in the direction opposite the competing strain or increased sporulation. Whereas the first possibility would contribute to an escape in space, the latter would allow an escape in time, leaving spores to germinate when nutrient stress is

relieved. These alternative responses might be anticipated if there are trade-offs between antibiotic production and other aspects of development, although these remain to be verified.

In summary, our results provide strong evidence that antibiotic production by streptomycetes is highly responsive to their social and resource environment. This is understandable given the likely costs of antibiotic production and the patchy distribution of these bacteria in nature (32). In addition to clarifying the role of BGC similarity on antibiotic induction, which builds on intuitive predictions of the "competition sensing" hypothesis, our results show that suppression and escape need to be more thoroughly considered as a response to interference competition. This is particularly true given the numerous mechanisms bacteria use to regulate inter- and intra-specific warfare (33). It will also be crucial to examine these responses in experiments that more closely approximate the natural environment, including an environment with increased spatial heterogeneity and decreased diffusion, and where local interactions are maintained over longer periods of time. Similarly, an important next step is determine how these social interactions influence competitive outcomes, as has been done for constitutive antibiotic production between competing species (1, 2, 34). Together, these approaches will lead to a fuller understanding of the role of antibiotic production in natural soils and the factors that maintain microbial diversity.

Methods

Strains and culturing conditions The panel of 24 *Streptomycetaceae* strains used in this study (Table S1) included 21 strains isolated from a single soil sample from the Himalaya Mountains collected at 5000 m near a hot water spring (35). These 21 strains were selected

due to their consistent phenotypes and the ability to sporulate in our lab growth conditions. The remaining three strains were well-characterized lab strains, *Streptomyces coelicolor* A3(2) M145, *Streptomyces griseus* IFO13350 and *Streptomyces venezuelae* ATCC 10712.

High density spore stocks were generated by culturing on Soy Flour Mannitol Agar (SFM) (20 g Soy Flour, 20 g Mannitol, 20 g Agar per liter) or on R5 Agar (103 g sucrose, 0.42 g K_2SO_4, 10.1 g $MgCl_2$, 50 g glucose, 0.1 g CAS amino acids, 5 g yeast extract, 5,7 g TES, 2 ml R5 trace element solution and 22 g agar per liter). After 3-4 days of growth, spores were harvested with a cotton disc soaked in 3 ml 20% glycerol, and spores were extracted from the cotton by passing the liquid through an 18g syringe to remove the vegetative mycelium. Resulting spore stocks were titred and stored at -20 °C.

Multi-well masterplates were prepared by diluting the high density spore stocks to 1 x 10^6 sp ml^{-1} in deionized water and these plates were stored at -20 °C. The glycerol concentration after the dilution of stocks was always lower than the concentration of glycerol added as a carbon source to the medium.

To perform the interaction assays approximately 1 µl of the focal strain, and when indicated 1 µl of the competitor strain, was replicated on a 25 grid plate (Thermo Fisher Scientific, Newport, UK) using a custom built multi-pin replicator (EnzyScreen BV, Heemstede, The Netherlands) from a frozen masterplate. Each well of the 25 grid plate contained 2 ml Minimal Medium (MM) (500 mg L-Asparagine (Duchefa Biochemie, The Netherlands), 500 mg KH_2PO_4 (Duchefa Biochemie, The Netherlands), 200 mg $MgSO_4.7H_2O$ (Duchefa Biochemie, The Netherlands), 10 mg $Fe_2SO_4.7H_2O$ (Sigma Aldrich, MO, USA) and 20 g agar (Company) per litre, pH 7.2 supplemented with either 0.05% or 0.5% glycerol). After 4 days of growth at 30 °C a 1 ml overlay (0.8% agar MM) containing 1.6 x 10^5 sp/ml was added on top. After 24 to 48 hours of incubation at 30 °C (depending on the growth speed of target

strain) 1 ml of the dye resazurin (Cayman Chemical Company, Michigan, USA) was added to each well at a concentration of 50 mg L^{-1} and incubated for half an hour before the surplus was removed. Change in colour of this redox dye from blue to pink was used as a measure of growth of the target strain, as resazurin (blue) is changed to resorufin (pink) by metabolically active cells. Pictures were taken of every plate and these were scored for the presence or absence of inhibition zones around the colony/colonies. Every interaction was assessed in duplicate. When the results of assays were inconsistent, the particular interaction was repeated a third time.

Whole genome sequencing Whole genome sequencing was performed for all strains for which a full genome sequence was not yet available to perform genome mining and to generate a phylogenetic tree. As described before (36) strains were grown in liquid culture containing 50% YEME/50% TSBS with 5mM $MgCl_2$ and 0.5% glycine at 30 °C, 250 rpm for 2 days. After centrifugation the pellet was resuspended in TEG-buffer with 1.5 mg ml^{-1} lysozyme and after 1 hour of incubation at 30 °C the reaction was stopped by adding 0.5 volume of 2M NaCl. DNA was extracted using a standard phenol/chloroform extraction, followed by DNA precipitation and washing in isopropanol and 96% ethanol. Dried DNA was resuspended in MQ water and then treated with 50 ug ml^{-1} of RNase and incubated at 37 °C for 1 hour. Following RNase treatment, the mixture was purified and cleaned as above, after which the purified DNA was washed with 70% ethanol and resuspended in MQ water. Paired-end sequence reads were generated using the Illumina HiSeq2500 system at BaseClear. *De novo* assembly was performed using the "De novo assembly" option of the CLC Genomics Workbench version 9.5.1 and the genome was annotated using the BaseClear

annotation pipeline based on the Prokka Prokaryotic Genome Annotation System (version 1.6).

Using the complete genomes, multilocus sequence typing was performed as described by (37). For this purpose we used the sequences of six housekeeping genes, *atpD, gyrB, recA, rpoB, trpB* and 16S rRNA that were shown to give good resolution for the *S. griseus* glade. For the already available sequenced genomes, the sequences for *S. coelicolor* (strain V) were downloaded from StrepDB (http://strepdb.streptomyces.org.uk) and used to blast against the genome sequences of *S. venezuelae* ATCC 10712 (txid 54571) (strain W), *S. griseus* supsp. griseus NBRC 13350 (txid 455632) and MBT66 (strain P) on the NCBI database. For all sequenced genomes the genes of interest were located from the annotated genome or were searched in a database constructed with the genomes in Geneious (Geneious 9.1.4). Each gene was aligned and trimmed before the six sequences for each strain were concatenated in frame and used to construct a neighbourjoining tree.

Analysis of biosynthetic gene clusters Biosynthetic gene clusters were identified within each genome with antiSMASH version 4.0 (19). BiG-SCAPE was used to calculate the pairwise distances between all BGCs, using a cutoff of 0.5 as a threshold for similarity (38). This generated a BGC presence/absence matrix that we used to calculate a Jaccard distance between each pair of genomes to define the BGC distance between the strains.

Resource use Carbon source utilization of each strain was tested using BiOLOG SFP2 plates (Biolog, Hayward, CA, USA) on which growth on 95 carbon sources can be assessed. Plates were inoculated as described by (39). Briefly, strains were grown on MM with 0.5% glycerol for 7 days before spores were swabbed into a 0.2% carrageenan solution and adjusted to

OD_{590} of 0.2 – 0.24. This solution was diluted 10 times in 0.2% carrageenan and 100 ul of this dilution was added to each well. Plates were incubated at 30 °C for 3 days before the absorbance of each well at 590 nm was measured using a Spark 10M plate reader (Tecan, Switzerland). All strains were assessed in triplicate. For the analysis the absorbance of the water control was subtracted for each well and the average was taken. If the average was not significantly different from 0 (one sample T-test), the value was adjusted to 0. The Pearson correlation coefficient was calculated between all possible pairwise combinations of the strains and the metabolic distance was calculated as 1 – correlation coefficient. Strain P showed extremely poor growth on the BiOLOG plates and was therefore excluded.

Statistics All statistics were performed in R. Correlation between phylogenetic distance, metabolic dissimilarity, secondary metabolite distance and inhibition and resistance phenotype was determined using Mantel tests. To establish whether antagonism and inhibition, induction and suppression are dependent, logistic regressions were performed. Logistic regression was also used to test for association between inhibition, induction or suppression and phylogenetic distance, metabolic distance or BGC distance. For the logistic regressions we excluded all self-self interactions, as these confound the analyses by having zero distance between the strains or test for self-inhibition.

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with the (bio)informatics; and SW, DER and GPvW wrote the manuscript. The authors declare there is no conflict of interest.

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FIGURES

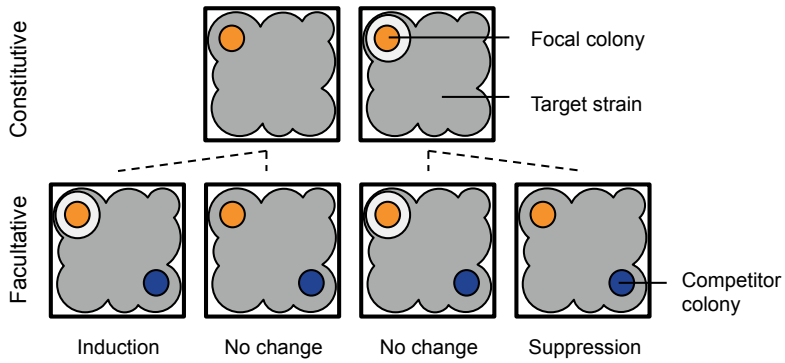

Fig. 1. Schematic of constitutive and facultative inhibition assays. Focal strains (orange) were tested for their capacity to inhibit each target strain (grey) inoculated on top of the focal colony in a soft agar overlay. Inhibition was detected as a zone of clearance surrounding the colony. All 24 strains were tested as both focal and target strains, leading to 576 possible assays for constitutive antibiotic production. For the facultative assays a second colony was inoculated one centimeter away, designated as the competitor, that could interact with the focal strain through diffusible molecules. All 24 strains were tested as the focal, competitor and target strain, resulting in 24 x 24 x 24 = 13,824 assays. Comparing the ability of the focal strain to inhibit the target in the constitutive and facultative assays revealed whether antibiotic production was induced, suppressed or unchanged.

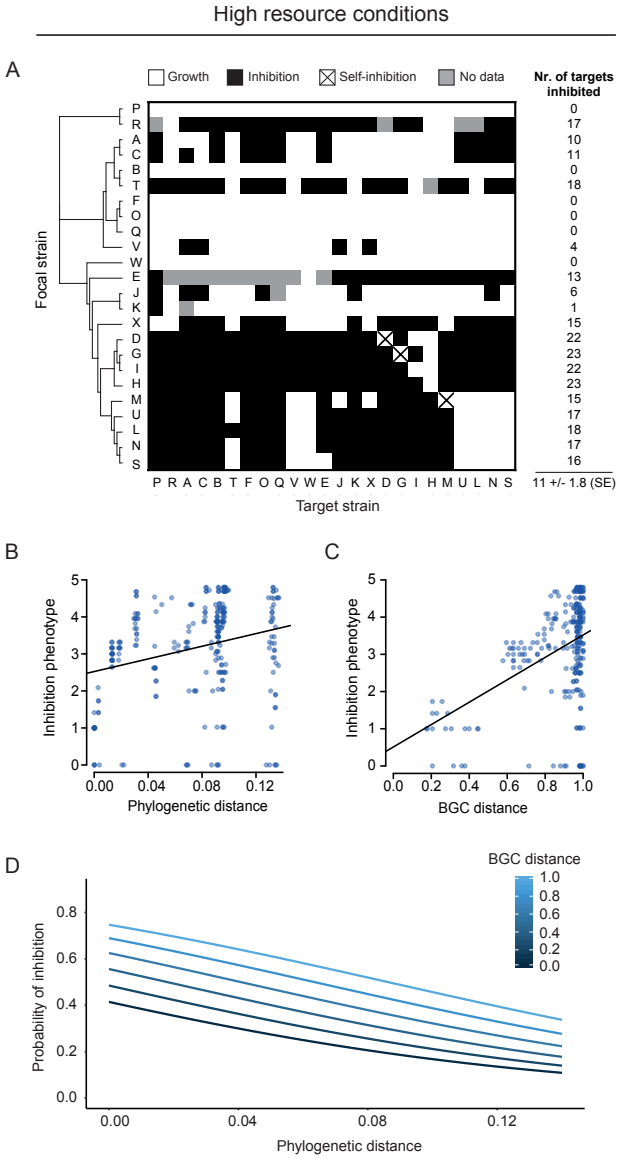

Fig. 2. Constitutive antagonism. (A) Interaction matrix sorted by MLST relatedness. Squares indicate whether a target strain showed growth (white) or was inhibited (black) by the focal strain. Self-inhibition is denoted by an X and missing data is shown in grey. (B) Correlation between inhibition phenotype (Euclidian distance) and phylogenetic distance (Mantel test, $P < 0.001$, r = 0.27 N = 552) or (C) biosynthetic gene cluster (BGC) distance (Mantel test, $P < 0.001$, r = 0.43, N = 552). (D) Logistic regression between the probability of inhibition and phylogenetic and biosynthetic gene cluster (BGC) distance ($P_{\text{phylogenetic distance}} < 0.001$, $P_{\text{BGC distance}} = 0.064$, McFadden $R^2 = 0.02$, N = 536).

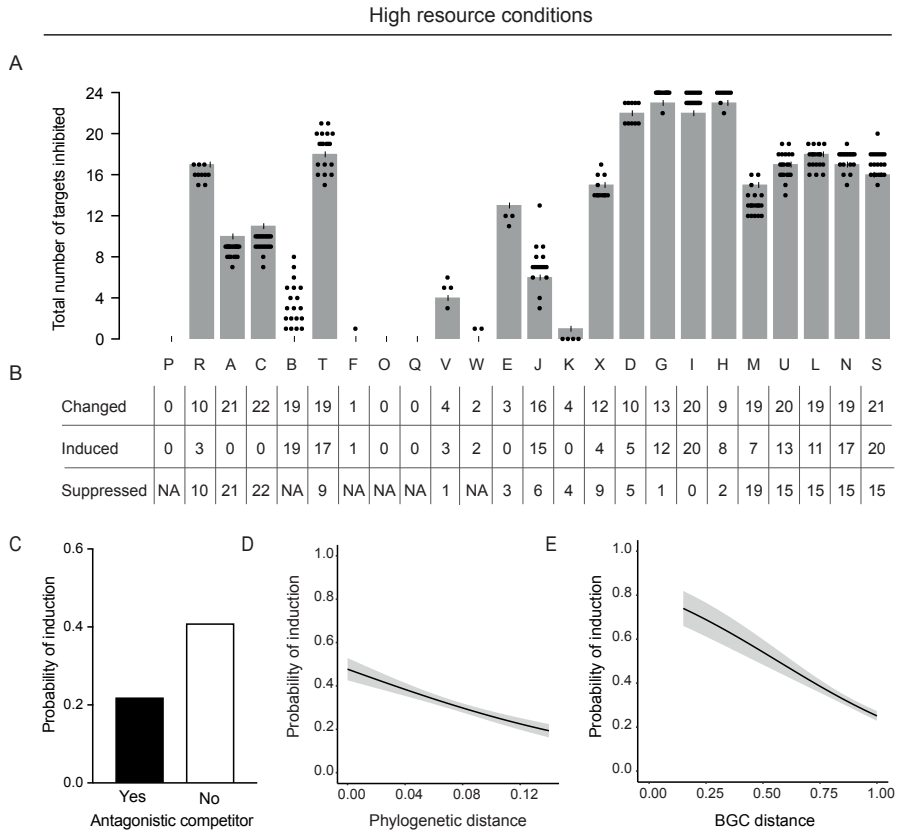

Fig. 3. Altered antagonism during co-culture. (A) Grey bars indicate the number of target strains inhibited by the focal strain when grown alone. Black dots indicate the number of target strains inhibited by the same focal strain when co-cultured with one of the 24 possible competitors. (B) Number of competitors that change, induce or suppress secondary metabolite expression for each focal strain. Cases where suppression is not possible due to the absence of constitutive inhibition are denoted as NA. (C) The probability of the focal strain showing induction is lower when the competitor is antagonistic to the focal strain (Logistic regression, $P < 0.001$, McFadden $R^2 = 0.06$, N = 354). (D) Logistic regressions between the probability of induction and phylogenetic ($P < 0.001$, McFadden $R^2 = 0.02$, N = 487) or (E) BGC distance ($P < 0.001$, McFadden $R^2 = 0.04$, N = 487). Ribbons indicate SE.

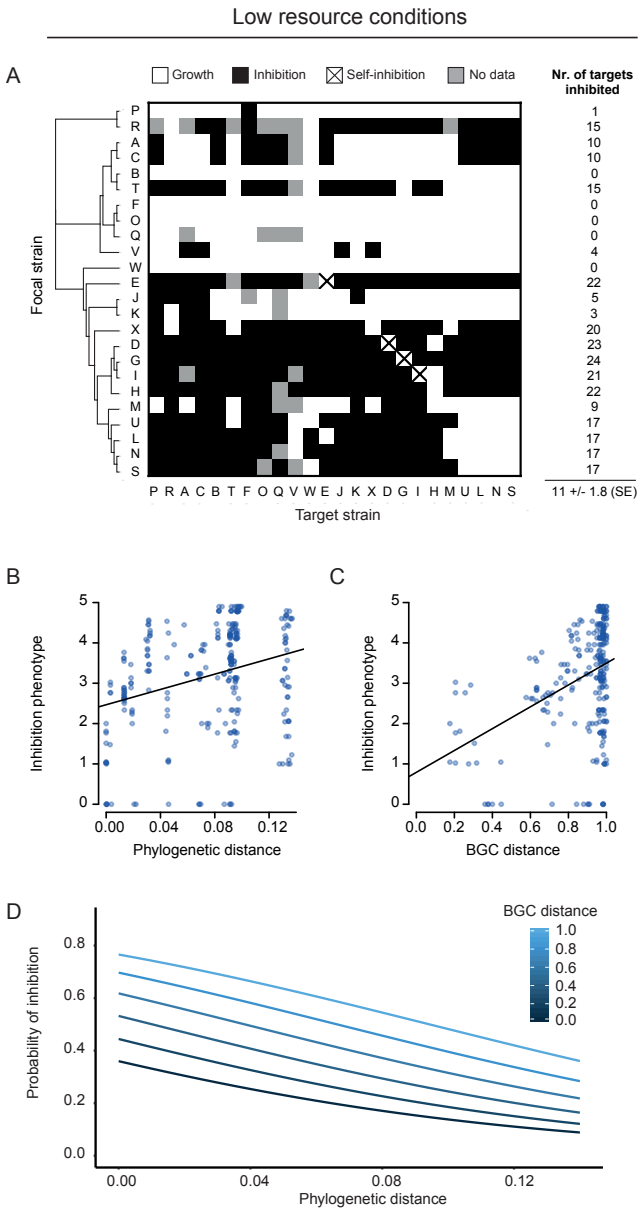

Fig. 4. Constitutive antagonism under low resource conditions (1/10 glycerol concentration). (A) Interaction matrix showing constitutive inhibition sorted by MLST relatedness. Shading is as in Figure 2. (B) Correlation between inhibition phenotype and phylogenetic distance (Mantel test, $P < 0.001$, r = 0.30, N = 552) or (C) biosynthetic gene cluster (BGC) distance (Mantel test, $P < 0.001$, r = 0.39, N = 552). (D) Logistic regression between the probability of inhibition and phylogenetic and biosynthetic gene cluster (BGC) distance ($P_{\text{phylogenetic distance}} < 0.001$, $P_{\text{BGC distance}} < 0.001$, McFadden $R^2 = 0.02$, N = 526).

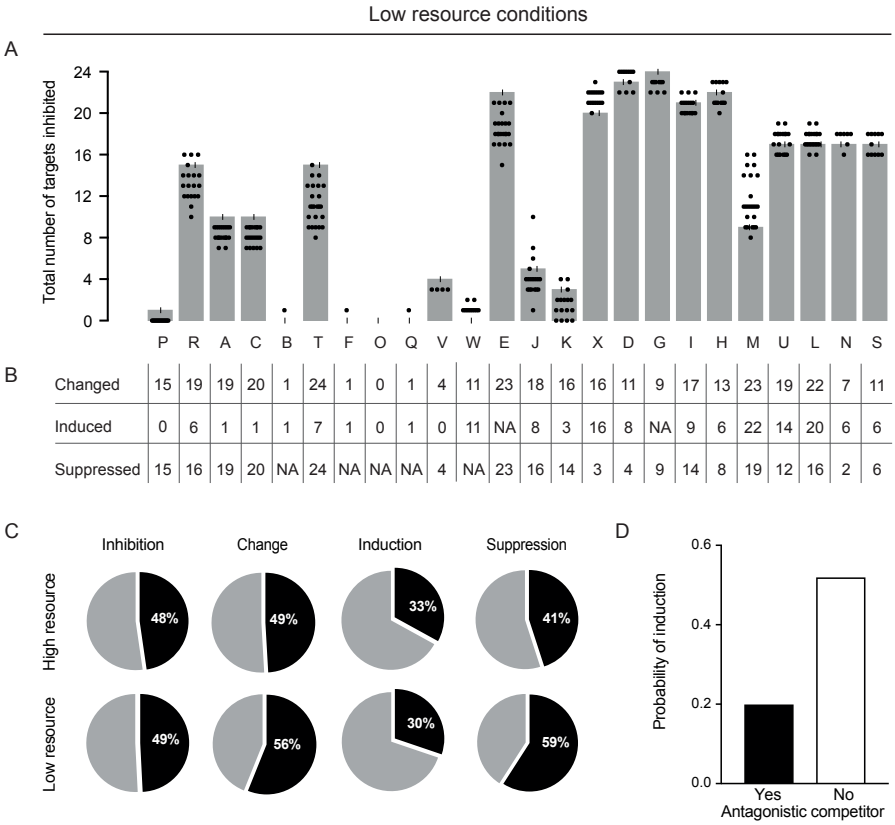

Fig. 5. Altered antagonism during co-culture under low resource conditions (1/10 glycerol concentration). (A) Grey bars indicate the number of strains inhibited by the focal strain when grown alone. Black dots indicate the number of target strains inhibited by the same focal strain when co-cultured with one of the 24 possible modifier strains. (B) Number of modifiers that change, induce or suppress secondary metabolite expression for each focal strain. Cases where suppression is not possible due to the absence of constitutive inhibition are denoted as NA. (C) Comparison of the total amount of inhibition, change in inhibition due to competition, induction and suppression found in low and high resource conditions. (D) The probability of the focal strain showing induction is lower when the competitor is antagonistic to the focal strain (Logistic regression, $P < 0.001$, McFadden $R^2 = 0.12$, N = 419).