Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Antibiotic discovery through microbial interactions

Chengxi Zhang and Paul D Straight

Microorganisms produce biologically active natural products, some of which are useful as antibiotics and other medicines. A great demand for new antibiotics exists due to the diversity of pathogens and their mechanisms of drug resistance. Antibiotics were discovered as natural metabolites that enable a microorganism to suppress the growth of a competitor. Although the pace of discovery has slowed dramatically, new approaches to identifying antibiotics show promise for the future. Among many modern approaches to discovery, co-culturing different species and understanding the molecular bases of their interactions is opening new windows to antibiotic discovery. Here we review several examples to illustrate how co-culturing as an approach is producing new insights into the biology of specialized metabolism. Understanding the varied functions of specialized metabolites, combined with use of innovative and advanced analytical tools, indicates that studies of microbial interactions will enhance the discovery of new antibiotics and other natural products.

Address
Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX, United States

Corresponding author: Straight, Paul D (paul_straight@tamu.edu)

Current Opinion in Microbiology 2019, **51**:64–71

This review comes from a themed issue on **Antimicrobials**

Edited by **Mattew I Hutchings, Andrew W Truman and Barrie Wilkinson**

For a complete overview see the Issue and the Editorial

Available online 22nd July 2019

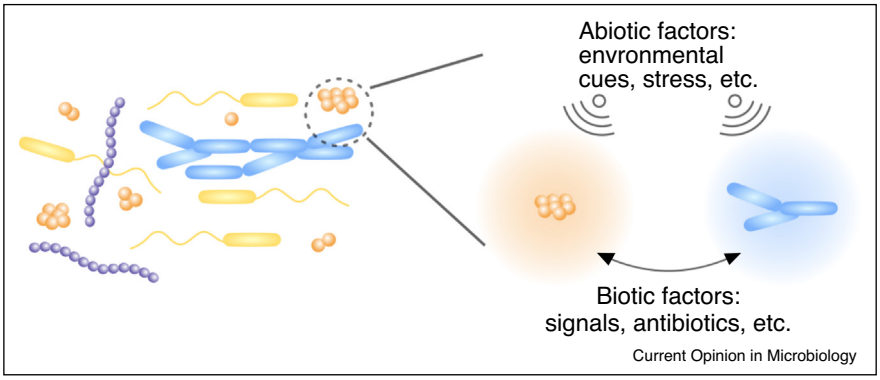
https://doi.org/10.1016/j.mib.2019.06.006

Introduction

Natural products are specialized metabolites for the producing organisms. As opposed to growth-related primary metabolic functions, specialized metabolites support environmental or physiological functions specific to the organisms [1]. Described functions of specialized metabolites include signals, siderophores, antibiotics, and other useful medicines. Among all therapeutically active antibiotics, approximately 90% are discovered from microbes [2–6]. Microbial genome-level studies reveal abundant and diverse biosynthetic gene clusters (BGCs) for the biosynthesis of specialized metabolites [7,8]. However, the vast majority of specialized metabolites are unknowns and have evaded detection due to their obscure functions or lack of production in laboratory cultures [7,9]. In addition to new BGCs, the detection and isolation of previously uncultured species further expand the potential for discovery of chemically diverse specialized metabolites [10,11••]. Given these revelations, the grand challenge for the field is to develop effective strategies for discovering new natural products among these many hidden molecules.

One promising strategy is to revisit the origins of natural product discovery in the same vein of Alexander Fleming, which is to focus on microbial interactions to identify specialized metabolites [12]. In general, the traditional approaches to discovery no longer yield sufficient novelty in antibiotics discovered and do not meet the demand driven by antibiotic resistance [13]. Simply stated, the traditional approach is to isolate metabolites from laboratory cultures and test for growth inhibition of target organisms as established by Waksman and coworkers [14–17]. To advance beyond this operating paradigm, many modern approaches have been explored. One is to consider the varying stimuli that may select for specialized metabolism. In their natural habitats, microbes encounter nutrient limiting conditions and challenges from neighboring species [18]. Microbes have mechanisms to sense and respond to diverse stimuli, such as abiotic environmental cues (e.g. pH, temperature, light, nutrients, and oxidative stress), and biotic stressors (e.g. bacteriocins, antibiotics, siderophores) arising from metabolism of neighboring organisms (Figure 1) [19]. The response mechanisms are many and include changes in specialized metabolism to mediate interactions between organisms [9,18]. Co-culturing species is one approach to identify bioactive metabolites and the relevant responses to them. However, inclusion of one or more additional species to a culture raises the complexity and challenge of isolating a single, active substance. In addition, observation of interspecies interactions commonly requires plate-based or otherwise structured-culture formats, which are cumbersome for traditional microbiology and natural product approaches [20]. Despite these challenges, recent studies of competitive interactions between species highlight their potential for enhancing access to new metabolites [21]. The molecular bases of the interactions are not always clear, but the evidence suggests that either activation of quiescent biosynthetic pathways or detection of competitive effects other than growth inhibition(e.g. changes in colony morphology, virulence, biofilm formation, sporulation) serve to reveal new candidate natural products [9].

Figure 1



Microbial communities as a source of environmental cues, stresses, and signals that influence specialized metabolism.
Microorganisms sense physical and chemical inputs from their surroundings and have diverse response mechanisms. Within the context of a microbial community, these inputs include both abiotic inputs (e.g. pH, temperature, light, nutrients) and biotic cues and signals (e.g. bacteriocins, antimicrobials, siderophores) that emerge from the metabolism of neighboring organisms. A focus on two selected species highlights the potential complexity of external inputs for microorganisms within a community. Contemporary approaches to microbial specialized metabolism include co-culturing selected species in order to expose biotic inputs, in particular specialized metabolites, and to provide new pathways to natural product discovery.

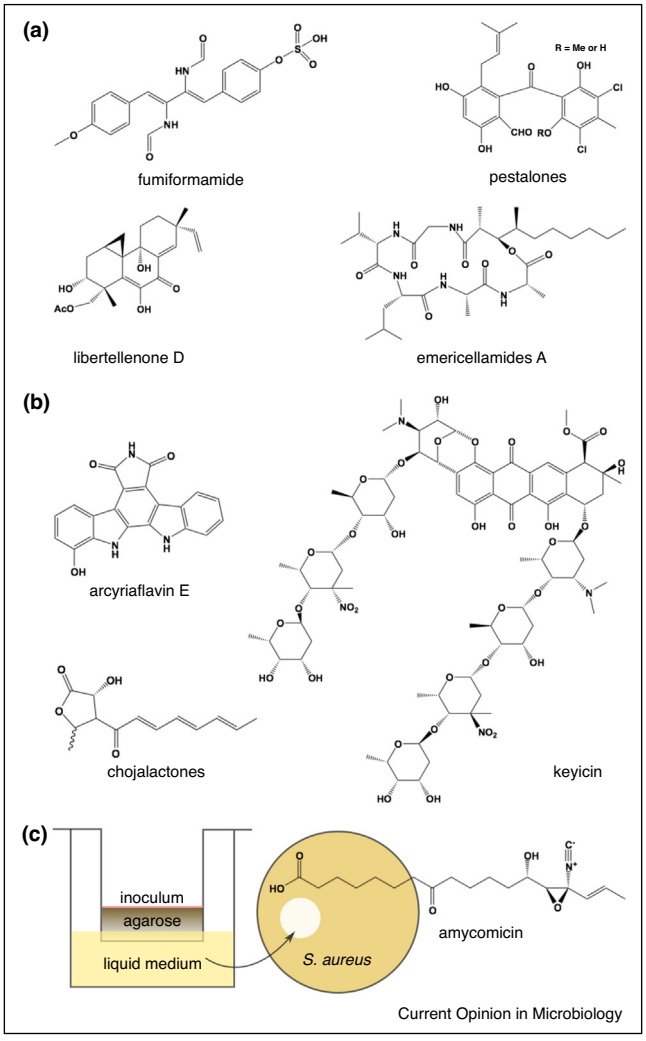
Recent studies indicate that exploration of interactions between microbial species may reveal new bacterial physiology and new specialized metabolites that will contribute to reinvigorated antibiotic discovery. A selection of contemporary review articles provides excellent coverage of several topics that converge on the interactions of species that influence natural product discovery. These articles include two recent reviews that emphasize bacterial–fungal interactions and innovative culturing and detection techniques for the induction of new specialized metabolites [22,23]. In addition, Molloy and Hertweck covered antibiotic discovery inspired by predator–prey interactions, pathogens, insect and other host protection mechanisms [24]. Cheverette and Currie provided a comprehensive review on evolutionary aspects of antibiotic discovery, and discussed the role of antibiotics in nature [25]. Nai and Meyer focused on technological advances in mixed-species culturing that impact specialized metabolism [21]. Ueda and Beppu reviewed instances where antibiotics were discovered using co-culturing methods through chemical stimulation of antibiotic production [26]. These reviews showcase the breadth of topics relevant to modern efforts to invigorate natural product discovery. Here, our intent is to provide a complement to these reviews, rather than a comprehensive review of this extensive field. The emphasis of this review is interactions between different species of bacteria. We will summarize some active specialized metabolites discovered from bacterial–fungal interactions, because this topic has been recently covered in depth [22,23]. We will also discuss innovative approaches to studying specialized metabolism in microbial interactions as means to discover new antibiotics and expand knowledge of specialized metabolism in microbiology.

Interspecies pairwise interactions for antibiotic discovery

Interaction-based assays follow a guiding concept that, as opposed to organisms cultured in isolation, complex interactions better approximate environmental conditions. As a case in point, one organism may serve to condition the environment for a second organism to produce antibiotics. In an approach similar to the process for discovering penicillin through a bacterial–fungal interaction, Zuck, Shipley and Newman co-cultured *Aspergillus fumigatus* with several other organisms on agar plates and showed the most significant inhibitory effect with *Streptomyces peucetius*. By following that competitive phenotype, mixed fermentation of the two organisms led to the isolation of two new natural products, fumiformamide (Figure 2a) and *N,N*'-((1Z,3Z)-1,4-bis(4-methoxyphenyl)buta-1,3-diene-2,3-diyl) diformamide, together with two known *N*-formyl derivatives and the xanthocillin analogue BU-4704 [27]. As an example, this study demonstrated that co-cultivation, creating a competitive environment, is a useful strategy to amplify the production of specialized metabolites. A detailed induction mechanism for these metabolites has yet to be reported.

There are many other active molecules discovered through bacterial–fungal co-culture formats (Figure 2a). For example, pestalones (potent bactericidal against drug-resistant bacteria) [28], libertellenones D (potent cytotoxicity against the HCT-116 human colon carcinoma cancer cell line) [29], emericellamides A (moderate antimicrobial against methicillin-resistant *Staphylococcus aureus*) [30], were identified from marine fungal–bacterial interactions. Glionitrin A, an anti-tumor metabolite, was discovered in the co-culture of a fungal strain with a

Figure 2



Active molecules identified from pairwise microbial interactions. **(a)** Chemical structures of active molecules representative of isolation from bacterial-fungal co-cultivation. **(b)** Chemical structures of molecules representative of isolation from bacterial interspecies interaction. **(c)** A transwell system for continuous microbial community growth (on an agarose plug supported by a permeable membrane), by drawing nutrients from liquid medium and exchange for natural products. The conditioned liquid medium was periodically sampled for antibiotic activity against *S. aureus*. Amycomicin was identified as an inhibitor of *S. aureus*.

bacterial strain from an unusual acidic environment [31], and secopenicillide C was discovered in a co-culture of two fungi from soil [32]. These findings suggest that continued use of bacterial–fungal interactions as a path to discovery will yield new potential antibiotics.

Induced production of natural products has also been observed with bacterial interspecies interactions. *Streptomyces* have been studied extensively for their prolific natural product production [33,34]. With the arrival of whole genome sequencing, new genomic evidence revealed the extent to which unexplored BGCs are embedded in streptomycete genomes [8,35]. The majority of these BGC products remain to be identified. A major goal for the field is to identify efficient methods to coax new specialized metabolites from apparently quiescent BGCs. Onaka and Mori showed *Streptomyces* spp. produced new specialized metabolites when they were co-cultured with the mycolic acid-containing bacterium *Tsukamurella pulmonis* [36••]. Several novel natural products, arcyriaflavin E [37], chojalactones A–C [38] (Figure 2b), niizalactams A–C [39], and 5-alkyl-1,2,3,4-tetrahydroquinolines [40] were identified in co-cultures of *Streptomyces* spp. with *T. pulmonis*. A specific stimulus or competitor may be required for activating cryptic BGCs in some organisms. Thus for some studies, species from the same natural microbial community have been prioritized as stimuli, based on perceived ecological functions of specialized metabolites. Adnani *et al.* identified keyicin, a new anthracycline antibiotic, from a *Micromonospora* sp. when co-cultivated with a sympatric *Rhodococcus* sp. from the sea squirt microbiome. Keyicin is active against some Gram-positive bacteria [41] (Figure 2b). These examples provide ample evidence that mixing two species in a single culture may yield new metabolites. However, rarely have the mechanisms leading to induction been deciphered, which would give a deeper understanding of the biology of specialized metabolism. The underlying mechanisms of interactions will likely inform new approaches to elicit specialized metabolites and enhance subsequent discovery efforts.

Co-culture cooperation-induced antibiotic metabolites

As more interaction studies emerge, it is increasingly clear that specialized metabolites have diverse and undiscovered functions. These functions extend beyond antibiotics and promote the fitness of the producer organisms in poorly understood ways. For example, diffusible metabolites from one organism may stimulate physiological transformations of nearby species. In what is a classic study for the field, Ueda *et al.* observed this type of stimulatory event among *Streptomyces* species [42]. By overlaying *Bacillus subtilis* as an indicator, they found that one *Streptomyces* spp. induced sporulation of a co-cultured *Streptomyces* spp. A zone of *B. subtilis* growth inhibition revealed the induction of antibiotic metabolite(s) only when the two species of *Streptomyces* were in close proximity. A more elaborate version of this experiment led to the recent discovery of a new antibiotic. Pischany *et al.* used multi-species cultivation to generate a large pool of molecules to screen for activity [43••]. Their study employed solid-phase cultures of nine Actinomycetes in transwell plates, which allowed for continuous monitoring without disrupting the community. In this experimental format, bacteria growing on agarose inside a transwell draw nutrients from liquid media and exchange metabolites, which diffuse into the surrounding liquid.

After mixed-species growth in the transwell, the liquid medium was tested for antibiotic activity on *S. aureus* plates (Figure 2c). Once antimicrobial activity was observed, they sampled the 16s rDNA to determine the species composition of the culture mixture. This method is straightforward and can be applied to many cultivable multispecies communities, potentially yielding new antimicrobial or signaling compounds. Indeed, in their study Pishchany *et al.* found that *Streptomyces coelicolor* M145 stimulated *Amycolatopsis* sp. AA4 to produce amycomicin, a new antibiotic that targets fatty acid biosynthesis and kills *S. aureus* in both *in vitro* and *in vivo* assays with submicromolar activity (MIC 30 nM) (Figure 2c). Subsequently, they found that alteration of the carbon source by *S. coelicolor* metabolism induces the production of amycomicin [43••]. These examples suggest that more complex patterns of interaction than binary chemical warfare reflect the dynamic interactions of multispecies microbial communities. Applying knowledge of the molecular bases of complex interactions into new discovery efforts may open the door to unpredicted new specialized metabolites.

Diverse biological functions of specialized metabolites

Specialized metabolites are expected to benefit the fitness of their producers, but in the majority of cases their functions are unknown. Some examples of non-antibiotic functions illustrate this point. For instance, siderophores are specialized metabolites that chelate environmental sources of iron for cellular uptake. Using a co-culture format, Traxler *et al.* found a siderophore, amychelin, produced by *Amycolatopsis* sp. AA4 that inhibits adjacent *S. coelicolor* development of aerial hyphae [44,45]. Because they are needed for iron uptake, some bacteria pirate siderophores produced by competitors [45,46], but this practice can have deleterious effects. Sideromycins are siderophore–antimicrobial conjugates that act by a Trojan horse strategy, where stealing iron may have lethal consequences [47]. Other examples of non-antibiotic activity from specialized metabolites include thiopeptides. Thiopeptides encompass a large and diverse family of ribosomally synthesized and post-translationally modified peptides (RiPPs). Some thiopeptides are antibiotics (e.g. thiocillins) and others are signaling molecules (e.g. goadsporin) that impact bacterial development [48,49]. The thiocillins, for example, were reported to alter development of other species, causing the induction of *B. subtilis* biofilm formation [50]. In our own lab, we have identified some surprising functions for antibiotics. Linearmycins are antibiotics produced by *Streptomyces* [51]. We recently reported that linearmycins are packaged into membrane vesicles and disrupt *B. subtilis* membranes [52,53]. The surprise result for these studies was that biogenesis of extracellular vesicles depends upon linearmycin biosynthesis, highlighting an unpredicted convergence of membranes and antibiotic biosynthesis. In another example, chloramphenicol, a classic antibiotic produced by *Streptomyces venezuelae*, was demonstrated to induce a *B. subtilis* mobile response at subinhibitory concentration [54]. The activation of motility is an example of the underlying principle of hormesis, wherein antibiotics are stimulatory metabolites at subinhibitory concentrations [55,56]. These observations indicate that even the activities of antibiotics as a subclass of specialized metabolites may have complex and unexpected functions, which may be exploited as an approach to discover useful natural products [57,58].

A recent study of human-associated bacteria provides a compelling example of the possibilities to develop new approaches to treat infectious disease based on microbial interactions. This study initiated with an observation of a negative correlation between *S. aureus* and *Bacillus* species in samples from a human population [59••]. Further exploration showed probiotic *Bacillus* abolished and eliminated colonization by *S. aureus* through inhibiting quorum sensing. Remarkably, the study demonstrated that fengycins from *B. subtilis*, commonly known for their antifungal activity, blocked the Agr quorum sensing system of *S. aureus*, thereby disrupting biofilm development and the ability to colonize the host. This study illustrates the capacity for specialized metabolism to influence bacterial pathogenesis in striking and effective, non-classical antibiotic modes. Other reported examples from microbial interactions also suggest a promising approach is to disrupt virulence among pathogens. For instance, 4-hydroxy-2-heptylquinoline-*N*-oxide (HQNO), a molecule promoted by *Pseudomonas aeruginosa* quorum sensing signaling system, suppresses the growth of *S. aureus*, and leads to formation of small-colony variants upon prolonged exposure [60]. As another example, *Streptococcus mutans* and *Streptococcus gordonii* are normally found in inverse proportions in dental plaque, because *S. gordonii* restrains several quorum sensing pathways in *S. mutans* [61,62]. Therefore, using non-growth-inhibiting natural products as medicines or adjuvants may be a broadly applicable tactic to combat infectious agents. The examples we have highlighted collectively point to an exciting and productive next wave of discovery for new natural products and new approaches to treat problematic microorganisms.

Novel strategies facilitate specialized metabolite discovery in microbial interactions

As we develop new interaction-based approaches for discovery, new tools are needed to make isolation and analysis more efficient (Table 1). Historically, antimicrobial drug discovery exploited cultivable soil microorganisms, but even these efforts captured only a fraction of the biological diversity of microbes in nature [35]. In a new development for culturing bacteria, Ling *et al.* used an iCHIP device to isolate previously uncultured bacteria out of complex communities [11••]. The antibiotic teixobactin, identified from this strategy, is the first member of a novel antibiotic class and is active against Gram-positive pathogens [11••,63]. In addition

Table 1

Novel strategies facilitate specialized metabolite discovery in microbial interactions

	Strategies	Molecules	References
Culturing formats	iChip	Teixobactin	[11••]
	Bioreactor system	Undecylprodigiosin	[71]
	Transwell system	Amycomicin	[43••]
	Microfluidic system	*N*-acyl homoserine lactone	[69]
	Transcriptome monitoring	Orsellinic acid	[72•]
Analytical tools	Reporter fusion	Thiocillin, albuquinone A	[50,73]
	IMS	Thiocillin, elaiophylin, efomycins A and G and desferrioxamines	[50,74•,75,83]
	Droplet probe	Dechloro-5′-hydroxygriseofulvin, hirsutatin A, and so on.	[77]
	Mass spec networking	Etimycin, elaiophylin, actinomycins D, X_2, X_{0b}, desferrioxamines	[74•,78,79,83]
	IDBac	Surfactin, plipastatin, desferrioxamine	[82]
	Biochemometric analysis	Altersetin, macrosphelide A	[80]

explorations of other environments, such as marine sediments, suggest that new natural products will follow discovery of new species [64,65]. Metagenomic methods expand views of bacterial genomes as sources for new natural products by previously understudied taxonomic groups [66•]. Along with recognition of the antibiotic-producing potential of microbes, advanced and creative culturing methods facilitate identifying interaction activities. For instance, microfluidics, a technique widely used in large-scale or high-throughput screening, enables focused study of small collections of cells, either single species or more elaborate interactions, during cultivation [20,67–69]. Transwell chambers are a method for maintaining separation of two or more species, or even a microbial community, while allowing their diffusible metabolites to pass through a membrane [43••,70]. For the same purpose, bioreactor systems provide a format for larger scale cultivation [71]. These culturing methods facilitate the study of molecular and chemical interactions between microorganisms at different scales, which may influence the modes of interaction detected and the metabolites available for isolation and further study.

Many new technological innovations show great promise for capturing and identifying new antibiotics. For example, metabolic transcriptome monitoring and other approaches to monitor biosynthetic gene expression may enhance discovery, as in the case of orsellinic acid [72•]. A high-throughput elicitor screening (HiTES) approach, with insertion of a reporter gene into the BGC of interest, provides a rapid read-out for gene expression, leading to the corresponding molecule(s) [73]. Imaging Mass Spectrometry (IMS) enables visualization of the spatial distribution of molecules during microbial interactions, which promote identification of relevant metabolites, as in the cases of elaiophylin [74•], bioconverted phenazines [75], and hydrolyzed surfactin [76]. Droplet-liquid microjunction-surface sampling probes (droplet probe) enable spatial microextraction of specialized metabolites from co-culture samples [77]. Mass spectrometry networking, a tandem mass spectrometry (MS/MS) data organizational approach, allows not only finding molecules with structural similarity to known molecules (same type of biosynthetic pathways), but also new molecules such as retimycin [74•,78,79]. Advanced computational approaches, such as Protein and Small Molecule MALDI BioInformatics (IDBac), MS-based principal component analysis, and other biochemometric analyses, help simplify otherwise complicated data analysis, enhancing efficiency in identifying chemical forms of natural products [80–82].

Given these and many other new technological advances, in combination with powerful computational tools for analyzing datasets and structural details of metabolites, the field is primed for microbial interactions to become a wellspring of new natural product discovery.

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank Reed Stubbendieck for feedback and support during the preparation of this manuscript. Support was provided by funding from the National Science Foundation [MCB-1253215]; and Texas A&M University and the Consejo Nacional de Ciencia y Tecnologia [2017-051].

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Davies J: **Specialized microbial metabolites: functions and origins**. *J Antibiot (Tokyo)* 2013, **66**:361-364.

2. Bérdy J: **Bioactive microbial metabolites**. *J Antibiot (Tokyo)* 2005, **58**:1-26.

3. Katz L, Baltz RH: **Natural product discovery: past, present, and future**. *J Ind Microbiol Biotechnol* 2016, **43**:155-176.

4. Stein T: ***Bacillus subtilis* antibiotics: structures, syntheses and specific functions**. *Mol Microbiol* 2005, **56**:845-857.

5. Broadbent D: **Antibiotics produced by fungi**. *Bot Rev* 1966, **32**:219-242.

6. Davies J, Ryan KS: **Introducing the parvome: bioactive compounds in the microbial world**. *ACS Chem Biol* 2012, **7**:252-259.

7. Baltz RH: **Strain improvement in actinomycetes in the postgenomic era**. *J Ind Microbiol Biotechnol* 2011, **38**:657-666.

8. Nett M, Ikeda H, Moore BS: **Genomic basis for natural product biosynthetic diversity in the actinomycetes**. *Nat Prod Rep* 2009, **26**:1362-1384.

9. Straight PD, Kolter R: **Interspecies chemical communication in bacterial development**. *Annu Rev Microbiol* 2009, **63**:99-118.

10. Lewis K: **Platforms for antibiotic discovery**. *Nat Rev Drug Discov* 2013, **12**:371-387.

11. •• Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schäberle TF, Hughes DE, Epstein S *et al.*: **A new antibiotic kills pathogens without detectable resistance**. *Nature* 2015, **517**:455-459.
This paper utilized iChip to expand the cultivable microbial pool, which leads to identification of a promising antibiotic, teixobactin.

12. Fleming A: **On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae***. *Br J Exp Pathol* 1929, **10**:226-236.

13. Davies J, Davies D: **Origins and evolution of antibiotic resistance**. *Microbiol Mol Biol Rev* 2010, **74**:417-433.

14. Schatz A, Bugie E, Waksman SA: **Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria**. *Proc Soc Exp Biol Med* 1944, **55**:66-69.

15. Waksman SA, Lechevalier HA: **Neomycin, a new antibiotic active against streptomycin-resistant bacteria, including tuberculosis organisms**. *Science (80-)* 1949, **109**:305-307.

16. Newton GGF, Abraham EP: **Cephalosporin C, a new antibiotic containing sulphur and D-α-aminoadipic acid**. *Nature* 1955, **175**:548.

17. Oxford AE, Raistrick H, Simonart P: **Studies in the biochemistry of microorganisms: griseofulvin, C(17)H(17)O(6)Cl, a metabolic product of *Penicillium griseofulvum* dierckx**. *Biochem J* 1939, **33**:240-248.

18. Stubbendieck RM, Straight PD: **Multifaceted interfaces of bacterial competition**. *J Bacteriol* 2016, **198**:2145-2155.

19. Schwartzman JA, Ruby EG: **Stress as a normal cue in the symbiotic environment**. *Trends Microbiol* 2016, **24**:414-424.

20. Goers L, Freemont P, Polizzi KM: **Co-culture systems and technologies: taking synthetic biology to the next level**. *J R Soc Interface* 2014, **11** 20140065.

21. Nai C, Meyer V: **From axenic to mixed cultures: technological advances accelerating a paradigm shift in microbiology**. *Trends Microbiol* 2018, **26**:538-554.

22. de Boer W: **Upscaling of fungal–bacterial interactions: from the lab to the field**. *Curr Opin Microbiol* 2017, **37**:35-41.

23. Netzker T, Flak M, Krespach MK, Stroe MC, Weber J, Schroeckh V, Brakhage AA: **Microbial interactions trigger the production of antibiotics**. *Curr Opin Microbiol* 2018, **45**:117-123.

24. Molloy EM, Hertweck C: **Antimicrobial discovery inspired by ecological interactions**. *Curr Opin Microbiol* 2017, **39**:121-127.

25. Chevrette MG, Currie CR: **Emerging evolutionary paradigms in antibiotic discovery**. *J Ind Microbiol Biotechnol* 2019, **46**:257-271.

26. Ueda K, Beppu T: **Antibiotics in microbial coculture**. *J Antibiot (Tokyo)* 2017, **70**:361-365.

27. Zuck KM, Shipley S, Newman DJ: **Induced production of N-formyl alkaloids from *Aspergillus fumigatus* by co-culture with *Streptomyces peucetius***. *J Nat Prod* 2011, **74**:1653-1657.

28. Cueto M, Jensen PR, Kauffman C, Fenical W, Lobkovsky E, Clardy J: **Pestalone, a new antibiotic produced by a marine fungus in response to bacterial challenge**. *J Nat Prod* 2001, **64**:1444-1446.

29. Oh DC, Jensen PR, Kauffman CA, Fenical W: **Libertellenones A-D: induction of cytotoxic diterpenoid biosynthesis by marine microbial competition**. *Bioorg Med Chem* 2005, **13**:5267-5273.

30. Oh DC, Kauffman CA, Jensen PR, Fenical W: **Induced production of emericellamides A and B from the marine-derived fungus *Emericella* sp. in competing co-culture**. *J Nat Prod* 2007, **70**:515-520.

31. Park HB, Kwon HC, Lee CH, Yang HO: **Glionitrin A, an antibiotic-antitumor metabolite derived from competitive interaction between abandoned mine microbes**. *J Nat Prod* 2009, **72**:248-252.

32. Nonaka K, Abe T, Iwatsuki M, Mori M, Yamamoto T, Shiomi K, Ômura S, Masuma R: **Enhancement of metabolites productivity of *Penicillium pinophilum* FKI-5653, by co-culture with *Trichoderma harzianum* FKI-5655**. *J Antibiot (Tokyo)* 2011, **64**:769-774.

33. Craney A, Ahmed S, Nodwell J: **Towards a new science of secondary metabolism**. *J Antibiot (Tokyo)* 2013, **66**:387-400.

34. Clardy J, Fischbach MA, Walsh CT: **New antibiotics from bacterial natural products**. *Nat Biotechnol* 2006, **24**:1541-1550.

35. Baltz RH: **Renaissance in antibacterial discovery from actinomycetes**. *Curr Opin Pharmacol* 2008, **8**:557-563.

36. •• Onaka H, Mori Y, Igarashi Y, Furumai T: **Mycolic acid-containing bacteria induce natural-product biosynthesis in *Streptomyces* species**. *Appl Environ Microbiol* 2011, **77**:400-406.
This paper first demonstrated that mycolic acid-containing bacteria could influence the biosynthesis of cryptic natural products in *Streptomyces* species. The following 4 references showed examples of induced natural products from this type of interaction.

37. Hoshino S, Zhang L, Awakawa T, Wakimoto T, Onaka H, Abe I: **Arcyriaflavin E, a new cytotoxic indolocarbazole alkaloid isolated by combined-culture of mycolic acid-containing bacteria and *Streptomyces cinnamoneus* NBRC 13823**. *J Antibiot (Tokyo)* 2015, **68**:342-344.

38. Hoshino S, Wakimoto T, Onaka H, Abe I: **Chojalactones A-C, cytotoxic butanolides isolated from *Streptomyces* sp. cultivated with mycolic acid containing bacterium**. *Org Lett* 2015, **17**:1501-1504.

39. Hoshino S, Okada M, Wakimoto T, Zhang H, Hayashi F, Onaka H, Abe I: **Niizalactams A-C, multicyclic macrolactams isolated from combined culture of *Streptomyces* with mycolic acid-containing bacterium**. *J Nat Prod* 2015, **78**:3011-3017.

40. Sugiyama R, Nishimura S, Ozaki T, Asamizu S, Onaka H, Kakeya H: **5-Alkyl-1,2,3,4-tetrahydroquinolines, new membrane-interacting lipophilic metabolites produced by combined culture of *Streptomyces nigrescens* and *Tsukamurella pulmonis***. *Org Lett* 2015, **17**:1918-1921.

41. Adnani N, Chevrette MG, Adibhatla SN, Zhang F, Yu Q, Braun DR, Nelson J, Simpkins SW, McDonald BR, Myers CL *et al.*: **Coculture of marine invertebrate-associated bacteria and interdisciplinary technologies enable biosynthesis and discovery of a new antibiotic, keyicin**. *ACS Chem Biol* 2017, **12**:3093-3102.

42. Ueda K, Kawai S, Ogawa H, Kiyama A, Kubota T, Kawanobe H, Beppu T: **Wide distribution of interspecific stimulatory events on antibiotic production and sporulation among *Streptomyces* species**. *J Antibiot (Tokyo)* 2000, **53**:979-982.

43. •• Pishchany G, Mevers E, Ndousse-Fetter S, Horvath DJ, Paludo CR, Silva-Junior EA, Koren S, Skaar EP, Clardy J, Kolter R: **Amycomicin is a potent and specific antibiotic discovered with a targeted interaction screen**. *Proc Natl Acad Sci U S A* 2018, **115**:10124-10129.

An intact antibiotic discovery paper inspired by microbial community interactions. It illustrated a new interaction monitoring system, activation of an inactivated BGC, and antibiotic activity against pathogens.

44. Seyedsayamdost MR, Traxler MF, Zheng SL, Kolter R, Clardy J: **Structure and biosynthesis of amychelin, an unusual mixed-ligand siderophore from *Amycolatopsis* sp. AA4**. *J Am Chem Soc* 2011, **133**:11434-11437.

45. Traxler MF, Seyedsayamdost MR, Clardy J, Kolter R: **Interspecies modulation of bacterial development through iron competition and siderophore piracy**. *Mol Microbiol* 2012, **86**:628-644.

46. Galet J, Deveau A, Hôtel L, Frey-Klett P, Leblond P, Aigle B: ***Pseudomonas fluorescens* pirates both ferrioxamine and ferricoelichelin Siderophores from *Streptomyces ambofaciens***. *Appl Environ Microbiol* 2015, **81**:3132-3141.

47. Ghosh M, Miller PA, Möllmann U, Claypool WD, Schroeder VA, Wolter WR, Suckow M, Yu H, Li S, Huang W *et al.*: **Targeted antibiotic delivery: selective siderophore conjugation with daptomycin confers potent activity against multidrug resistant *Acinetobacter baumannii* both in vitro and in vivo**. *J Med Chem* 2017, **60**:4577-4583.

48. Wieland Brown LC, Acker MG, Clardy J, Walsh CT, Fischbach MA: **Thirteen posttranslational modifications convert a 14-residue peptide into the antibiotic thiocillin**. *Proc Natl Acad Sci U S A* 2009, **106**:2549-2553.

49. Igarashi Y, Kana Y, Fujiib K, Fujitaa T, Haradab K-I, Naokia H, Tabata H, Onaka H, Furumai T: **Goadsporin, a chemical substance which promotes secondary metabolism and morphogenesis in *Streptomycetes***. *J Antibiot (Tokyo)* 2001, **54**:1045-1053.

50. Bleich R, Watrous JD, Dorrestein PC, Bowers AA, Shank EA: **Thiopeptide antibiotics stimulate biofilm formation in *Bacillus subtilis***. *Proc Natl Acad Sci U S A* 2015, **112**:3086-3091.

51. Stubbendieck RM, Straight PD: **Escape from lethal bacterial competition through coupled activation of antibiotic resistance and a mobilized subpopulation**. *PLoS Genet* 2015, **11**:1-27.

52. Stubbendieck RM, Brock DJ, Pellois JP, Gill JJ, Straight PD: **Linearmycins are lytic membrane-targeting antibiotics**. *J Antibiot (Tokyo)* 2018, **71**:372-381.

53. Hoefler BC, Stubbendieck RM, Josyula NK, Moisan SM, Schulze EM, Straight PD: **A link between linearmycin biosynthesis and extracellular vesicle genesis connects specialized metabolism and bacterial membrane physiology**. *Cell Chem Biol* 2017, **24**:1238-1249.

54. Liu Y, Kyle S, Straight PD: **Antibiotic stimulation of a *Bacillus subtilis* migratory response**. *mSphere* 2018, **3**:1-13.

55. Davies J, Spiegelman GB, Yim G: **The world of subinhibitory antibiotic concentrations**. *Curr Opin Microbiol* 2006, **9**:445-453.

56. Bernier SP, Surette MG: **Concentration-dependent activity of antibiotics in natural environments**. *Front Microbiol* 2013, **4**:1-14.

57. Romero D, Traxler MF, López D, Kolter R: **Antibiotics as signal molecules**. *Chem Rev* 2011, **111**:5492-5505.

58. Yim G, Wang HH, Davies J: **Antibiotics as signalling molecules**. *Philos Trans R Soc B Biol Sci* 2007, **362**:1195-1200.

59. •• Piewngam P, Zheng Y, Nguyen TH, Dickey SW, Joo H-S, Villaruz AE, Glose KA, Fisher EL, Hunt RL, Li B *et al.*: **Pathogen elimination by probiotic Bacillus via signalling interference**. *Nature* 2018, **562**:532-537.

This paper initiated with an observation of bacterial colonization in human hosts, followed by detailed elucidation of action and mechanism, and proposed additional roles for fengycins.

60. Hoffman LR, Deziel E, D'Argenio DA, Lepine F, Emerson J, McNamara S, Gibson RL, Ramsey BW, Miller SI: **Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa***. *Proc Natl Acad Sci* 2006, **103**:19890-19895.

61. Wang BY, Kuramitsu HK: **Interactions between oral bacteria: inhibition of *Streptococcus mutans* bacteriocin production by *Streptococcus gordonii***. *Appl Environ Microbiol* 2005, **71**:354-362.

62. Kuramitsu HK, He X, Lux R, Anderson MH, Shi W: **Interspecies interactions within oral microbial communities**. *Microbiol Mol Biol Rev* 2007, **71**:653-670.

63. Homma T, Nuxoll A, Gandt AB, Ebner P, Engels I, Schneider T, Götz F, Lewis K, Conlon BP: **Dual targeting of cell wall precursors by teixobactin leads to 2 cell lysis**. *Antimicrob Agents Chemother* 2016, **60**:6510-6517.

64. Mincer TJ, Jensen PR, Kauffman CA, Fenical W: **Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments**. *Appl Environ Microbiol* 2002, **68**:5005-5011.

65. Tuttle RN, Demko AM, Patin N, Kapono CA, Donia MS, Dorrestein P, Jensen PR: **The detection of natural products and their producers in ocean sediments**. *Appl Environ Microbiol* 2019, **85** e02830-18.

66. • Crits-Christoph A, Diamond S, Butterfield CN, Thomas BC, Banfield JF: **Novel soil bacteria possess diverse genes for secondary metabolite biosynthesis**. *Nature* 2018, **558**:440-444.

This paper showed combined metagenomic and metatranscriptomic analyses for novel soil bacteria and their specialized metabolic potentials.

67. Kim HJ, Boedicker JQ, Choi JW, Ismagilov RF: **Defined spatial structure stabilizes a synthetic multispecies bacterial community**. *Proc Natl Acad Sci U S A* 2008, **105**:18188-18193.

68. El-Ali J, Sorger PK, Jensen KF: **Cells on chips**. *Nature* 2006, **442**:403-411.

69. Bai Y, Patil SN, Bowden SD, Poulter S, Pan J, Salmond GPC, Welch M, Huck WTS, Abell C: **Intra-species bacterial quorum sensing studied at single cell level in a double droplet trapping system**. *Int J Mol Sci* 2013, **10570–10581**.

70. Miki Y, Ono K, Hata S, Suzuki T, Kumamoto H, Sasano H: **The advantages of co-culture over mono cell culture in simulating in vivo environment**. *J Steroid Biochem Mol Biol* 2012, **131**:68-75.

71. Luti KJK, Mavituna F: **Elicitation of *Streptomyces coelicolor* with *E. coli* in a bioreactor enhances undecylprodigiosin production**. *Biochem Eng J* 2011, **53**:281-285.

72. • Schroeckh V, Scherlach K, Nützmann H-W, Shelest E, Schmidt-Heck W, Schuemann J, Martin K, Hertweck C, Brakhage AA: **Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans***. *Proc Natl Acad Sci U S A* 2009, **106**:14558-14563.

This paper showed bacterial–fungal physical contact is required for induction of a molecule. It also described the usage of transcriptome monitoring for BGC expressions.

73. Xu F, Nazari B, Moon K, Bushin LB, Seyedsayamdost MR: **Discovery of a cryptic antifungal compound from *Streptomyces albus* j1074 using high-throughput elicitor screens**. *J Am Chem Soc* 2017, **139**:9203-9212.

74. • Boya CA, Fernández-Marín H, Mejiá LC, Spadafora C, Dorrestein PC, Gutiérrez M: **Imaging mass spectrometry and MS/MS molecular networking reveals chemical interactions among cuticular bacteria and pathogenic fungi associated with fungus-growing ants**. *Sci Rep* 2017, **7**:1-13.

This paper utilized a combination of IMS and molecular networking strategies to study and demonstrate the chemistry during bacterial interactions.

75. Bandeira N, Dorrestein PC, Wu C-H, Duggan BM, Cornett DS, Phelan VV, Moree WJ: **Interkingdom metabolic transformations captured by microbial imaging mass spectrometry**. *Proc Natl Acad Sci U S A* 2012, **109**:13811-13816.

76. Hoefler BC, Gorzelnik KV, Yang JY, Hendricks N, Dorrestein PC, Straight PD: **Enzymatic resistance to the lipopeptide surfactin as identified through imaging mass spectrometry of bacterial competition**. *Proc Natl Acad Sci U S A* 2012, **109**:13082-13087.

77. Knowles SL, Raja HA, Wright AJ, Lee AML, Caesar LK, Cech NB, Mead ME, Steenwyk JL, Ries LNA, Goldman GH *et al.*: **Mapping the fungal battlefield: using in situ chemistry and deletion mutants to monitor interspecific chemical interactions between fungi**. *Front Microbiol* 2019, **10**:285.

78. Duncan KR, Crüsemann M, Lechner A, Sarkar A, Li J, Ziemert N, Wang M, Bandeira N, Moore BS, Dorrestein PC *et al.*: **Molecular**

networking and pattern-based genome mining improves discovery of biosynthetic gene clusters and their products from salinispora species. *Chem Biol* 2015, **22**:460-471.

79. Quinn RA, Nothias LF, Vining O, Meehan M, Esquenazi E, Dorrestein PC: **Molecular networking as a drug discovery, drug metabolism, and precision medicine strategy**. *Trends Pharmacol Sci* 2017, **38**:143-154.

80. Raja HA, Kellogg JJ, Egan JM, Todd DA, Kvalheim OM, Oberlies NH, Cech NB: **Biochemometrics for natural products research: comparison of data analysis approaches and application to identification of bioactive compounds**. *J Nat Prod* 2016, **79**:376-386.

81. Hou Y, Bugni T, Wyche T, Braun D, Chanana S, Thomas C: **Natural product discovery using planes of principal component analysis in R (PoPCAR)**. *Metabolites* 2017, **7**:34.

82. Clark CM, Costa MS, Sanchez LM, Murphy BT: **Coupling MALDI-TOF mass spectrometry protein and specialized metabolite analyses to rapidly discriminate bacterial function**. *Proc Natl Acad Sci U S A* 2018, **115**:4981-4986.

83. Traxler MF, Watrous JD, Alexandrov T, Dorrestein PC: **Interspecies interactions stimulate diversification of the *Streptomyces coelicolor* secreted metabolome**. *mBio* 2013, **4**:1-12.