SHORT COMMENTARY

Biological hotspots in oceans as unique reservoirs for novel antibiotics

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ABSTRACT

Oceans span over 70% of the Earth's surface. The wide range of environments coupled with the large diversity of organisms have creatively-shaped unique biological interactions. Antibiotics are forms of natural products synthesized mostly by bacteria to influence or terminate other bacteria, and whose production is stimulated through biological interactions. Antibiotics are best-known for their clinical and agricultural purposes. However, their effectiveness is rapidly diminishing because overuse and misuse has driven a proliferation of resistance and resulted in an emerging crisis of multidrug-resistant (MDR) pathogens. A critical requisite for controlling MDR pathogens is through discovery of new antibiotics having novel mechanisms of action (MOA). Since most present-day antibiotics are derived from a relatively narrow range of soil bacteria isolated decades ago, there has been recent emphasis to isolate novel antibiotics from unique environments among the Earth's vast microbiome.

Ocean systems offer a strong potential as reservoirs for novel antibiotics. This commentary argues that this will occur primarily in areas of intense biological interactions; where microbes interact either with each other or with plants and animals. Such interactions include animal symbioses; microbe-microbe interactions in hypersaline microbial mats; surface biofilms of marine plants and vertebrate/invertebrate animals; and the internal microbiomes of host animals. Discovery has progressed from screening of cultured bacterial-isolates to a growing arsenal of more-recent approaches: 1) probing metagenomes and transcriptomes; 2) induction of "silent genes"; 3) exploiting ecological relationships of natural bacteria- those not observed under pure-culture conditions; and 4) chemical genomics targeting conserved pathways in bacterial physiology. Given recent advances in sequencing, bioinformatics, and gene manipulation, these capabilities are becoming increasingly feasible. The relatively untapped microbial reservoirs of oceans offer a potentially-limitless source for isolation of novel antibiotics, and whose ecological interactions may be used to advance our fundamental understanding of bacteria.

Key Words: *Biofilms; Microbial mats; Antibiotics; MDR; EPS*

INTRODUCTION

The Earth's entire microbiome has been estimated on the order of 1030 cells, having a biomass of 1017 g (1) and consisting of over a trillion species (2). This microbiome produces a wide range of natural products having potential bioactivity. Antibiotics are a form of small-molecule 'natural products', most often produced by bacteria to limit growth or even kill other bacteria (3). Surprisingly, our present-day arsenal of antibiotics, which are used to treat human infections, are derived from only a small fraction of the Earth's bacterial species that were isolated from soils many decades ago. This small arsenal is now failing and is predicted to result in over 10 million human deaths annually by year 2050 (4). This has encouraged researchers to look elsewhere in the Earth's vast microbiome for new antibiotics having novel mechanisms of action (5,6). Accomplishing this will require us to enhance our fundamental understanding of how this vast microbial reservoir interacts under natural conditions.

Ocean systems contain an incredible diversity of plants and animals. Oceans also encompass a seemingly unlimited reservoir of known, and mostly-unknown, microbial species. Estimates propose over 10^{13} bacteria are present in oceans. Since bacteria produce antibiotics, though not under all conditions, this represents a tremendous genetic potential for antibiotics (7-9). In open ocean environments, bacteria exist as single-cells or in aggregate-associated communities. Yet, owing to nutrient limitations in open waters, many bacteria live in concentrated microbial 'hotspots'; areas where occur intense biological interactions. These may include close associations with mobile (e.g. fish, squid, mammals etc.) and attached animals (e.g. corals, tunicates,), as well as plants (both macro- and micro-algae), and in dense aggregations known as microbial mats. Here, it is argued that these microbial hotspots by their ecological nature will predispose bacteria to produce localized reservoirs of new antibiotics, many that may possess novel mechanisms of action, when compared with our small current arsenal of antibiotics.

Microbial hotspots and antibiotic production

Environments, which contain large diversities of microorganisms, or specialized environments such as symbiotic relationships, have creatively shaped unique biological interactions. Antibiotics production is results from these biological interactions because bacteria are attempting to limit other bacteria and their activities.

Certain bacterial isolates may be grown planktonically (i.e. suspended in liquid culture) in the laboratory and may produce antibiotics under these conditions. However, under natural conditions many bacteria grow as attached biofilms where cells are surrounded in a secreted matrix of extracellular polymer substances or EPS (10-12). This is a common strategy used by bacteria to localize and concentrate extracellular enzymes, plasmids, chemical signals etc., where they can be utilized in an efficient manner. The diffusion-slowing properties can also localize important proximal molecules, such as antibiotics, close to the cells that have secreted them (13,14).

Microbial mats: Microbial mats are larger-scale examples of biofilms, where high diversities of bacteria and archaea occur in proximity and are often horizontally stratified, often in response to light gradients. While they coexist over relatively small spatial scales, the coexistence is not entirely complementary, and much antagonism is present. In microbial mats living under the extreme conditions of hypersaline ponds, for example, salinities can exceed 300 g L^{-1} (NaCl), and the mats are often subjected to intermittent desiccation (15). Species richness of bacteria in these mats often exceeds thousands of OTU's (i.e. operational taxonomic units) per cm^3. This type of diversity living in proximity generates a great deal of chemical interaction, including quorum sensing, and antibiotic production. The resident microbial flora in these environments likely harbor the genetic capabilities to produce many natural product compounds having antimicrobial properties (16,17). Microbial mats also occur in other near-shore intertidal environments, as epipontic communities on the underside of ice in arctic and Antarctic environments, and deep sea hydrothermal vent communities.

Biofilms: Biofilms, a more condensed for of microbial mat, form on most surfaces in seawater (18) and served important roles on the surfaces of host animals (e.g. fish scales) and plants (e.g. macroalgae). They form protective biofilms that prevent other (pathogenic) bacteria from entering the host through their surfaces. Human skin has protective biofilm communities that serve similar protective effects against invasion by pathogens (11). These host surface communities represent likely places to detect unique growth-inhibiting compounds, which are produced and localized by the protective commensal biofilms.

Inhibition of bacterial quorum sensing: The surfaces of many macroalgae have limited biofilm coverage on their outer surfaces (19). While some marine macroalgae-associated microbial communities contain diverse and abundant

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sources of antifouling and other bioactive compounds (20,21). Antimicrobial compounds that prevent biofilm formation can be produced by the surfaces of or within the macroalgae itself (22). It is now realized that certain natural product compounds that interfere with bacterial chemical communication (i.e. quorum sensing) may be responsible for biofilm inhibition, either directly or indirectly. Studies are addressing environmentally-friendly antifouling compounds that include fatty acids, lipopeptides, amides, alkaloids, lactones, steroids, terpenoids and pyrroles (22). Therefore, biofilms will be a primary location for antibiotic production and discovery.

Bacteria living within animals: It is also a unique source of antibiotics (23). The host environment (e.g. gut, light organs, interstitial tissues) provides locations for commensal, and in some cases, symbiotic relationships with microorganisms. The production of antibiotics is thought to maintain specific populations of bacteria, which work efficiently for the host animal. This has been observed in a wide range of marine animals, including sponges (24-26), tunicates (27-30), corals, Mollusca (31), bryozoans, and crustaceans.

Ecological multifunctional roles of antibiotics: Antibiotics are most-commonly known for their clinical properties to kill or limit infections, they are also produced for additional ecological purposes under natural conditions. When a natural product molecule, such as an antibiotic, is produced under natural conditions, it will likely have other ecologically-relevant roles to the bacterium besides that, which is obvious to investigators. Recent explorations of sub-lethal concentrations of certain antibiotics indicate they can have roles in cell-cell signaling and even exert changes in gene expression in the responding bacteria cell, like those of quorum sensing (32-34). For this reason, it has been hypothesized that antibiotics may have evolved (in bacteria) as cell-cell chemical signals. Similarly, well studied quorum sensing signals, such as AHLs (acylhomoserine lactones), which mediate cell-cell signaling in many Gram-negative bacteria, have been shown to have lethal (i.e. antibiotic-like) effects on certain Gram-positive cells (35). At present, limited major conclusions can be drawn from these studies, but certainly highlight the co-evolving ecological roles of antibiotics and cell-cell chemical signaling.

Approaches for discovery of novel antibiotic compounds

The classical approach for finding new antibiotics has been to isolate cultures of bacteria, then examine their exudates for antimicrobial activities against other bacterial strains. Once a compound is identified and its structure determined as unique (i.e. compared to known compounds), then it is tested against known pathogens using minimum inhibitory concentration (MIC) tests (36).

It is not surprising, therefore, that several major limiting steps occur in the antibiotic discovery process. First, we are limited in isolating, culturing, and testing many natural bacteria, since the vast majority of natural bacterial species or strains are unculturable using present-day approaches. Second, we have not understood the full antibiotic-producing capabilities of a given bacterium because that reflects its natural environment (i.e. a given bacterium may produce different antibiotics in response to different conditions). A third limitation has occurred in understanding the potential mechanism(s) of action (MOA) of new antibiotics. Together, this has largely limited what is known regarding the genes and antibiotic-producing capabilities of the bugs.

Major mechanism of action: A truly novel antibiotic, to be used against human infections, must ideally inhibit a critical bacterial pathway(s) in a new and different manner, compared with presently-used antibiotics, so resistant pathogens will be susceptible. Antibiotics are categorized into several (approximately five) major groupings, depending on their compositions and/or mechanisms of action (MOA) on the bacterial cell. These are: (1) Beta-Lactams; (2) Macrolides; (3) Fluoroquinolones; (4) Tetracyclines; and (5) Aminoglycosides. This has been reviewed extensively elsewhere (37). There are five major bacteria targets of attack by antibiotics: 1 splitting of cell membranes; 2 inhibition of cell wall synthesis; 3 inhibitions of RNA syntheses; 4 Inhibition of protein syntheses; and 5 inhibition of DNA syntheses. These have been reviewed, in detail elsewhere (3). There are also other mechanisms of action, some of which are currently outside the realm of immediate resistance in human infections. However, since bacteria produce antibiotics, the same bacterium will inherently possess a resistance mechanism to those antibiotics, which can later be passed to pathogens. Molecular-based approaches continue to be refined and are offering a new window into the workings of antibiotic production by bacteria (38). Chemical genomics approaches are a relatively new and rapidly-evolving area, which help to determine how genetic interactions occur in a bacterium when exposed to a potentially new antibiotic or another bioactive compound (39,40). This group of approaches can involve fluorescent promoter-reporter libraries (41), next-generation deep sequencing of transcriptomes using RNA-seq (42) to determine changes in gene expression, through both coding- and non-coding RNA, in response to an antibiotic, and other approaches. These will become less costly over the next several years. These approaches can reveal physiological responses of essential cell processes to antibiotic exposure and will hasten the discovery of antimicrobial compounds having novel MOA. It is now possible to isolate genomes from single cells, or to examine the metagenomes of entire microbial communities in nature. This allows probing for known antibiotic-producing gene clusters. The products of *in situ* bacterial communities can also be separated from cells and examined relatively quickly (43). High-throughput screening approaches can screen hundreds to thousands of potential compounds for antimicrobial activity within days (44,41).

Metagenomic studies: Metagenomic studies of community-associated genes, has allowed the search for the genetic potential to produce compounds similar to those that are already known or understood. However, bacteria, through the selective pressures of natural conditions, have been the architects of unanticipated antimicrobial molecular designs (45). Therefore, it has been difficult to assess and understand the full potential of genes in complex natural communities, simply because many are unrecognized at present.

Ecological roles of antibiotics under natural conditions and the silent genome

Understanding the ecological role(s) of antibiotics under *in situ* conditions will improve our fundamental understanding of bacteria. Antibiotics are most often produced by bacteria to inhibit the growth and/or destroy bacterial and other microorganisms. There is growing evidence that low concentrations of some antibiotics can act as signaling molecules, which can coordinate the activities of bacteria within complex biofilms (33). It is the ambient conditions and ecological interactions of bacteria which often trigger differential expression of antibiotics (46,47).

It is now recognized that a given bacterial strain, when placed under different conditions, will often produce different natural product compounds compared with those obtained under standard culture conditions (48). Exposure of a bacterium to sublethal concentrations of a given antibiotic often responds by the upregulation and production of a different, often novel antibiotic. This has led to the idea of the 'silent genome'; upregulation of a portion of chromosomal genes that are not typically expressed under standard culture conditions.

Stimulating new secondary metabolites via silent genes upregulation using exposures of bacteria to stressors is a relatively new area of investigation. A growing consensus is that many or most bacteria possess inherent, but seldom expressed, capabilities of antibiotic production. This likely represents an ecological strategy for surviving in the presence of other microbes, within their community. It is not surprising, therefore, that when bacteria are cultured (in the laboratory) in the presence of other bacteria, their metabolite production often changes (49). New approaches, such as stimulation by rare-earth metal supplements have been used to activate silent genes (50). It is now realized that most antibiotic-producing bacteria, only produce a subset of their potential. In the absence of the proper environmental cues, many natural product genes are down-regulated or silenced entirely. This is because natural bacteria contain a reservoir of antibiotics that can be called upon as needed (51). A major challenge in harnessing ocean microbes will be in how to stimulate upregulation of new antibiotics.

REFERENCES

1. McMahon S, Parnell J. Weighing the deep continental biosphere. FEMS Microbiol Ecol. 2014;87:113-20.
2. Locey KJ, Lennon JT. Scaling laws predict global microbial diversity. Proc Natl Acad Sci. 2016;113:5970-75.
3. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74:417-33.
4. World Health Organization (WHO). Global Priority list of Antibiotic-resistant Bacteria to Guide Research, Discovery and Development of New Antibiotics. 2017.
5. Demain AL. Importance of microbial natural products and the need to revitalise their discovery. J Ind Microbiol Biotechnol. 2014;41:185-201.
6. Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. Nature. 2016;529:336-43.

7. Faulkner DJ. Marine natural products. Nat Prod Rep. 2001;18:1-49.
8. Fenical W, Jensen PR. Developing a new resource for drug discovery: Marine actinomycete bacteria. Nat Chem Biol. 2006;2:666-73.
9. Blunt JW, Copp BR, Keyzers RA, et al. Marine natural products. Nat Prod Rep. 2013;30:237-323
10. Decho AW. Microbial exopolymer secretions in ocean environments: their role (s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev. 1990;28:73-153.
11. Hall-Stoodley L, Costerton JW, Stoodley P, et al. Bacterial biofilms: from the natural environment to infectious disease. Nat Rev Microbiol 2004;2:95-108.
12. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8:623-33.
13. Decho AW. Localization of quorum sensing by extracellular polymeric substances (EPS): considerations of *in situ* signaling. In: The physical basis of bacterial quorum communication. S.J. Hagen (ed.), Biological and Medical Physics, Biomedical Engineering. 2015;1:105-21.
14. Decho AW, Gutierrez T. Microbial Extracellular Polymeric Substances (EPS) in ocean systems. Front Microbiol. 2017;8:922.
15. Paerl HW, Pinckney JL, Steppe TF. Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environ Microbiol. 2000;2:11-26.
16. Kim J, Shin D, Kim SH, et al. Borrelidins C–E: New Antibacterial Macrolides from a Saltern-Derived Halophilic Nocardiopsis sp. Mar Drugs. 2017;15:166.
17. Duangmal K, Suksaard P, Pathom-aree W, et al. Actinopolyspora salinaria sp nov a halophilic actinomycete isolated from solar saltern soil. Int J Syst Evol Microbiol. 2016;66:1660-65.
18. Vogel MB, Des Marais DJ, Turk KA, et al. The Role of Biofilms in the Sedimentology of Actively Forming Gypsum Deposits at Guerrero Negro, Mexico. Astrobiology. 2009;9:875-93.
19. Holmström C, Egan S, Franks A, et al. Antifouling activities expressed by marine surface associated Pseudoalteromonas species. FEMS Microbiol Ecol. 2002;41:47-58.
20. Egan S, Harder T, Burke C. The seaweed holobiont: understanding seaweed-bacteria interactions. FEMS Microbiol Rev. 2013;37:462-76.
21. Singh RP, Kumari P, Reddy CRK. Antimicrobial compounds from seaweed-associated bacteria and fungi. Appl Microbiol Biotechnol. 2015;99:1571-86.
22. Dahms HU, Dobretsov S. Antifouling compounds from marine macroalgae. Mar Drugs. 2017;15:265.
23. König GM, Kehraus S, Seibert SF, et al. Natural products from marine organisms and their associated microbes. ChemBioChem. 2006;7:229-38.
24. Esquenazi E, Coates C, Simmons L, et al. Visualizing the spatial distribution of secondary metabolites produced by marine cyanobacteria and sponges via MALDI-TOF imaging. Mol Biosys. 2008;4:562–70.
25. Erwin PM, Olson JB, Thacker RW. Phylogenetic diversity, host-specificity and community profiling of sponge-associated bacteria in the northern Gulf of Mexico. PLoS ONE. 2011;6:e26806.
26. Inbaneson SJ, Ravikumar S. *In vitro* antiplasmodial activity of bacterium RJAUTHB 14 associated with marine sponge Haliclona Grant against Plasmodium falciparum. Parasitol Res. 2012;110:2255-62.
27. Hirose E, Uchida H, Murakami A. Ultrastructural and microspectrophotometric characterization of multiple species of cyanobacterial photosymbionts coexisting in the colonial ascidian Tridemnum clinides (Tunicata, Ascidiacea, Didemnidae). Eur J Phycol. 2009;44:365-75.
28. Hirose E, Hirose M. Morphological process of vertical transmission of photosymbionts in the colonial ascidian Tridemnum miniatum Kott. Mar Biol. 2007;150:359-67.
29. Martinez-Garcia M, Diaz-Valdes M, Wanner G, et al. Microbial community associated with the colonial ascidian Cystodytes dellechiajei. Environ Microbiol. 2007;9:521-534.
30. Schmidt EW, Donia MS. Life in cellulose houses: Symbiotic bacterial biosynthesis of ascidian drugs and drug leads. Curr Opin Biotechnol. 2010;21:827-833.
31. Gromek SM, Suria A, Fullmer MS, et al. Leisingera sp. JC1, a bacterial isolate from Hawaiian bobtail squid eggs, produces indigoidine and differentially inhibits vibrios. Front Microbiol. 2016;7:1342.
32. Shank EA, Kolter R. New developments in microbial interspecies signaling. Curr Opin Microbiol. 2009;12:205-14.
33. Romerro D, Traxler MF, Lopez D, et al. Antibiotics as signal molecules. Chem Rev. 2011; 11:5492-5505.
34. Townsley L, Shank EA. Natural-Product antibiotics: cues for modulating bacterial biofilm formation. Trends Microbiol. 2017;25:12.
35. Kaufmann GF, Sartorio R, Lee SH, et al. Revisiting quorum sensing: Discovery of additional chemical and biological functions for 3-oxo-N-acylhomoserine lactones. Proc Natl Acad Sci USA. 2005;102:309-14.
36. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protocols 2008;3:163-75.
37. Giedraitien A, Vitkauskien A, Naginien R, et al. Antibiotic resistance mechanisms of clinically important bacteria". Medicina. 2011;47:137-46.
38. Lane AL, Moore BS. A sea of biosynthesis:Marine natural products meet the molecular age. Nat Prod Rep. 2011;28:411-28.
39. French S, Mangat C, Bharat A, et al. A robust platform for chemical genomics in bacterial systems. Mol Biol Cell. 2016;27:1015-25.
40. French S, Ellis MJ, Coutts BE, et al. Chemical genomics reveals mechanistic hypotheses for uncharacterized bioactive molecules in bacteria. Curr Opin Microbiol. 2017;39:42-47.
41. Elad T, Seo HB, Belkin S, et al. High-throughput prescreening of pharmaceuticals using a genome-wide bacterial bioreporter array. Biosens Bioelectron. 2015;68:699-704.
42. Tjaden B. De novo assembly of bacterial transcriptomes from RNA-seq data. Genome Biol 2015;16:1.
43. Bose U, Hewavitharana AK, Ng YK. LC-MS-Based Metabolomics Study of Marine Bacterial Secondary Metabolite and Antibiotic Production in Salinispora arenicola. Mar Drugs. 2015;13:249-66.
44. Milshteyn A, Schneider JS, Brady SF. Mining the metabiome: identifying novel natural products from microbial communities. Chem Biol. 2014;21:1211-23.
45. Gontang EA, Gaudencio SP, Fenical W, et al. Sequence-based analysis of secondary-metabolite biosynthesis in marine actinobacteria. Appl Environ Microbiol 2010;76:2487-99.
46. Ng YK, Hodson MP, Hewavitharana AK, et al. Effects of salinity on antibiotic production in sponge-derived Salinispora actinobacteria. J Appl Microbiol. 2014;117:109-25.
47. Offret C, Desriac F, Le Chevalier P, et al. Spotlight on antimicrobial metabolites from the marine bacteria Pseudoalteromonas: chemodiversity and ecological significance. Mar Drugs. 2016:14.
48. Bertrand S, Bohni N, Schnee S, et al. Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. Biotechnol Adv. 2014;32:1180-204.
49. Shentu X, Liu N, Tang G, et al. Improved antibiotic production and silent gene activation in Streptomyces diastatochromogenes by ribosome engineering. J Antibiotics. 2016;69:406-10.
50. Xu D, Han L, Li C, et al. Bioprospecting Deep-Sea Actinobacteria for Novel Anti-infective Natural Products. Front. Microbiol. 2018;9:787.
51. Molloy EM, Hetweck C. Antimicrobial discovery inspired by ecological interactions. Curr Opin Microbiol 2017;39:121–127.