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Natural Products from Anaerobes

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Abstract

Natural product discovery in the microbial world has historically been biased toward aerobes. Recent in silico analysis demonstrates that genomes of anaerobes encode unexpected biosynthetic potential for natural products, however, chemical data on natural products from the anaerobic world are extremely limited. Here, we review the current body of work on natural products isolated from strictly anaerobic microbes, including recent genome mining efforts to discover polyketides and non-ribosomal peptides from anaerobes. These known natural products of anaerobes have demonstrated interesting molecular scaffolds, biosynthetic logic, and/or biological activities, making anaerobes a promising reservoir for future natural product discovery.

Keywords

Secondary metabolite; anaerobic organism; genome mining; antibiotic

Introduction

Natural products, often known as secondary metabolites, are structurally diverse small molecules possessing diverse and potent biological activities. Due to the metabolic burden inherent to natural products biosynthesis, these compounds are believed to be shaped by evolutionary pressure to fulfill specific biological functions [34]. For example, natural products produced by microbes have been demonstrated to influence processes such as virulence, motility, stress response, biofilm formation, morphological differentiation, nutrient acquisition, and defense [40]. Elucidating the identity and function of these microbial natural products may yield valuable biological insights or tools for modulating critical biological processes and promoting biotechnological applications of producing microbes. Historically, natural products are also valuable as medicinal compounds, and have been used to treat various human health conditions such as cancer, infectious disease, autoimmune disorder, cardiovascular disease, and neurological disease [33]. However, modern natural product-based drug discovery is often challenged by high incidence of

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compound rediscovery, which may be partially attributed to screening bias toward well-known natural product producers including filamentous actinomycetes and fungi. This has spurred recent investigations into new reservoirs of natural products, such as anaerobes, pathogens, and symbionts of humans, insects, and nematodes [14]. Anaerobes especially have been largely neglected in natural product discovery efforts, with few compounds identified, despite the fact that antibiotic production by anaerobic bacteria was first reported more than a half century ago [46].

The idea of anaerobes as promising natural product producers is supported by genomic analysis [28, 29], although their total genetic capacity for secondary metabolite biosynthesis lags behind that of the most “gifted” aerobes [5]. Nonetheless, a recent survey of 211 complete anaerobic bacterial genomes revealed that natural product biosynthetic gene clusters (BGCs), in particular those involved in polyketide and non-ribosomal peptide biosynthesis, could be found in 33% of the analyzed genomes [28]. As summarized in Table 1, some BGCs contain over 60 kb of genes encoding polyketide synthases (PKSs) and/or non-ribosomal peptide synthetases (NRPSs) [28], making them comparable in size to well-known antibiotic BGCs characterized in aerobes. It is notable that natural product BGCs are not equally distributed in various phyla, with Firmicutes of the genus *Clostridium* and Deltaproteobacteria possessing a relatively greater potential in anaerobes for natural product biosynthesis (Table 2). Interestingly, genomic potential for secondary metabolism is also correlated with the isolation site of the anaerobic organism; strains with the most BGCs predominantly originated from soil [28]. However, despite established genomic potential, chemical data on natural products from the anaerobic world are very limited and most gene clusters identified from genomic analysis do not have an associated product. To date, only a handful of natural products have been isolated from anaerobes and structurally characterized. In this mini-review, we present examples of several known natural products isolated from anaerobic organisms, highlighting recent genome mining efforts to discover unique natural products, in particular polyketides and non-ribosomal peptides with diverse activities.

Genomics-independent compound discovery

Genomic analysis suggests that nearly all major families of natural products, such as polyketides, non-ribosomal peptides, ribosomally synthesized and post-translationally modified peptides (RiPPs), and terpenes, could be produced by anaerobes [28]. However, very few metabolites have been isolated, perhaps because the lower efficiency of fermentative metabolism in anaerobes precluded the biosynthesis of secondary metabolites under typical laboratory conditions [8]. This section summarizes a few known natural products discovered from anaerobes through traditional methods regardless of genome availability, with a particular focus on their structures, biological activities, and biosynthesis. We exclude examples of RiPPs although quite a few have been isolated and characterized from anaerobes [16, 22].

Methanophenazine

The phenazine family of natural products is known to be synthesized by diverse bacterial genera, including many pseudomonads and actinomycetes [24]. Additionally, several anaerobic archaeal *Methanosarcina* species are also known phenazine producers. The first such compound, methanophenazine, was isolated from lyophilized membranes of *Methanosarcina mazei* Gö1 by extraction with isooctane [1]. Methanophenazine is a 2-hydroxyphenazine derivative that is connected to a polyisoprenoid tail via an ether bridge (Figure 1). Proposed to play an important role in membrane-bound electron transport, methanophenazine represents the first example of such bioactivity by a phenazine compound [1, 11, 10]. Although phenazine biosynthesis in bacteria has been linked to the *phz* operon, *M. mazei* Gö1 does not have any identifiable *phz* homologs, suggesting that methanophenazine biosynthesis might proceed via a mechanism different to that in bacteria [39, 12, 24]. The biosynthetic pathway of methanophenazine currently remains unclear despite the availability of genome sequences of the producers [24].

Naphthalecin

One of the early studies on an obligate anaerobic bacterium isolated from a symbiotic growth with an aerobic bacterium in soil led to discovery of a new small molecule antibiotic [20]. In particular, the producing anaerobe was identified as a new species of the genus *Sporotalea*, and activity-guided isolation of a cell-associated compound led to the discovery of naphthalecin, a naphthalene derivative substituted with hydroxyl, propionyl, and acetyl groups (Figure 1). This compound demonstrated broad antibacterial activity against Gram-positive bacteria, including the symbiotic aerobic bacterium, but not against tested Gram-negative bacteria or fungi. The biosynthetic pathway for naphthalecin was not reported.

Ammonificins

Deep-sea hydrothermal vent systems are a promising source of new natural products because these systems contain a broad diversity of microbial organisms that have adapted their biochemical machinery to cope with extremely harsh environment conditions, likely resulting in altered metabolic pathways to produce structurally unusual metabolites. Aerobic isolates from these systems have already yielded interesting new natural products with antibiotic activities [17, 44]. Additionally, one family of novel secondary metabolites, named ammonificins, have been purified from *Thermovibrio ammonificans*, an anaerobic and chemolithoautotrophic bacterium isolated from a hydrothermal vent system on the East Pacific Rise [47]. A total of four ammonificins, A-D, were purified from apoptosis-inducing cell extracts [3, 4]. Ammonificins A-D were structurally determined to be novel chroman (A and B) or chromene (C and D) derivatives substituted with hydroxyethylamine and phenol or brominated phenol (Figure 1). Interestingly, ammonificins C and D induced apoptosis at micromolar concentrations while ammonificins A and B were inactive in an apoptosis induction assay. The biosynthetic pathway for ammonificin biosynthesis was not elucidated.

Closthioamide

The genus *Clostridium* occurs widely in soil and in the gastrointestinal tract of higher organisms and includes several notorious human pathogens as well as non-pathogenic

species useful for industrial biotechnology [35]. Although recent genomic analysis has revealed that natural product biosynthetic genes are widespread among clostridia [28], no secondary metabolite had been isolated from clostridia until 2010. Closthioamide was the first natural product antibiotic purified from *Clostridium cellulolyticum* (recently renamed to *Ruminiclostridium cellulolyticum*), a cellulose-degrading organism isolated from decayed grass compost [36]. The initial discovery of closthioamide involved extensive culture condition screening, and was only successful upon addition of soil extracts to the culture medium to mimic its natural habitat [30]. The production of closthioamide was further improved by overexpression of an anti-terminator gene (*nusG*) in *C. cellulolyticum* that induced the biosynthesis of closthioamide and related thioamides without the need for soil extracts [9]. Closthioamide possesses an unprecedented structure with multiple thioamide groups (Figure 1) that are critical for its potent antibiotic activity toward methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and drug-resistant *Neisseria* in a variety of disease models [32, 30]. The mode of action of closthioamide was shown to inhibit DNA gyrase activity, with a different molecular mechanism from that of the quinolones and aminocoumarins [15]. The biosynthesis of closthioamide was proposed to go through stepwise assembly using a hydroxybenzoate starting unit followed by elongation with β-alanine units and thionation of the intermediates, finished through the fusion of two intermediates via a diaminopropane linker [9]. The recently identified BGC for closthioamide suggests that instead of typical modular NRPSs, free-standing enzymes such as an ATP-grasp enzyme, an AMP-dependent ligase, and an amide synthase may be involved in activation and ligation of building monomers in a thio-templated assembly line [19]. In addition, a homolog of alpha-adenine nucleotide hydrolase has been predicted to promote the incorporation of sulfur into amide bonds [19].

Clostrubins

Clostrubin A was initially isolated as a deep red to purple pigment from cultures of *Clostridium beijerinckii* HKI0724 [37]. Its production was also detected in cultures of the potato pathogen *Clostridium puniceum*, along with a related compound clostrubin B [43]. Both compounds feature the same unusual pentacyclic polyphenol scaffold, and clostrubin B contains an extra sugar-like linear side chain (Figure 1). The biosynthesis of clostrubins has been linked to a type II PKS gene cluster that is rare in anaerobes, and the polyphenol scaffold seems to emerge from a non-canonical polyketide folding, distinct from the conserved folding patterns of aerobic bacteria. Activity assays demonstrated potent antibiotic activity of clostrubin A against human pathogens, with minimum inhibitory concentrations (MIC) of 0.12 μM against MRSA, 0.97 μM against VRE, and 0.12–0.48 μM against various mycobacteria [37]. In addition, clostrubins A and B demonstrated antibacterial activity against a few common microbial potato pathogens, with MIC values in the range of 14–95 nM, suggesting that clostrubins may be used as chemical weapons to fight against competitors in a resource-limited environment. Intriguingly, these aromatic polyketides were also shown to enable the plant pathogen *C. puniceum* to survive in an oxygen-rich environment, adding to the growing number of examples of antibiotics with dual functions [21].

Genomics-driven compound discovery

Considering the occurrence of many putative secondary metabolite BGCs in anaerobes, genome mining to identify these BGC-associated metabolites can be a powerful approach to rapidly access new compounds [51]. In the following examples, we describe three recent applications of genome mining to discover new anaerobic natural products that are associated with PKS and/or NRPS gene clusters. Dependent on strain availability, strain cultivability, and available genetic tools, these natural products were identified and characterized by different methods including combinations of bioinformatics, gene expression studies, biosynthetic gene disruption, heterologous expression, comparative metabolomics, biochemical characterization of biosynthetic enzymes, and chemical synthesis.

Dipeptide aldehydes

The human gut harbors a broad diversity of anaerobes, but they are often difficult to culture, hindering metabolic and functional study of gut residents in isolation [50]. Nevertheless, metagenomic analysis supports the idea that the gut microbiome, collectively, possesses significant biosynthetic coding potential with thousands of identified BGCs, although it is challenging to ascertain whether these genes truly correspond to secondary metabolism due to the use of ClusterFinder in the analysis [18]. One family of NRPS gene clusters was studied because it is widely distributed among healthy humans based on stool analysis and resides nearly exclusively in gut bacterial genome sequences [23]. The core NRPS enzymes typically have domains organized into A-T-C-A-T-R (A, adenylation; T, thiolation; C, condensation; R, reduction), although an extra module or domain (in particular an *N*-terminal C domain) can be present (Figure 2). These gene clusters are prevalent in gut anaerobic clostridia and a few other organisms. A total of 14 of these clusters were selected for metabolite analysis through heterologous expression in *Escherichia coli* and *Bacillus subtilis*, seven of which yielded detectable compounds belonging to the family of pyrazinones and dihydropyrazinones (Figure 2). At least one native strain harnessing this gene cluster was able to produce the same metabolites as the engineered heterologous host, confirming that heterologous expression was a valid means to probe the metabolites produced by these gene clusters. Intriguingly, as the dipeptide aldehydes are plausible biosynthetic precursors to the isolated pyrazinones and dihydropyrazinones, and *N*-acylated metabolites (promoted by the first C domain of NRPS) retain the predicted *C*-terminal aldehyde moiety generated by the R domain, the dipeptide aldehydes immediately released from the NRPS assembly were proposed to be the active metabolites found in the gut under physiological conditions (Figure 2). Since peptide aldehydes are well known to inhibit proteases [48, 49, 25], a few dipeptide aldehydes were tested and confirmed to be potent and selective protease inhibitors. A further unbiased target identification of the metabolite Phe-Phe-H using the isotopic tandem orthogonal proteolysis-activity-based protein profiling (isoTOP-ABPP) approach identified the cathepsins (specifically cathepsin L) as the principal targets [23].

The list of dipeptide aldehydes produced by gut anaerobes was further expanded by recent work to identify the product of a conserved NRPS gene cluster from the abundant gut

commensal *Ruminococcus bromii* [42]. It is notable that this cluster failed to yield any product during the heterologous expression in *B. subtilis* [23]. The core NRPS enzyme has domains organized into C-A-T-C-A-T-R, and bioinformatics coupled with biochemical analysis of the NRPS suggested that an *N*-acylated dipeptide aldehyde (named ruminopeptin) could be the active metabolite, although the function of the R domain could not be reconstituted in vitro; nor could the proposed product be isolated from *R. bromii* cultures. Nonetheless, putative ruminopeptin scaffolds were chemically synthesized and several of them inhibited *Staphylococcus aureus* endoproteinase GluC (SspA/V8 protease), homologs of which were found in gut commensals, opportunistic pathogens, and human gut metagenomes [42].

Barnesin

Anaerobic proteobacteria may also be promising sources for future natural product discovery based on genomic analysis [28]. A recent genome mining effort in *Sulfurospirillum barnesii*, an arsenate-reducing Epsilonproteobacterium isolated from a freshwater marsh, demonstrated for the first time that bioactive natural products could be produced from this genus [41]. In this study, an NRPS/PKS hybrid biosynthetic gene cluster found in *S. barnesii* was targeted for metabolite discovery (Figure 2). A homologous unexplored NRPS/PKS cluster was also found on the genome of *Geovibrio* sp. L21-Ace-BES, but not in any other sequenced *Sulfurospirillum* species. After confirming gene expression under laboratory growth conditions, metabolite comparison between *S. barnesii* and two closely related *Sulfurospirillum* spp. that lack the NRPS/PKS cluster led to the identification of a new metabolite, named barnesin A, which is unique to *S. barnesii.* Barnesin A was revealed to be an *N*-acylated dipeptide carboxylate containing a vinylogous side chain (Figure 2). In addition to antibiotic activity against a few human pathogens, barnesin A showed selective and nanomolar inhibitory activity against cysteine proteases including cathepsin B. Considering that small peptides and vinylogous systems are well known to inhibit proteases [38, 27, 45], it was reasonable to propose that barnesin A has a mode of action via a 1,4-Michael-type addition mechanism, which was supported by structure-activity-relationship studies [41].

Clostrienose

In contrast to clostridia from the gastrointestinal tract of higher organisms that tend to have few secondary metabolite BGCs, soil and other environmental isolates of clostridia have greater natural product biosynthetic potential according to genomic analysis (Table 1 and 2) [28]. Many of these *Clostridium* spp. are useful for industrial biotechnology, such as biomass degradation and industrial-scale production of organic acids or solvents [31, 35]. However, despite extensive research and industrial application of these organisms, knowledge of the molecular identity and function of their secondary metabolites is extremely limited. Modern genome mining of secondary metabolites could be a promising approach to accelerate the discovery of new natural products from these anaerobes, provide insights in biological functions of secondary metabolites, and possibly improve these strains for industrial applications by manipulating their secondary metabolism. A recent genome mining effort in *Clostridium acetobutylicum* exemplified such opportunities [26].

C. acetobutylicum is an organism well-known for its historical use as an industrial producer of the organic solvents acetone, butanol, and ethanol (ABE) through a process known as ABE fermentation [31]. One PKS gene was identified in all sequenced *C. acetobutylicum* strains and the encoding enzyme has domains organized into KS-AT-DH-KR-ACP (KS, ketosynthase; AT, acyltransferase; DH, dehydratase; KR, ketoreductase; ACP, acyl carrier protein) (Figure 2). Expression of this PKS gene was significantly upregulated during early stationary phase, suggesting that the corresponding polyketide product could be associated with morphological development and/or solventogenesis [2]. Metabolomic comparison between the wild-type and mutant with the disrupted *pks* gene led to the identification of the polyketide metabolite, named clostrienose. The structure of clostrienose was revealed to be a 2-hydroxy-5,8,10-tetradecenoic acid linked to a disaccharide, α-D-galactofuranosyl(1−>2)-α-L-rhamnopyranoside, via an ester linkage (Figure 2). This molecular scaffold could never be predicted by bioinformatics due to the iterative activity of this single module PKS as well as the apparent non-clustered nature of the biosynthetic genes on the genome. This also raised concerns about using heterologous expression to probe BGC-associated metabolites since all required biosynthetic genes may not be clustered together. Clostrienose was shown to be important in stimulating sporulation and granulose accumulation in *C. acetobutylicum*, and the *pks* deletion strain exhibited improved traits for industrial ABE fermentation such as reduced sporulation, reduced granulose accumulation, and increased butanol titer and productivity [26].

Conclusions

Genomic analyses had previously demonstrated that anaerobic organisms could produce secondary metabolites, and suggested good sources, such as clostridia from soil, for new natural product discovery [28]. Novel compounds with unique molecular scaffolds or functionalities have already been isolated from anaerobes, and more examples are expected to come from application of genome mining to link BGCs to their metabolites. In addition to new chemical space possibly offered by anaerobes, the biosynthesis of these unique molecules likely involves interesting enzymes and pathways, some of which remain to be discovered. When one considers the limited energy that an anaerobic lifestyle provides, the diversion of precious nutrients into secondary metabolism suggests that these natural products probably play important roles in the lifestyle of these organisms. Compounds with promising pharmaceutical properties, such as potent antibiotic activities toward multi-drug resistant pathogens, have also been found to be produced by anaerobes. Given the recent and rapid progress in natural product discovery from anaerobes, we look forward to seeing the continued growth of this exciting field.

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References

1. Abken HJ, Tietze M, Brodersen J, et al. (1998) Isolation and characterization of methanophenazine and function of phenazines in membrane-bound electron transport of Methanosarcina mazei Gö1. J Bacteriol 180:2027–32 [PubMed: 9555882]
2. Alsaker KV, Papoutsakis ET (2005) Transcriptional program of early sporulation and stationary-phase events in Clostridium acetobutylicum. J Bacteriol 187:7103–18. doi: 10.1128/JB.187.20.7103-7118.2005 [PubMed: 16199581]
3. Andrianasolo EH, Haramaty L, Rosario-Passapera R, et al. (2009) Ammonificins A and B, hydroxyethylamine chroman derivatives from a cultured marine hydrothermal vent bacterium, Thermovibrio ammonificans. J Nat Prod 72:1216–1219. doi: 10.1021/np800726d [PubMed: 19507867]
4. Andrianasolo EH, Haramaty L, Rosario-Passapera R, et al. (2012) Ammonificins C and D, hydroxyethylamine chromene derivatives from a cultured marine hydrothermal vent bacterium, Thermovibrio ammonificans. Mar Drugs 10:2300–2311. doi: 10.3390/md10102300 [PubMed: 23170085]
5. Baltz RH (2017) Gifted microbes for genome mining and natural product discovery. J Ind Microbiol Biotechnol 44:573–588. doi: 10.1007/s10295-016-1815-x [PubMed: 27520548]
6. Baltz RH (2017) Molecular beacons to identify gifted microbes for genome mining. J Antibiot 70:639–646. doi: 10.1038/ja.2017.1 [PubMed: 28119518]
7. Baltz RH (2018) Synthetic biology, genome mining, and combinatorial biosynthesis of NRPS-derived antibiotics: a perspective. J Ind Microbiol Biotechnol 45:635–649. doi: 10.1007/s10295-017-1999-8 [PubMed: 29288438]
8. Behnken S, Hertweck C (2012) Anaerobic bacteria as producers of antibiotics. Appl Microbiol Biotechnol 96:61–67. doi: 10.1007/s00253-012-4285-8 [PubMed: 22854892]
9. Behnken S, Lincke T, Kloss F, et al. (2012) Antiterminator-mediated unveiling of cryptic polythioamides in an anaerobic bacterium. Angew Chemie Int Ed 51:2425–2428. doi: 10.1002/anie.201108214
10. Beifuss U, Tietze M (2005) Methanophenazine and other natural biologically active phenazines. In: Mulzer J (ed) Natural products synthesis II. Topics in current chemistry, vol 244 Springer, Berlin, Heidelberg, pp 77–113 doi:10.1007/b96889
11. Beifuss U, Tietze M, Bäumer S, Deppenmeier U (2000) Methanophenazine: structure, total synthesis, and function of a new cofactor from methanogenic archaea. Angew Chemie Int Ed 39:2470–2472. doi: 10.1002/1521-3773(20000717)39:14<2470::AID-ANIE2470>3.0.CO;2-R
12. Blankenfeldt W, Parsons JF (2014) The structural biology of phenazine biosynthesis. Curr Opin Struct Biol 29:26–33. doi: 10.1016/J.SBI.2014.08.013 [PubMed: 25215885]
13. Blin K, Wolf T, Chevrette MG, et al. (2017) antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res 45:W36–W41. doi: 10.1093/nar/gkx319 [PubMed: 28460038]
14. Challinor VL, Bode HB (2015) Bioactive natural products from novel microbial sources. Ann NY Acad Sci 1354:82–97. doi: 10.1111/nyas.12954 [PubMed: 26509922]
15. Chiriac AI, Kloss F, Krämer J, et al. (2015) Mode of action of closthioamide: the first member of the polythioamide class of bacterial DNA gyrase inhibitors. J Antimicrob Chemother 70:2576–2588. doi: 10.1093/jac/dkv161 [PubMed: 26174721]
16. Dabard J, Bridonneau C, Phillipe C, et al. (2001) Ruminococcin A, a new lantibiotic produced by a *Rumi*nococcus gnavus strain isolated from human feces. Appl Environ Microbiol 67:4111–8. doi: 10.1128/AEM.67.9.4111-4118.2001 [PubMed: 11526013]
17. Ding C, Wu X, Auckloo B, et al. (2016) An unusual stress metabolite from a hydrothermal vent fungus *Aspergill*us sp. WU 243 induced by cobalt. Molecules 21:105. doi: 10.3390/molecules21010105 [PubMed: 26784166]
18. Donia MS, Cimermancic P, Schulze CJ, et al. (2014) A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. Cell 158:1402–1414. doi: 10.1016/J.CELL.2014.08.032 [PubMed: 25215495]

19. Dunbar K, Büttner H, Molloy E, Dell M, Kumpfmueller J, Hertweck C (2018) Genome Editing Reveals Novel Thiotemplated Assembly of Polythioamide Antibiotics in Anaerobic Bacteria. Angew Chem Int Ed doi: 10.1002/anie.201807970
20. Ezaki M, Muramatsu H, Takase S, et al. (2008) Naphthalecin, a novel antibiotic produced by the anaerobic bacterium, Sporotalea colonica sp. nov. J Antibiot (Tokyo) 61:207–212. doi: 10.1038/ja.2008.30 [PubMed: 18503199]
21. Fajardo A, Martínez JL (2008) Antibiotics as signals that trigger specific bacterial responses. Curr Opin Microbiol 11:161–167. doi: 10.1016/j.mib.2008.02.006 [PubMed: 18373943]
22. Gonzalez DJ, Lee SW, Hensler ME, et al. (2010) Clostridiolysin S, a post-translationally modified biotoxin from Clostridium botulinum. J Biol Chem 285:28220–8. doi: 10.1074/jbc.M110.118554 [PubMed: 20581111]
23. Guo C-J, Chang F-Y, Wyche TP, et al. (2017) Discovery of reactive microbiota-derived metabolites that inhibit host proteases. Cell 168:517–526.e18. doi: 10.1016/J.CELL.2016.12.021 [PubMed: 28111075]
24. Guttenberger N, Blankenfeldt W, Breinbauer R (2017) Recent developments in the isolation, biological function, biosynthesis, and synthesis of phenazine natural products. Bioorg Med Chem 25:6149–6166. doi: 10.1016/J.BMC.2017.01.002 [PubMed: 28094222]
25. Hans-Hartwig O, Schirmeister T (1997) Cysteine proteases and their inhibitors. Chem Rev 97:133–172. doi: 10.1021/CR950025U [PubMed: 11848867]
26. Herman NA, Kim SJ, Li JS, et al. (2017) The industrial anaerobe *Clostridium acetobutylicum* uses polyketides to regulate cellular differentiation. Nat Commun 8:1514. doi: 10.1038/s41467-017-01809-5 [PubMed: 29138399]
27. Konno H (2012) Synthesis of bioactive natural products as protein inhibitors. Biosci Biotechnol Biochem 76:1257–1261 [PubMed: 22785468]
28. Letzel A-C, Pidot SJ, Hertweck C (2013) A genomic approach to the cryptic secondary metabolome of the anaerobic world. Nat Prod Rep 30:392–428. doi: 10.1039/C2NP20103H [PubMed: 23263685]
29. Letzel A-C, Pidot SJ, Hertweck C (2014) Genome mining for ribosomally synthesized and post-translationally modified peptides (RiPPs) in anaerobic bacteria. BMC Genomics 15:983. doi: 10.1186/1471-2164-15-983 [PubMed: 25407095]
30. Lincke T, Behnken S, Ishida K, et al. (2010) Closthioamide: An unprecedented polythioamide antibiotic from the strictly anaerobic bacterium Clostridium cellulolyticum. Angew Chemie 122:2055–2057. doi: 10.1002/ange.200906114
31. Lütke-Eversloh T, Bahl H (2011) Metabolic engineering of *Clostridium acetobutylicum*: recent advances to improve butanol production. Curr Opin Biotechnol 22:634–647. doi: 10.1016/J.COPBIO.2011.01.011 [PubMed: 21377350]
32. Miari VF, Solanki P, Hleba Y, et al. (2017) In vitro susceptibility to closthioamide among clinical and reference strains of *Neis*seria gonorrhoeae. Antimicrob Agents Chemother 61:e00929–17. doi: 10.1128/AAC.00929-17 [PubMed: 28784667]
33. Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79:629–661. doi: 10.1021/acs.jnatprod.5b01055 [PubMed: 26852623]
34. O'Brien J, Wright GD (2011) An ecological perspective of microbial secondary metabolism. Curr Opin Biotechnol 22:552–558. doi: 10.1016/J.COPBIO.2011.03.010 [PubMed: 21498065]
35. Paredes CJ, Alsaker KV., Papoutsakis ET (2005) A comparative genomic view of clostridial sporulation and physiology. Nat Rev Microbiol 3:969–978. doi: 10.1038/nrmicro1288 [PubMed: 16261177]
36. Petitdemange E, Caillet F, Giallo J, Gaudin C (1984) *Clostridium cellulolyticum* sp. nov., a cellulolytic, mesophilic species from decayed grass. Int J Syst Bacteriol 34:155–159
37. Pidot S, Ishida K, Cyrulies M, Hertweck C (2014) Discovery of clostrubin, an exceptional polyphenolic polyketide antibiotic from a strictly anaerobic bacterium. Angew Chemie Int Ed 53:7856–7859. doi: 10.1002/anie.201402632
38. Powers JC, Asgian, Asgian JL, Ekici OD, Özlem Doğan Ekici A, James KE (2002) Irreversible inhibitors of serine, cysteine, and threonine proteases. Chem Rev 102:4639–4750 doi: 10.1021/CR010182V [PubMed: 12475205]

39. Price-Whelan A, Dietrich LEP, Newman DK (2006) Rethinking “secondary” metabolism: physiological roles for phenazine antibiotics. Nat Chem Biol 2:71–78. doi: 10.1038/nchembio764 [PubMed: 16421586]
40. Raaijmakers JM, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annu Rev Phytopathol 50:403–424. doi: 10.1146/annurev-phyto-081211-172908 [PubMed: 22681451]
41. Rischer M, Raguž L, Guo H, et al. (2018) Biosynthesis, synthesis, and activities of barnesin A, a NRPS-PKS hybrid produced by an anaerobic epsilonproteobacterium. ACS Chem Biol Article ASAP doi: 10.1021/acschembio.8b00445
42. Schneider BA, Balskus EP (2018) Discovery of small molecule protease inhibitors by investigating a widespread human gut bacterial biosynthetic pathway. Tetrahedron 74:3215–3230. doi: 10.1016/J.TET.2018.03.043
43. Shabuer G, Ishida K, Pidot SJ, et al. (2015) Plant pathogenic anaerobic bacteria use aromatic polyketides to access aerobic territory. Science 350:670–4. doi: 10.1126/science.aac9990 [PubMed: 26542569]
44. Shi Y, Pan C, Auckloo BN, et al. (2017) Stress-driven discovery of a cryptic antibiotic produced by *Streptomyces* sp. WU20 from Kueishantao hydrothermal vent with an integrated metabolomics strategy. Appl Microbiol Biotechnol 101:1395–1408. doi: 10.1007/s00253-016-7823-y [PubMed: 27730337]
45. Siklos M, BenAissa M, Thatcher GRJ (2015) Cysteine proteases as therapeutic targets: does selectivity matter? A systematic review of calpain and cathepsin inhibitors. Acta Pharm Sin B 5:506–519. doi: 10.1016/J.APSB.2015.08.001 [PubMed: 26713267]
46. Sturgen NO, Casida LE Jr. (1962) Antibiotic production by anaerobic bacteria. Appl Microbiol 10:55–9 [PubMed: 13918037]
47. Vetriani C, Speck MD, Ellor SV., et al. (2004) *Thermovibrio ammonificans* sp. nov., a thermophilic, chemolithotrophic, nitrate-ammonifying bacterium from deep-sea hydrothermal vents. Int J Syst Evol Microbiol 54:175–181. doi: 10.1099/ijs.0.02781-0 [PubMed: 14742477]
48. Westerik JO, Wolfenden R (1972) Aldehydes as inhibitors of papain. J Biol Chem 247:8195–8197 [PubMed: 4640942]
49. Woo J-T, Sigeizumi S, Yamaguchi K, et al. (1995) Peptidyl aldehyde derivatives as potent and selective inhibitors of cathepsin L. Bioorg Med Chem Lett 5:1501–1504.
50. Zhu B, Wang X, Li L (2010) Human gut microbiome: the second genome of human body. Protein Cell 1:718–725. doi: 10.1007/s13238-010-0093-z [PubMed: 21203913]
51. Ziemert N, Alanjary M, Weber T (2016) The evolution of genome mining in microbes – a review. Nat Prod Rep 33:988–1005. doi: 10.1039/C6NP00025H [PubMed: 27272205]

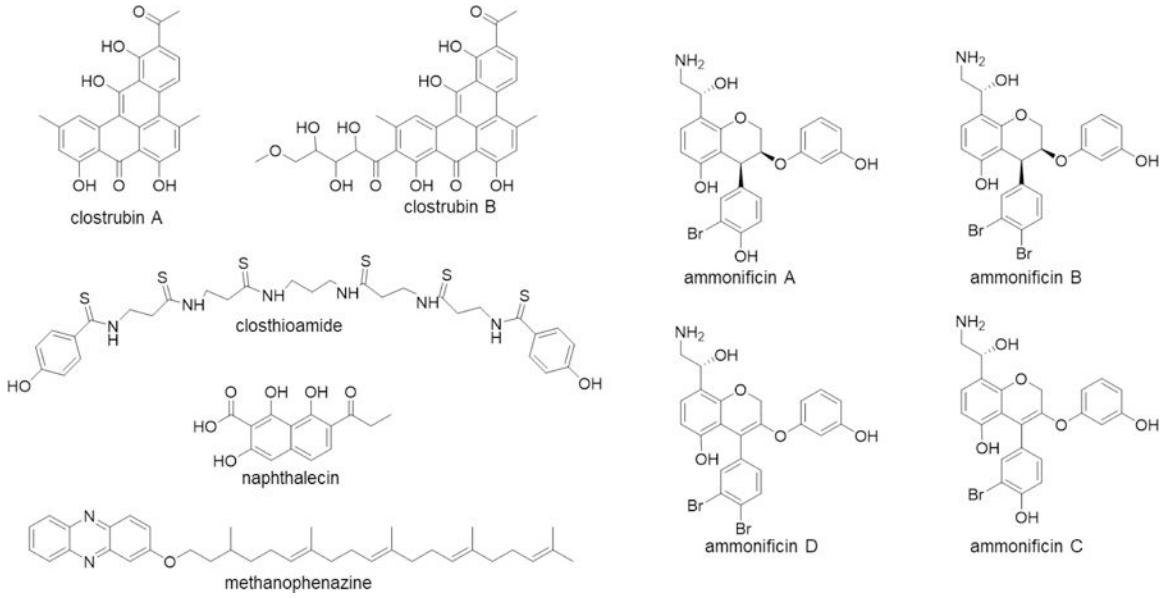

Figure 1.
Examples of compounds isolated from anaerobes.

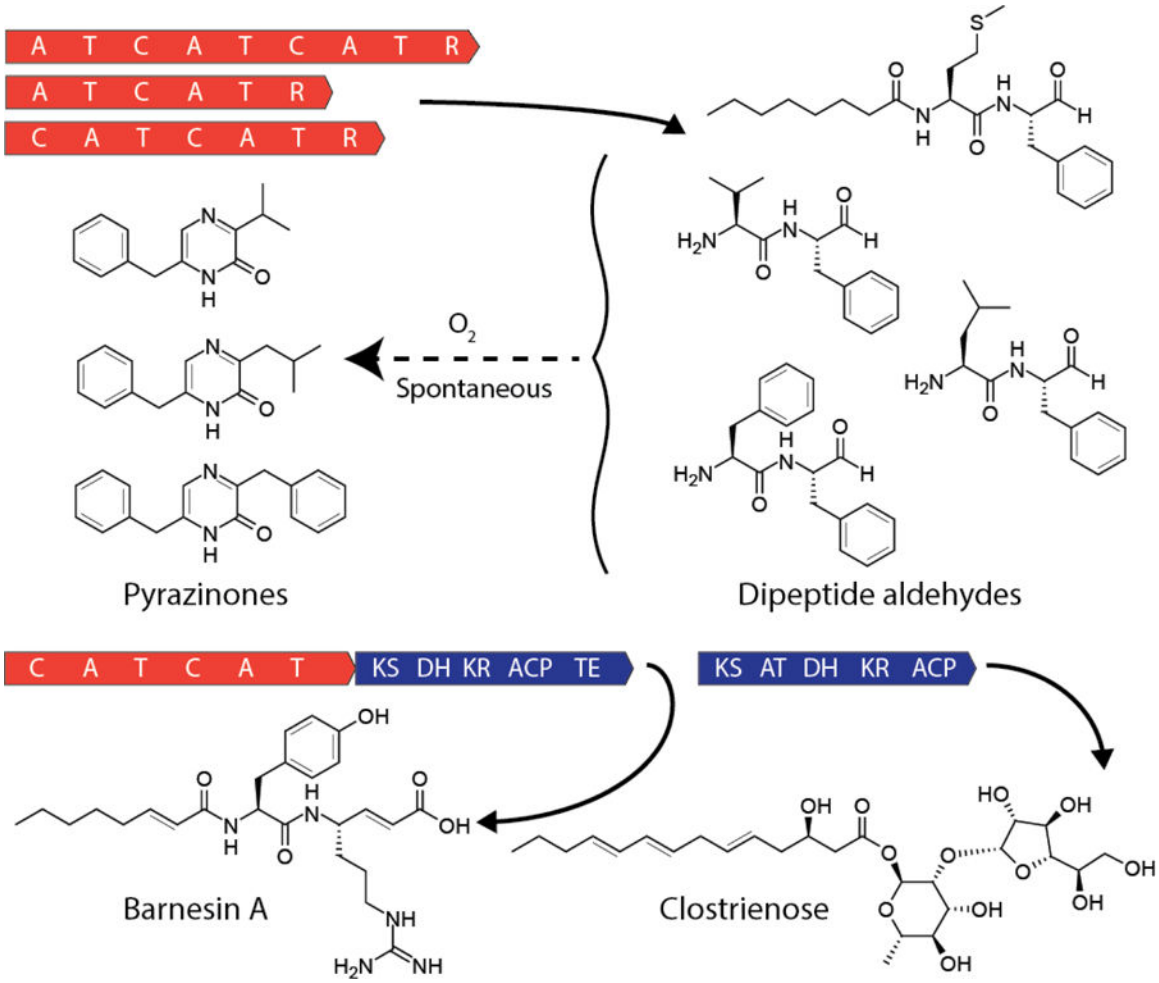

Figure 2.

Examples of polyketides/non-ribosomal peptides discovered from anaerobes using genome mining. Domain abbreviations: A, adenylation; T, thiolation; C, condensation; R, reduction; KS, ketosynthase; AT, acyltransferase; DH, dehydratase; KR, ketoreductase; ACP, acyl carrier protein; TE, thioesterase.

Table 1.

Examples of large PKS- and/or NRPS-encoding BGCs detected in the genomes of anaerobes [28]. PKS, polyketide synthase. NRPS, non-ribosomal peptide synthetase. BGC, biosynthetic gene cluster.

organism	BGC Type	# of PKS Modules	# of NRPS Modules	Total PKS/NRPS Sequence (kb)
Clostridium cellulolyticum H10[a]	Hybrid	14	1	63.75
	Hybrid	3	8	48.10
	Hybrid	6	9	60.98
Clostridium cellulovorans 743B	NRPS	-	18	65.71
	Hybrid	1	9	33.72
Clostridium botulinum A2 BoNT/Kyoto-F	NRPS	-	9	36.83
Clostridium botulinum H04402 065	NRPS	-	7	29.53
Clostridium kluyveri DSM 555	Hybrid	2	6	35.99
Ruminococcus albus 7 ATCC 27210	Hybrid	3	6	35.16
Geobacter uraniireducens R_f4	PKS	9	-	35.12
Opitutus terrae PB90–1	Hybrid	6	11	68.88

[a]As *Clostridium* is highly polyphyletic, this species has been reassigned to the suggested genus *Ruminiclostridium* according to the National Center for Biotechnology Information

Table 2.

Examples of secondary metabolite biosynthetic potential in anaerobic bacteria. BGCs from published genomes were detected using AntiSMASH 4.0 with ClusterFinder "off" [13]. The selected species are from two of the most promising phyla, the Firmicutes and Deltaproteobacteria. PK, polyketide. NRP, non-ribosomal peptide. RiPP, ribosomally produced and post-translationally modified peptide. BGC, biosynthetic gene cluster. SM, secondary metabolite.

Organism	Genome Size (Mb)	Predicted PK/NRP BGCs	PK/NRP BGCs >50 kb	Predicted RiPP BGCs	Other BGCs	Predicted Total SM BGCs	Total BGC Sequence (Mb)	%of Genome
Clostridium papyrosoivens DSM 2782[a,b]	4.92	13	7	3	2[d]	18	0.77	15.7
Clostridium roseum DSM 6424[a]	4.94	8	3	3	2[c]	13	0.4	8.0
Clostridium sp. BNL1100	4.61	9	6	4	1[c]	14	0.73	15.9
Clostridium aurantibutyricum DSM 793[a]	4.92	9	2	3	1[c]	13	0.43	8.7
Clostridium termitidis CT1112, DSM 5398[a,b]	6.42	14	7	3	1[d]	18	0.77	12.0
Clostridium cellulovorans 743B, ATCC 35296	5.26	7	5	10	0	17	0.67	12.7
Clostridium cellobioparum ATCC 15032[a,b]	6.13	11	4	2	1[c]	14	0.47	7.7
Clostridium saccharoperbutylacetonicum N1–4	6.67	6	2	2	1[d]	9	0.41	6.1
Clostridium beijerinckii NRRL B-598	6.19	3	3	3	0	6	0.26	4.1
Desulfofaba hansenii DSM 12642[a]	6.71	15	1	4	4[e]	23	0.52	7.8
Desulfospira joergensenii DSM 10085[a]	6.12	3	1	1	2[f,g]	6	0.22	3.6

[a] Denotes BGCs for PK/NRPs identified from draft genomes. Note that these are often fragmented or misassembled in draft genomes so the number of BGCs can be overrepresented [6, 7].

[b] As *Clostridium* is highly polyphyletic, these species have been reassigned to the suggested genus *Ruminiclostridium* according to the National Center for Biotechnology Information.

[c] Putative ladderane biosynthesis clusters.

[d] Putative AMP-binding domain containing clusters.

[e] Putative arylpolyene biosynthesis clusters.

[f] Putative acyl amino acid biosynthesis cluster.

[g] Putative homoserine lactone biosynthesis cluster.