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Exploiting the human gut microbiome for novel therapeutics

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Abstract

Within the human body, there exists a diverse community of microbes that have meaningful contributions to the health of their host. Throughout evolution, many of these bacteria have developed mechanisms in the form of chemical compounds to thrive successfully in the human body. These compounds can include antimicrobials to inhibit competition, as bacteria are in a state of constant warfare for nutrients. As mankind grapples with problems such as antibiotic resistant pathogens like methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococci, novel therapies are urgently needed to treat these diseases. Advanced computational approaches as well as experimental validation and isolation can be used to discover compounds produced by the symbiotic bacteria that exist in the human gut, that can be exploited for new drugs. The microbiome's extreme diversity, if harnessed, could provide another solution to treat mankind's most pressing challenges, in the form of live therapeutics. This review will cover pre-existing research that has successfully identified novel compounds and bacteria from the human gut microbiota as well as look at where the future of this field may be headed.

Keywords

Antibiotics, Bacterial Natural Products, Biochemistry, Bioinformatics, Biotechnology, Microbial Engineering, Microbiome, Biosynthetic gene clusters, Bacteriocin, Dysbiosis

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Introduction

"Never has the threat of antimicrobial resistance been more immediate and the need for solutions more urgent." Those were the words of Dr. Ghebreyesus, Director-General of the World Health Organization, in 2020. Although the need for antibiotics to treat bacterial infections remains greater than ever, discovery and production of new antibiotics is lacking, creating a serious problem; many types of bacteria have developed resistance to commonly used drugs, including to some antibiotics deemed to be those of last resort. As bacteria evolve and further resistance is generated, the need for new antimicrobial compounds will only grow in the coming years. Thus, researchers have turned to bacteria that live naturally in diverse places to obtain sources of novel compounds. One of these environments where bacteria live in rich communities is inside the human gastrointestinal tract.

The human gut microbiome is a community of microbes — mostly bacteria, along with protozoa, archaea, eukaryotes, and viruses — that inhabit the body's gastrointestinal (GI) system, predominantly found in the intestines and colon. The microbiota found in the gut microbiome varies between individuals, with each individual's unique combination formed from birth (1). The size of this microbiome is enormous, outnumbering human cells by a ratio of 1.3 to 1 (2). Most of these diverse microorganisms have a symbiotic relationship with the human body, aiding us in vital functions such as food digestion, vitamin and neurological signaling, molecule production, and immune protection, while our body in turn provides them with their habitat. The bacteria in the gut are so important that disturbances or perturbations to the gut microbiome have been linked to various diseases, including obesity (3,4), metabolic syndrome (5), multiple sclerosis (6), and inflammatory bowel diseases like Crohn's disease and ulcerative colitis (7). In addition to changes in diet, exposure to antibacterial or antifungal agents, pharmaceuticals, and other types of drugs can influence the microbiome. The composition and balance of our gut microbiome is vital to maintaining health and inhibiting pathogenic species.

Most of the bacteria that make up the gut microbiome are from the phyla Bacteroidetes and Firmicutes, out of the five main phyla that inhabit the gut (Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia) (8). The specific species, however, vary; a study conducted in China, for example, revealed that those living in the northern regions tended to have guts dominated by *Prevotella copri*, while *Bacteroides vulgatus* was more prevalent in the south (9). Even birth methods affect microbiota composition, as shown in a study (10) that found infants born vaginally to have a large number of *Bifidobacterium longum* and *Bifidobacterium catenulatum*, different in infants born *via* Cesarean section. Other commonly found genera of bacteria are *Escherichia*, *Staphylococcus, Bacteroides,* and *Streptococcus* (8).

Due of the diversity of the gut microbiome, naturally, bacteria find themselves competing against one another for the limited resources in the gut. Throughout evolution, many bacterial species have evolved complex ways to gain an edge over their competitors — one of those being the use of toxic molecules to target other

bacteria. This includes bacteriocins (antimicrobial peptides produced by bacteria) such as ribosomally synthesized and post-translationally modified peptides (RiPPs) and non-ribosomally synthesized proteins (NRPs), small chain fatty acids (SCFAs), siderophores, and other toxic metabolites (11). Because of how diverse the microbiome is — it includes 9,879,896 different bacterial genes (12) — if these mechanisms could be discovered, characterized, and produced on a large scale, they could be transformed into a formidable source of novel therapeutics capable of meeting the demands of modern and future medicine.

Techniques for Molecule Discovery

Traditionally, researchers have used an activity-oriented approach to discover new molecules, meaning products from lab-grown bacteria are isolated and analyzed for potential activity. However, this presents a problem — bacteria grown in a laboratory environment are not always subject to the environmental conditions needed to activate certain genes, leading to some genes being unexpressed and their protein products staying "hidden." This means that not all compounds that are encoded by the organism may be discovered using this approach. To circumvent this problem and access the full diversity of potential natural products encoded by bacteria, scientists have turned to bioinformatic tools, such as genome sequencing, to identify clusters of genes that may encode for a potential drug candidate.

This approach to molecule discovery has been made possible by major advancements in DNA sequencing technology. When Fred Sanger first developed his chain termination method of sequencing in 1977, it wasn't until 1995 — 18 years later — that *Haemophilus influenzae,* the first bacteria sequenced, had its DNA fully documented (13). Obviously, at the time, using genetics to discover new products would have been extremely costly and inefficient, and thus the activity-oriented approaches were favored. However, with the advent of new technologies in the field over time, costs have decreased and made gene sequencing much more accessible to everyone — with modern technology, bacterial genomes can be sequenced in a matter of days; even hours. As a result, online databases of sequenced microbes, such as the one found in the National Library of Medicine, have grown enormously, providing free and easy access to bacterial genomes for anyone across the world. Many bacteria found in the gut microbiome have their DNA sequences stored in such databases, thereby facilitating the discovery of new metabolites.

However, this presents a problem: how does one sort and identify unknown molecules of interest through the millions of base pairs found in bacterial DNA? Even bacteria with relatively small genomes, such as *E. coli*, have genomes that are too long to sort through every nucleotide. Instead, researchers have developed ways to computationally identify genes responsible for natural product synthesis.

While aimlessly scanning through the genome may provide some answers, it is extremely ineffective and inefficient. Modern techniques, as a result, scan for Biosynthetic Gene Clusters (BGCs) — groups of DNA sequences that code for commonly known biosynthetic molecule-related enzymes. Many of the enzymes for natural product synthesis are conserved, meaning that the same enzymes can create different products when used in a different order or when slightly modified. This allows us

to use knowledge of known enzymes to search for similar enzymes in other genomes. AntiSMASH, PRISM, and Rodeo are three tools commonly used by researchers to scan through microbial genomes (14). Both AntiSMASH, an international collaboration between researchers from the University Tübignen in Germany, the University Groningen in the Netherlands, and the University of Manchester in England, and PRISM, developed by Adapsyn Bioscience, scan genomes for potential BGCs that make a plethora of secondary metabolites (15). RODEO focuses specifically on searching for ribosomally synthesized and post-translationally modified peptides, which will be described later, by searching for genetic motifs indicative of their biosynthetic enzymes (16). Other approaches that have yielded results include synthetic-Bioinformatic Natural Product (syn-BNP) (17), phylogeny mining (18), and resistance gene mining (19).

Bacterial Natural Product Biosynthesis

A bacteriocin, by definition, is a bacteria-produced peptide that exhibits antimicrobial activity (20). With modern tools, researchers have been able to identify many of these promising molecules, such as RiPPs and NRPs. The biosynthesis of these classes of molecules will be described briefly here, with references to sources that describe them in more detail for those interested. These bacterial natural products and their derivatives have been the sources of many pharmaceuticals on the market today. Streptomycin from *Streptomyces griseus* (21) and aureomycin from *Streptomyces aureofaciens* (22) are both antibiotics that have been derived from bacteria.

RiPPs

Ribosomally synthesized and post-translationally modified peptides, or RiPPs, are a type of natural product produced by bacteria. Their final structures vary greatly, but all are synthesized in the same manner. First, a precursor peptide, usually around 20-110 residues in length, is synthesized, following the flow of the central dogma of biology: the DNA sequence gets transcribed into an mRNA, which is translated into a peptide chain at a ribosome. After translation, the precursor peptide undergoes modifications that transform it into its functional form. The leader peptide is usually cleaved off, and the core peptide is further modified. These modifications can include isopeptide-bond linkages (23), thioether linkages (24), and cyclization (25,26)

Lasso Peptides

One major type of RiPPs that has been investigated for its antibiotic potential is lasso peptides. These unique peptides are characterized by their shape, which includes a macrolactam ring threaded by the C-terminus, resembling the shape of a lasso (Figure 1A) (28). The gene clusters of lasso peptides contain those genes that modify the peptide into its lasso shape (Figure 1B). All lasso peptides require at least two enzymes to create — one that functions as a leader peptidase (to cleave off the leader strand), and one responsible for threading the core peptide through the macrolactam ring (28). Lasso peptides vary in function, but many exhibit antimicrobial properties.

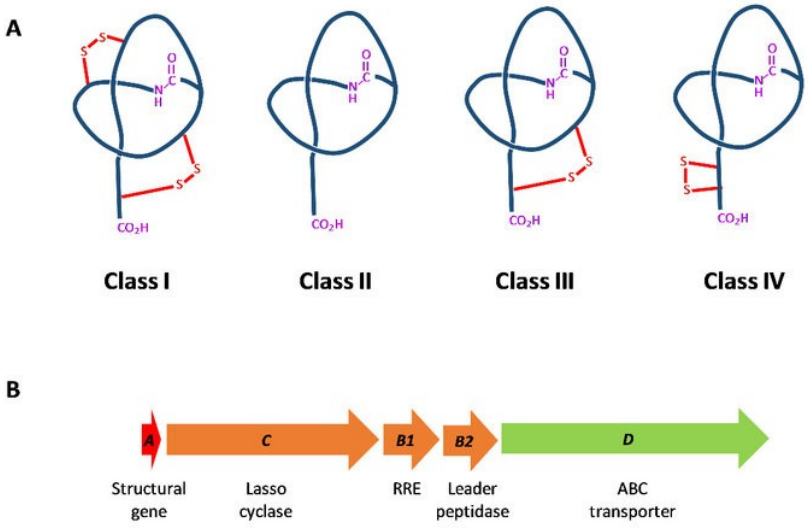

Figure 1: A:the four classifications of lasso peptides, shown with their structures (variations shown in orange). B: the typical sequence of genes for lasso peptide synthesis, each represented by a letter and arrow (27)

Due to their threaded shape, these peptides are able to evade proteases (enzymes in bacteria that cleave foreign peptides for immune protection) in bacteria, making them promising potential drugs. For instance, microcin J25, a well-studied lasso peptide produced by a strain of *Escherichia coli* discovered in a stool sample, was shown to inhibit RNA polymerase in target bacteria (29). Microcin J25 is also resistant to heat, being able to withstand the heat of an autoclave (30) making it a suitable drug candidate, as it needs to be able to survive the many different environments of the human body in order to reach its target and work as intended. Capistruin (23), streptomonomicin (31), and lassomycin (32) are other examples of antimicrobial lasso peptides. Studies have suggested that lasso peptide enzymes have a high tolerance for substitutions in their peptide structure, allowing for activity to be altered through engineering (33).

Plantazolicin

Plantazolicin, or PZN, is a linear azol(in)e-containing RiPP (Figure 2) identified for its antibiotic potential. A linear azoline structure is characterized by thiazol(in)e and (methyl)oxazol(in)e heterocycles (which have cysteine and serine/threonine residues). Created by *Bacillus methylotrophicus* and *Bacillus pumilus*, PZN has been shown to systematically target the membrane and cause lysis of a specific strain of bacteria, *Bacillus anthracis* (34). It has been proposed that PZN targets weak points in the cell membrane of target cells (34). Though PZN peptides have shown little capacity for substitutions, its ability to target a specific strain of bacteria is interesting. Targeting specific strains of bacterial species may be a suitable alternative to the broad-spectrum antibiotics currently on the market, due to the former circumventing bacterial resistance mechanisms.

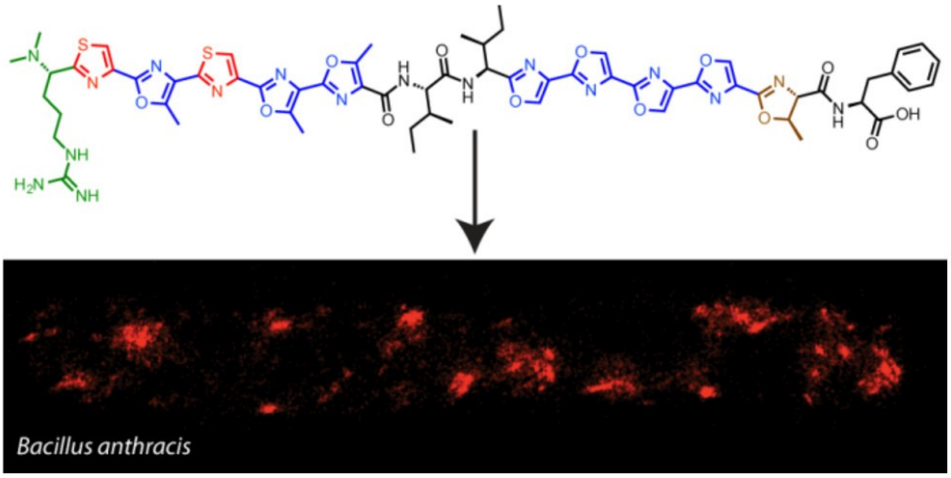

Figure 2: chemical structure of plantazolicin molecule, and its effect on B. anthracis, colocalizing to the cell membrane (34)

Lanthipeptides

Lanthipeptides, characterized by their lanthionine residues (a nonproteinogenic amino acid formed by two alanine residues crosslinked by a thioether linkage, Figure 3), are another type of RiPPs that possess antibiotic functionality. Those that do are referred to as lantibiotics, and usually kill target bacteria by sequestering lipid II, forming pores in the membrane, and/or binding to phosphatidylethanolamine (36). Nisin, a widely used food preservative, is one such lantibiotic, both sequestering lipid II and causing pores to form in targets. Enzymes involved in lanthipeptide formation, most notably dehydrases, are very tolerant of substrates. As a result, lanthipeptide structures are flexible, allowing for the creation of novel molecules by fusing cores of different lanthipeptide precursors (37). Using such techniques to create new molecules could be a promising source of novel antibiotics.

Thiopeptides

Thiopeptides are another class of macrocyclic RiPPs, featuring a six-membered N-heterocycle and a diverse set of functional groups, thus classifying it as a natural "hybrid" RiPP (38). They have also been shown to be antibacterial, mostly against Gram-positive bacteria, by inhibiting protein synthesis. Specifically, some have been shown to have nanomolar activity against pathogenic Firmicutes, while others have been shown to be highly selective towards *Propionibacterium acnes* (39). However, thiopeptides have low solubility in aqueous solutions (38), thereby making their development into drugs particularly challenging, despite their high bacterial selectivity.

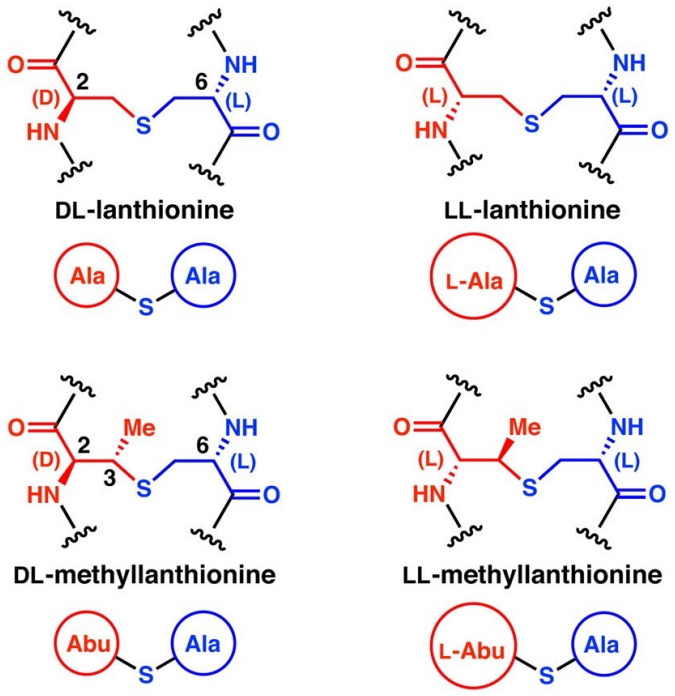

Figure 3: Chemical structure of various lanthipeptides. Serine/threonine residues are shown in red, cysteine residues are shown in blue (35)

Sactipeptides

A fifth type of antibiotic RiPPs is sactipeptides, characterized by their sactionine modification. This involves a thioether linkage (which is a sulfur linkage between two R groups) between a cysteine residue and an alpha carbon of another amino acid. Those that display antibiotic activity, such as thuricin CD, usually disrupt target cells by attacking the cell membrane (40).

Non-ribosomally Synthesized Proteins

A second type of bacteriocin with promising potential is non-ribosomally synthesized proteins, or NRPs. Unlike RiPPs, these peptides are not synthesized according to the central dogma. Rather, modular multidomain enzymes called nonribosomal peptide synthetases (NRPSs) take the place of ribosomes, catalyzing the reactions necessary for peptide synthesis. NRPSs are relatively large enzymes (their genes can be on the order of millions of base pairs long), and they function by adding amino acids (the building block of NRPs) onto a growing peptide chain while potentially adding peptide modifications onto each amino acid (41). The specific domains required for addition of an amino acid are an adenylation domain, thiolation domain, condensation domain, and a release/cyclization domain. Many major antibiotics that are in use today were first discovered as NRPs, such as penicillin, vancomycin, teixobactin, actinomycin D, and bacitracin (42).

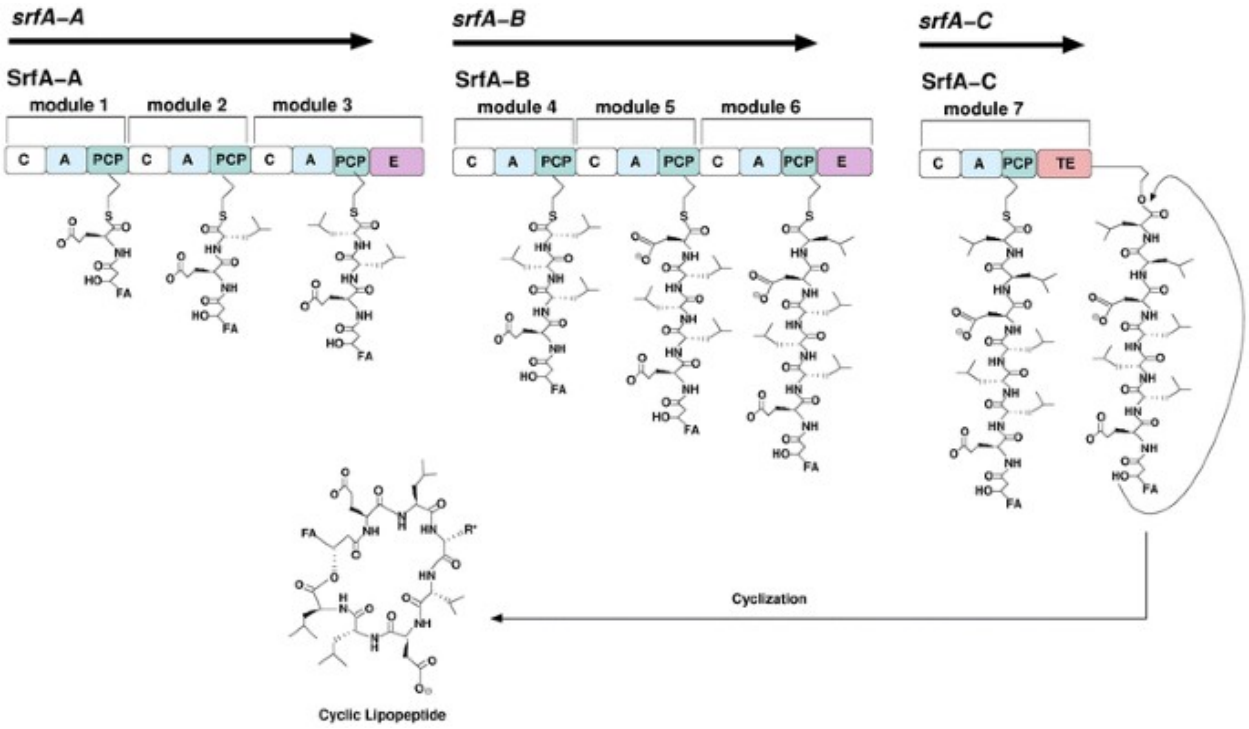

Figure 4: Modular pathway of a non-ribosomal peptide synthetase generating a non-ribosomal peptide (in this case, a cyclic lipopeptide) (41)

The domain-centered organization of NRPSs also provides the potential for engineering. Through recombinant genes, researchers have worked on creating hybrid NRPSs by module and domain fusions, which could potentially create novel peptides with functions not observed naturally. For example, one group of researchers attempted to use domain substitution and random mutagenesis to alter the Peptidyl Carrier Protein domains of NRPSs, using an evolution approach that ended in new, functional NRPSs (43). As DNA technologies continue to improve, we could see more novel NRPs in the future.

Short-chain Fatty Acids

Short chain fatty acids, or SCFAs, are the main metabolites of bacteria found in the large intestines, which ferment fibers and starch to create them (44). They are distinguished by their short length, ≤ six carbon atoms. The activity of SCFAs cover a wide range, one of which is their antimicrobial properties. The small structure of these molecules allows them to diffuse through target membranes easily, thereby reducing intracellular pH (45). In a study that compared the SCFAs acetic acid, butyric acid, propionic acid, and valeric acid against the common foodborne pathogen *Salmonella enterica*, for instance, the presence of SCFAs with a concentration of ~ 3750 µg/mL was enough to inhibit bacterial growth (46): Sub inhibitory concentrations also showed signs of bacterial inhibition, significantly decreasing motility and bioflim

formation, and disrupting quorum sensing (46). Due to their membrane penetrating ability, SCFAs could become a viable source or a starting template of antibiotics in the future.

Problems and limitations with genome mining

Although our knowledge of the gut microbiome has advanced exponentially in the past few decades, there are still many obstacles that remain to be overcome in order for these advancements to be applicable in a realistic sense.

First and foremost, the number of gut bacteria that have been discovered and sequenced are vastly outnumbered by those still undiscovered. While it is true that bacterial genomes have become more accessible and the gut microbiome has great biodiversity, we have only merely scratched the surface of gut bacterial discovery. Most species known to us today have come from analyzing stool samples and intestinal fluids. These, however, do not contain all bacteria. There are more accurate tests, such as biopsies, but they are rather invasive for a patient to undergo. Additionally, most genome mining techniques used today rely on already known information. As mentioned before, researchers use bioinformatics tools to scan for enzyme-coding sequences conserved among many known antibiotics. However, this method may not account for compounds which do not follow this pattern or are synthesized in unique ways in the organism. Unknowingly, we may be overlooking many promising bacteriocins. And, even if all bacteria were sequenced and thousands of promising new antimicrobial molecules were discovered, these would still need to be manufactured at a high enough yield, and then delivered in vivo to the intended target, to be usable as drugs. Finally, in general, there remain many uncertainties regarding the ecological roles of many bacteria and how they interact with their surroundings, both in terms of bacterial and non-bacterial species. To fully unlock the potential of microbial genomes, we must have a better understanding of this complex environment and its network of interactions. For example, we must learn what environmental cues trigger a bacterium to express "silent" BGCs (not expressed in the lab).

Live Biotherapeutics

In addition to searching bacterial genomes for antibacterial molecules, researchers have also utilized gut bacteria themselves in order to treat diseases.

Live Therapeutic Development

Though it may seem intuitive to populate the human gut microbiome with beneficial bacteria, much of the development process is devoted to the drug's research, manufacturing and in vivo delivery mechanisms. This can be seen with Oxabact®, a drug developed by the pharmaceutical company OxThera to treat Primary Hyperoxaluria (PH). PH is a rare genetic condition where the body secretes excessive amounts of oxalate, a molecule that can only be eliminated by the kidneys. *Oxalobacter formigenes*, the bacteria discovered to metabolize oxalate (using oxazyme) in the gut microbiome of sheep, is the basis for Oxabact® (47). In order to turn *O. formigenes* into a usable product, for example, researchers needed to maintain specific anaerobic conditions to preserve its function during manufacturing. To deliver the medication, the lyophilized drug was enteric

coated for protection from stomach acid. As these constraints are common to many drugs derived from anaerobic gut bacteria, there are numerous other ways bacterial protection has been approached. One such way is the use of a self-assembling coating of metal–phenolic networks (a coordination structure of metal ions and natural polyphenols), which can protect cells and enzymes from oxygen exposure and lyophilization, two common stresses during bacteria manufacturing (48).

Gut Microbiome Dysbiosis

As discussed previously, dysbiosis in the gut microbiome can be detrimental to human health. *Clostridium difficile* (CD) infections, which affect around 500,000 persons annually (49), are a big contributor. Antibiotics have been the traditional way of combating this disease, but their effectiveness has decreased over time (50). Instead, research has shown that using therapeutic microbes to fight disease could potentially be far more effective than modern antibiotics. A study in 2014, of using a fecal matter transplant (which introduces microbes from a donor to the GI tract of the patient) to treat CD infections, found that all patients given the transplant were cured of the disease, without the same being said for the group given conventional antibiotics (51). Fecal transplants, however, are not the most convenient; recently, researchers have developed easier methods to restore a balanced microbiome. Orally taken medications like RBX7455, a live biotherapeutic treatment developed by Rebiotix to treat CD infections may be a potential alternative (52). Furthermore, because RBX7455 is stable at room temperature (52), it has the potential to increase the accessibility and use of such medications. Seres Therapeutics, another biotechnology company, uses a unique blend of purified Firmicute spores in its SER-109 therapeutic, which was able to decrease CD recurrence in patients by 28% in a clinical trial (53). Among others, Finch therapeutics (CP101), Vedanta Biosciences (VE303), and MaaT Pharma (54) are all companies developing live biotherapeutic products for the gut microbiome.

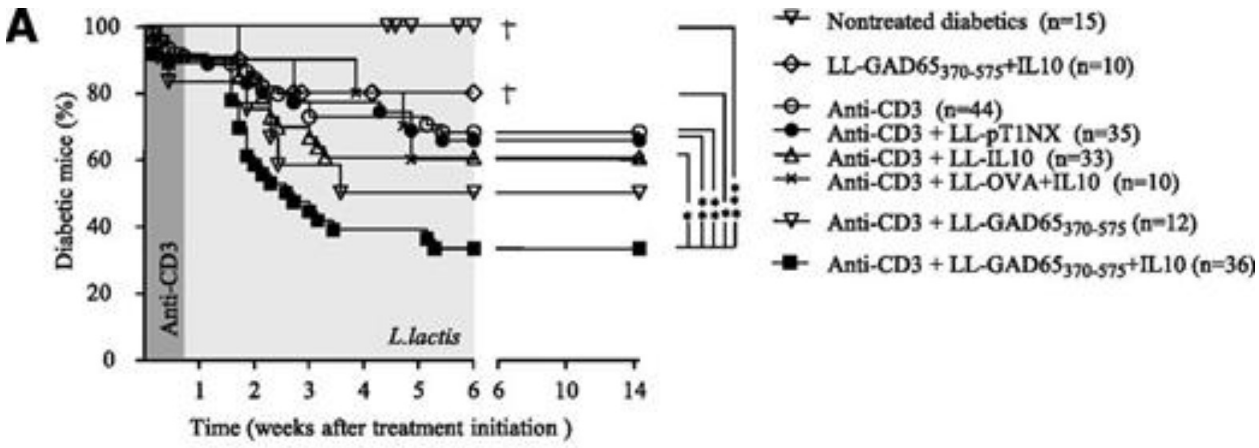

Figure 5: The effect of Anti-CD3, LL-GAD65, and IL-10 on diabetes in mice (figure adapted from reference 55).

Engineered Microbes

Though the gut microbiome contains thousands of bacteria with vastly unique abilities, to achieve the specific results they want, researchers have had to turn to genetic engineering. One approach to manipulating gut bacteria is by attempting to make them secrete specific enzyme(s). This is especially useful in therapeutics aimed at reversing metabolic disorders. An example of this can be found in type I diabetes. *Lactococcus lactis*, a strain originating from the human gut, was engineered to secrete the Anti-CD3 (an antibody), LL-GAD65 (an autoantibody), and the molecule IL-10. When fed to diabetic mice, a synergy of the two molecules reversed diabetes in ⅔ of the animals, as shown in Figure 5 (55). Using this method has also worked for other metabolic disorders like phenylketonuria (56, 57), hyperammonemia (58, 59), and metabolism of Levodopa (a drug used to treat Parkinson's disease) (60).

Often, bacteria form biofilms, or communities of microbes, after causing an infection, thereby decreasing the effectiveness of antibiotics and the semi or impermeable biofilm acts as a barrier to molecules. Engineered probiotic treatments do not have this problem, as there are several ways bacterial infections could be countered, including using antibacterial chemicals, activating the human immune system, and removing resources through competition. Many pathogenic bacteria like *Pseudomonas aeruginosa* use a system known as quorum sensing, which allows the species to communicate with those cells around it and sense the population density of similar cells. This system is vital to the pathogenicity of many bacterial species, and hence can be targeted for bacteriostatic or bactericidal effect. For instance, *E. coli* equipped with synthetic genetic quorum sensing and lysis were effective in getting rid of a *Pseudomonas aeruginosa* infection, inhibiting the formation of a biofilm by nearly 90% (61). A separate study was able to engineer the probiotic *Escherichia coli* Nissle 1917, or ECN, to inhibit biofilm formation by secreting DegP, a periplasmic protein that functions both as a protease and a chaperone (62). The DegP helps the ECN bacteria out compete the target biofilm, which could be composed of bacteria like enterohemorrhagic *E. coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, among others. A third study also used a strain of ECN, this time equipped with microcin H47 production and a tetrathionate sensing system, and found that it was effective against *Salmonella* infections (63). The possibilities of microbe engineering have continued to grow, possibly even replacing or becoming a substitute for antibiotic drugs.

Molecule Absorbents

Although many gut related health issues can be solved by secreted molecules, in some cases, it is the lack of a molecule that is beneficial. One of such situations occurs with autism spectrum disorder, or ASD. In addition to limiting social skills, individuals with ASD commonly have issues with their GI system (64). The relationship between the gut microbiome and ASD is still unproven. However, since the gut microbiome has been shown to affect the immune system (65) and behavior (66), it may exert an influence on ASD as well. One molecule in particular, 4-ethylphenylsulfate (4EPS), has been linked to autism (67).Through their drug AB-2004, Axial Therapeutics was able to reduce anxiety in mice affected by ASD (66). AB-2004 – a

precisely engineered form of spheronized carbon - has a high binding affinity for ucremic toxins, such as phenols and 4EPS produced by gut bacteria, and therefore absorbs these molecules from the gut microbiome (68). This principle could be applied to engineered microbes for another hypothetical therapeutic using a similar mechanism of action. Bacteria could be engineered to produce absorbers such as AB-2004, but a more interesting and possibly more efficient way would be to engineer bacteria not to produce toxins like 4EPS in the first place. These engineered microbes could then be used to repopulate the gut, replacing their natural counterparts. This method has not been tested, and many unknowns like the engineering and repopulating processes still hide within a knowledge gap.

Stool Sample Diagnosis

Finally, outside of therapeutic treatment, bacteria have also been engineered for disease diagnosis in the human body. Researchers have been able to exploit the quorum sensing-abilities of bacteria to identify infections (69). For instance, *L. lactis* was engineered to secrete β-lactamase after detecting a signaling molecule produced by an infective *Vibrio cholerae*, which would alter the color of the feces of the patient and alert them (69). This could potentially help decrease the number of serious, sometimes fatal, misdiagnoses, potentially saving lives.

Table 1: Engineered gut bacterial functions

Engineered bacteria can be made to:	**References**
Secrete Autoantigens	55
Absorb metabolites and/or small molecules from the plasma	47, 4
Correct dysbiosis	49-54
Secrete predefined secondary metabolites (antibiotics, antivirals, SCFAs)	56-60
Not secrete predefined secondary metabolites and used to repopulate the gut.	64-68
Modulate quorum sensing and inhibit biofilm formation.	61-63
Diagnose infections by stool visual change.	69

Future directions

If products from the gut microbiome become the next generation of antibiotics, new technologies to help solve current problems will be the key to getting there. One of these is the intersection of machine learning and scientific research. While modern tools have discovered thousands of new molecules, AntiSMASH alone having identified over 147,000 BGCs sequences (70), this does not necessarily mean that molecules with usable functions are being discovered, as BGCs are identified based simply on structure. With better algorithms to search for more BGCs, such as DeepBGC, false positive identification rates in could go down as more molecules with antibiotic potential are discovered (71). This is still in it infancy, however, as the small training

size (370) of the DeepBGC algorithm limits its accuracy. One study reported accuracies of 57%-79% with their method (72) which is expected to increase over time. The number of sequenced bacteria from the gut microbiome is expected to increase with time as well, implying that more molecules will be discovered with antibiotic functionality.

With all of these new molecules discovered, a possible way to enable them to perform a desired function is through directed evolution. In this process, many iterations of a certain molecule are produced and tested against a certain trait, often reflective of a final target (such as being lethal to a specific strain of bacteria or having a desired molecular target). Similar to Charles Darwin's theory of natural selection in the natural world, the molecule with the best performance is selected to make more iterations of, and so on, until the target is eventually reached (73). Recently, directed evolution has been used to reveal the mechanisms that are used to develop antibiotic resistance, specifically by studying the rRNA methylating enzyme Cfr (74). Scientists have begun to apply these techniques to bacterial natural products, one of them being the "phage-assisted continuous evolution" (PACE) method to facilitate improved production of the antibiotic bicyclomycin (a transcription terminator inhibitor) in a heterologous host (75). This could be one way to solve many of the aforementioned problems with current techniques — production yield, transportation and effectiveness— depending on the "target."

Conclusion

Although the threat of bacterial antibiotic resistance remains greater than ever, with the advent of new technologies, there may be a potential solution concealed within the diverse human gut microbiome. Genome mining techniques and growing global libraries have enabled enhanced potential for the discovery of thousands of promising bacterial natural products, many of them being RiPPs and NRPs. Engineered gut bacteria also offer an optimistic future, as both a disease detector and fighter. The advances covered in this paper may not lead to an omnipotent solution for bacterial infections, but over time, the large number of species comprising the gut microbiome is expected to yield at least some novel antibiotics and therapeutic compounds. Databases will continue to grow as more bacterial genomes are sequenced, sequencing technologies are expected to become cheaper and faster, and machine learning algorithms will become more sensitive and specific in their ability to detect and predict bacteriocins from BGCs; this has the potential to unearth entirely new classes of peptides and other molecules. Using the directed evolution approach of synthesizing new molecules has been promising, and would be a good way to proceed in the future. Though not mentioned in this review, many gut bacteria produce antivirals as well; as seen during the Covid-19 pandemic (76), many genome mining and live biotherapeutic techniques could help combat deadly viruses as well. The day may not be far when a plethora of novel gut-derived antibiotics could change the course of modern medicine.

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