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Mini Review

Antibiotic Discovery from the Human Microbiome

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ABSTRACT

The emergence of drug resistant microorganisms causes a lot of concern particularly in this era marked by a growing array of new microbial infections. A good number of existing antibiotics has been rendered ineffective and as a consequence there is an aggressive search for novel antibiotics with properties that tackle this rapidly growing menace. The human microbiome is a source for the discovery of such antibiotics with rare properties. This paper seeks to review the existing body of knowledge on the human microbiome as a source for antibiotic discovery. The human microbiome is the complete set of genes of all microorganisms that live in or around the body. Because most of these organisms are uncultivable, culture-independent methods such as metagenomics are employed for the functional study of their genomes. Metagenomics, a fast and rapidly growing field of research in Microbiology, is the application of modern genomic techniques to study communities of microorganisms directly in their natural environment, circumventing the need for laboratory cultivation of individual species and it could take any of two approaches: sequence-based or functional approach. With the Metagenomic study of the human microbiome, some antibiotics such as Lactocillin(sequence-based genome mining), Commendamide (functional genome mining), Lugdunin (functional screening) and Humimycins (bioinformatics modeling and chemical synthesis) have been discovered and these antibiotics have been proved useful. Hence, the human microbiome presents an interesting frontier for antibiotics discovery. Although this field of research is still in its infancy, it has prospects of developing with time.

Keywords: *Antibiotic, microbiome, metagenomics, microorganisms, resistance, human*

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INTRODUCTION

As multi-drug resistant pathogens continue to emerge, the current increase in the number of new diseases and pathogens has become a threat to public health globally. There is an urgent need to combat these microorganisms and/or diseases and one direct course of action to take is to discover novel antibiotics with efficient action potential against pathogenic microorganisms. Previously, since the mid-20th century, antibiotics discovery was achieved by screening cultivable soil microorganisms, especially Actinomycetes for bioactivity (Katz and Baltz, 2016). The advances in DNA sequencing, molecular biology and improved cultivation methods have also facilitated the discovery of antibiotics. Recent trends in research suggest that the human microbiome which represents the vast diversity of microbial communities, including unculturable ones contains a broad diversity of small molecules and other products that deserve attention for their possible use as antibiotics (Aziz *et al*., 2018; Zimmermann *et al*., 2019; Du *et al*., 2019; Nichols *et al*., 2019; Cully, 2019), and hence can be a source of antibiotics (Lewis *et al*.,2010) .The human microbiome is defined as the complete set of genes of all microorganisms that live in or around the body. However, throughout the history of Microbiology, we have only been able to culture a small fraction of the diverse microbial population for different purposes including discovery of antibiotics (Xiong, 2016). Majority of microbial species has not been explored since they do not grow in synthetic media in vitro. This has greatly hindered our capability to exploit the rich microbial population as a source of antibiotics (Sherpa *et al*., 2015). There is therefore a need for an approach that will bypass the cultural method of assessing bioactive metabolites from microorganisms.

ANTIBIOTICS: HISTORY AND DEVELOPMENT

Antibiotics are the scaffold of modern medicine, and have made significant impact on the life expectancy and the quality of life, which divide the history of medicine into pre- and post-antibiotic era (Ling *et al*., 2015). The term “antibiotics” refers to a chemical substance of microbial origin that inhibits growth or obstructs metabolic pathways and are used therapeutically to overcome invading infections caused by pathogens (Waksman, 2015). Its origin is from microorganisms themselves, driven by the force of natural

selection to compete for resources with other microorganisms in their environment. These bioactive substances have been in existence in the natural world long before 1928 when Alexander Fleming accidentally encountered a Petri dish of bacterial colonies that had been killed by mould contamination (Ligon, 2004). This was the birth of Penicillin, an antibiotic derived from Penicillium genus of Ascomycetous fungi (Sherpa *et al*., 2015). The discovery of penicillin and its benefit to human beings precipitated the search for other antibiotics present in the natural world. Thus, the "Golden Age" of antibiotics started, a period from 1950 to 1960, when half of the antibiotics commonly used today were discovered (Singh, 2014). By 1941, there was a high demand for penicillin. The aim was to use it to protect soldiers against infections from battle field injuries and this required that the scale of production of penicillin be increased. Following this development, two scientists, Dr. Florey and his assistant Dr. Heatley came to United States to search for new sources of penicillin. They joined forces with the Department of Agriculture which also had interest in the subject and their joint effort led to the discovery of the organism, Penicillium chrysogenum which yielded 200 times more penicillin than any other source tested before then (Robert, 2017).

As the demands for new antibiotics increased, several antibiotics discovery approaches such as genomics, high-tech chemical approaches, and high- throughput screening were launched in the 1990s. Since then the number of companies involved in antibiotic research has decreased significantly and this has affected the number of antibiotics in development (Sherpa *et al*., 2015). Because of factors such as high reduction rate in clinical trials and non-lucrative economic prospect surrounding antibiotics, many pharmaceutical companies have directed their interest into the greener pastures of lifestyle drugs that provide a favourable return on their investment (Sherpa *et al*., 2015).

THE HUMAN MICROBIOME

The term "Human Microbiome' can be defined as the complete set of genes contained in the entire collection of all the microorganisms that live in and on the human body (Chu *et al*., 2016). From deep ocean habitat to radioactive surroundings, microorganisms not only occupy the outside world but they also exist in large numbers in the internal organs of human beings such as the gastrointestinal tract (Jebbar *et al*., 2015). The ecological community of commensal, symbiotic and pathogenic microorganisms that share our body space is referred to as the Human Microbiota (Yang *et al*., 2011). Many of these microorganisms play important role in the human system. They digest certain foods, assist in production of vitamins (B and K) (Cooke *et al*., 2006; LeBlanc *et al*., 2011), and also help to protect the intestinal mucosa against unwanted pathogenic organisms (Zhang *et al*., 2017). The regulation of gastrointestinal T-lymphocytes balance (regulatory T-cell/T helper type 17(Treg/TH 17) ratio), which is critical in maintaining intestinal homeostasis, discriminating between pathogens and commensal microbes through developing "immune tolerance-productive immune response" status is found to be involving the gut microbiota (El Aidy *et al*., 2012; Lawley and Walker, 2013; Khosravi *et al*., 2014; Laparra *et al*., 2012; Lee *et al*. 2004; Duchmann *et al*., 1999). Dysbiosis in the human gut microbiota impairs host health as it induces the selective enumeration of certain microbiota members including pathobionts, leading to dysregulated production of microbial products or metabolites which might be harmful to the host, causing a wide range of diseases including inflammatory bowel disease, celiac disease, obesity, Clostridium difficile infection, colorectal cancer etc (Duboc *et al*., 2012; Lerner *et al*., 2016; Lessa *et al*., 2015; Ley *et al*., 2005; Ley *et al*. 2006; Erny *et al*., 2015; Adams *et al*., 2011; Briton and Young, 2012; Castellarin *et al*., 2012; Collins *et al*., 2014; De Palma *et al*., 2010; Konturek *et al*., 2016; Landers *et al*., 2002).

The search for the understanding of the structure of the human microbiome began with the creation of the Human Microbiome Project in 2007. The Human Microbiome Project (HMP) was a five-year-long international effort to characterize the microbial communities found in the human body and to identify each microorganism's role in health and disease. The project leveraged on the decreasing cost of whole genome sequencing technology, which allows organisms to be identified from samples by-passing the need for culturing them in the laboratory. The technology also facilitates the process of comparing DNA sequences of microorganisms isolated from different parts of the human body and from different people. This project was funded by National Institute of Health (NIH), and launched by a consortium of about 80 Universities and Scientific Institutions from different parts of the world.

The researchers analyzed 242 Individuals (129 male, 113 female) from the United State, collecting tissues from 15 body sites in men and 18 body sites in women. The body sites from which the tissues were collected were mouth, nose, skin, lower intestine (stool), and vagina; 3 samples were collected from each site (Human Microbiome Project Consortium, 2012). By 2012, the HMP researchers had come up with the composition of the human microbiome of a healthy individual. In the first three years of the project, scientists came out with the result that trillions of microorganisms (microflora) colonize the human body and have significant influence on health and diseases. These organisms are collectively known as the human microbiota and possess 100-fold more genes than the human genome (Human Microbiome Project Consortium, 2012). They discovered new members of the human microbiota and characterized nearly 200 different bacterial member species.

According to the researchers, at the phylum level, humans have remarkably the same microbiome while at the genus, species and strains levels, microbiome diversity are highly specific for each individual (National Institute of Health, 2012). The human microbiota consists of mainly bacteria, but also includes fungi, algae, protozoa and non-living viruses (bacteriophages) that reside in and on different body niches such as oral cavity, throat, esophagus, stomach, colon, urogenital tract, respiratory tract, and skin (Cenit *et al*., 2014). The colonic microbiota constitutes the most abundant microbial domain within the human body with the vast majority belonging to bacterial phyla; Firmicutes and Bacteroidetes. The relative diversity, composition and abundance of these phyla vary between the different body sites

and also among individuals with respect to age, diet, and geographical distribution (Yatsuendo *et al*., 2012). These microorganisms have greater complexity than the human genome itself. Microbial cells in the body outnumber human cells by a ratio that is yet to be fully elucidated (Gilbert *et al*., 2018), although because of their small size they make up about 1 to 3 percent of the body mass (Joice *et al*., 2014).The human microbiome, however, is a dynamic system unlike the host genome, which is relatively constant; the microbiome is dynamic and changes with early development, environmental factors such as diet (Ursell *et al*., 2012) and use of antibiotics, especially in response to disease (Briton and Young, 2012; Blum, 2017; Kho and Lal, 2018).The human microbiome also has extensive functions which includes development of immunity, defense against pathogens, host nutrition, synthesis of essential amino acids and vitamins, and storage of fat (Qin *et al*., 2010).

ANTIBIOTICS FROM THE HUMAN MICROBIOME

Lactocillin: Lactocillin was discovered from the human microbiome using genome mining approach. Genome mining approach for antibiotics discovery from the human microbiome is based on the understanding that the genes responsible for synthesizing secondary metabolites are typically co-localized in the biosynthetic gene clusters (BGCs). These human associated BGCs have been predicted to synthesize a broad range of natural product classes including saccharides, Non-ribosomal Proteins (NRPs), Polypeptides and ribosomally encoded and post translationally modified peptides (RiPPs). With the development of algorithm programs such as Cluster Finder BGCs can be rapidly and accurately identified from DNA sequenced data (Sharon *et al*., 2014). With this strategy, Fishbach and co-workers discovered lactocillin (Fig.1) (Cimermancic, 2014). These scientists reasoning that RiPPs are widely distributed across the body sites, searched the Human Microbiome Project shotgun metagenomic assemblies for biosynthetic pathways that encode thiopeptides, a group of RiPPs. They identified thirteen (Singh, 2014) thiopeptide BGCs but chose the thiopeptide bcg66 for further studies because they observed that it had the features of the BGC that produces thiocillin, an antibiotic. Cross referencing of the BGCs with shotgun metagenomic sequences generated from healthy volunteers with focus on gene clusters that were widely distributed across body sites and samples showed that a wild type vaginal isolate, Lactobacillus gasseri was the bacterium harbouring the bgc66 cluster. Comparative metabolomics was carried out between the Lactobacillus gasseri and an isogenic mutant and this gave rise to Lactocillin. An antibiotic assay of the Lactocillin showed that Lactocillin was active against Gram +ve pathogens including colonizers of the urinogenital tract such as Staphylococcus aureus, Enterococcus faecalis, Corynebacteruim aurimucosum. Also, Lactocillin did not show any antibacterial activity against other vaginal Lactobacillus strains suggesting that Lactocillin production by Lactobacillus gasseri is used to prevent the colonization of harmful organisms without affecting nearby beneficial bacteria (Donia *et al*., 2014).This work by Fishbach and co-workers shows how sequence-based metagenomic approach which can be used to rapidly identify BGCs from metagenomes can be employed for antibiotics discovery from the human microbiome. With advances in DNA sequencing and synthesis, and also improved ways for homologous expression of BGCs, this approach is a promising strategy for more antibiotics discovery from the human microbiome.

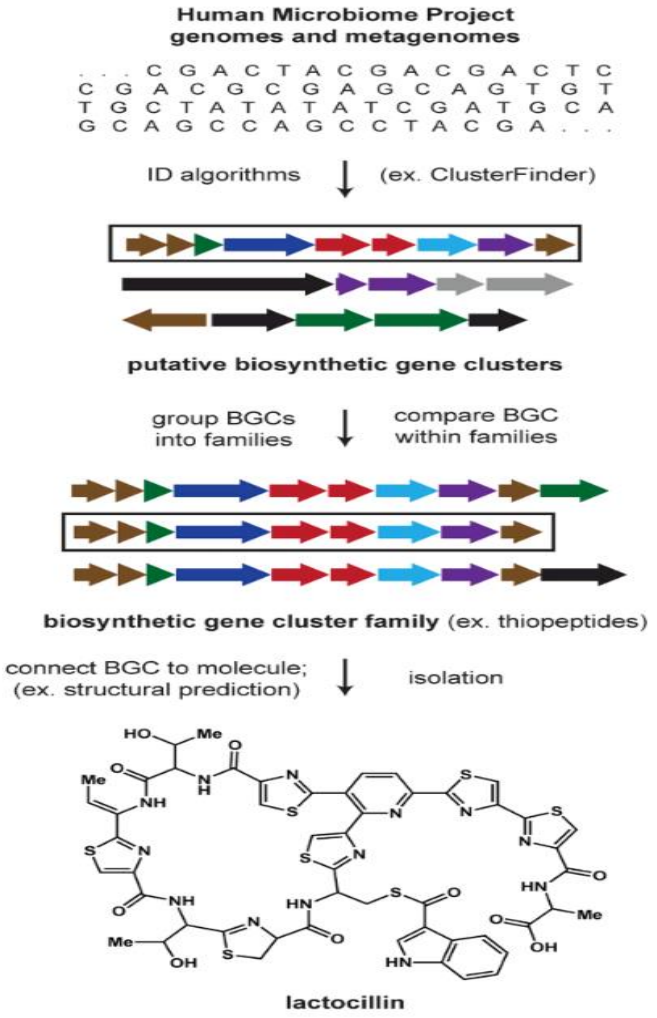

Figure 1:
Overview of the sequence-based metagenomic analysis used to discover Lactocillin (Cimermancic *et al*., 2014; Donia *et al*., 2014).

Commendamide: Although Functional Metagenomics has been used for the discovery of bioactive products from the soil it has not been applied to the human microbiome until recently when Functional Metagenome Mining was shown to be a veritable approach towards the discovery of antibiotics from the human microbiome (Wilson *et al*., 2017). This approach involves the extraction of DNA from an environmental sample (eDNA), and then the genetic information is heterologously expressed in an easily cultivable host such as Escherichia coli. This will result to cloned libraries which are screened for a particular phenotype (in this case antibacterial activity) or the presence of specific genes (biosynthetic genes). The clones of

interest are further analyzed for the production of clone-specific metabolites (Donia *et al*., 2014).

Using this approach, a group of researchers, Bradly and co-workers in 2015 announced the discovery of Commendamide (Fig.2), a microbial metabolite with antibiotic ability isolated from the human gut. They identified this metabolite by screening Escherichia coli clone libraries created from human stool metagenomic DNA (Xiong, 2013).

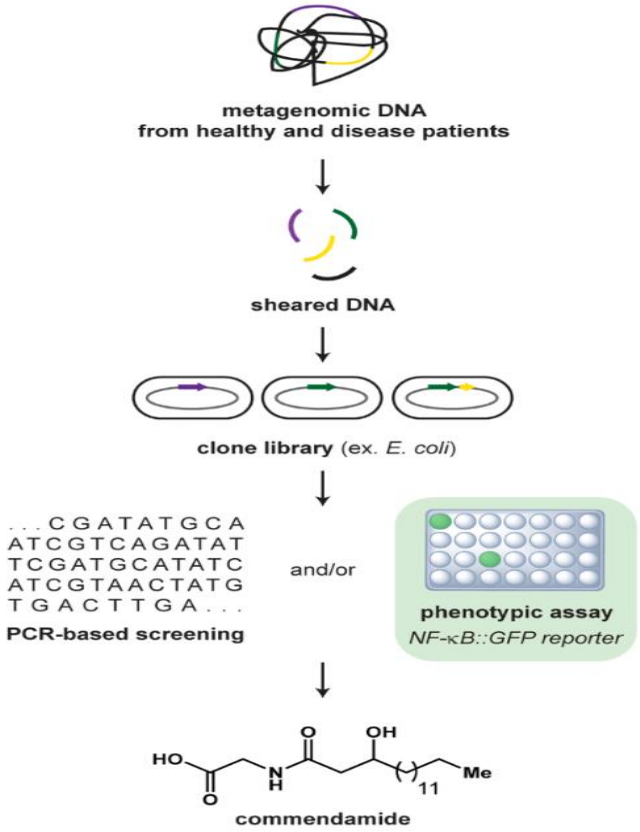

Figure 2:
Overview of the functional metagenomic analysis used to discover Commendamide (Xiong, 2013).

Using a cell-based reporter assay, clones that modulated nuclear factor- kB (NF-KB) were identified. NF-KB is a transcription factor that responds to a variety of cellular processes. On examining a 75,000 member cosmid library, they identified a total of 26 unique bacterial effector genes (Cbegs). A particular effector gene, Cbeg12 was discovered to encode the production of N-acyltransferase. Homologous expression of Cbeg12 and metabolic profiling were carried out and this led to the production of N-acyl-3-hydroxy palmitolglycine, a new natural metabolite which they named commendamide. Upon further research, Cbeg12 was identified in several strains of commensal Bacteroides genomes and the production of this metabolite was confirmed on cultivation of Bacteroides vulgatus. It could be suggested that Bacteroides vulgatus produces Commendamide to interact with host immune system (Cohen *et al*., 2015). Functional Metagenomics as strategy for antibiotics discovery from the human microbiome reveals the ability to rapidly connect a single open reading frame present in stool metagenomic DNA to a biologically active metabolite (Donia *et al*., 2014). The efficiency of this strategy will improve significantly with the development of heterologous hosts with features best suited for the expression of DNA from major members of the human microbiome.

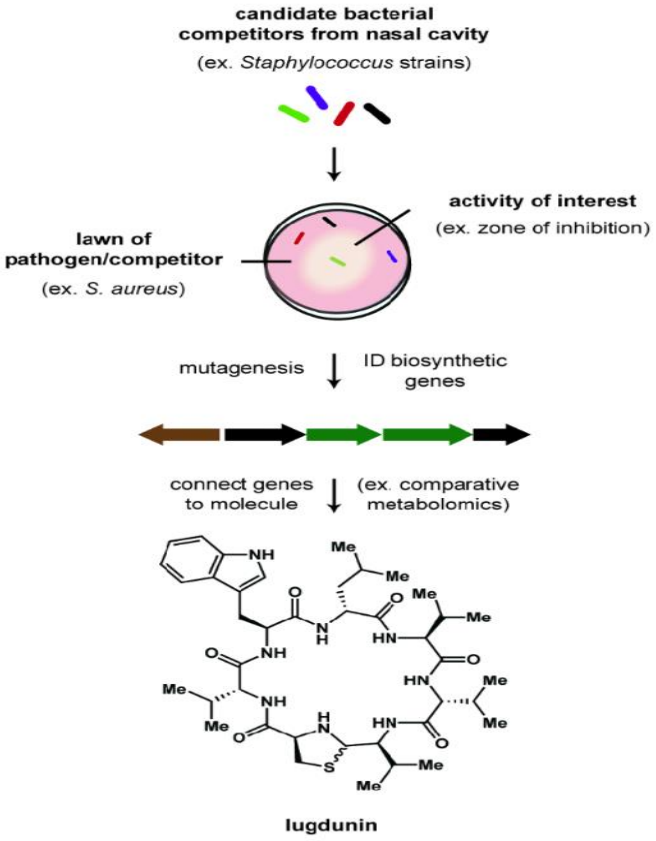

Figure 3:
Schematic diagram describing the Functional screening approach that enabled the isolation and characterization of Lugdunin (Donia *et al*., 2014).

Ludgunin: A study on the chemical ecology of members of the human microbiome is another strategy for antibiotics discovery from the human microbiome. Identifying the biochemical entities that mediate interactions in their environment can be exploited for the production of antibiotics. In this approach, instead of isolating a natural product and then searching for its relevant biological activity, a relevant biological activity is first done and then there is a subsequent search for the molecule or molecules responsible for the activity (Donia *et al*., 2014). Functional screening of nasal bacteria using this approach led to the discovery Lugdunin (Fig.3), an antibiotic produced by the commensal bacterium, Staphylococcus lugdunensis.This research was carried out by Zipper and co-workers (Zipperer *et al*., 2016) who were the first to elucidate the potentially beneficial properties of Staphylococcus lugdunensis. They observed that Staphylococcus lugdunensis had strong antagonism for the human pathogen Staphylococcus aureus. With transposon mutagenesis, this activity was linked to 30-kb gene cluster that encodes an NRPS, and additional biosynthetic enzymes, which suggests that a non-ribosomal peptide was likely responsible for the activity.

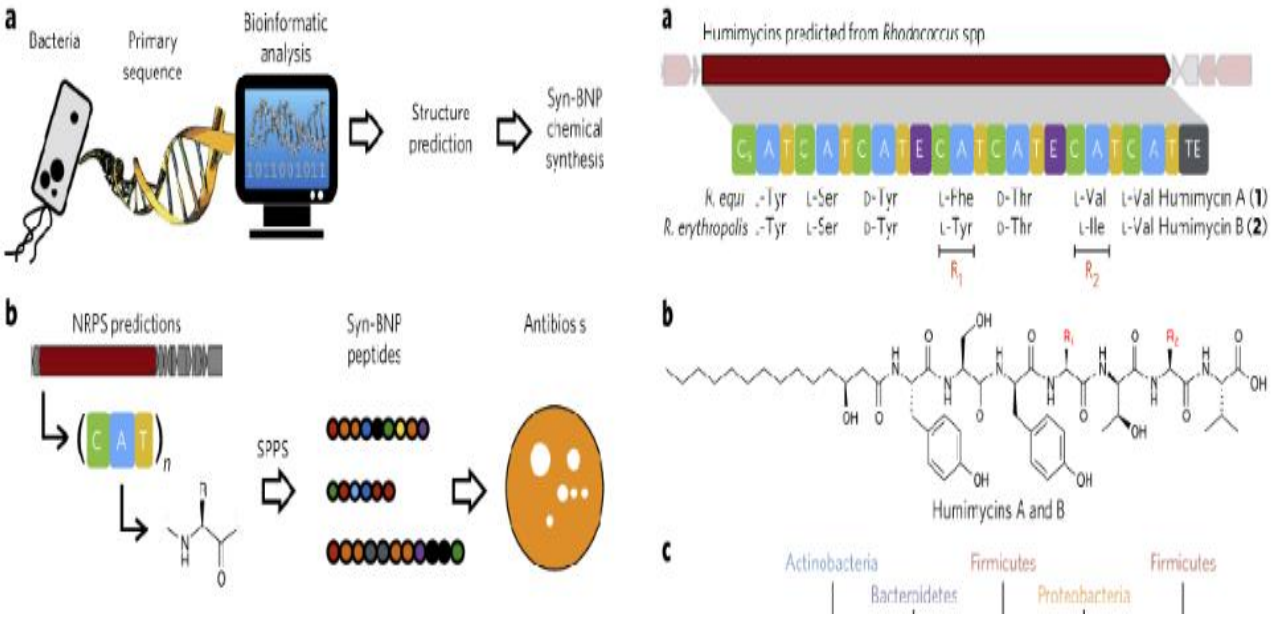

Figure 4:
Overview of the bioinformatic modeling and chemical synthesis used to discover Humimycins [15].

Transposon mutagenesis is a biological process that allows genes to be transferred to a host organism or chromosome, interrupting or modifying the function of an extant gene on the chromosome and causing mutation. On cultivation of S. lugdunensis, they isolated Ludgunin after overcoming initial cultivation hurdles. Antibiotic assay demonstrated that Ludgunin has potent antimicrobial activity against Gram +ve bacteria including Vancomycin and Methicilin resistant Staphylococcus aureus and Enterococcus species without any sign of resistance. In further studies, Lugdunin completely eradicated Staphylococcus aureus from both skin and animal infection models. Colonization of the nasal region by Staphylococcus lugdunensis leads to low population of Staphylococcus aureus (Donia *et al*., 2014). Following this strategy, further or future comparisons of antagonism between human associated microbes, and analyzing the composition of these microbial communities could reveal more bioactive metabolites from the human microbiome.

Humimycins: Basically, in this approach, natural product-like structures are bioinformatically predicted from primary sequence, and then chemically synthesized(synthetic bioinformatic natural products, syn-BNPs). This results in small molecules that are generated by bacterial biosynthetic system but however, not physically isolated from the bacteria (Chu *et al*., 2016). Chu and his co-workers (Chu *et al*., 2016) used this approach to discover a new antibiotic molecule named Humimycin (Fig.4). Because in nature, natural products often appear as families of related structures having the same biological activity, scientists reasoned that even if the structural predictions were not particularly perfect, many syn-BNPs would be sufficiently accurate representations of nature to obtain the intended bioactivities. They called these bioinformatically inspired compounds syn-BNPs. After bioinformatically predicting a pool of non-ribosomal peptide (NRP) structures from the human microbiome-associated BGCs, and using solid phase peptide synthesis to produce these peptides, Chu et.al screened the resulting molecules for antibiotic activity against a range of human commensals and human associated pathogenic bacteria and this led to the discovery of two novel antibiotics, Humimycin A and Humimycin B which are linked to the NRPS gene clusters encoded by Rhodococcus equi and Rhodococcus erythropolis respectively. Rhodococcus.erythropolis is found as a part of the normal human nasal, mouth and eye microbiota, while Rhodococcus equi is found as a normal flora of the gut microbiota. The antibiotics were potent against Firmicutes and some Actinobacteria, with efficient activity on methicillin resistant Staphylococcus aureus and Streptococcus pneumoniae.

CHALLENGES AND FUTURE SPECULATIONS

Our understanding and exploration of the human microbiome and the structure and functions of microbiome-derived antibiotics is still in its early stage, with prospects of improving steadily. The case studies here present the utilization of multiple approaches for identification and characterization of bioactive metabolites derived from the human microbiome. Although the studies here illustrate the high potential of antibiotics discovery from the human microbiome, they also showcase some challenges. Considering the overwhelming amount of sequenced data, the question before researchers is, how do we prioritize BGCs from the human microbiome for antibiotics discovery? Considering the case of lactocillin, researchers can focus specifically on BGCs that encode secondary metabolites with structures similar to known natural products possessing well

defined bioactivities. Also if analysis of BGCs distribution across humans (both healthy and patient populations) is done, it may help to identify the pathways most relevant to human biology (Donia *et al*., 2014). Another major challenge facing researchers is to confirm whether human-associated microbes actually produce these antibiotics while in and on the human body. In all the processes illustrated here, culturable strains were used to isolate and characterize antibiotics outside of a host context. However, it remains unknown if these metabolites are produced in the human body. If sensitive analytical techniques such as MALDI-imaging mass spectrometry is applied, it could proffer solution to this issue (Biteen *et al*., 2016). Previously, these tools have been used to confirm the presence of bioactive metabolites on the skin (Bouslimani *et al*. 2015), in lungs sputum (Quinn *et al*., 2016), and tissues of various organs (Xiong, 2016). The development of better techniques that can help access body sites such as the gastrointestinal tract and respiratory tract will help to identify natural bioactive metabolites present in these environments.

CONCLUSION

From the foregoing discussion, the human microbiome represents the vast metabolic potential of uncultivated microbes, including entirely novel microbes and novel metabolic pathways. It also presents an exciting frontier for antibiotics discovery. The bacteria producing these metabolites could also serve as probiotics therapy (Trippett, 2020; Guthrie and Kelly, 2019; Kuntz and Gilbert, 2017).

Concurrently, it highlights substantial challenges to researchers; with time these challenges could be surmounted. The efficient discovery and characterization of antibiotics from the human microbiome will require the synergistic efforts of biochemists, genetic engineers, and microbiologists for the development of approaches and strategies suitable for the exploration and exploitation of this complex microhabitat.

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