Pakistan Academy of Sciences

Review Article

Biosynthetic Gene Clusters in Bacteria: A Review

Rimsha Dilshad, Nazia Jamil, and Rida Batool*

Institute of Microbiology and Molecular Genetics, University of the Punjab,
Quaid e Azam Campus, Lahore, Pakistan

Abstract: Soil is a nutrient-rich environment that harbors billions of microbial species. The diversity of microbes in an environment varies with the change in edaphic factors. To survive these environmental changes, microbes produce secondary metabolites which are not directly associated with their growth and reproduction. Bacterial genomes possess biosynthetic gene clusters (BGC) which regulate the synthesis of these secondary metabolites. These BGCs encode for megasynthases such as nonribosomal peptide synthases (NRPS) and polyketide synthases (PKS) which produce metabolites such as antimicrobial compounds, which are the most common metabolites produced by these megasynthases. They help bacteria to survive in the competitive environment by killing surrounding microbes. As chemical drugs may pose immense damage to human health and the environment, so antibiotics produced by natural sources are of major attention these days. The extraction of antimicrobial compounds from bacterial sources also provides scaffolds for new synthetic drugs. Bacteria maintain strict genetic control over antibiotic production. They have particular quorum sensing pathways that help to trigger the surrounding cells to produce antibiotics when required. Biosynthetic gene clusters need to be explored widely under various culture conditions so that more useful products can be extracted from a single type of bacterium. This review focuses on the secondary metabolite production, extraction, and biosynthetic gene clusters which encode megasynthases responsible for the production of antimicrobial compounds.

Keywords: Secondary metabolites, Biosynthetic gene clusters, Megasynthases, Antimicrobial Compounds, Quorum Sensing.

1. INTRODUCTION

Soil is a rich source of microbes as per gram of soil harbors billions of species of various microorganisms [1]. The growth of a bacterium requires certain compounds to be metabolized for fulfilling its needs. The starting, intermediate, or end products of the metabolism of bacteria are known as metabolites. These metabolites can be of two types i.e. primary and secondary metabolites [2]. Primary metabolites are those metabolites that are essential for the normal growth, development, and reproduction of a bacterium. While secondary metabolite is the type of metabolite that is not directly involved in any of such activities but it has some other ecological functions [3]. The uncountable interactions between microbes or amongst microbes and plants including, mutualism, predation, competition occur within the soil

ecosystem due to the presence of secondary metabolites secreted by these microbes [4].

Bacteria are useful to mankind due to their ability to produce diverse nature of metabolites. Bacteria are the catalytic factories that possess the potential to undergo evolutionary changes in their genome over a short period and thus introducing a new diversity of metabolites having specialized applications. These metabolites get structurally optimized when bacteria use them against different targets for their defense mechanism. Thus, bacterial metabolite production potential, evolutionary adaptation, and the aptitude of response to external stimuli enhance their potential to be used in industries, agriculture, and medicines [5].

Single bacterial strain can yield different types of secondary metabolites depending upon their

nature. Secondary metabolites are produced by different biosynthetic pathways which are regulated by biosynthetic gene clusters. These biosynthetic gene clusters encode various megasynthases such as Polyketide Synthases and Non-Ribosomal Peptide Synthases which yield polyketides and non-ribosomal peptides respectively. These are the most copious families of secondary metabolites consisting of diverse compounds with numerous functions. The functions of polyketides and non-ribosomal peptides include protection against stress factors by pigments, iron scavenging by siderophores, antimicrobials, and communication molecules [6].

These secondary metabolites are produced in great quantities by bacteria but the major issue arises when they cannot be extracted properly from the bacterial growth medium or from inside the cells. Bader et al. [7] have reported a supercritical fluid extraction method by using various co-solvents to extract various secondary metabolites from bacteria. Nevertheless, to identify novel compounds plenty of different solvents will have to be used. Hence, there is a need to explore more convenient culture-dependent and culture-independent methods to identify and extract secondary metabolites.

A major class of secondary metabolites produced by bacteria is antimicrobial drugs. The need to explore natural antibiotics increased due to the over and misuse of antibiotics leading to the persistence of antibiotic-resistant bacteria and antibiotic resistance genes in our environment. These antibiotics are released in wastewater of healthcare services, industrial plants, agriculture, and the general population. Even after treating this wastewater by its treatment plants, these antibiotics remain in the plants, and as soon as this treated water is released these antibiotics also get released into the environment. Some of the antibiotics like sulfonamides, fluoroquinalones, and tetracyclines get attached to the soil particles and thus hinder the process of their biodegradation. These issues urge scientists to discover natural products to avoid health and environmental problems [8].

This study focuses on the culture-dependent and independent methods for the extraction of secondary metabolites from bacteria, and the overview of PKS and NRPS megasynthases

involved in the production of these metabolites. The most common category of compounds being produced by these megasynthases i.e. antimicrobial compounds will also be discussed.

2. SECONDARY METABOLITE BIOSYNTHETIC GENE CLUSTERSS

Secondary metabolites are of great concern as a specific type of secondary metabolite is produced by only a narrow range of species. Comprehensive analytical techniques result in a better understanding of the complex secondary metabolome of species. Besides, it can help in evaluating the deviations in the metabolite profile and their morphological changes under variable culture conditions [9]. Microorganisms possess secondary metabolite biosynthetic gene clusters (BGC) in their genomes. Biosynthetic gene clusters (BGCs) are a locally clustered group of two or more genes that function together to encode a biosynthetic pathway for particular secondary metabolite production [10]. For instance, various BGCs present in Bacillus include polyketide synthases (PKS), non-ribosomal peptide synthases (NRPS), siderophores, phosphonates, ectoines, terpenes, thiopeptides, lanthipeptides, bacteriocins, and other non-traditional, hybrid BGCs [11]. Conventionally, bacterial strains are focused to obtain a particular novel metabolite from them which is being produced in large quantities. But, this metabolite is generally a low-hanging fruit of that particular bacteria so, for getting a better fruit it must be explored further by modifying the culture conditions in which the strain is being grown. This technique is known as the "one strain many compounds" (OSMAC) approach where one strain is studied on a deeper level to investigate various compounds being produced by it [12].

This hunt for more compounds from a single strain was stimulated by the rise in genome sequencing and BGC annotation. This advancement revealed the presence of more biosynthetic gene clusters in strains which shows that the particular strain is explored for characterization of lesser biosynthetic pathways as compared to the biosynthetic genes present in it. This variation in characterization and presence of biosynthetic gene clusters can be because many of the BGCs either become cryptic (un-expressed) or even if they are expressed the expression is so low that it

cannot be detected in the growth conditions being analyzed [13]. As studied by a group of researchers, *Streptomyces albus* has genes to produce about 14 anticancerous and antifungal compounds but they remain silent until a specific elicitor is added to the culture [14].

These BGCs are responsible for encoding megasynthases which are large multi-enzymatic proteins involved in the production of various important natural compounds. These megasynthases use the precursor units and do their condensations and modifications to get diversified natural products [15]. Examples of megasynthases include fatty acid synthases, polyketide synthases, non-ribosomal peptide synthases, etc. These metabolites are valuable in various industries as well because they are known to provide aid as competitive weapons against other organisms like bacteria, fungi, plants, or animals; metal transporters; symbiotic agents between bacteria and plants, insects, nematodes, and other higher animals; differentiation effectors and as sexual hormones [16]. The identification of these secondary metabolites can be done by culture-based and culture-independent methods. Culture-based methods give us the idea of metabolites that can be produced by various bacteria under different culture conditions while culture-independent methods help to identify the potential of bacteria to produce secondary metabolites based upon the biosynthetic gene clusters present in them. These metabolites may not be identified by culture-based methods because of the lesser production or unsuitable conditions for better yield.

2.1 Culture-Based Methods for Identifying Secondary Metabolites

For decades, scientists have isolated bacteria and optimized them for their better growth and secondary metabolite production by changing temperature, time of incubation, pH, and nutritional requirements i.e. carbon, nitrogen, iron, and trace elements sources [17]. If the growth conditions in which bacteria are surviving are not met by the artificial media, or there occurs any competition for the nutrients in bacteria, it hinders the proper growth. Other than growth medium, the incubation conditions may vary for bacteria or some bacteriocins might be produced in the medium by other bacteria thus inhibiting the growth of some

important bacterial strains [18]. According to a study by Bode *et al. Streptomyces* sp. experience a significant shift in streptazoline production by the addition of supplements, like $CaCO_3$ and Al_2O_3, in the medium. Production of rubromycin was also affected by changes in pH. Rubromycins were produced only in slightly acidic pH conditions while under neutral pH i.e. 7.3, different compounds were observed [19].

Sometimes bacteria are thriving in nutrient-deprived environments thus when they are provided with nutrient-rich culture media they do not grow. This limitation can be breached by diluting the nutrient medium. Researchers have used this technique to isolate bacteria from aquatic and terrestrial habitats [20]. Other important techniques may involve a physical reduction in the number of mixed bacterial colonies by filtration methods, density-gradient centrifugation, elutriation, and extinction dilution whereby samples are diluted to isolate single colonies [21].

Diffusion chambers are the devices in which semi-permeable membranes are enclosed, to ensure the flow of external nutrients into the chamber. These devices were developed about 20 years ago for the isolation of unknown novel marine isolates [22]. Researchers took the marine sediment samples and serially diluted them and inoculated them in diffusion chambers containing agar. The natural marine environment was mimicked by reinoculating it in marine aquaria. This experiment ended up in 300 times increased growth of known as well as novel bacterial taxa as compared to the standard plate methods. Some other groups of scientists have also used this approach to cultivate bacteria of soil and forests that could not be isolated by previous traditional methods [23]. Other than diffusion chambers, difficult to culture bacteria that can also be isolated by developing growth media that imitate soil conditions. As methanol can be used to extract metabolites from the soil that can be supplemented in growth media to ensure better growth of uncultured microbes. This culture media is known as Intensive Soil Extract Medium (ISEM) [24]. According to a study on *Streptomyces* spp. it was observed that by using soil enriched medium about 4 novel secondary metabolites were obtained which were otherwise not produced in the medium [19]. This approach is a better choice for

the cultivation of unknown bacteria because it is inexpensive and easy to perform in the laboratory. This approach does not require technical expertise or special equipment that's why it can be utilized for an extensive range of applications. This growth media adjustment can be used to activate cryptic or silent BCGs. Most of the unexpressed genes in cryptic or silent BCGs help to avoid unnecessary energy costs as the secondary metabolites produced by these genes are not a prerequisite for the survival of bacteria [25]. This concept was demonstrated by a group of researchers that when *Streptomyces* sp. is co-cultured with Bacillus subtilis or Methicillin-resistant *Staphylococcus aureus*, it causes the activation of Granaticin cluster in *Streptomyces* thus enabling the production of granaticin, granatomycin D, and dihydrogranaticin B [26].

Development in culture-based methods for natural compounds' isolation has been very modest. While contrary to this, culture-independent methods are getting major concerns of scientists. This shift in isolation methods is mainly because of the challenges associated with *in vitro* and in vivo environment differences. Sometimes, it becomes very difficult to imitate in-vivo conditions in a laboratory where traditional culture media are used to isolate bacteria from samples. It results in the rediscovery of the same natural products, repeatedly. It can be attributed to the fact that typical growth media comprise a limited range of nutrient concentrations, carbon sources, oxygen saturation, and pH. Besides, these media favor the growth of fast-growing bacteria only, and metabolic interactions between bacteria are not considered [18]. Bioprospecting, (systematic search for natural products from bioresources) novel compounds has urged the development of unconventional culturing techniques known as culturomics which is the culturing and identification of unknown bacteria employing non-conventional and novel approaches. This technique circumvents many of the restrictions posed due to culture-based techniques by simulating nutritional or environmental conditions that are found in soil [27]. Advances in research have also helped to express silent biosynthetic gene clusters, those were unable to express under normal laboratory conditions, and thus, the innovation of novel products in known bacteria has become easier [25].

2.2 Culture-Independent Methods for Secondary Metabolites

Culture-independent methods for the identification of biosynthetic gene clusters emerged with the development of high throughput sequencing techniques. This development has facilitated the discovery of most of the BGC's that remain unexplored due to the inability to culture certain bacterial strains. Bioinformatics tools use various algorithms that are a necessity for investigating different domains and clusters of genes within sequenced datasets. These algorithms are also used for the identification of regulatory sequences, or to predict molecular structures of synthesized chemicals and discover homologous structures [28]. To access these bioinformatics tools, researchers have developed a web portal that comprises various databases and tools. It is known as the Secondary Metabolites Bioinformatics Portal (SMBP, www.secondarymetabolites.org) [29].

Mining tools use the curated reference data fingerprints of metagenomes and assembled genomes to analyze gene clusters in sequenced data. AntiSMASH is the most commonly used for this analysis. AntiSMASH utilizes Hidden Markov Models (HMM) to detect secondary metabolite coding gene clusters in the bacterial and fungal genomes [30]. Other commonly used tools for the prediction of BGC include Cluster Finder and Prediction Informatics for Secondary Metabolome (PRISM). Cluster finder identifies the BGC based on statistical analysis. Decisions are based on the probability of each converted nucleotide sequence domain to be part of a gene cluster, depending on the frequencies at which this domain occurs in BGC and non-BGC reference training sets, and the identities of neighboring domains [31]. PRISM also works on the principle of AntiSMASH i.e. it uses HMM and reference genomes to identify biosynthetic gene clusters but it focuses on the structural identification of metabolites being produced by the clusters [32].

These tools are used for the already available genomes which are well assembled. But for the case of unassembled short reads or PCR products these tools are not suitable. For such samples, another web portal has been designed which is known as "Environmental Surveyor of Natural Product Diversity (eSNaPD)". This platform has made it

easier to access functions of short PCR amplicons having sequence tags of an adenylation (AD) and ketosynthase (KS) domain by relating them to the reference dataset of gene clusters. Other tools such as Natural Products Domain Seeker (NaPDoS) and SBSPKS v2 are also helpful for short sequences that focus on pathways and their chemical products; and sequence and structure-based analysis of secondary metabolites respectively [33]. These approaches have led scientists to discover BGC for nargenicin macrolides which are anti-Staphylococcus aureus antibiotics, first isolated in the 1970s. Its BGC was rediscovered by genome sequencing in Nocardia species after being hidden for about 40 years [13]. To store these numerous data of BCG tools there are 3 major repositories including MIBiG (Minimum Information about Biosynthetic Gene cluster), ABC (Atlas of Biosynthetic Gene Clusters), and antiSMASH-DB [33]. Where, MIBiG contains data of about 1700 gene clusters, AntiSMASH contains 7800 NRPS and 4500 PKS clusters while ABC consists of 2400 experimentally validated secondary metabolites and 1,000,000 non-verified entries [17]. These databases help researchers worldwide to upload or access the data submitted in these repositories.

3. MEGASYNTHASES INVOLVED IN SECONDARY METABOLITE PRODUCTION

Secondary metabolites in bacteria are produced by multi enzymatic and multi-domain megasynthases.

These megasynthases are the multienzyme protein products of biosynthetic gene clusters. They include various structural types but here nonribosomal peptide synthases (NRPSs) and polyketide synthases (PKSs) are being considered which are responsible for the production of nonribosomal peptides and polyketides. These secondary metabolites are a great source of valuable biological activities as well as clinical applications such as antifungal, antimicrobial, antitumor, antiparasitic, and immunosuppressive agents. These domains further have their core catalytic domains that aid in the biosynthesis of polyketide or nonribosomal backbone moieties. These catalytic domains are adenylation (A), an acyl carrier protein (ACP), acyltransferase (AT), condensation (C), dehydratase (DH), enoylreductase (ER), ketoreductase (KR), ketosynthase (KS), and thiolation (T) [34]. Other than these catalytic domains, several auxiliary functional domains called tailoring domains also support providing a diverse range of chemical alterations to the backbone moieties of these secondary metabolites to enhance their structural diversity [34]. The scheme of these megasynthases is shown in figure 1 while the various types of compounds being synthesized from these domains are given in table 1.

3.1 Nonribosomal Peptide Synthetases (NRPSs)

NRPSs are the megasynthases that are recognized to be the largest known enzymes having a

Table 1. Examples of Non-ribosomal peptides and Polyketides derived from bacteria

Compound	Known Function
Non-ribosomal Peptides [38]	
Pyochelin	Antibacterial
Bactobolins	Antibacterial
Xylocandin	Antifungal
Glidobactins	Anticancer
Polyketides [40]	
Actinorhodin	Antibacterial
Tetracycline	Antibacterial
Amphotericin	Antifungal
Anthracycline	Anticancer

molecular weight of up to 2.3MDa. They consist of several units each of which is a set of enzymatic domains that determines the primary structure of the corresponding peptide product in terms of their specificity, number, and organization [35]. To get a final peptide a cascade of reactions takes place along a particular line of direction. NRPSs provide this assembly line so that proper functional peptides can be produced. In the first step, the primary sequence of the peptide is defined by the arrangements of recurring units of an NRPS. This minimal repetitive module comprises three domains known as adenylation domain (A-domain), condensation domain (C-domain), and peptidyl carrier domain (PCP-domain). All three domains are responsible for different tasks. A domain is involved in the recruitment of amino acids that are to be incorporated in the final product. Each type of amino acid is recruited by a particular A-domain substrate because there are several hundred A-Domain substrates with their specific specialties.

A domain activates the amino acids for peptide synthesis. PCP domain then takes these activated amino acids and acts as a scaffold for adding amino acids and establishing covalent bonds in them. The condensation domain is responsible for amide bond formation between nascent peptides and the amino acids it adds to the chain [36, 37]. Some examples of NRPs include lipopeptides (xylocandin, cepacidin, occidiofungin, and burkholdins, etc), siderophores (ornibactin, malleobactin, pyochelin, and cepaciachelin, etc), and hybrid PKS-NRPS (thailandamides, thailanstatins, bactobolins, glidobactins, rhizoxin, and rhizonin, etc) [38].

3.2 Polyketide Synthases

Polyketides are a group of natural products which contain diverse carbon skeletons that comprise enediynes, macrolides, polyenes, polyethers, and polyphenols. The exact function of these compounds is still to be known but their estimated
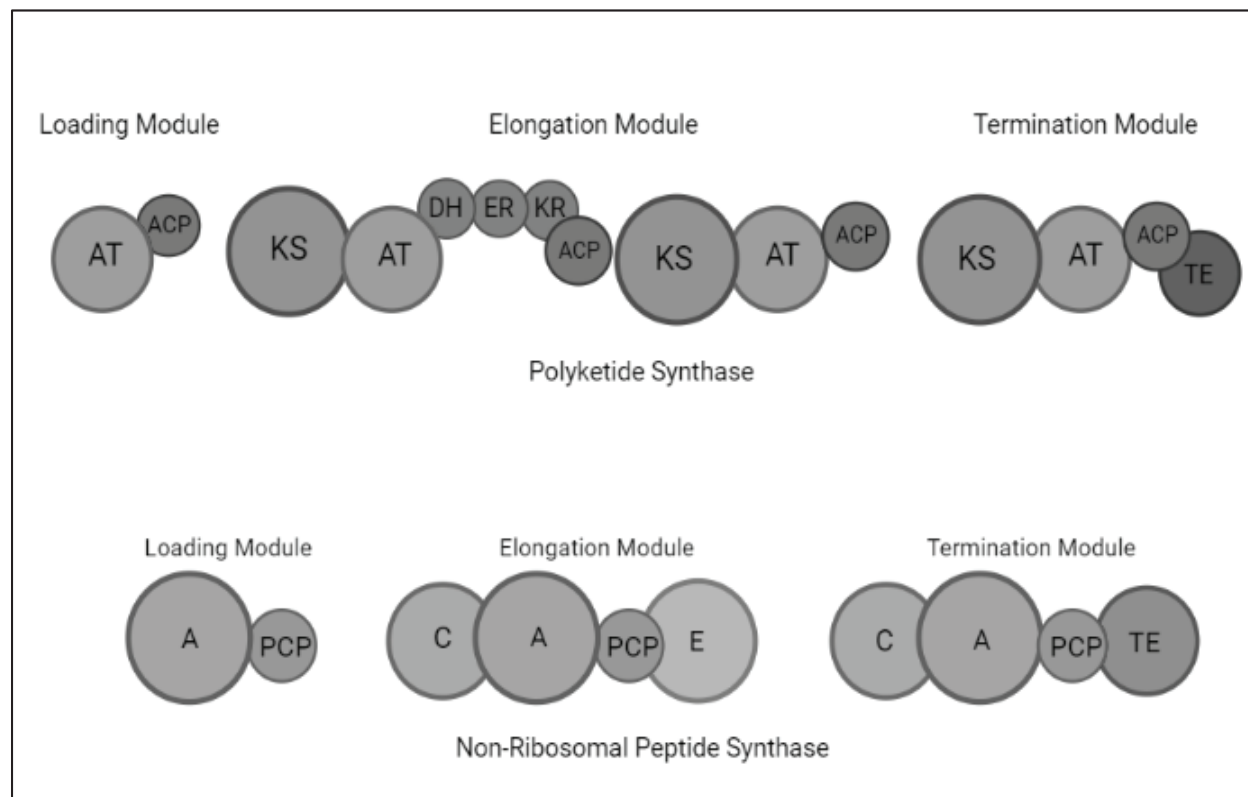

Fig. 1. Schematic representation of PKS and NRPS megasynthases. In Polyketide synthase, a minimal module is made up of Ketosynthase (KS), Acyltransferase (AT) and Acyl Carrier Protein (ACP) domains. DH (dehydrogenase), ER (enoyl reductase) and KR (ketoreductase) are additional domains these are optional. PKS proteins may contain more than one module. These interactions occur between the C terminal of ACP and N terminal of KS. In Non- ribosomal peptide synthases, C (condensation), A (Adenylation) and PCP (Peptidyl Carrier Protein) domains make the minimal module. E (epimerization) domain is optional here.

functions include virulence factors, infochemicals, pigments, or defense mechanisms. Polyketides are known as a potential source of novel therapeutic drugs, in pharmacology. In medicines, they can be used as an immunosuppressant, antibiotics, antiparasitic, antitumor, and cholesterol-lowering agents. Polyketides are classified based on their biosynthetic enzyme products. A minimal module in them is made up of different domains including the ketoacyl synthase (KS) domain, an acyltransferase (AT) domain, and an acyl carrier protein (ACP) domain [39].

There are three kinds of polyketide synthases. Multimodular PKSs contain either one or more multidomain polypeptides. In this type of synthase, the polyketide chain passes serially from one active site to another. With the change in catalytic domains of these megasynthases the variety of chemicals being produced by them and their complexity also alters in a stepwise manner [36]. While the other class of polyketides that is, iterative PKSs consists of a single set of catalysts that aims at assembling a polyketide of controlled chain length by consuming active sites repetitively. The third type of polyketide synthases which is called type III PKSs is not the same as the rest of the two types of PKSs. In this synthase, the growing polypeptide chain is not directly involved with a protein [40]. Bacteria are an abundant source of polyketides that act as an antibiotic. The major example of these polyketide antibiotics is erythromycins, tylosin, tetracyclines, monensin, tiacumicin, rifamycin, and streptogramins [41]. Antimicrobial compounds being produced by bacteria are of major concern these days.

4. ANTIMICROBIAL COMPOUNDS

According to the definition of the English dictionary, antibiotics are defined as "a substance produced by a microorganism and able, in dilute solution, to inhibit or kill another microorganism." Lietman altered this definition by adding that human beings are also a producer of antibiotics as they modify the drugs by chemical changes. According to another group of researchers "antibiotics encompass a chemically heterogeneous group of organic, low-molecular-weight compounds produced by microorganisms that are deleterious to the growth or metabolic activities of other microorganisms"

[42].

The need for the discovery of new antimicrobials is increasing tremendously due to the increasing antimicrobial resistance by bacteria which may lead to the insufficiency of the already discovered antimicrobials. The majority of the compounds being used for anti-infective purposes are the derivatives of naturally occurring compounds. Antimicrobial production is a general phenomenon of most bacteria. Bacteria are well-known to yield a diverse range of antimicrobials that can be formed either by a specific bacterial group while others may be produced by a broad range of species. Major antibiotic categories such as tetracyclines, beta-lactams, and macrolides depend on the natural product scaffold [43]. Both gram-negative and gram-positive bacteria produce secondary metabolites. Among gram-positive strains, *Streptomyces* produces cypemycin, bottromycins, eomycin, grisemycin, and chloramphenicol while *Bacillus* species are known to produce four non-ribosomal antibiotics including bacilysin, bacitracin, surfactin, and plipastatin; three ribosomal TasA, sublancin, and subtilosin, antibiotics [44]. While, among gram-negative strains, Proteobacteria such as *Burkholderiales, Myxobacteria, Photorhabdus, Pseudomonads*, and *Xenorhabdus* produce a large and underexploited variety of secondary metabolites [45]. Different types of antimicrobials produced by bacteria include classical broad-spectrum antibiotics, bacteriocin, protein exotoxins, metallic by-products, and other lytic agents.

Bacterial secondary metabolites can be a potential source of leads for new drugs such as cytostatic and antibiotics. These compounds are synthesized in a stepwise manner. The process begins with the synthesis of building blocks by using primary metabolites such as amino acids or acyl CoA derivatives as educts. The use of primary metabolites is dependent on the structure and class of the metabolites being produced. In the next step, precursor molecules are gathered either by modular mega enzymes like polyketide synthases or nonribosomal synthases. While in the final step, the assembled molecules are further altered by extremely specific reactions such as hydroxylation, ring formation, or glycosylation [46].

More than 75 % of all antibacterial while about

50 % of anticancer drugs are being produced from natural products [47]. As apratoxin A, produced by *Lyngbya boulloni* is used for the treatment of cancer [48]. The rate of antibiotic resistance is getting increased with the widespread use of antibiotics. This increase in resistance does not have any limitation because of emerging mutations and genetic transfers among pathogens thus making them least prone to the available antibiotics [49]. The fight for new drug discovery must be continued at all times by the pharmacies but because of the difficulty and expense of this process scientists rely on synthetic chemicals to treat humans. For this reason, the interest in finding new lead structures for drug discovery has reduced in past years [50]. This decrease in interest can be attributed to various disadvantages that a natural product discovery possesses over synthetic chemicals:

• The discovery of new drugs is a laborious task.
• It requires much more time compared to the synthesis of new chemical drugs.
• Natural products are produced by some biological agents which require proper handling by experts and specialized conditions and equipment for growth and maintenance [51]

But the advantages that we get from these natural compounds can always set back the disadvantages posed by them. One of the major advantages is that natural compounds possess a wider range of chemical space as compared to synthetic compounds. Both of the compounds are aimed at producing biological matter that can help to treat the ailment but the synthesis process is different for both of the products [52]. Naturally, in the biosynthetic process, a very limited amount of building blocks is exploited while in the case of chemical synthesis we have tens of thousands of chemicals available for use. Consequently, we get a massive number of different products just by changing the input. While in the case of natural products limited building blocks are fed into different pathways to achieve diversity of products. Other than the difference in building blocks the other difference lies in the synthetic transformation of the products. The biosynthetic process easily accomplishes site-selective C_H activation to introduce oxygen and distinguish between various functional groups while chemical synthesis involves nitrogen or sometimes uses additional atoms such as sulfur and halogens which are infrequent. A final difference in these pathways is their stereochemistry. The second major advantage of natural products over chemical compounds is their ability to be amended into other useful compounds. It is obvious from the fact that natural product extracts or the pure form extracted from these compounds are further modified to be used. In some other cases, it can be used in its crude or pure form as well [53].

This finding made scientists think about "natural products like" compounds synthesis strategies. They focus on finding new lead compounds by identifying the natural product scaffolds or by synthesizing the analogs of these compounds. This approach leads to the fusion of natural product research and combinatorial chemistry. Both of these disciplines now support, fertilize and rely on each other [54].

Bacteria have developed multiple strategies to defend themselves against predators and competitors in soil microhabitat. Among all of the defense strategies being adopted by these bacteria, antibiotics act as a weapon in numerous conflicts as shown in Figure 2 [42]. Many bacterial species and genera involved in antibiotic production had been isolated from diverse soil environments. Streptomycetes that belong to the family of Actinomycetes are a primary source for clinical antibiotics scaffolds. Other than *Streptomyces*, Myxobacteria are also a potential source of antibiotic discovery. These antibiotic makers arise from the soil but can originate in microbial accumulations in or on plants and insects, as well [55].

In nature, antibiotics act as a weapon or a shield but this potential of antibiotics is not the sole benefit of antibiotics being produced by bacteria in the soil. This notion came from the fact that antibiotic resistance occurs in bacteria but this resistance is also counteracted by these bacteria. So this concept leads to the discovery of a new phenomenon that is "hormesis". Hormesis refers to the ability of metabolites to act differently at varying concentrations [56]. According to various research groups antibiotics act in a concentration-dependent manner i.e. they act as an inhibitor at high concentrations while at low concentrations they function as a mediator of intracellular cell responses [57].

4.1 Antibiotics Produced in Secondary Metabolism

As discussed earlier, the soil is best known to possess organisms that produce antimicrobial agents. Among these diverse species *Streptomyces*, bacilli, and myxococci are known to be the best producer of antibiotics [58]. Antibiotics are mostly produced by secondary metabolic pathways in various growth conditions. *Streptomyces* species produce griseofulvin, erythromycin, anthracyclines, tetracyclines, nystatin, and curvularin, etc as a product in their secondary metabolism. While other organisms are known to produce mycotoxins, alkaloids, terpenes, glycosides, steroids, and other secondary metabolic products that act as antibiotics [59]. Antibiotics can be different in their structure and activity, based upon their production pathway [60]. Growth dynamics of bacterial cells indicate the metabolic activity of strains at various stages. For example, at the high nutrient level, the production of proteins, nucleic acids, and other macromolecules that are necessary for survival as well as the exponential growth of the bacterial population increases. While at a low concentration of nutrients, cells stop dividing and enter into the stationary phase. This limitation in nutrient concentration causes the metabolic routes to open which leads to the synthesis of secondary metabolites. These antibiotics may possess activities ranging from killing or inhibiting competitors to controlling cell growth or modulating colony morphology [57].
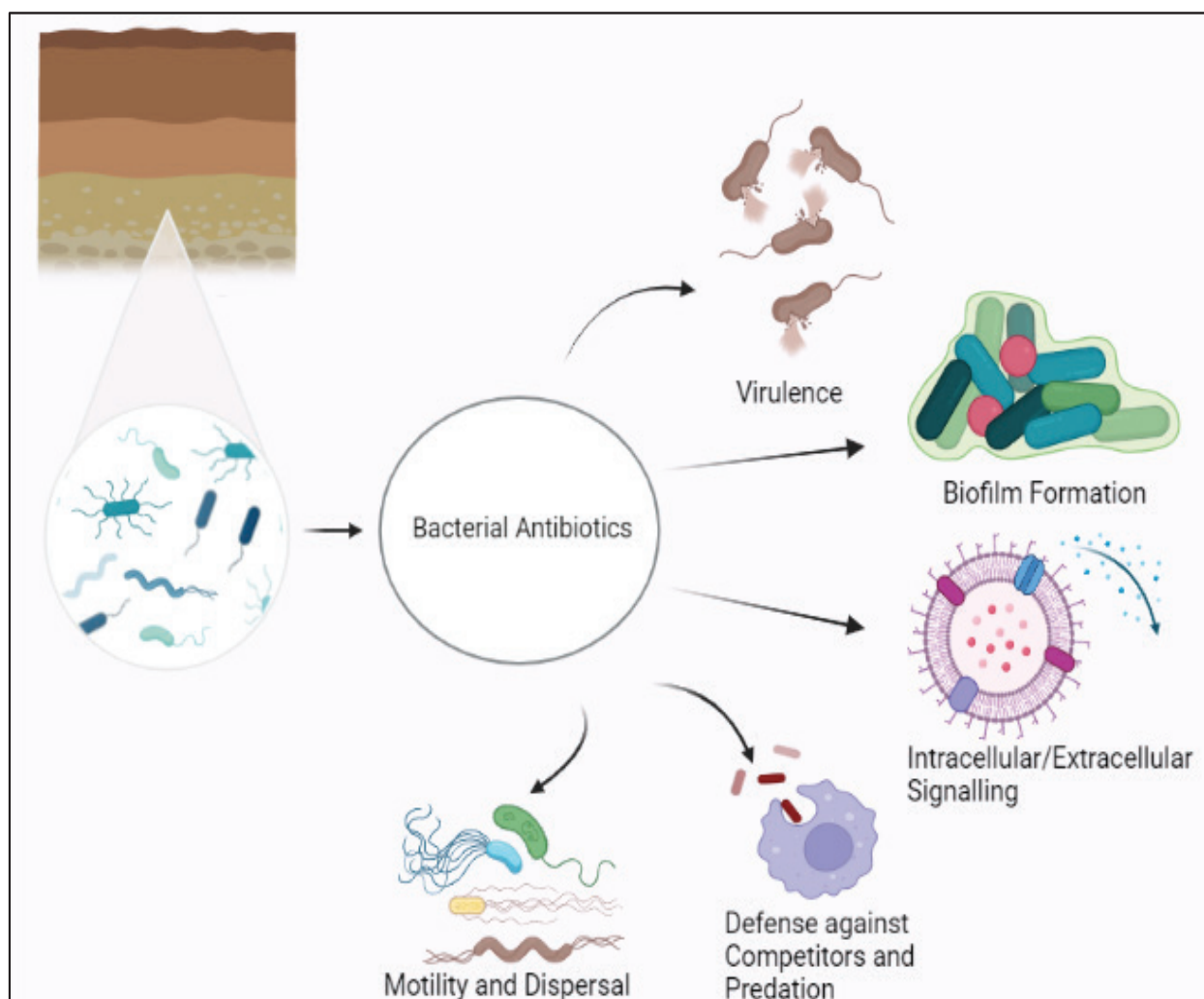

Fig. 2. Functions of antibiotics produced by bacteria. Bacteria isolated from soil produce various antibiotics which not only help to kill the bacteria in their surroundings for survival, but also aid in biofilm formation, intracellular or extracellular signaling, defense against competitors and predators and in motility and dispersal of cells.

4.2 Quorum Sensing: Antibiotic Production Regulation

Antibiotic production is under stringent genetic control. It usually starts in the stationary phase of bacterial cell growth. By various bacterial systems, we get information regarding the presence of these complicated signaling routes which pave the way for communication between the cells of the same or different species [61]. This kind of signaling makes it necessary for a transduction system to be present in bacteria so that the external information could induce the production of antibiotics at an accurate time and in proper quantity by a particular subpopulation of cells. This phenomenon is known as "Quorum Sensing". Quorum means the least number of board members required to make a decision. Here, microbiologists, refer to this term as the decision-making ability of bacteria to regulate their expression of genes based upon the population size. This process helps bacteria to count population members in culture to control the production of antibiotics. Self-produced signaling molecules or auto-inducers are released in the medium. When these molecules reach the threshold value, it induces the quorum-sensing response. The molecule which is triggering the response determines the specificity of the receptor and guarantees the appropriate recognition and genetic response in that particular population [62]. As shown in Figure 3, gram-positive and gram-negative strains have different signaling pathways. In gram-positive bacteria, the signal transduction system is a two-component system, which comprises small and post-translationally modified peptide signal molecules. An ABC exporter system (Ex) secretes these peptides which bind to the receptor and in return trigger the auto-phosphorylation of the sensor kinase (SK). Then the expression of the gene is modified by the response regulator (RR) which is activated by the transfer of the phosphate group. While in gram-negative bacteria, quorum sensing usually consists of the LuxI-LuxR system. LuxI acts to synthesize and export N-acyl homoserine lactone (AHL). When the threshold value of AHL is reached, it gets bound to the LuxR, a transcription regulator, which consequently modifies the expression of genes. [63].

Pseudomonas aeruginosa contains two kinds of quorum system AHLs i.e. Las and Rhl. Both of these systems produce their specific AHLs i.e. LasI and RhlI respectively. These AHL signal molecules bind to their cognate receptors LasR and RhlR respectively and trigger the gene expression. These genes express and perform various physiological
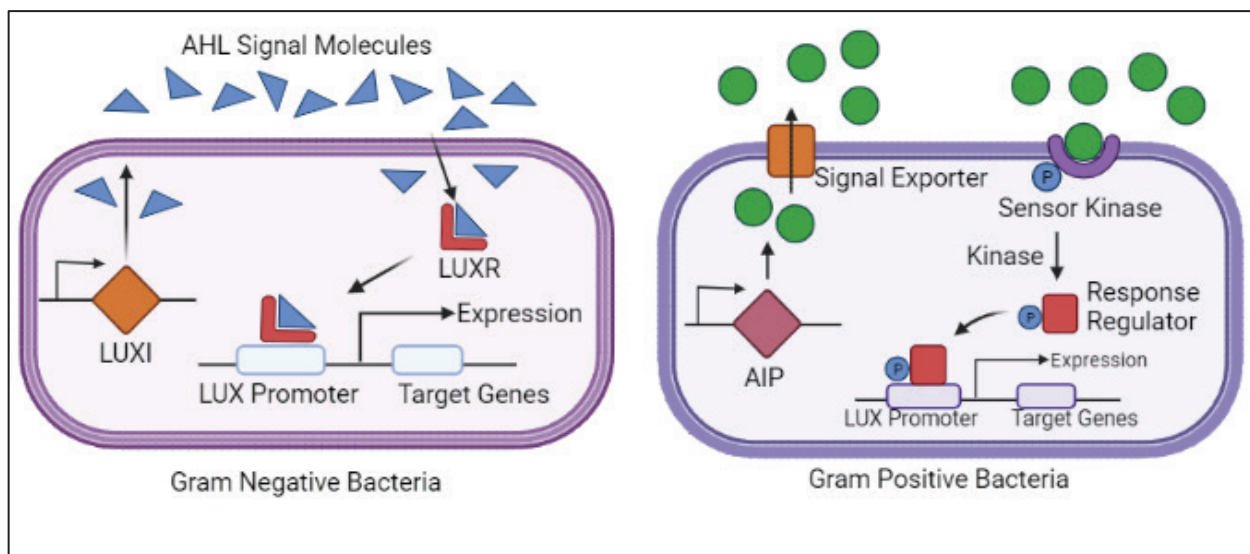

Fig. 3. Quorum sensing in gram positive and gram negative bacteria. In gram negative bacteria, LUXI produces N-acyl Homoserine Lactone (AHL) signal molecules. When threshold level of these molecules is reached, they attach to the LUXR receptors which ultimately binds to the LUX promoter and starts the expression of target genes such as biofilm formation. While in gram positive bacteria, Auto-inducer peptides are secreted by signal exporter (EX). These peptides get bound to the receptor sensor kinase and cause its auto-phosphorylation. It then helps to phosphorylate response regulator which ultimately enhances the expression of target genes such as virulence processes.

processes like biofilm formation or virulence [64]. In gram-positive bacteria, different signal molecules which are altered by post-translational changes are used for quorum sensing. *Bacillus subtilis* uses ComX or CSF and CSP in *Streptococcus pneumoniae* to control the gene's expression which is associated with different processes such as competence, sporulation, or virulence [65].

5. CONCLUSION

Production and extraction of biomolecules from bacteria have been an area of tremendous research for scientists. Researchers have utilized bioactivity-based, culture-based, metabolome based and genome-based strategies for the production and isolation of bacterial secondary metabolites Use of culture-dependent methods usually provides only the low-hanging fruit produced by these bacteria. The major challenges associated with these methods include the use of excessive chemicals or nutrients to identify a variety of metabolites being produced. Thus, mining the bacterial genome for secondary metabolite biosynthetic gene clusters provides rapid and limited resources using the approach to determine most of the metabolites producing potential in one go. In the future, studies should be targeted towards novel methods for isolations of secondary metabolites.

6. ACKNOWLEDGMENTS

7. CONFLICT OF INTEREST

The authors declared no conflict of interest.

8. REFERENCES

1. W. Islam, A. Noman, H. Naveed, Z. Huang and Y.H.H. Chen. Role of environmental factors in shaping the soil microbiome. *Environmental Science and Pollution Research* 27: 41225-41247 (2020).

2. D.B. Kell. Metabolomics and systems biology: making sense of the soup. *Current Opinion in Microbiology* 7(3): 296-307 (2004).

3. D. Thirumurugan, A. Cholarajan, S.S.S. Raja, and R. Vijayakumar. An introductory chapter: secondary metabolites. In: Secondary Metabolites - Sources and Applications. *University of California, San Diego,* (2015).

4. Y. Moënne-Loccoz, P. Mavingui, C. Combes, P. Normand, and C. Steinberg. Microorganisms and biotic interactions. *Environmental Microbiology: Fundamentals and Applications* 29: 395–444 (2014).

5. J.J. Hug, D. Krug, and R. Müller. Bacteria as genetically programmable producers of bioactive natural products. *Nature Reviews Chemistry* 4: 172–193 (2020).

6. N.J. Tobias, B. Mishra, D.K. Gupta, R. Sharma, M. Thines, T.P. Stinear, and H.B. Bode. Genome comparisons provide insights into the role of secondary metabolites in the pathogenic phase of the Photorhabdus life cycle. *BMC Genomic*s 17(1): 1-11 (2016).

7. B.D. Chantal, M. Neuber, F. Panter, D. Krug, and R. Müller. Supercritical fluid extraction enhances discovery of secondary metabolites from myxobacteria. *Analytical Chemistry* 92(23): 15403-15411 (2020).

8. L. Serwecińska. Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. *Water* 12(12): 3313 (2020).

9. C.D. Bader, F. Panter, and R. Müller. In depth natural product discovery-Myxobacterial strains that provided multiple secondary metabolites. *Biotechnology Advances* 39: 107480 (2020).

10. M. Medema, R. Kottmann, and P. Yilmaz. et al. Minimum Information about a biosynthetic gene cluster. *Nature Chemical Biology* 11: 625–631 (2015).

11. K.J. Grubbs, R.M. Bleich, K.C. Santa Maria, S.E. Allen, S. Farag, AgBiome Team, E.A. Shank, and A.A. Bowers. Large-scale bioinformatics analysis of Bacillus genomes uncovers conserved roles of natural products in bacterial physiology. *mSystems* 2(6): e00040-17 (2017).

12. C. Hemphill, P. Sureechatchaiyan, M.U. Kassack, R.S. Orfali, W. Lin, G. Daletos, and P. Proksch. OSMAC approach leads to new fusarielin metabolites from Fusarium tricinctum. *The Journal of Antibiotics* 70(6): 726–732 (2017).

13. E. Kenshole, M. Herisse, M. Michael, and S. J. Pidot. Natural product discovery through microbial genome mining. *Current Opinion in Chemical*

Biology 60: 47-54 (2021).

14. X. Zhang, and M.A. Elliot. Unlocking the trove of metabolic treasures: activating silent biosynthetic gene clusters in bacteria and fungi. *Current Opinion in Microbiology* 51: 9-15 (2019).

15. M. Fischer, and M. Grininger. Strategies in megasynthase engineering - fatty acid synthases (FAS) as model proteins. *Beilstein Journal of Organic Chemistry* 13: 1204-1211 (2017).

16. A. Haruna, and S. M. Yahaya. Recent Advances in the Chemistry of Bioactive Compounds from Plants and Soil Microbes: a Review. *Chemistry Africa* 4: 231-248 (2021).

17. B. Dror, E. Jurkevitch, and E. Cytryn. State-of-the-art methodologies to identify antimicrobial secondary metabolites in soil bacterial communities-A review. *Soil Biology and Biochemistry* 147: 107838 (2020).

18. H.Tamaki, Y. Sekiguchi, S. Hanada, K. Nakamura, N. Nomura, M. Matsumura, and Y. Kamagata. Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. *Applied Environmental Microbiology* 71: 2162–2169 (2005).

19. H.B. Bode, B. Bethe, R. Höfs, and A. Zeeck. Big effects from small changes: possible ways to explore nature's chemical diversity. *ChemBioChem* 3(7): 619-27 (2002).

20. S.A. Connon, and S. J. Giovannoni. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Applied Environmental Microbiology* 68: 3878–3885 (2002).

21. Y.Wang, F. Hammes, N. Boon, M. Chami, and T. Egli. Isolation and characterization of low nucleic acid (LNA)-content bacteria. *ISME Journal* 3: 889–902 (2009).

22. T. Alkayyali. Towards the cultivation of'uncultivable'microbes: The design and development of a microbial domestication pod containing microencapsulated marine sediment bacteria. [dissertation]. *University of Prince Edward Island* (2019).

23. D.K. Chaudhary, A. Khulan, and J. Kim. Development of a novel cultivation technique for uncultured soil bacteria. *Scientific Reports* 9(1): 6666 (2019).

24. T.M. Nguyen, C. Seo, M. Ji, M-J. Paik, S-W. Myung, and J. Kim. Effective soil extraction method for cultivating previously uncultured soil bacteria. *Applied Environmental Microbiology* 84(24):

e01145-18 (2018).

25. K. Shostak, C. Bonner, A. Sproule, I. Thapa, S.W.J. Shields, B. Blackwell, J. Vierula, D. Overy, and R. Subramaniam. Activation of biosynthetic gene clusters by the global transcriptional regulator TRI6 in Fusarium graminearum. *Molecular Microbiology* 114(4): 664-680 (2020).

26. H.A. Tomm, L. Ucciferri, and A.C. Ross. Advances in microbial culturing conditions to activate silent biosynthetic gene clusters for novel metabolite production. *Journal of Industrial Microbiology and Biotechnology* 46(9-10): 1381-400 (2019).

27. L. Huo, J.J. Hug, C. Fu, X. Bian, Y. Zhang, and R. Müller. Heterologous expression of bacterial natural product biosynthetic pathways. *Natural Product Reports* 36(10): 1412-36 (2019).

28. L. Foulston. Genome mining and prospects for antibiotic discovery. *Current Opinion in Microbiology* 51:1-8 (2019).

29. L. Meunier, P. Tocquin, L. Cornet, D. Sirjacobs, V. Leclère, M. Pupin, P. Jacques, and D. Baurain. Palantir: a springboard for the analysis of secondary metabolite gene clusters in large-scale genome mining projects. *Bioinformatics* 36(15): 4345–4347 (2020).

30. K. Blin, S. Shaw, K. Steinke, R. Villebro, N. Ziemert, S.Y. Lee, M.H. Medema, and T. Weber. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Research* 47: 81-87 (2019).

31. P. Cimermancic, M.H. Medema, J. Claesen, K. Kurita, L.C.W. Brown, K. Mavrommatis, A. Pati, P. A. Godfrey, M. Koehrsen, and J. Clardy. Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. *Cell* 158: 412-421 (2014).

32. M.A. Skinnider, N.J. Merwin, C.W. Johnston, and N.A. Magarvey. Prism 3: expanded prediction of natural product chemical structures from microbial genomes. *Nucleic Acids Research* 45: 49-54 (2017).

33. R. Hengqian, C. Shi, and H. Zhao. Computational tools for discovering and engineering natural product biosynthetic pathways. *Iscience* 23(1): 100795 (2020).

34. S. Hwang, N. Lee, S. Cho, B. Palsson, and B.K. Cho. Repurposing modular polyketide synthases and non-ribosomal peptide synthetases for novel chemical biosynthesis. *Frontiers in Molecular Biosciences* 7:87 (2020).

35. C. Beck, J.F.G. Garzón, and T. Weber. Recent advances in re-engineering modular PKS and NRPS

assembly lines. *Biotechnology and Bioprocess Engineering* 7:1-9 (2020).

36 D. Harwani, S. Barupal, J. Begani, and J. Lakhani. Genetic diversity of polyketide synthases and nonribosomal peptide synthetases in fungi. In New and Future Developments in Microbial Biotechnology and Bioengineering. *Elsevier* (2020) p11-21.

37. K. Bloudoff, and T.M. Schmeing. Structural and functional aspects of the nonribosomal peptide synthetase condensation domain superfamily: discovery, dissection and diversity. *Biochim Biophys Acta (BBA) - Proteins Proteom* 1865(11B): 1587-604 (2017).

38. Q. Esmaeel, M. Pupin, P. Jacques, and V. Leclere. Nonribosomal peptides and polyketides of Burkholderia: new compounds potentially implicated in biocontrol and pharmaceuticals. *Environmental Science and Pollution Research* 25: 29794–29807 (2018).

39. S.Y. Hou, M.Y. Zhang, H.D. Wang, and Y.X. Zhang. Biosynthesis gene cluster and oxazole ring formation enzyme for inthomycins in *Streptomyces* sp. strain SYP-A7193. *Applied and Environmental Microbiology* 86(20): e01388-20 (2020).

40. J. Wang, R. Zhang, X. Chen, X. Sun, Y. Yan, X. Shen, and Q. Yuan. Biosynthesis of aromatic polyketides in microorganisms using type II polyketide synthases. *Microbial Cell Factories* 19: 1-11 (2020).

41. H.L. Robertsen, and E.M. Musiol-Kroll. Actinomycete-derived polyketides as a source of antibiotics and lead structures for the development of new antimicrobial drugs. *Antibiotics* 8(4): 157 (2019).

42. J.M. Raaijmakers, and M. Mazzola. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annual Reviews of Phytopathology* 50: 403-24 (2012).

43. K.I. Mohr. History of antibiotics research. How to Overcome the Antibiotic Crisis: *Springer* (2016). p. 237-72.

44. E.A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, J.P. Meier-Kolthoff, H.P. Klenk, C. Clément, Y. Ouhdouch, and G.P. Van Wezel. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiology and Molecular Biology Review* 80(1): 1-43 (2016).

45. N.J. Tobias, Y.M. Shi, and H.B. Bode. Refining the natural product repertoire in entomopathogenic bacteria. *Trends in Microbiology* 26(10): 833-40 (2018).

46. T. Weber, C. Rausch, P. Lopez, I. Hoof, V. Gaykova, D.H. Huson, and W. Wohlleben. CLUSEAN: A computer-based framework for the automated analysis of bacterial secondary metabolite biosynthetic gene clusters. *Journal of Biotechnology* 140(1): 13-7 (2009).

47. F. Pereira. Have marine natural product drug discovery efforts been productive and how can we improve their efficiency? *Expert Opinions in Drug Discovery* 14(8): 717-722 (2019).

48. N. Srinivasan, S. Dhanalakshmi, and P. Pandian. Encouraging Leads from Marine Sources for Cancer Therapy A Review Approach. *Pharmacognosy Journal* 12(6): 1475-1481 (2020).

49. B. Raymond. Five rules for resistance management in the antibiotic apocalypse, a road map for integrated microbial management. *Evolutionary Applications* 12(6): 1079-1091 (2019).

50. B. David, J.L. Wolfender, and D.A. Dias. The pharmaceutical industry and natural products: historical status and new trends. *Phytochemical Review* 14(2): 299-315 (2015).

51. M.A. Farha, and E.D. Brown. Strategies for target identification of antimicrobial natural products. *Natural Product Reports* 33(5): 668-680 (2016).

52. H. Abozenadah, A. Bishop, S. Bittner, O. Lopez, C. Wiley, and P.M. Flatt. Consumer Chemistry: How Organic Chemistry Impacts Our Lives. CC BY-NC-SA. (2017).

53. A. Ganesan. The impact of natural products upon modern drug discovery. *Current Opinion in Chemical Biology* 12(3): 306-17 (2008).

54. M.I. Hutchings, A.W. Truman, and B. Wilkinson. Antibiotics: past, present and future. *Current Opinion in Microbiology* 51: 72-80 (2019).

55. O. Genilloud, I. González, O. Salazar, J. Martín, J.R. Tormo, and F. Vicente. Current approaches to exploit actinomycetes as a source of novel natural products. *Journal of Industrial Microbiology and Biotechnology* 38(3): 375-89 (2011).

56. I. Iavicoli, L. Fontana, E. Agathokleous, C. Santocono, F. Russo, I. Vetrani, M. Fodele, and E.J. Calabrese. Hormetic dose responses induced by antibiotics in bacteria: A phantom menace to be thoroughly evaluated to address the environmental risk and tackle the antibiotic resistance phenomenon. *Science of the Total Environment* 798: 149255 (2021).

57. D. Romero, M.F. Traxler, D. López, and R. Kolter. Antibiotics as signal molecules. *Chemical Reviews* 111(9): 5492-505 (2011).

58. K.J. Weissman, and R. Müller. Myxobacterial secondary metabolites: bioactivities and modes-of-action. *Natural Product Report* 27(9): 1276-95 (2010).

59. W. Kurzątkowski, and J. Kuczerowska. Antibiotic biosynthesis and secondary metabolism in high-yielding strains of *Streptomyces, Penicillium chrysogenum and Acremonium chrysogenum. Postęp Mikrobiologii- Advancements of Microbiology* 56(4): 422-428 (2019).

60. B. Ruiz, A. Chávez, A. Forero, Y. García-Huante, A. Romero, M. Sánchez, D. Rocha, B. Sánchez, R. Rodríguez-Sanoja, S. Sánchez, and E. Langley. Production of microbial secondary metabolites: regulation by the carbon source. *Critical Reviews in Microbiology* 36(2):146-67 (2010).

61. S. Atkinson, and P. Williams. Quorum sensing and social networking in the microbial world. *Journal of the Royal Society Interface* 6(40): 959-78 (2009).

62. S. Mukherjee, and B.L. Bassler. Bacterial quorum sensing in complex and dynamically changing environments. *Nature Reviews Microbiology* 17: 371–382 (2019).

63. A.R. Horswill, P. Stoodley, P.S. Stewart, and M.R. Parsek. The effect of the chemical, biological, and physical environment on quorum sensing in structured microbial communities. *Analytical and Bioanalytical Chemistry* 387(2): 371-80 (2007).

64. K.M. Sandoz, S.M. Mitzimberg, and M. Schuster. Social cheating in Pseudomonas aeruginosa quorum sensing. *Proceedings of National Academy of Science USA.* 104(40): 15876-81 (2007).

65. C.M. Waters, and B.L. Bassler. Quorum sensing: cell-to-cell communication in bacteria. *Annual Review of Cell and Developmental Biology* 21: 319-46 (2005).