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Review

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Innovations in Microbial Biodiscovery, Targeting Silent Metabolism and New Chemical Diversity

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ABSTRACT

It is quite evident that microbial (bacteria or fungi) crude extracts contain several important bioactive compounds and some have already shown their therapeutic activity. Unfortunately, most of the compounds have not properly been evaluated for the exploration of new lead molecule. Moreover, some of the mechanisms of actions of few bioactive compounds have not been identified so far. Hence, extensive research is required to find out the activity of compounds in the microbial crude extracts and to exploit their therapeutic potential to unlock silent secondary metabolites. Therefore, this review article raise the importance of activating microbial secondary metabolites noting the need for new tools to access the full microbial genome.

KEYWORDS: Microbial biodiscovery; Antibiotics; Anticancer; Silent metabolism; Lipopolysaccharide.

ABBREVIATIONS: MRSA: Methicillin-resistant staphylococcus aureus; VRE: Vancomycin-resistant enterococcus; CCR5: C-C chemokine receptor type 5; CD4: Cluster of differentiation 4; AIDS: Acquired immunodeficiency syndrome; DSB: Derivative of betulinic acid; DNA: Deoxyribonucleic acid; LPS: Lipolysaccharide; FDA: Food and Drug Administration.

INTRODUCTION TO MICROBIAL BIODISCOVERY

Historical Impact of Microbial Natural Products

Natural products continue to play a crucial role in the discovery of new drugs and drug leads.[1] Biodiscovery from microbial resources can be defined as the exploration of microbial metabolic products that provide important benefits to the fields of medicine, agriculture and biotechnology.[2] It has been reported that approximately 50% of approved drugs in the market are derived from microbial origin (Table 1).[3] Actinomycetes and fungi are very important groups of microorganisms that are known for their ability to produce secondary metabolites with therapeutic activities.[4] Actinobacteria produce over half of the bioactive compounds that are present in the antibiotic literature.[5] These compounds include well-known antibacterials such as aminoglycosides and tetracyclines, antifungals such as amphotericin B (1) anticancer agents such as adriamycin (2) immunosuppressants such as tacrolimus (3) and anthelmintics such as avermectin (4) plus numerous other valuable therapeutic drugs. Fungi also play a vital role in the microbial discovery, with the genus *Penicillium* providing several well-known drugs (e.g. penicillins). Therefore, the drug discovery process is considered as a very critical issue in the industrial field as it is a very expensive process and it requires multiple steps to produce new drug potentials to target specific disease.[6]

Despite this great success in the discovery of valuable antibiotics and other pharmaceutical active agents, there has been a dramatic decline in the discovery of new antibiotics

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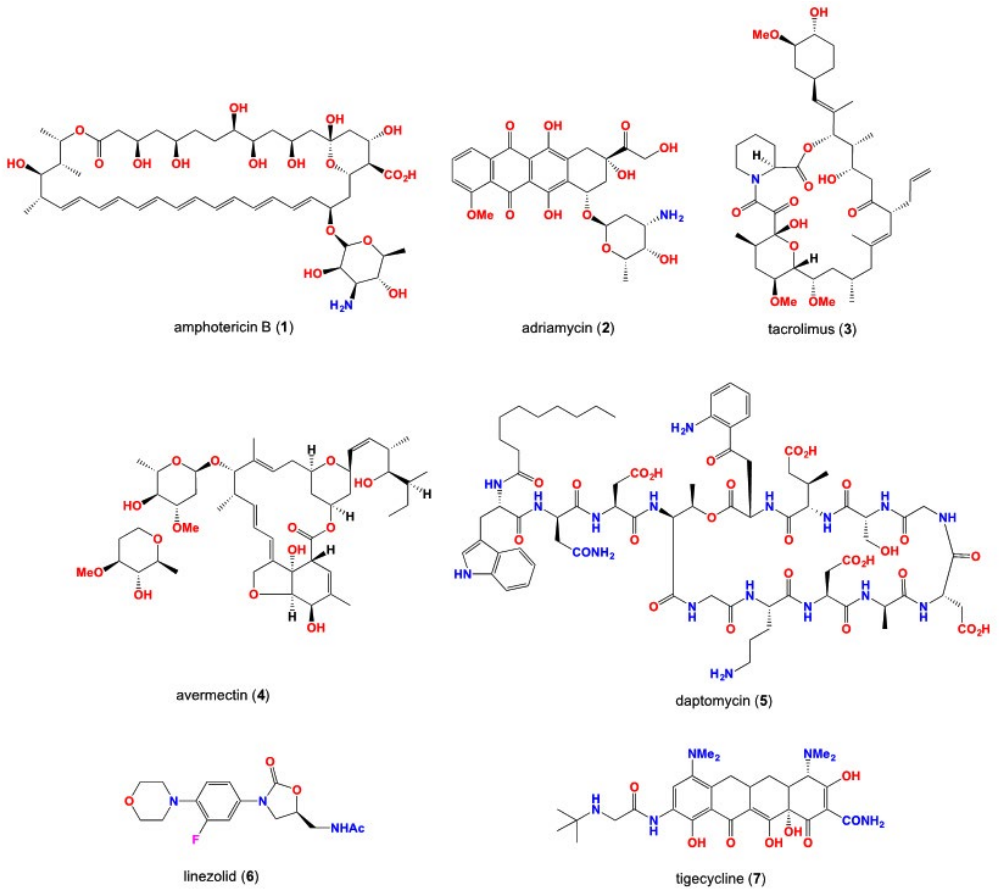
Original metabolite	Commercial products	Producing organism
Penicillins	Penicillin G, V, Ampicillin, Methicillin	*Penicillium* spp., *Aspergillus* spp.
Cephalosporins	Mefoxin, Ceclor	*Acremonuim* spp.
Thienamycin	Primaxin, Invanz	*Streptomyces cattleya*
Erythromycin	Erythrocin, Zithromax	*Saccharopolysporaerythraea*
Vancomycin	Vancocin	*Streptomyces orientalis*
Fosfomycin	Monuril	*Streptomyces fradiae*
Daptomycin	Cubicin	*Streptomyces roseosporus*

Table 1: Examples of marketed antibiotics originated from microbial origin.[3]



in the 21st century. Despite a 2007 review[7] revealing that 70% of hospital-acquired infections in the US are resistant to one or more antibiotics, with the exception of the narrow spectrum antibiotic daptomycin (5) and linezolid (6) very few new classes of clinically relevant antibiotics have been approved over the last 40 years. Tigecycline (7) is one of a new class of antibiotics derived from the tetracycline nucleus. This broad-spectrum antibiotic was approved for the treatment of complicated skin infections and is also active against methicillin-resistant staphylococcus aureus (MRSA), vancomycin-resistant *Enterococcus faecium* and beta-lactamase-producing bacteria as *Escherichia coli* and *Klebsiella pneumonia*.[8,9]

Therapeutic Fields Responsive to Natural Products

Microbial secondary metabolites are highly potent and selective for the treatment of many diseases. Many of these metabolites can be interpreted to be a signal molecule or defense mechanism against competitors or pathogens, an aid to the survival of the microorganism.

Bacterial pathogens: Natural products are still the main source of promising new antibiotics for the treatment of bacterial diseases. Platensimycin (8) was first reported in 2006 from *Streptomyces platensis* by Merck researchers, followed by the isolation of platencin (9).[11-13] In 2008, another platensimycin analogues B_1-B_3 (10-12)[14] and platensimycin B_4 (13).[15] This class of antibiotics demonstrated a novel mode of action against gram-positive bacteria through inhibition of cellular lipid biosynthesis. Because of this unique role, it shows no cross resistance to MRSA, vancomycin-resistant enterococcus (VRE) or other antibiotic resistant microbes.

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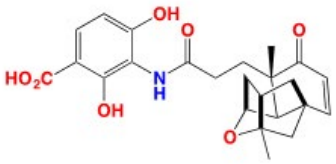
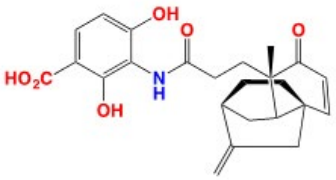
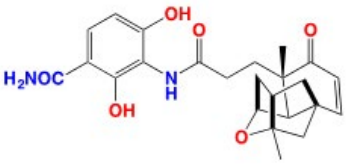


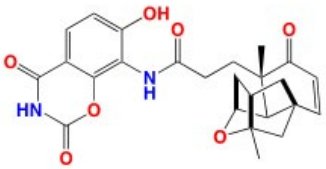
platensimycin (**8**)



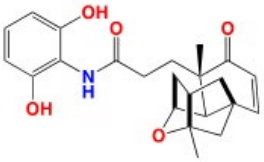
platencin (**9**)



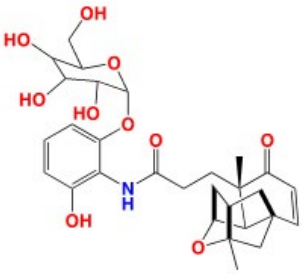
platensimycin B_1 (**10**)



platensimycin B_2 (**11**)



platensimycin B_3 (**12**)



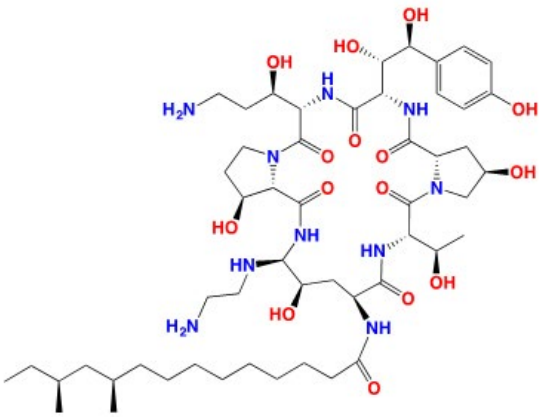
platensimycin B_4 (**13**)

Fungal pathogens: The need for the discovery of new antifungal agents continues, as we seek to treat the opportunistic infections in immune-compromised patients and the emerging resistance to existing antifungal agents. Jacob et al,[16] recently discussed the whole cell screening techniques targeting natural products and their mode of action including the use of genetically modified fungal strains in which the antifungal drug is targeting the cell wall and cell membrane. Caspofungin (14) is a promising new antifungal agents derived from pneumocandin, a natural product produced by *Glarealozoyensis*.[17]

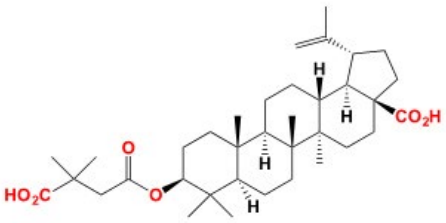
Viral pathogens: New anti-HIV (human immunodeficiency virus) drugs are targeting the point of entrance of the virus into the cell such as interferon signaling and cell surface receptors as C-C chemokine receptor type 5 (CCR5)-human cytokine receptor and cluster of differentiation 4 (CD4).[18] Many antiviral lectins are from algal origin and they are mainly small proteins that bind to carbohydrates found on the viral envelopes and prevent the transmission of HIV.[19] In addition, viral polymerases are the key for viral replication and are considered as promising targets for anti-viral drugs, with inhibitors for viral polymerase and proteases in clinical use for the treatment of acquired immunodeficiency syndrome (AIDS) and chronic hepatitis. All drugs that are in the market for the treatment of HIV are synthetic in origin, although the activities of many natural products have been explored in the recent years.[20] Yu et al,[21] discovered a plant-derived modified betulinic acid derivative, DSB (15) as a first in class HIV maturation inhibitor. This natural product is currently in Phase II clinical trial.[21]

Resistance to Microbial Natural Products

Antibiotics and the resistance in nature: The discovery of antibiotics more than 70 years ago initiated an era of drug innovation in human and animal health. These discoveries were interrupted by the emergence of antibiotic resistance, largely due to the widespread of overuse of antibiotics in both medicine and agriculture.[22] Not withstanding the outstanding list of antibiot-



caspofungin (**14**)



DSB (**15**)

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ics that were discovered from microbial sources, especially soil microbes, resistance to these antibiotics evolved in the environment long before they were developed as commercial antibiotics.[23] The resistance we see in the clinic today is to a great extent similar to the environmental one. Although the resistance in both contexts are similar, clinical resistance tends to increase substantially with time. Therefore there should be a certain mechanism that keeps control of resistance in the environment.[24-26]

Anticancer resistance: Cancer cells become resistant to anticancer drugs by several mechanisms. One way is to pump drugs out of cells by increasing the activity of efflux pumps, such as ATP-dependent transporters. Alternatively, resistance can occur as a result of reduced drug influx–a mechanism reported for agents that interact with intracellular carriers or enter the cell by means of endocytosis. In cases where drug accumulation is unchanged, activation of detoxifying proteins, such as cytochrome P450 mixed-function oxidases, can promote drug resistance. Cells can also activate mechanisms that repair drug-induced deoxyribonucleic acid (DNA) damage. Finally, disruptions in apoptotic signalling pathways (e.g. p53 or ceramide) allow cells to become resistant to drug-induced cell death.[27]

In order to overcome these problems, we need to better understand the microbial genomics. In the last 10 years, advances in genomics have revealed new knowledge of a silent microbial secondary metabolism, offering a glimpse of a new source of potentially valuable biomedical agents and tools. To access this genetic resource requires molecular tools capable of activating latent secondary metabolism gene clusters, to build knowledge of microbial systems biology, and facilitate access to new microbial natural products.[28-30]

Silent Resources

After 100 years of discovering microbes, there is great evidence that microbes are capable of producing different classes of bioactive secondary metabolites.[31] However, strong evidence revealed that we are only scratching the surface of microbial genome. Microbes share and accumulate multiple secondary metabolite gene clusters, which are capable of producing different bioactive metabolites, with a wide range of biological activities.[32] Two challenges limit our ability to access the microbial genome; the first one is that both the bacteria and fungi harbour massive number of genes. These genes usually remain dormant and do not produce any metabolites under normal laboratory conditions until they become activated. It is very probable that these genes are activated by chemical stimuli produced by other competing microbes. Such chemical stimuli represent valuable molecular tools that could be used to unlock the silent secondary metabolism, improving prospective for next generation antibiotics.[33]

Detecting of silent secondary gene cluster: A 2001 study by Omura et al, on the genome of *Streptomyces avermitilis*, the microbial source of anthelmintic drug avermectins, revealed an 8.7 Mbp linear chromosome with 25 recognizable secondary metabolite gene clusters accounting for 6.4% of the genome. This study concluded that there are many uncharacterized genes involved in the secondary metabolism. Twenty-five secondary metabolite gene clusters were found in the genome of *S. avermitilis.* Four of them are responsible for the production of melanin pigment on solid medium, two are derived from tyrosine and one is an aromatic polyketide. Another melanin is an ochronotic pigment, which is derived from homogentiginic acid and produced, in both solid and liquid media.[34] *S. avermitilis* has the highest number of secondary metabolite gene clusters of all bacterial genomes sequenced. The production of different metabolites can be attributed to the presence of many gene clusters, which can encode for enzymes for the activation of different secondary metabolic pathway. The report concluded that there are "*many other uncharacterized genes involved n secondary metabolism*".

In 2002, Bentley et al[35] reported that the genome of *Streptomyces coelicolor* contained 20 secondary metabolite gene clusters and metabolic enzymes of known or predicated secondary metabolites. Furthermore, the genome contained an unprecedented proportion of regulatory genes that are involved in response to external stimuli and stresses. *S. coelicolor* contains duplicated genes that operate in different phases during colonial development, which if stimulated could lead to new pharmaceutical compounds. The report concluded by noting "*The abundance of previously uncharacterized metabolic enzymes, particularly those likely to be involved in the production of natural products, is a resource of enormous potential values*". In 2005, McAlpine et al[36] reported the genome analysis of *Streptomyces aizunensis* to detect the presence of hitherto unexpressed natural product in which different media where used to express microbial secondary metabolites. Udwary et al,[37] analysed the genome of *Salinospora tropica* to show a large percentage of its genome is dedicated to natural product assembly. In addition, this study identified secondary metabolic biosynthetic gene clusters from the complete genome sequence of *S. tropica* revealing an unrealised secondary metabolism potential and the importance of the understanding and the control for the secondary metabolite pathway.

Therefore, modern genomics has redefined our understanding of microbial secondary metabolism. The computational analysis of microbial metabolism provides the sequencing of thousands of microbial genomes. This knowledge will provide a new pathway to access the microbial molecular diversity and also will help to develop more tools and methodologies to take advantage of this potential. In addition, the previous articles noted a growing appreciation of a global microbial genome that encompasses molecular discovery value than previously appreciated. On the other hand, while learning that microbes possess a secondary metabolism, the challenge is how to activate and benefit from that the activation of silent secondary gene clusters and produce new pharmaceutical compounds.

Activating of Silent Genes

Effect of culturing conditions on secondary metabolite production: The choice of the cultivation parameters is crucial for the

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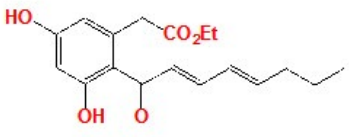
production of secondary metabolites by microorganisms. It is well known fact that a change in culture conditions (temperature, pH, oxygen etc.) can affect secondary metabolites production. Any small change in the culture medium may impact not only the level of production of certain compounds, but also the diversity.[38] Paranagama et al[39] studied the effect of tap water and distilled water on the metabolite profiling of 2 plant associated fungus, *Para phaeosphaeria quadrisep tata* by the changing the water used in fermentation from tap to distilled resulted in the production of 6 new compounds, cytosporones F-I (16-19), 5′-hydroxymonocillin (20) and quadriseptin A (21).

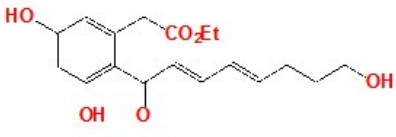
The second fungus, *Chaetomium chiversii*, produce-dradicicol (22) on solid phase media, but shifted to chaetochromin (23) in liquid phase.[39]

In addition to various culture conditions, Ayer et al have studied the impression of the diverse stress conditions on secondary metabolite production. They were able to isolate jadomycin B (24), a glycosylated nezoxazolophenanthridine antibiotic from *Streptomyces venezuelae*. The aglyconejadomycin was produced in a galactose-isoleucine medium at 37 °C.[40] Increasing the temperature to 42 °C increased the level of production of jadomycin B (24). Other parameters were found to increase the production of jadomycin B such as addition of ethanol as well as bacteriophage infection.[41] Also, Overy et al[42] cultivated different strains of necrotrophic *Penicillium* strains in different macerated host tissue media. This led to the stimulation of production of corymbiferone (25) and corymbiferan lactones I-IV (26-29).

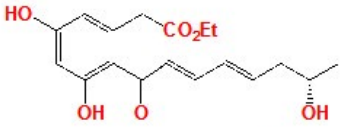
Novel metabolites in co-cultures: The functional role of natural products in microbes has long been a topic of discussion. It was found that some natural products result from the interaction of the organism with its own environment. Cueto et al cultured a marine *Pestalotia* sp. with an unidentified antibiotic resistant marine bacterium, resulting in the biosynthesis of pestalone



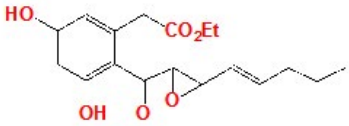
cytosporone F (**16**)



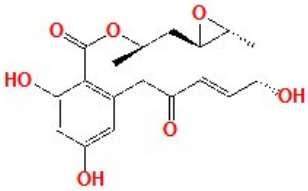
cytosporone G (**17**)



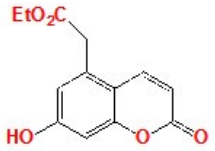
cytosporone H (**18**)



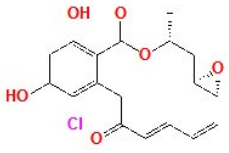
cytosporone I (**19**)



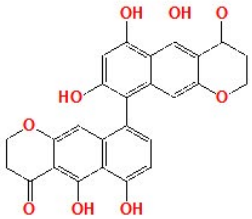
5'-hydroxymonocillin (**20**)



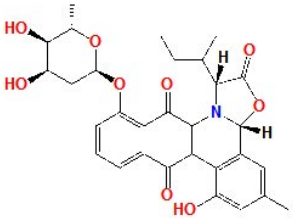
quadriseptin A (**21**)



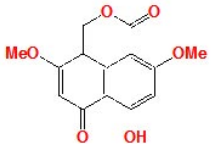
radicicol (**22**)



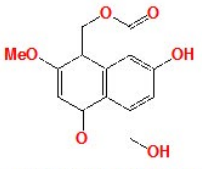
chaetochromin (**23**)



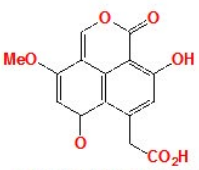
jadomycin B (**24**)



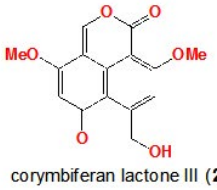
corymbiferone (**25**)



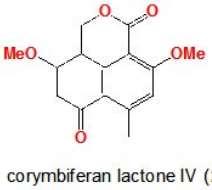
corymbiferan lactone I (**26**)



corymbiferan lactone II (**27**)



corymbiferan lactone III (**28**)



corymbiferan lactone IV (**29**)

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(30), a new benzophenone. This compound could not be detected when each strain was cultivated independently. Pestalone was found to be active against MRSA and vancomycin-resistant *Enterococcus faecium*.[43] The marine derived fungus *Emericella* sp. was challenged with the actinomycete *Salinispora arenicola*. This co-culture activated the production of two new cyclic depsipeptides, emericellamide A (31) and emericellamide B (32).[44] Latifiet al. reported another interesting example of the synergistic function of co-culturing in 2006. A co-culture between *Pseudomonas aeruginosa* and *Enterobacter* sp. stimulated *P. aeruginosa* to produce the blue pigment pyocyanin, (33).[45] To establish whether any other microorganism can induce the production of pyocyanin, *P. aeruginosa* was cultivated with different microorganisms. These results showed that *P. aeruginosalost* its ability to produce pyocyanin when co-cultured with other microbes.[46] These related examples highlight the role that co-culture can play in the regulation of secondary metabolite production.

Although these strategies have been studied and can lead to promising results, it is nevertheless a time consuming and unpredictable process that while it can express silent metabolites, is not up to the challenge of activating the broad array of silent metabolites hidden within the global microbial genome. In order to access the microbial genome, new approaches informed by the microbial evolution need to be acquired.[47]

Autoregulators: Autoregulators are compounds that act as triggers to improve cellular development and activate silent secondary metabolite genes, leading to the production of secondary metabolites. Knowing that a microbe is genetically far more capable than previously suspected, reveals a potential, but activating that potential is another challenge entirely. Even without detailed knowledge of the microbial genome, early researchers enjoyed some success in stimulating microbial secondary metabolites. For example, it had been recognized that morphological differentiation during the growth of *Streptomyces* sp. was closely associated with the secondary metabolites production. More specifically, the transition from primary to secondary metabolism occurred simultaneously with the formation of the aerial hyphae. In exploring these phenomena, a selection of low molecular weight metabolites were found to act as regulators of secondary metabolism.

γ--Butyrolactones of actinomycetes: The potential value of autoregulators as tools to regulate antibiotic production was quickly recognized, such that γ--butyrolactones came under intense investigation. An early example, of such a regulator (called an autoregulator) discovered in 1979 was A-factor (34), a γ--butyrolactone produced by Streptomyces griseus. A-factor induced both aerial mycelium formation in *S. griseus* and production of the antibiotic streptomycin, with A-factor appearing prior to streptomycin production and disappearing before streptomycin reached its maximum level.[48]

In addition to its effect on streptomycin (35) production,[49] other achievements of A-factor included inducing the production of pristinamycins in a mutant strain of *S. pristinaespiralis*, in which pristinamycins biosynthesis was otherwise blocked, and initiating the production in *S. griseus* (under phosphate depletion) of the parasiticidal metabolites grixazones A and B (36 and 37). Other closely related naturally occurring microbial **γ-**butyrolactone autoregulators included the virginiaebutenolides (38) (i.e. VB-A), which activated the production of virginoamycin that consists of 75% virginamycin M_1 (39) and 25% virginamycin S_1 (40), IM-2 (41), which activated the production of showdomycin (42) and minimycin (43), and SCB1 (44), which activated the production of actinorhodine (45) and undecylprodigiosin (46). IM-2 is noteworthy in that it both activates and suppress secondary metabolism. For instance, biosynthesis of the antituberculosis antibiotic D-cycloserine by *S. lavendulae* was completely suppressed in the presence of IM-2. Further investigations into the autoregulation of microbial secondary metabolism revealed that this capability was not limited to γ--butyrolactones.

A limited selection of *Streptomyces* metabolites have been described as promoters of morphogenesis and secondary metabolism, and include the thiazole/oxazole peptide, the polyether pamamycin-607 (47) and the siderophore desferrioxamine E (48). Pamamycin-607 (47) plays an important role in the regulation of aerial mycelium production in 67% of *Streptomyces*.[50] Desferrioxamine E (48) simulates secondary metabolites production in different strains of actinomycetes such as *Streptomyces coelicolor*.[51]

However there are some non-butyrolactone *Streptomyces* metabolites that have been discovered as promoters of secondary metabolites, including thiazole/oxazole peptide goadsporin. Goadsporin (49) is an oligopeptide consisting of 19 amino acids, which acts on the sporulation pathway and regulates secondary metabolites production for *Streptomyces*.[52]

Homoserine lactones of gram-negative bacteria: Acyl homoserine lactones (acyl-HSLs) are important intercellular signalling molecules used by many bacteria to monitor their population density in quorum-sensing control of gene expression.[53,54] These signals are synthesized by members of the LuxI family of proteins.[53] Homoserine lactones (HSLs) function by "quorum sensing" in which they reach a particular extracellular concentration due to high cell density. They act in bioluminescence, antibiotic biosynthesis, animal pathogenicity, plant pathogenicity and extracellular enzyme synthesis.[55] HSLs are similar in structure to A-factor. They can also induce exoenzymes, which are plant and animal virulence defense against *E. carotovora* and *P. aeruginosa* respectively.[56]

Genomics-Inspired Screening for Novel Natural Products

The idea of screening the extract libraries on different types of microorganisms has emerged as an important aspect to determine new metabolites.[57] Microbial metabolites (gene activators) may be capable of eliciting the expression of silent secondary metab-

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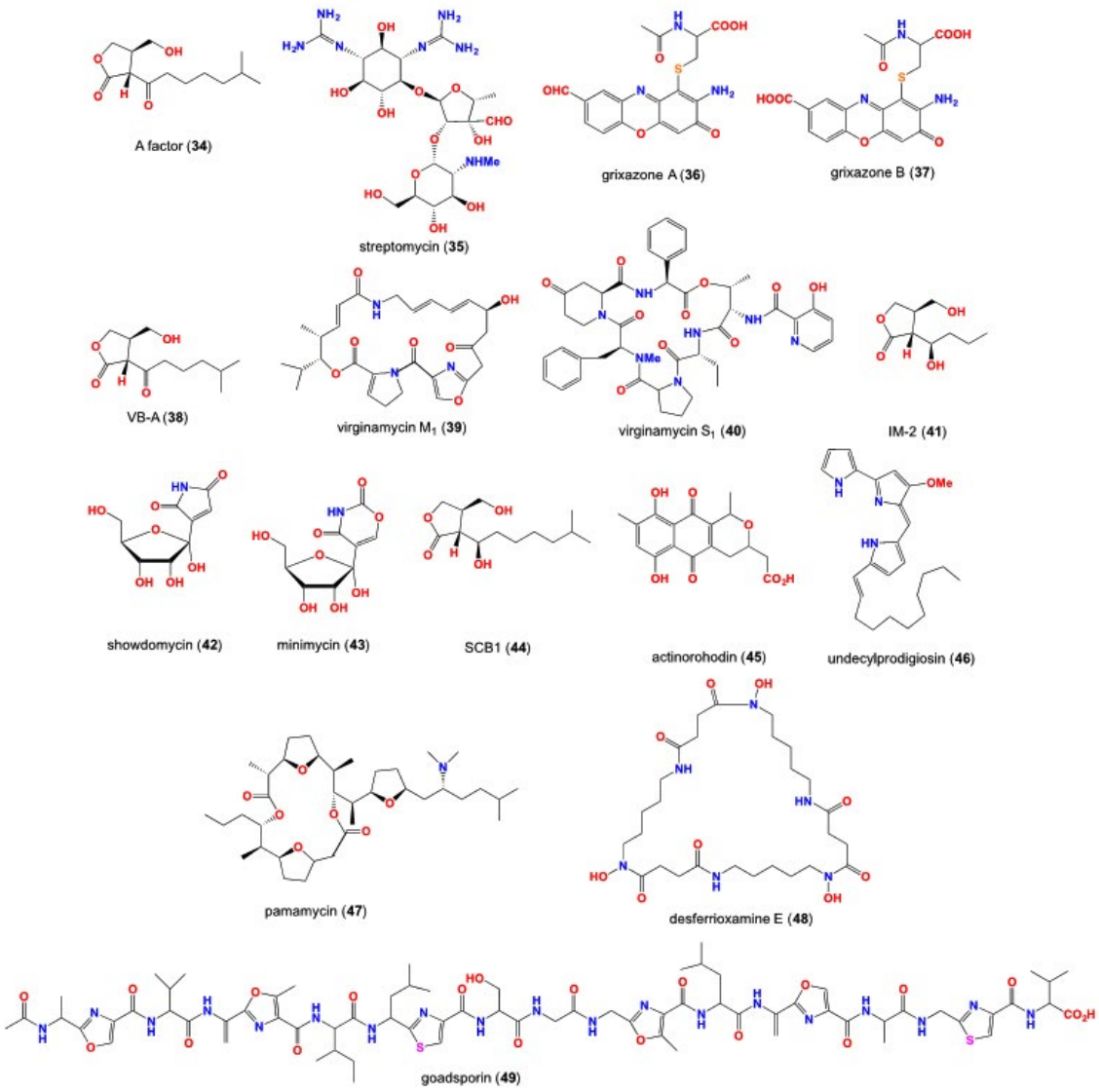

olism on other microbes. The activation of silent gene clusters will advance the discovery of next generation antibiotics.[58,59] Zazopoulos et al[60] analyzed the genome of different actinomycetes looking for the genes that were responsible for the production "enydiyne" class of antitumor agents. These loci remain inactive until certain chemical or physical signals activate them. After optimizing the growth conditions, Zazopoulos et al[60] were able to induce the expression of the gene cluster and leading to production of "enydiyne". In addition, McAlpine et al. scanned the genome of *Streptomyces aizunensis*. The genome scanning identified 11 gene clusters coding for the biosynthesis of wide range of bioactive metabolites. One of these gene clusters was responsible for the production of type I polyketide synthase generating a novel polyketide with characteristic UV absorbance at 300 nm. Varying the culture conditions helped trigger the biosynthesis of compound ECO-02301 (50).[36] Scanning the genome sequence of *Aspergillus nidulans* revealed the presence of three copies of genes that codes for proteins with high similarity to anthranylate synthases (ASs). These enzymes are responsible for the conversion of chorismate to anthranilic acid, which is important for the synthesis of tryptophan. By altering the cultivation conditions, four new prenylated quinoline alkaloids aspoquinolones I, II and III (51 -54) were produced.[61]

Epigenetic modifiers: The strategy of epigenetic modifiers (gene activators) was further studied on different fungi. Some of these studies reported that *Aspergillus* sp. contains nuclear transcriptional regulator LaeA, which controls secondary metabolite production, suggesting the existence of different regulatory mechanisms that ensure secondary metabolites production at certain developmental stages or under specific environmental conditions.[62-64] Cichewicz et al[65] treated 12 fungi with several DNA methyltransferase and histone deacetylase inhibitors. Eleven strains were found to respond, with the production of new or enhancement of known natural products. One of these strains, *Cladosporium cladosporioides*, was treated with 5-azacytidine,

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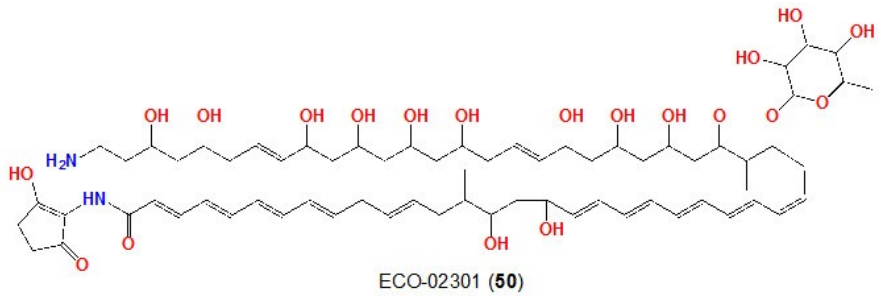
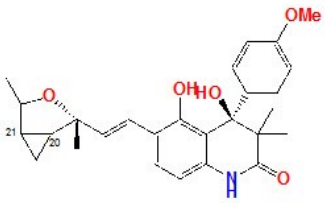
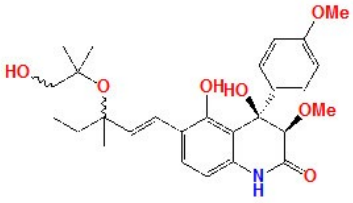


ECO-02301 (**50**)



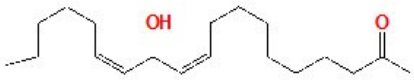
aspoquinolone I (**51**) 20 (*S*); 21 (*R*)

aspoquinolone II (**52**) 20 (*R*); 21 (*S*)

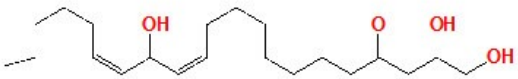


aspoquinolone III (**53/54**)

oxylipin I (**55**)



oxylipin II (**56**)



oxylipin III (**57**)

and led to the production of oxylipins I, II and III (55-57).

All these observations listed above highlight that the microbial genome offers for greater molecular potential that previously imagined, and that molecular tools are required to access this resource.

Lipopolysaccharide: The lipolysaccharide (LPS) is considered as the main component of the cell wall of all the gram-negative bacteria. The composition of the cell wall of the gram-negative bacteria is made up of an outer membrane, inner plasma membrane and a peptidoglycan layer in the periplasm. The inner cell wall is mainly considered of phospholipids while the outer surface of the outer membrane is composed of 90% LPS, plus the addition of some phospholipids and proteins. LPS consists of phospholipids in which the hydrophilic portion contains different polysaccharide made of core and outer portion.[66]

LPS is a tripartite molecule comprising a membrane-anchored lipid A moiety, a core oligosaccharide and an O-antigen polysaccharide made up of repeating units. 3-deoxy-D-manno-2-octulosonate (KDO) residues link lipid A to the core oligosaccharide, which can also be decorated with other (often non-stoichiometric) substituents, such as phosphate and phosphoethanolamine. LPS is only found in gram-negative bacteria.[67]

Bacteria with rough LPS usually have more penetrable cell membranes to hydrophobic antibiotics, since a rough LPS is more hydrophobic.[66] The hydrophobic, membrane-anchoring region of LPS is called lipid A. This part of the LPS is well conserved among bacterial species, and is considered as the most active moiety of LPS, responsible for many of the pathophysiological effects associated with infection. Lipid A is composed of phosphorylated glucosamine disaccharide decorated with 6 or even saturated acyl chains linked through amide and ester bonds. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhoea, and even fatal endotoxic shock.[67]

Khalil et al[68] reported on a preliminary investigation of the use the gram-negative bacterial cell wall constituent lipopolysaccharide (LPS) as a natural chemical cue to stimulate and alter the expression of fungal secondary metabolism. Integrated

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HTP micro-cultivation and micro-analysis methods determined that 6 of 40 (15%) of fungi tested responded to an optimal exposure to LPS (0.6 ng/mL) by activating, enhancing, or accelerating secondary metabolite production. To explore possible mechanisms behind this effect, we employed light and fluorescent microscopy in conjunction with a nitric oxide (NO) sensitive fluorescent dye and an NO scavenger to provide evidence that LPS stimulation of fungal secondary metabolism coincided with LPS activation of NO. Several case studies demonstrated that LPS stimulation can be scaled from single microplate well (1.5 mL) to preparative (>400 mL) scale cultures. For example, LPS treatment of *Penicillium* sp. (ACM-4616) enhanced pseurotin A, and activated pseurotin A_1 and pseurotin A_2 biosynthesis, whereas LPS treatment of *Aspergillus* sp. (CMB-M81F) substantially accelerated and enhanced the biosynthesis of shornephine A and a series of biosynthetically related ardeemins, and activated production of neoasterriquinone. As an indication of broader potential, we provide evidence that cultivations of *Penicillium* sp. (CMB-TF0411), *Aspergillusniger* (ACM-4993F), *Rhizopusoryzae* (ACM-165F) and *Thanatephorus cucumeris* (ACM-194F) were responsive to LPS stimulation, the latter 2 examples being particular noteworthy as neither are known to produce secondary metabolites. Our results encourage the view that LPS stimulation can be used as a valuable tool to expand the molecular discovery potential of fungal strains that have either been exhaustively studied by, or that are unresponsive to traditional cultivation methodology.

CONCLUSION

Nature is a good source and producer of small molecules that have a great interaction with biological targets. Despite that natural products continue to provide more than half of all new drugs approved by the US Food and Drug Administration (FDA) during the last century, there is a huge shift in finding a new molecules to overcome the threat coming from multi-drug resistance bacteria and cancer resistant cells.

The decline in the pipeline for the discovery of new microbial metabolites can be attributed to the continuous rediscovery of known metabolites and we believe that the main reason for the decline in the discovery of new biologically active compounds can be that many of the microbial genes can remain dormant under normal laboratory conditions. Therefore, the microbe may require an external stimuli in order to start behave in different way and try to produce different chemistry. In fact, modern genomics have opened the tools and brighten our understanding of the microbial secondary metabolism. Medema et al[69] have demonstrated a computational analysis of the microbial metabolism through comprising approximately 300 microbial genome, which lead to the prediction of thousands of microbial genome sequencing. This great outcome provides the opportunity for the acquisition of the full microbial genome and the availability of high throughput sequencing technologies with modern analytical instrumentation allowed to determine or postulate the type of metabolites that can be produced by any microorganisms.

Therefore, we found that it is worthwhile to try to find a new paradigm for drug discovery to help to overcome the limited laboratory conditions and to establish a new technique to target the production of antibiotics. In order to perform such paradigm, we established a combinatorial chemical libraries in order to cover much chemical space to increase the probability of wakening up the silent genes and turning on new secondary metabolites.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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