We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Diverse Survival Functions of Secondary Metabolites in Nature

Ayush Mandwal

Abstract

Secondary metabolites are low molecular mass products of secondary metabolism which are usually produced by microorganisms experiencing stringent conditions. These metabolites are not essential for growth but serve diverse survival functions in nature. Besides offering survival advance to the producing organisms, they have several medicinal uses such as antibiotics, chemotherapeutic drugs, immune suppressants, and other medicines which benefited human society immensely for more than a century. This chapter provides an overview of various functions these secondary metabolites offer in nature from single-cell organisms to multicellular organisms. Furthermore, this chapter also discusses the underlying mechanisms behind their diverse functions and how these are regulated and synthesized under non-viable environmental conditions.

Keywords: secondary metabolites, antibiotics, *Streptomyces*, resistance/tolerance, cluster-situated regulators

1. Introduction

Secondary metabolites are biologically active small molecules that are not required for growth and development but which provide a competitive advantage to the producing organism [1]. These are small organic molecules that consist of unusual chemical structures which include β -lactam rings, cyclic peptides, depsipeptides containing unnatural and non-protein amino acids, unusual sugars and nucleosides, unsaturated bonds of polyacetylenes and polyenes, covalently bound chlorine and bromine; nitro-, hydroxamic acids, and so on. Their enormous diversity includes 22,000 terpenoids as well [2]. These complex molecules are commonly obtained from molds which make 17% of all antibiotics and actinomycetes make 74% [3]. The bacterial secondary metabolites are a source of many antibiotics, chemotherapeutic drugs, immune suppressants, and other medicines. Some species are relatively more prolific in secondary metabolism, for example, strains of *Streptomyces hygroscopicus* produce more than 180 different secondary metabolites [4]. It is estimated that the total number of microbial secondary metabolites so far discovered vary from 8000 up to 50,000 [5–7].

As microorganisms are rarely found in isolation, the presence of secondary metabolites in a microbial community exerts evolutionary pressure on both secondary metabolite-producing and non-producing members to develop means to withstand

them. They either use secondary metabolites to have competitive advances or support the intra/interspecies communities against stressful environmental conditions. As these molecules play a vital role in the survival of various microbes, biosynthesis of these molecules is tightly regulated via various transcriptional factors which got triggered under stringent conditions.

This chapter is dedicated to the role of secondary metabolites as antibiotics by various species. The first section of the chapter begins with a discussion on how these molecules are utilized by the various organisms to ensure their survival in the environment. The second section of the chapter goes into the details of how secondary metabolites affect the metabolism of the organism and alter their own or other's organism susceptibility against antibiotics. And the last section discusses how these secondary metabolites as antibiotics are regulated and synthesized with a special focus on *Streptomyces* as they are a rich source of natural antibiotics due to their prolific secondary metabolism.

2. Secondary metabolites in nature

In recent years, the view that secondary metabolites facilitate the survival of the producer in competition with other living species has been expressed more widely [8, 9]. Some common arguments behind such a view are as follows: (1) Organisms that lack an immune system are prolific producers of these compounds which act as an alternative defense mechanism. (2) The compounds have sophisticated structures, mechanisms of action, and complex and energetically expensive pathways which can only exist if they provide survival advantage to the organism [10]. (3) They are produced in nature and used in competition between microorganisms, plants, and animals [11, 12]. (4) Biosynthetic genes of secondary metabolites are clustered, which would only be selected for if the product conferred a selective advantage, and the absence of non-functional genes in these clusters. (5) The presence of resistance and regulatory genes in these clusters, and lastly by not least the non-producers have clustering of resistance genes.

Besides providing a survival advantage to microbes, secondary metabolites with antibacterial and antifungal properties can cause public health problems if found in soil, straw, and agricultural products. These are usually considered to be mycotoxins, but they are nevertheless antibiotics. And the natural production of such toxic metabolites is one of our major public health problems in the field and during the storage of crops. Natural soil and wheat straw contain patulin [13] and aflatoxin is known to be produced on corn, cottonseed, peanuts, and tree nuts in the field [14]. These toxins can cause hepatotoxicity, teratogenicity, immunotoxicity, mutation, cancer, and death [15].

Below list provide some of the functions of secondary metabolites with examples found across various species from single cell microbes to multicellular organisms.

1. Agents of chemical warfare in nature

- According to Cavalier-Smith [16], secondary metabolites are most useful to the organisms producing them as competitive weapons. Antibiotics are more effective than macromolecular toxins such as animal venoms because of their higher diffusibility into cells and broader modes of action and diverse molecular structure varieties possible.

- Microbe vs. microbe:
 - In nature, competition between various fungi has been demonstrated in virtually every type of fungal ecosystem including coprophilous, carbinocolous, lignicolous, fungicolous, phylloplane, rhizosphere, marine, and aquatic [17].
 - Bacteria produce antibiotics when they need them for survival. For instance, myxobacteria can grow on *E. coli* only if the cell density is more than 10^7 myxobacteria/ml [18]. Such high cell density in the local environment produces high concentrations of lytic enzymes and antibiotics needed to grow on *E. coli*.
- Bacteria vs. amoebae:
 - As eukaryotes cells such as amoebae, a protozoans cell, feed on bacteria [19], bacteria found their ways to protect themselves against amoebae and other protozoans in general. Both *Serratia marcescens* and *Chromobacterium violaceum* bacteria produce antibiotically-active pigments namely prodigiosin and violacein, respectively to protect themselves from amoebae. These molecules can either encyst the protozoa or kill them.
- Microorganisms vs. higher plants:
 - Over 150 microbial compounds called phytotoxins have been reported that are active against plants [20]. Several such phytotoxins (e.g., phaseolotoxin, rhizobitoxine, and syringomycin) show typical antibiotic activity against other microorganisms and are thus belong to a class of antibiotics.
 - Plants produce antibiotics called phytoalexins after being exposed to plant pathogenic microorganisms in order to protect themselves [21]. They are of low molecular weight, weakly active, and indiscriminate, that is, they inhibit both prokaryotes and eucaryotes including higher plant cells and mammalian cells.
- Microorganisms vs. insects:
 - Certain fungi produce secondary metabolites for entomopathogenic activity: infecting and killing insects. *Beauveria bassiana* fungus produces one such compound called bassianolide, a cyclodepsipeptide, which elicits atonic symptoms in silkworm larvae [22]. Another pathogen, *Metarrhizium anisophae*, produces the peptidolactone toxins known as destruxins [23].
 - Similarly, to fight back against bacterial infections, insects produce antibacterial proteins [24]. Some of these proteins are lysozyme, sarcotoxins, cecropins, and defensins. These proteins are either bactericidal or bacteriostatic by nature.

- Microorganisms vs. higher animals:
 - It is beneficial for microbes to make fresh food as objectionable as possible to large organisms as quickly as possible [25]. They produce secondary metabolites such as antibiotics and toxins which are toxic to large animals such as livestock. Thus, large animals will refuse to consume moldy feed which ensures the availability of food sources for various microbes.
 - If in case, animals and plants do get infected from microbes, they produce various peptides which kill microbes by permeabilizing their cell membranes as a way to defend against microbial infection [26].

2. Metal transport agents

- Certain secondary metabolites can act as metal transport agents. Siderophores (also known as sideramines) containing molecules function in the uptake, transport, and solubilization of iron. Siderophores are complex molecules that solubilize ferric ion which has a solubility of only 10^{-18} mol/L at pH 7.4 and have an extremely high affinity for iron ($K_d = 10^{-20}$ – 10^{-50}). Another group of molecules includes the ionophoric antibiotics, for example, macrotetrolide antibiotics, which function in the transport of certain alkali-metal ions such as potassium and affect its permeability through the cell membranes.
- Iron-transport factors in many cases are antibiotics by nature. They are on the borderline between primary and secondary metabolites since they are usually not required for growth but do stimulate growth under iron-deficient conditions. Antibiotic activity is due to the ability of these compounds to starve other species of iron when the latter lack the ability to take up the Fe-sideramine complex. Such antibiotics include nocardamin [27] and desferritriacetylfusigen [28].

3. Effects of microbial secondary metabolites on antibiotic tolerance and resistance

Secondary metabolites can bring profound variation in microbial physiology, metabolism, and stress responses [29]. Several evidence suggest that these molecules can modulate microbial susceptibility to commonly used antibiotics. This section explores which types of secondary metabolites alter antimicrobial susceptibility, and how and why this phenomenon occurs.

In a given environment, microorganisms are rarely found in isolation. Due to such reasons, the presence of secondary metabolites in a microbial community exerts evolutionary pressure on both secondary metabolite-producing and non-producing members to develop means to withstand them. Generally, secondary metabolites alter the state of metabolism which directly has an impact on an organism's ability to withstand antibiotics assault by either increasing its tolerance or making itself more resistant.

Although antibiotics tolerance and resistance are often treated in a similar manner, they offer different abilities to the microorganism. The phenomenon of antibiotic

tolerance is the ability of organism to survive transient antibiotic exposure while resistance is the ability of organism to grow in the presence of antibiotics at a given concentration [30–32]. Another generic term commonly used is antibiotic resilience which refers to the ability of a bacterial population to be refractory to antibiotic treatment, which can arise from an increase in tolerance and/or resistance.

There are common modes of action through which secondary metabolites molecules can alter antibiotic efficacy in both single-species and polymicrobial communities. Specifically, secondary metabolites can regulate multidrug resistance efflux systems, can modulate the toxicity of antibiotics through interactions with reactive oxygen species (ROS) and can induce antibiotic tolerance to provide an overlooked route for the evolution of antibiotic resistance.

The knowledge of such interactions between secondary metabolites and antibiotic efficacy is beneficial as this could be applied to optimize the use of existing antimicrobial drugs and generate targets for novel therapeutic strategies.

3.1 Induction of efflux systems

One common mechanism bacteria use to tolerate clinical antibiotic treatment is by activation of efflux pumps that export toxins out of the cell [33, 34]. However, efflux pumps exist long before human use of synthetic antibiotics and therefore are presumed to have originally evolved to transport other, naturally occurring, substrates, such as secondary metabolites [35, 36]. Importantly, efflux pumps vary in their specificity, with regard to both their regulation and their substrate affinity [37]. Therefore, it is essential to understand how the secondary metabolite interacts with the transcriptional regulation of the efflux system, as well as which classes of drug the efflux system can transport before one predict whether a secondary metabolite will increase antibiotic resilience in its producer through the induction of a particular efflux system or not. A well-known example of a secondary metabolite that affects the efflux system is indole. Indole is a signaling molecule self-produced by *E. coli* that triggers the expression of certain multidrug efflux pumps in enteric bacteria [38, 39]. It is also found that subpopulation of mutants in *E. coli* which is more resilient towards norfloxacin and gentamicin antibiotics treatment produces high levels of indole which give population-level resistance [40]. This behavior is characterized as a “charity” as the subpopulation responds in favor to support the rest of the community. It was also found that indole achieve such a role by upregulating the MdtEF-TolC efflux system [40].

As mentioned earlier, efflux pumps are evolved to transport in general various molecules including secondary metabolites, efflux pumps can also provide protection against secondary metabolites that are toxic to their producers. For instance, phenazines are redox-active and toxic secondary metabolites produced by the *Pseudomonas aeruginosa*, which activates expression of the MexGHI-OpmD efflux system via the redox-sensing transcription factor SoxR [41–43]. In addition, phenazines also serve other important roles both for the microbes and other species as it can increase *P. aeruginosa* virulence in the lungs of patients with cystic fibrosis, or help in protecting plants against fungal pathogens [44, 45].

A related phenomenon has been observed in the Gram-positive bacterium *Streptomyces coelicolor*. This bacterium produces a natural antibiotic, actinorhodin, that stimulates the expression of a transporter similar to those that export tetracycline [46, 47].

3.2 Modulation of oxidative stress

The idea of oxidative stress become well known when it was proposed that regardless of the specific cellular targets of various antibiotic classes, bactericidal antibiotics exert their lethal effects in part by inducing oxidative stress [48]. Although this hypothesis resulted in major controversies [49, 50], evidence reviewed elsewhere [51] suggests that bactericidal antibiotics do impact cellular redox states and that the resulting increase in ROS and oxidative stress can contribute to cell death. Such phenomena relate well with secondary metabolites as they also interface with cellular redox homeostasis and oxidative stress responses. Below three different modes of action are discussed by which secondary metabolites can potentially antagonize or potentiate the toxicity of antibiotics: upregulation of oxidative stress response genes; direct detoxification of ROS; and increased endogenous ROS generation.

3.2.1 Upregulation of defenses against oxidative stress

Secondary metabolites that upregulate the expression of oxidative stress responses can prime bacterial cells for tolerance and/or resistance to antibiotics, which is similar to the protective effects of exposure to sublethal concentrations of oxidants such as H_2O_2 [52]. Among this class of metabolites, indole is a non-toxic molecule produced by *E. coli*, which activates various genes such as thioredoxin reductase, DNA-binding protein (Dps), and alkyl hydroperoxide reductases that activate only during oxidative stress response [53]. Furthermore, the frequency of *E. coli* persisters can increase by at least an order of magnitude if it is exposed to indole to three different classes (fluoroquinolones, aminoglycosides, and β -lactams) antibiotics. In addition, deletion of *oxyR* substantially diminishes this effect, which demonstrates that upregulation of oxidative stress responses by a secondary metabolite can contribute to bacterial persistence [53].

Another example of the secondary metabolite is pyocyanin (PYO) produced by *P. aeruginosa*. PYO increases superoxide dismutase activity [54] and upregulates the transcription of several other oxidative stress response genes, including those encoding alkyl hydroperoxide reductases, thioredoxin reductase, catalase, and iron-sulfur cluster biogenesis machinery [43]. Interestingly, PYO has been shown to increase the frequency of gentamicin-resistant mutants in *P. aeruginosa* cultures that is independent of drug efflux, as PYO does not upregulate aminoglycoside-transporting efflux pumps [55]. A plausible explanation for this phenomenon is that PYO-induced oxidative stress responses counteract ROS-related gentamicin toxicity. The reasoning behind the explanation is as follows: (1) Gentamicin is known to promote increased intracellular ROS levels [56, 57]. (2) Pretreating cells with oxidants can prime them to tolerate antibiotics [52]. This consequently could decrease the rate at which spontaneous mutants are lost from the population [58], thus the frequency of resistant mutants increases as observed experimentally. PYO has been demonstrated to promote the growth of *P. aeruginosa* in the presence of other antibiotics such as a β -lactam antibiotic carbenicillin and other aminoglycosides such as kanamycin, streptomycin, and tobramycin [59]. These antibiotics have been shown to perturb cellular redox states [60, 61] rather than as substrates for PYO-regulated efflux systems [55, 62]. This suggests that the observed decreases in antibiotic efficacy could be related to PYO-induced oxidative stress responses.

3.2.2 Detoxification of ROS

Another way to protect against antibiotic assaults of oxidative stress is by directly detoxifying antibiotic-induced ROS by upregulating the antioxidant activity. Ergothioneine, is one of two major sulfur-containing redox buffers in mycobacteria, along with mycothiol which help in detoxifying ROS during the stress response. This molecule is so important for the *Mycobacterium tuberculosis* that loss of ergothioneine biosynthesis genes decreases minimum inhibitory concentrations (MICs) for various clinical antibiotics such as rifampicin, bedaquiline, clofazimine, and isoniazid. Additionally, loss of gene also decreases the survival rate by at least 30–60% under treatment at the MICs compared to wild type [63].

3.2.3 Synergistic interactions between secondary metabolites and antibiotics

So far secondary metabolites have been demonstrated to decrease antibiotic efficacy by attenuating oxidative stress. However, they can also amplify the toxicity of antibiotics by increasing ROS generation. 2-heptyl-3-hydroxy-4-quinolone is one example, also known as the *Pseudomonas* quinolone signal (PQS) produced by *P. aeruginosa*. PQS is a redox-active molecule that possesses both antioxidant properties and pro-oxidant activity as it can reduce not only free radicals but also metal ions. Reduction in metal ions concentration such as iron is lethal for the cell as it facilitates ROS formation through the Fenton reaction [64].

3.3 Interspecies antibiotic resilience

So far, we have discussed examples of how secondary metabolites affect their producers susceptibility to antibiotics. However, secondary metabolites can also modulate interspecies antibiotic resilience. Such study is beneficial as it will potentially show how interactions among members of a polymicrobial infection might affect antibiotic treatment outcomes [65, 66]. For example, one study has demonstrated that indole, which is produced by *E. coli*, can increase antibiotic tolerance of *Salmonella enterica subsp. enterica serovar Typhimurium* [38, 67]. *S. Typhimurium* does not produce indole, yet it becomes more than threefold tolerant against ciprofloxacin in the presence of exogenously added indole, as well as in the case where it is co-cultured with indole-producing *E. coli* [67]. Indole induces the same OxyR-regulated oxidative stress responses in *S. Typhimurium*, as in *E. coli*, and the deletion of oxyR decreases tolerance against ciprofloxacin in *S. Typhimurium* mediated by it [67]. Thus, indole acts like an interspecies modulators of antibiotic resilience. Besides indole, putrescine is another secondary metabolite that acts similar to indole. For example, *Burkholderia cenocepacia* produces putrescine to protect itself from polymyxin B but it also protects its neighboring species in the co-cultures, including *E. coli* and *P. aeruginosa* [68]. There are definitely more such secondary metabolites that exist which have the potential of being interspecies modulators of antibiotic resilience.

Importantly, although the above examples suggest that certain secreted secondary metabolites have the potential to raise the community-wide level of antibiotic resilience in polymicrobial communities, it may not be this case always. As mentioned earlier that secondary metabolites can be toxic or non-toxic and can increase or decrease resilience against antibiotics, it is important to consider whether the stress caused by the secondary metabolite is tolerable to the non-producing species. If the molecule-caused toxicity outweighs the benefits it provides against antibiotics, the

non-producing species would not gain a benefit. In such a case, the secondary metabolite might even act synergistically with the clinical antibiotic [69].

4. The regulation of the secondary metabolism of *Streptomyces*

So far, we have discussed what different kinds of secondary metabolites exist, how they affect cellular metabolism and help in survival in competitive and stressful environments. This last section focuses on the factors on which the biosynthesis of secondary metabolites is regulated. This will show how different conditions lead to the secretion of secondary metabolites which leads to phenomena discussed in the previous two sections. As the exploration of regulation of secondary metabolites among various species is still in its early stages except for Streptomycetes, this section will focus on its regulatory mechanisms behind biosynthesis of secondary metabolites.

Streptomycetes and other actinobacteria are renowned as a rich source of natural products of clinical, agricultural, and biotechnological value. Sequencing genomes of numerous streptomycetes has revealed that they all possess the capacity to produce multiple secondary metabolites [70, 71] implies that it can repel a large number of competitors using either individual or combination of molecules using their synergistic characteristics [72]. The genes of enzymes responsible for the production of individual secondary metabolites are found clustered. Furthermore, these clustered genes are commonly associated with regulatory gene/s that regulate their transcription or resistance genes.

This section discusses some of the factors which regulate the production of secondary metabolites in *Streptomyces*.

4.1 Cluster-situated regulators (CSRs)

Generally, a single regulatory gene regulates several gene clusters associated with secondary metabolites production. This way multiple chemical signals which can trigger activation of the regulatory gene can activate specific or multiple genes clustered corresponding to secondary metabolite production. Some of the gene clustered include the clusters for streptomycin in *S. griseus* and actinorhodin (ACT) in *S. coelicolor*. Their corresponding transcription factors are called StrR and ActII-ORF430 respectively [73]. Both StrR and ActII-ORF430 transcription factors directly activate the transcription of genes of the corresponding clusters that encode biosynthetic enzymes. Moreover, evidence suggests that the cellular level of a CSR is the principal factor that determines the level of transcription of the biosynthetic genes it targets. This correlates closely with the level of secondary metabolite produced [74, 75]. Thus, factors that control the production of ActII-ORF4 and StrR will ultimately regulate the production of ACT and streptomycin respectively. Both these transcription factors are under regulation with many activators, repressors, and inducers which are explained well in detail here [76].

4.2 The stringent response and nutrient deprivation

Stringent response is an stress response of bacteria in reaction to amino-acid starvation, fatty acid limitation, iron limitation, heat shock, and other stress conditions. During stringent response, accumulation of (p)ppGpp enables bacteria to survive sustained periods of nutrient deprivation. For several *Streptomyces* species,

mutations that block the synthesis of (p)ppGpp (guanosine tetra- and penta-phosphate) have been found to alter antibiotic production and hinder morphological development [77]. In general, the stringent response enhances transcription of numerous genes associated with the stationary phase of batch culture and stress responses. Stimulation of (p)ppGpp synthesis, either by subjecting growing cultures to amino acid starvation [78] or inducing expression of a truncated version of relA that confers ribosome independent (p)ppGpp synthetase activity [79, 80] increases the level of actII-ORF4 transcription and production of the corresponding antibiotics.

4.2.1 Regulation of secondary metabolism by carbon

The availability and source of carbon have a substantial effect on the production of antibiotics and morphological development [81]; for example, glucose blocks production of ACT by *S. coelicolor* [82]. Lack of carbon source triggers a stringent response which as explained above leads to accumulation of (p)ppGpp which increases the level of actII-ORF4 transcription and production of the corresponding antibiotics.

4.2.2 Regulation of secondary metabolism by nitrogen

Numerous studies have shown that the source of nitrogen can influence the production of antibiotics. In the presence of sources of nitrogen that are favorable for growth, production of many, but not all, secondary metabolites is reduced [83–85]. One interpretation of this tendency is that by supplying a good source of nitrogen, the available carbon can be used for growth and generating biomass. Thus cell does not experience or experience of lesser extent of the stringent condition in the presence of suitable nitrogen source.

4.3 Upsetting of zinc and iron homeostasis

Zinc is an essential trace element and cofactor required for the structure and function of many proteins. Being important, it is under tight regulation by Znr, a zinc-responsive transcriptional repressor that regulates genes encoding a high-affinity uptake system for zinc, as well as zinc-free paralogues of ribosomal proteins in many bacteria, including streptomycetes [86, 87]. Znr also directly represses a promoter within the cluster of coelibactin, a non-ribosomally synthesized peptide predicted to have siderophore (metal-chelating) activity in *S. coelicolor* [88, 89]. AbsC, a pleiotropic regulator which is required for the production of ACT and RED (chromosomally-encoded antibiotics, the prodiginine complex RED, which is red in color), represses promoters of the coelibactin cluster under the specific condition of low zinc [88]. Although the underlying regulatory mechanism is still unknown, under low zinc concentration if upregulation of genes encoding a high-affinity uptake system for zinc via Znr does not work, AbsC potentially becomes active which increases the production of ACT and RED for antibiotics production.

Iron is another essential metal that is under tight regulation [90]. Members of the DmdR (divalent metal-dependent) family i.e., DmdR1 and DmdR2 are the key regulatory components of iron homeostasis in *S. coelicolor* [91, 92]. The dmdR1 gene overlaps with adm gene on the opposite strand and disruption of the overlapping gene increases the production of RED and ACT which leads to antibiotics production [91]. Although the details of the DmdR1/Adm system remain to be uncovered, it is likely that physiological cellular stress indirectly affects antibiotic production.

4.4 Extracellular signaling molecules

One extracellular signaling molecule γ -butyrolactones has been shown to regulate antibiotic production in many streptomyces cultures [93–95]. Such a mechanism is beneficial for the community survival as extracellular signaling molecules are diffusible in solid media. This way even a single actinomycete can stimulate antibiotic production in another when grown next to each other on an agar plate [96], thus protecting the entire community against competitive microbes.

5. Conclusions

Although secondary metabolites are not considered essential for the growth and development of microorganisms, they serve diverse survival functions in nature. These molecules allowed both antagonistic and symbiotic relationships between various species from single-cell organisms to multicellular organisms. Without such relationships, the natural ecosystem whether it is soil, or lake, or forest would not be filled with rich and diverse life forms that we find on this planet. Thus, it can be said that secondary metabolites are as essential as primary metabolites in a environment with multi-species communities coexisting together.

Author details

Ayush Mandwal
University of Calgary, Calgary, Canada

*Address all correspondence to: ayushmandwal5@gmail.com

IntechOpen

© 2022 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Demain AL, Fang A. The natural functions of secondary metabolites. In: Fiechter A, editor. History of Modern Biotechnology I. Advances in Biochemical Engineering/Biotechnology. Vol. 69. Berlin, Heidelberg: Springer; 2001. pp. 1-39. DOI: 10.1007/3-540-44964-7_1
- [2] Connolly JD, Hill RA, et al. Dictionary of terpenoids. London, UK: Chapman & Hall; 1991
- [3] Miyadoh S. Research on antibiotic screening in Japan over the last decade: A producing microorganism approach. Actinomycetologica. 1993;7(2):100-106. DOI: 10.3209/saj.7_100
- [4] Zedan H. The economic value of microbial diversity. SIM News. 1993;43:178-185
- [5] Lechevalier HA, Lechevalier MP. Biology of actinomycetes. Annual Reviews in Microbiology. 1967;21(1):71-100
- [6] Omura S. Trends in the search for bioactive microbial metabolites. Journal of Industrial Microbiology. 1992;10(3-4):135-156. DOI: 10.1007/bf01569759
- [7] Reichenbach H, Höfle G. Biologically active secondary metabolites from myxobacteria. Biotechnology Advances. 1993;11(2):219-277. DOI: 10.1016/0734-9750(93)90042-1
- [8] Stone MJ, Williams DH. On the evolution of functional secondary metabolites (natural products). Molecular Microbiology. 1992;6(1):29-34. DOI: 10.1111/j.1365-2958.1992.tb00834.x
- [9] Williams DH, Stone MJ, Hauck PR, Rahman SK. Why are secondary metabolites (natural products) biosynthesized? Journal of Natural Products. 1989;52(6):1189-1208. DOI: 10.1021/np50066a001
- [10] Katz E, Demain AL. The peptide antibiotics of *Bacillus*: Chemistry, biogenesis, and possible functions. Bacteriological Reviews. 1977;41(2):449-474
- [11] Demain A. Do antibiotics function in nature. Search. 1980;11(5):148-151
- [12] Demain AL, Solomon NA. Biology of industrial microorganisms. vol. 6. Oxford, UK: Butterworth-Heinemann; 1985
- [13] Norstadt FA, McCalla TM. Microbial populations in stubble-mulched soil. Soil Science. 1969;107(3):188-193. DOI: 10.1097/00010694-196903000-00006
- [14] Hesseltine CW, Rogers RF, Shotwell OL. Aflatoxin and mold flora in North Carolina in 1977 corn crop. Mycologia. 1981;73(2):216. DOI: 10.2307/3759642
- [15] Trail F, Mahanti N, Linz J. Molecular biology of aflatoxin biosynthesis. Microbiology. 1995;141(4):755-765
- [16] Cavalier-Smith T. Origins of secondary metabolism. In: Secondary Metabolites: Their Function and Evolution. Chichester, UK: John Wiley & Sons; 1992. pp. 64-87
- [17] Gloer JB. The chemistry of fungal antagonism and defense. Canadian Journal of Botany. 1995;73(S1):1265-1274. DOI: 10.1139/b95-387
- [18] Rosenberg E. Myxobacteria: development and cell interactions. Berlin/Heidelberg, Germany: Springer Science & Business Media; 2012

- [19] Habte M, Alexander M. Further evidence for the regulation of bacterial populations in soil by protozoa. *Archives of Microbiology*. 1977;**113**(3):181-183. DOI: 10.1007/bf00492022
- [20] Fischer H, Bellus D. Phytotoxins from microorganisms and related compounds. *Pesticide Science*. 1983; **14**(3):334-346. DOI: 10.1002/ps.2780140316
- [21] Darvill AG, Albersheim P. Phytoalexins and their Elicitors-A Defense Against Microbial Infection in Plants. *Annual Review of Plant Physiology and Plant Molecular Biology*. 1984;**35**(1):243-275. DOI: 10.1146/annurev.pp.35.060184.001331
- [22] Kanaoka M, Isogai A, Suzuki A. Syntheses of bassianolide and its two homologs, enniatin C and decabassianolide. *Agricultural and Biological Chemistry*. 1979;**43**(5): 1079-1083
- [23] Lee S, Izumiya N, Suzuki A, Tamura S. Synthesis of a cyclohexadepsipeptide, protodestruxin. *Tetrahedron Letters*. 1975;**16**(11):883-886. DOI: 10.1016/S0040-4039(00)72010-7
- [24] Kimbrell DA. Insect antibacterial proteins: Not just for insects and against bacteria. *BioEssays*. 1991;**13**(12):657-663. DOI: 10.1002/bies.950131207
- [25] Janzen DH. Why fruits rot, seeds mold, and meat spoils. *The American Naturalist*. 1977;**111**(980):691-713. DOI: 10.1086/283200
- [26] Nissen-Meyer J, Nes IF. Ribosomally synthesized antimicrobial peptides: Their function, structure, biogenesis, and mechanism of action. *Archives of Microbiology*. 1997;**167**(2-3):67-77. DOI: 10.1007/s002030050418
- [27] Keller-Schierlein W, Prelog V. Stoffwechselprodukte von Actinomyceten. 30. Mitteilung. Über das Ferrioxamin E; ein Beitrag zur Konstitution des Nocardamins. *Helvetica Chimica Acta*. 1961;**44**(7):1981-1985. DOI: 10.1002/hlca.19610440721
- [28] Anke H. Metabolic products of microorganism. 163 Desferritriacetilfusigen, an antibiotic from *Aspergillus deflexus*. *The Journal of Antibiotics*. 1977;**30**(2):125-128
- [29] Perry EK, Meirelles LA, Newman DK. From the soil to the clinic: The impact of microbial secondary metabolites on antibiotic tolerance and resistance. *Nature Reviews Microbiology*. 2021;**19**:1-14. DOI: 10.1038/s41579-021-00620-w
- [30] Kester JC, Fortune SM. Persisters and beyond: Mechanisms of phenotypic drug resistance and drug tolerance in bacteria. *Critical Reviews in Biochemistry and Molecular Biology*. 2013;**49**(2):91-101. DOI: 10.3109/10409238.2013.869543
- [31] Brauner A, Fridman O, Gefen O, Balaban NQ. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nature Reviews Microbiology*. 2016;**14**(5):320-330. DOI: 10.1038/nrmicro.2016.34
- [32] Balaban NQ, Helaine S, Lewis K, Ackermann M, Aldridge B, Andersson DI, et al. Definitions and guidelines for research on antibiotic persistence. *Nature Reviews Microbiology*. 2019;**17**(7):441-448. DOI: 10.1038/s41579-019-0196-3
- [33] Li XZ, Plésiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in gram-negative bacteria. *Clinical Microbiology Reviews*. 2015; **28**(2):337-418. DOI: 10.1128/cmr.00117-14

- [34] Piddock LJV. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clinical Microbiology Reviews*. 2006; **19**(2):382-402. DOI: 10.1128/cmr.19.2.382-402.2006
- [35] Piddock LJV. Multidrug-resistance efflux pumps? Not just for resistance. *Nature Reviews Microbiology*. 2006; **4**(8):629-636. DOI: 10.1038/nrmicro1464
- [36] Martinez JL, Sánchez MB, Martínez-Solano L, Hernandez A, Garmendia L, Fajardo A, et al. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. *FEMS Microbiology Reviews*. 2009; **33**(2): 430-449. DOI: 10.1111/j.1574-6976.2008.00157.x
- [37] Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, et al. Multidrug efflux pumps: Structure, function and regulation. *Nature Reviews Microbiology*. 2018; **16**(9):523-539. DOI: 10.1038/s41579-018-0048-6
- [38] Nikaido E, Giraud E, Baucheron S, Yamasaki S, Wiedemann A, Okamoto K, et al. Effects of indole on drug resistance and virulence of *Salmonella enterica* serovar Typhimurium revealed by genome-wide analyses. *Gut Pathogens*. 2012; **4**(1):5-5. DOI: 10.1186/1757-4749-4-5
- [39] Nishino K, Nikaido E, Yamaguchi A. Regulation of multidrug efflux systems involved in multidrug and metal resistance of *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*. 2007; **189**(24):9066-9075. DOI: 10.1128/jb.01045-07
- [40] Lee HH, Molla MN, Cantor CR, Collins JJ. Bacterial charity work leads to population-wide resistance. *Nature*. 2010; **467**(7311):82-85. DOI: 10.1038/nature09354
- [41] Dietrich LEP, PriceWhelan A, Petersen A, Whiteley M, Newman DK. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Molecular Microbiology*. 2006; **61**(5): 1308-1321. DOI: 10.1111/j.1365-2958.2006.05306.x
- [42] Sakhtah H, Koyama L, Zhang Y, Morales DK, Fields BL, Price-Whelan A, et al. The *Pseudomonas aeruginosa* efflux pump MexGHI-OpnD transports a natural phenazine that controls gene expression and biofilm development. *Proceedings of the National Academy of Sciences*. 2016; **113**(25):E3538-E3547. DOI: 10.1073/pnas.1600424113
- [43] Meirelles LA, Newman DK. Both toxic and beneficial effects of pyocyanin contribute to the lifecycle of *Pseudomonas aeruginosa*. *Molecular Microbiology*. 2018; **110**(6):995-1010. DOI: 10.1111/mmi.14132
- [44] Lau GW, Hassett DJ, Ran H, Kong F. The role of pyocyanin in *Pseudomonas aeruginosa* infection. *Trends in Molecular Medicine*. 2004; **10**(12):599-606. DOI: 10.1016/j.molmed.2004.10.002
- [45] Mavrodi DV, Blankenfheldt W, Thomashow LS. Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation*. *Annual Review of Phytopathology*. 2006; **44**(1): 417-445. DOI: 10.1146/annurev.phyto.44.013106.145710
- [46] Tahlan K, Ahn SK, Sing A, Bodnaruk TD, Willems AR, Davidson AR, et al. Initiation of actinorhodin export in *Streptomyces coelicolor*. *Molecular Microbiology*. 2007; **63**(4):951-961. DOI: 10.1111/j.1365-2958.2006.05559.x
- [47] Willems AR, Tahlan K, Taguchi T, Zhang K, Lee ZZ, Ichinose K, et al.

Crystal structures of the *Streptomyces coelicolor* TetR-like protein ActR alone and in complex with actinorhodin or the actinorhodin biosynthetic precursor (S)-DNPA. *Journal of Molecular Biology*. 2008;**376**(5):1377-1387. DOI: 10.1016/j.jmb.2007.12.061

[48] Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell*. 2007; **130**(5):797-810. DOI: 10.1016/j.cell.2007.06.049

[49] Liu Y, Imlay JA. Cell death from antibiotics without the involvement of reactive oxygen species. *Science*. 2013; **339**(6124):1210-1213. DOI: 10.1126/science.1232751

[50] Keren I, Wu Y, Inocencio J, Mulcahy LR, Lewis K. Killing by bactericidal antibiotics does not depend on reactive oxygen species. *Science*. 2013;**339**(6124):1213-1216. DOI: 10.1126/science.1232688

[51] Dwyer DJ, Collins JJ, Walker GC. Unraveling the physiological complexities of antibiotic lethality. *Annual Review of Pharmacology and Toxicology*. 2015;**55**(1): 313-332

[52] Mosel M, Li L, Drlica K, Zhao X. Superoxide-mediated protection of *Escherichia coli* from antimicrobials. *Antimicrobial Agents and Chemotherapy*. 2013;**57**(11):5755-5759. DOI: 10.1128/aac.00754-13

[53] Vega NM, Allison KR, Khalil AS, Collins JJ. Signaling-mediated bacterial persister formation. *Nature Chemical Biology*. 2012;**8**(5):431-433. DOI: 10.1038/nchembio.915

[54] Hassett DJ, Charniga L, Bean K, Ohman DE, Cohen MS. Response of *Pseudomonas aeruginosa* to pyocyanin:

Mechanisms of resistance, antioxidant defenses, and demonstration of a manganese-cofactored superoxide dismutase. *Infection and Immunity*. 1992;**60**(2):328-336. DOI: 10.1128/iai.60.2.328-336.1992

[55] Meirelles LA, Perry EK, Bergkessel M, Newman DK. Bacterial defenses against a natural antibiotic promote collateral resilience to clinical antibiotics. *PLoS Biology*. 2021;**19**(3):e3001093. DOI: 10.1371/journal.pbio.3001093

[56] Priuska EM, Schacht J. Formation of free radicals by gentamicin and iron and evidence for an iron/gentamicin complex. *Biochemical Pharmacology*. 1995;**50**(11):1749-1752. DOI: 10.1016/0006-2952(95)02160-4

[57] Prayle A, Watson A, Fortnum H, Smyth A. Side effects of aminoglycosides on the kidney, ear and balance in cystic fibrosis. *Thorax*. 2010;**65**(7):654-658. DOI: 10.1136/thx.2009.131532

[58] Alexander HK, MacLean RC. Stochastic bacterial population dynamics restrict the establishment of antibiotic resistance from single cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2020; **117**(32):19455-19464. DOI: 10.1073/pnas.1919672117

[59] Zhu K, Chen S, Sysoeva TA, You L. Universal antibiotic tolerance arising from antibiotic-triggered accumulation of pyocyanin in *Pseudomonas aeruginosa*. *PLoS Biology*. 2019;**17**(12):e3000573. DOI: 10.1371/journal.pbio.3000573

[60] Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, et al. Antibiotics induce redox-related physiological alterations as part of their lethality. *Proceedings of the National Academy of Sciences*. 2014;**111**(20): E2100-E2109

- [61] Belenky P, Ye J, Porter CM, Cohen N, Lobritz M, Ferrante T, et al. Bactericidal antibiotics induce toxic metabolic perturbations that lead to cellular damage. *Cell Reports*. 2015;**13**(5):968-980 DOI: 10.1016/j.celrep.2015.09.059
- [62] Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews*. 2009;**22**(4):582-610. DOI: 10.1128/cmr.00040-09
- [63] Saini V, Cumming B, Guidry L, Lamprecht DA, Adamson J, Reddy V, et al. Ergothioneine maintains redox and bioenergetic homeostasis essential for drug susceptibility and virulence of *Mycobacterium tuberculosis*. *Cell Reports*. 2016;**14**(3):572-585. DOI: 10.1016/j.celrep.2015.12.056
- [64] Häussler S, Becker T. The *Pseudomonas* quinolone signal (PQS) balances life and death in *Pseudomonas aeruginosa* populations. *PLoS Pathogens*. 2008;**4**(9):e1000166. DOI: 10.1371/journal.ppat.1000166
- [65] Bottery MJ, Pitchford JW, Friman VP. Ecology and evolution of antimicrobial resistance in bacterial communities. *The ISME Journal*. 2021;**15**(4):939-948. DOI: 10.1038/s41396-020-00832-7
- [66] Welp AL, Bomberger JM. Bacterial community interactions during chronic respiratory disease. *Frontiers in Cellular and Infection Microbiology*. 2020;**10**:213. DOI: 10.3389/fcimb.2020.00213
- [67] Vega NM, Allison KR, Samuels AN, Klempner MS, Collins JJ. *Salmonella typhimurium* intercepts *Escherichia coli* signaling to enhance antibiotic tolerance. *Proceedings of the National Academy of Sciences*. 2013;**110**(35):14420-14425. DOI: 10.1073/pnas.1308085110
- [68] El-Halfawy OM, Valvano MA. Chemical communication of antibiotic resistance by a highly resistant subpopulation of bacterial cells. *PLoS One*. 2013;**8**(7):e68874. DOI: 10.1371/journal.pone.0068874
- [69] Wood KB, Cluzel P. Trade-offs between drug toxicity and benefit in the multi-antibiotic resistance system underlie optimal growth of *E. coli*. *BMC Systems Biology*. 2012;**6**(1):48. DOI: 10.1186/1752-0509-6-48
- [70] Corre C, Challis GL. New natural product biosynthetic chemistry discovered by genome mining. *Natural Product Reports*. 2009;**26**(8):977-986. DOI: 10.1039/b713024b
- [71] Nett M, Ikeda H, Moore BS. Genomic basis for natural product biosynthetic diversity in the actinomycetes. *Natural Product Reports*. 2009;**26**(11):1362-1384. DOI: 10.1039/b817069j
- [72] Challis GL, Hopwood DA. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proceedings of the National Academy of Sciences*. 2003;**100**(Suppl. 2):14555-14561. DOI: 10.1073/pnas.1934677100
- [73] Vujaklija D, Horinouchi S, Beppu T. Detection of an A-factor-responsive protein that binds to the upstream activation sequence of strR, a regulatory gene for streptomycin biosynthesis in *Streptomyces griseus*. *Journal of Bacteriology*. 1993;**175**(9):2652-2661
- [74] Gramajo HC, Takano E, Bibb MJ. Stationaryphase production of the antibiotic actinorhodin in *Streptomyces coelicolor* A3(2) is transcriptionally

regulated. *Molecular Microbiology*. 1993; 7(6):837-845. DOI: 10.1111/j.1365-2958.1993.tb01174.x

[75] Tomono A, Tsai Y, Yamazaki H, Ohnishi Y, Horinouchi S. Transcriptional control by A-factor of strR, the pathway-specific transcriptional activator for streptomycin biosynthesis in *Streptomyces griseus*. *Journal of Bacteriology*. 2005;187(16):5595-5604. DOI: 10.1128/jb.187.16.5595-5604.2005

[76] van Wezel GP, McDowall KJ. The regulation of the secondary metabolism of *Streptomyces*: New links and experimental advances. *Natural Product Reports*. 2011;28(7):1311-1333. DOI: 10.1039/c1np00003a

[77] Hopwood DA. Forty years of genetics with *Streptomyces*: From in vivo through in vitro to in silico. *Microbiology*. 1999;145(9):2183-2202

[78] Strauch E, Takano E, Baylts HA, Bibb MJ. The stringent response in *Streptomyces coelicolor* A3(2). *Molecular Microbiology*. 1991;5(2):289-298. DOI: 10.1111/j.1365-2958.1991.tb02109.x

[79] Hesketh A, Sun J, Bibb M. Induction of ppGpp synthesis in *Streptomyces coelicolor* A3(2) grown under conditions of nutritional sufficiency elicits actIIORF4 transcription and actinorhodin biosynthesis. *Molecular Microbiology*. 2001;39(1):136-144. DOI: 10.1046/j.1365-2958.2001.02221.x

[80] Sun J, Hesketh A, Bibb M. Functional analysis of relA and rshA, two relA/spoT homologues of *Streptomyces coelicolor* A3(2). *Journal of Bacteriology*. 2001;183(11):3488-3498. DOI: 10.1128/jb.183.11.3488-3498.2001

[81] Sánchez S, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, et al. Carbon source regulation of

antibiotic production. *The Journal of Antibiotics*. 2010;63(8):442-459. DOI: 10.1038/ja.2010.78

[82] Lee HN, Im JH, Lee MJ, Lee SY, Kim ES. A putative secreted solute binding protein, SCO6569 is a possible AfsR2-dependent down-regulator of actinorhodin biosynthesis in *Streptomyces coelicolor*. *Process Biochemistry*. 2009; 44(3):373-377. DOI: 10.1016/j.procbio.2008.12.002

[83] Merrick M, Edwards R. Nitrogen control in bacteria. *Microbiological Reviews*. 1995;59(4):604-622

[84] Shapiro S. Regulation of Secondary Metabolism in Actinomycetes. CRC Press; 1989

[85] Sanchez S, Demain AL. Metabolic regulation of fermentation processes. *Enzyme and Microbial Technology*. 2002;31(7):895-906. DOI: 10.1016/s0141-0229(02)00172-2

[86] Shin JH, Oh SY, Kim SJ, Roe JH. The zinc-responsive regulator Zur controls a zinc uptake system and some ribosomal proteins in *Streptomyces coelicolor* A3(2). *Journal of Bacteriology*. 2007; 189(11):4070-4077. DOI: 10.1128/jb.01851-06

[87] Panina EM, Mironov AA, Gelfand MS. Comparative genomics of bacterial zinc regulons: Enhanced ion transport, pathogenesis, and rearrangement of ribosomal proteins. *Proceedings of the National Academy of Sciences*. 2003;100(17):9912-9917. DOI: 10.1073/pnas.1733691100

[88] Kallifidas D, Pascoe B, Owen GA, Strain-Damerell CM, Hong HJ, Paget MSB. The zinc-responsive regulator Zur controls expression of the coelibactin gene cluster in *Streptomyces coelicolor*. *Journal of Bacteriology*. 2009;

192(2):608-611. DOI: 10.1128/
 jb.01022-09

Part A: overview articles and peptides.
 Cambridge, Massachusetts: Academic
 Press; 2009;458

[89] Hesketh A, Kock H, Mootien S,
 Bibb M. The role of absC, a novel
 regulatory gene for secondary
 metabolism, in zincdependent antibiotic
 production in *Streptomyces coelicolor* A3
 (2). Molecular Microbiology. 2009;
74(6):1427-1444. DOI: 10.1111/
 j.1365-2958.2009.06941.x

[96] Ueda K, Kawai S, Ogawa HO,
 Kiyama A, Kubota T, Kawanobe H, et al.
 Wide distribution of interspecific
 stimulatory events on antibiotic
 production and sporulation among
Streptomyces species. The Journal of
 Antibiotics. 2000;**53**(9):979-982

[90] Hantke K. Iron and metal
 regulation in bacteria. Current
 Opinion in Microbiology. 2001;**4**(2):
 172-177. DOI: 10.1016/s1369-5274(00)
 00184-3

[91] Tunca S, Barreiro C, Coque JJR,
 Martin JF. Two overlapping antiparallel
 genes encoding the iron regulator
 DmdR1 and the Adm proteins
 control sidephore and antibiotic
 biosynthesis in *Streptomyces coelicolor* A3
 (2). The FEBS Journal. 2009;**276**(17):
 4814-4827

[92] Tunca S, Barreiro C, Sola-Landa A,
 Coque JJR, Martn JF. Transcriptional
 regulation of the desferrioxamine gene
 cluster of *Streptomyces coelicolor* is
 mediated by binding of DmdR1 to an
 iron box in the promoter of the desA
 gene. The FEBS Journal. 2007;**274**(4):
 1110-1122

[93] Willey JM, Gaskell AA.
 Morphogenetic signaling molecules of the
 streptomycetes. Chemical Reviews. 2011;
111(1):174-187. DOI: 10.1021/cr1000404

[94] Nishida H, Ohnishi Y, Beppu T,
 Horinouchi S. Evolution of
 butyrolactone synthases and receptors in
Streptomyces. Environmental
 Microbiology. 2007;**9**(8):1986-1994.
 DOI: 10.1111/j.1462-2920.2007.01314.x

[95] Hopwood DA. Complex enzymes in
 microbial natural product biosynthesis,