

# Bioactive Metabolites Obtained from Endophytes Bacterial Isolate

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**ABSTRACT:** Microbial metabolism encompasses the biochemical basis of study and as such spans all biological disciplines. The history of specific characteristics and opportunity of research of microbial metabolites, including antibiotics and other bioactive molecules have been considered. The microbial origin and diversity of producing species, functions and various bioactivities of metabolites, exclusive features of their chemical structures are discussed, mainly on the basis of arithmetical data. The probable numbers of metabolites may be discovered in the upcoming, the problems of dereplication of newly isolated compounds as well as the new trends and prospects of the research are also discussed. On the conclusion of a detailed understanding of components and relations that comprise metabolism will come the ability to generate a comprehensive numerical model that describes the coordination. The goal of this review is to highlight that in silico, in vitro, and in vivo approaches must be used in mixture to achieve a full accepting of microbial metabolism.

**KEYWORDS:** Bioactive metabolites, Endophytic bacteria, biological origin, chemical features, the number of known compounds, future chances.

## I. INTRODUCTION

The want of this study was to isolate and identify endophytic bacteria from stems and root of *endophyte bacteria* and to estimate there antifungal and antibacterial properties. The present investigation was carried out to analyse the cultural, physiological, biochemical and, morphological features diversity in the bacterial. The antimicrobial and antifungal activity shows against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Penicillium chrysogenum*, *Lactobacillus lactis*, *Bacillus subtilis* and *Candida albican*. Minimum inhibitory concentration (MIC) of crude extract against performed microorganisms was determined. Microorganisms live in a world of chemical signals. They small molecular weight compounds (<2,500 amu), are identified as metabolites, to regulate their own growth and development, to encourage other organisms valuable to them and suppress organisms that are injurious. To control competitors and microbes are produce antibiotics, such as erythromycin, streptomycin and penicillin, antifungal, such as nystatin, amphotericin and cycloheximide, antiprotozoan metabolites including trichostatin, salinomycin and monensin and herbicides like herbicidin and bialophos. They larger organisms are produce nematocides, such as the avermectins and paraherquamide, and insecticides such as the milbemycins, piericidins and spinosads. To encourage plants and animals they produce growth stimulants and metabolites that inhibit pathogens. Most microbial metabolites are elegantly selective; others are broadly active against many species. Organisms resistant to the effects of metabolites thrive; sensitive organisms falter. Microbes use metabolites to adjust the environment in which they survive and from this platform they organize the function and figure of much of the world's biodiversity. Microbial metabolites represent a tremendously various array of chemistry. Microbes can make molecules that synthetic chemists cannot admittance. While over 25,000 bacterial metabolites have been mentioned in the scientific literature, fewer than 2% of these have ever readily existed to the brief research area. A large no of bacterial metabolites have only ever existed in little quantities in the research laboratory and they were discovered and their microbiological activity has never been fully investigated. Most bacterial physiologists and biological and biochemical geneticists would have the same opinion that what they possess is not a textbook-style catalog of metabolic proof, but rather a capability to integrate diverse

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experimental results with past interpretation and published data to generate insight into the metabolic complex. In the past, this ability was facilitated by accumulated explanation. The decline in researchers who focus on metabolism has unfortunately decreased this resource.

## II. SHORT HISTORY OF THE ANTIBIOTIC RESEARCH

The antibiotics are also called antibacterial, are a type of antimicrobial [1] drug used in the treatment and prevention of bacterial infection. [2, 3] They may either kill or reduce the growth of bacteria. A small group of antibiotics also acquire antiprotozoal activity [4, 5]. Antibiotics are not efficient against viruses such as the common cold or influenza, and may be harmful when taken inappropriately. Antibiotics revolutionized medicine in the 20th century, and have together with vaccination led to the near eradication of diseases such as tuberculosis in the developed world. Their effectiveness and easy access led to overuse, especially in livestock raising, prompting bacteria to develop resistance. This has led to widespread problems with antimicrobial and antibiotic resistance, so much as to prompt the World Health Organization to classify antimicrobial resistance as a "serious threat [that] is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country". The antibiotic research from the discovery of Fleming to our days has been a fascinating, exciting, continuously changing and developing adventure. As a result of the frenzied research of the past more than 50 years, in our days ten thousands of natural products derived from microbial sources are known. Interest towards the field were generally increasing, although sometimes declining, interest and the whole story shows some cyclic features with successes and failures and evolved around changing clinical needs and new enabling technology. Metabolism is far more than the sum of its parts and thus understanding metabolism will not be achieved simply by defining and cataloguing metabolic processes. Recent technological advances have facilitated efforts to consider metabolism as a system. Mathematical models and metabolic maps generated from various in silico analyses allow visualization of known metabolic connections and attempt to predict behaviours on the basis of these models [6, 7, 8, and 9]. Notwithstanding the failures, the almost exponential increasing of the total number of discovered compounds in the last decades surprisingly became constant. In 1940 only 1020, in 1950 300400, in 1960 approximately 800 1000 and in 1970 already 2500 antibiotics were known. From that time the total number of known bioactive microbial metabolites has doubled in every ten years. In 1980 about 5000, in 1990 10000 and in 2000 already almost 20000 antibiotic compounds were known. By the end of 2002 over 22000 bioactive secondary metabolites (including antibiotics) were published in the scientific and patent literature. On Fig. 1 the total number of discovered antibiotics is summarized chronologically from 1950 to 2000 in five years period intervals, indicating their main sources, such as *Streptomycin*, rare *Actinomycete*, fungi and bacteria (1a), as well as their percentages indicated in the same periods (1b). The majority of natural products is derived—besides the microbial products isolated from prokaryotic bacteria and eukaryotic microorganisms (protists), where almost all of the antibiotic producing microbes (except the animal protozoa) are belonging to—from higher plants and various animal organisms. Higher plant metabolites represent at least 500000 to 600000 compounds, covering a great number of common plant compounds, such as alkaloids, flavonoids, terpenoids, steroids, carbohydrates etc. In other words, they may show some kind of biological activity. The activity may be highly specific, exhibiting usually in low concentration (representing the usual bioactivities), or may be very unspecific (toxic) action. The exact number of bioactive natural products (compounds with discovered bioactivity or toxicity), is almost undeterminable, but this figure by all means is at least 200000 to 250000, – including more than 20000 microbial metabolites

**MODEL ORGANISM(S) FOR METABOLIC STUDIES** Historically, model systems and model organisms have been used to generate knowledge of general biological paradigms. This representative approach compensates for the fact that the number of organisms worthy of study far exceeds available resources. An ideal organism for studying microbial metabolism has two essential characteristics: (a) sequenced genome and (b) rigorous in vivo genetic capabilities. For decades *Escherichia coli* and its close relative *Salmonella typhimurium* have been used as the model bacteria for metabolic studies [10]. Metabolic paradigms differ enough between gram-positive (i.e., *Bacillus subtilis*) and gram-negative (i.e., *E. coli*, *S. typhimurium*) bacteria that a model for each is desirable [11].

**CORE METABOLISM** In contrast to stoichiometric-based models, the development of large-scale kinetic models of metabolism has been hindered by the challenge of identifying kinetic parameter values and kinetic rate laws applicable

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to a wide range of environmental and/or genetic perturbations. As such there is virtue in studying fewer systems in more detail to define network integration. This strategy is defensible particularly with the highly conserved central metabolism that includes components of the central dogma, pathways for carbon catabolism, biosynthesis of amino acids, nucleotides, vitamins, and cofactors.

**SPECIFIC FEATURES OF MICROBIAL METABOLITES** The most important, inherent characteristics of the bioactive microbial metabolites are their microbial origin, that is to say their specific microbial producers; their interaction with the environment, namely their various biological activities and last but not least their unique chemical structures.

**ORIGIN THE PRODUCERS OF MICROBIAL METABOLITES (TAXONOMIC DIVERSITY) DISTRIBUTION OF BIOACTIVE NATURAL PRODUCTS** Antibiotics and similar natural products, being secondary metabolites can be produced by almost all types of living things. They are produced by prokaryotic (Prokaryote, Monera) and eukaryotic organisms belonging to the Plant and Animal Kingdom, alike. The secondary metabolite producing ability, however, is very uneven in the species of living world. In the Prokaryote and Plant Kingdom there are distinct groups of organisms, namely unicellular bacteria, eukaryotic fungi and first of all filamentous actinomyces being the most frequent and most versatile producers.

**APPROXIMATE NUMBER OF KNOWN NATURAL PRODUCTS**

| Source                                          | All known compounds        | Bioactive        | Antibiotics      |
|-------------------------------------------------|----------------------------|------------------|------------------|
| Antibiotics                                     | over 1.5 million           | 200000 to 250000 | 25000 to 30000   |
| Plant Kingdom                                   | 250,000 -                  | 200000 to 250000 | Nearly 27000     |
| Microbes                                        | More than 50000            | 22000 to 23000   | Near about 16500 |
| Algae, Lichens                                  | 2500 to 4500               | 1000 to 2000     | 950              |
| Higher Plants                                   | 500000 to 600000           | Nearly 150000    | 10000 to 15000   |
| Animal Kingdom                                  | 250000 to 400000           | 50000 to 100000  | 4900             |
| Protozoa                                        | More than several hundreds | Several hundreds | ?                |
| Invertebrates:                                  | Approximate 100000         | ?                | 450              |
| Marine animals                                  | 20000 to 25000             | 6000 to 8000     | 2500 to 4000     |
| Vertebrates (mammals, fishes, amphibians, etc.) | 200000 to 300000           | 50000 to 70000   | 1500             |

**Table. 1**

## III. DEFINITIONS OF NATURAL PRODUCT

The antibiotics and similar compounds form perhaps the most exciting, most energetically increasing, and one of the practically most significant groups of the natural products. The usual definition of natural products in the widest sense emphasizes that they “are chemical (carbon) compounds isolated from various living things”. These compounds may derive by primary or rather than secondary metabolism of living organisms. A natural product is a chemical compound or substance produced by a living organism that is, found in nature. In the broadest sense, natural products include any substance produced by life. Natural products can also be prepared by chemical synthesis (both semi synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients. The primary metabolites (polysaccharides, proteides, nucleic and fatty acids) are common in all biological systems. The

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secondary metabolites are, however, low molecular (MW- 3000), chemically and taxonomically extremely diverse compounds with obscure function, characteristic mainly to some specific, distinct types of organisms. Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components not only of most traditional medicines but also many modern medicines. Furthermore, because the structural diversity of natural products exceeds that readily achievable by chemical synthesis, and synthetic analogs can be prepared with improved potency and safety, natural products are often used as starting points for drug discovery. In fact, natural products are the inspiration for approximately one half of U.S. Food and Drug Administration-approved drugs. The exact definition and the real position and function/s of secondary metabolites for long time is the most disputed and most obscure field in the whole area of microbiology. They seem to be absolutely needless for the producers, having no any apparent function in their life cycle. Most characteristic features are their incredible array of unique chemical structures and their very frequent occurrence and versatile bioactivities [12]. A bioactive compound is a compound that has an effect on a living organism, tissue or cell. In the field of nutrition bioactive compounds are distinguished from essential nutrients. While nutrients are essential to the sustainability of a body, the bioactive compounds are not essential since the body can function properly without them, or because nutrients fulfil the same function. Bioactive compounds can have an influence on health. This activity may appear at the *invitro* molecular level or may be studied at the *in vivo* level, the activity on the whole organism. Taking into consideration the possible differences and specific features surely manifesting in these different levels, in the following this term will be used generally for any types of interactions between chemicals and any kinds of molecular targets or living organisms. Secondary metabolites are known from the ancient times, and they were mainly botanicals. The first crystalline fungal product from *Penicillium glaucum* considered as microbial secondary metabolite was penicillin, discovered in 1896 by Gosio. The secondary metabolites isolated from microbes and exhibit either antimicrobial (antibacterial, antifungal, antiprotozoal), antitumor and/or antiviral activities, used to be called as antibiotics. Secondary metabolites have a broad range of functions. These include pheromones that act as social signalling molecules with other individuals of the same species, communication molecules that attract and activate symbiotic organisms, agents that solubilise and transport nutrients (siderophores etc.), and competitive weapons (repellents, venoms, toxins etc.) that are used against competitors, prey, and predators. For many other secondary metabolites, the function is unknown. One hypothesis is that they confer a competitive advantage to the organism that produces them. An alternative view is that, in analogy to the immune system, these secondary metabolites have no specific function, but having the machinery in place to produce these diverse chemical structures is important and a few secondary metabolites are therefore produced and selected for [13]. This broadest definition should cover besides the so-called "classical antibiotics" (microbial compounds exhibiting antimicrobial and/or antitumor and/or antiviral activities) practically all bioactive compounds obtained either from microbes or from any other living things. In the followings, the term antibiotic/s sometimes used, in a logical sense, instead of the term bioactive microbial metabolite/s. Further on, I try to call consistently the non-antibiotic bioactive compounds exhibiting exclusively some kind of other biological activities as "other bioactive" metabolites/compounds. It is possible to name all of these compounds, however, somewhat more correctly but less inclusively, by the terms as (natural) bio regulators or biochemical modulators.

## IV. THE RESEARCH OF MICROBIAL METABOLITES (STATISTICAL EVALUATIONS)

the short history, specific features and future prospects of research of microbial metabolites, including antibiotics and other bioactive metabolites, are summarized. The microbial origin, diversity of producing species, functions and various bioactivities of metabolites, unique features of their chemical structures are discussed, mainly on the basis of statistical data. The possible numbers of metabolites may be discovered in the future, the problems of dereplication of newly isolated compounds as well as the new trends and prospects of the research are also discussed [14]. Metabolism is all of an organism's chemical processes (an emergent property that arises from interactions of molecules in the orderly environment of the cell) Metabolism is very important for the management of cellular material and energy resources. The significance and frequency of these main types of microbes as producers of bioactive metabolites had varied significantly during the last decades. In the beginning of the antibiotic era the fungal (penicillin, griseofulvin) and bacterial (gramicidin) species were in the foreground of the interest, but after the discovery of streptomycin and later chloramphenicol, tetracycline's and macrolides the attention turned to the *Streptomyces* species. In the fifties and sixties the majority (~70%) of antibiotics were discovered from these species. In the next two decades the significance

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of the non-*Streptomyces* actinomycetales species (rare actions) were increased, up to a 25\_30% share of all antibiotics. The early nineties the number of bioactive compounds isolated from various filamentous and other microscopic and higher fungal species had continuously increased up to more than 50% by the turn of the millennium (2000). The interest to bacteria in the recent years had only slightly increased. Simultaneously, the ratio of Actinomycete compounds naturally had definitely decreased.

| Number of actinomycetales species producing bioactive microbial metabolites |         |                                      |     |
|-----------------------------------------------------------------------------|---------|--------------------------------------|-----|
| <b>Streptomycetaceae:</b>                                                   |         | <b>Micromonosporaceae:</b>           |     |
| <i>Streptomyces</i>                                                         | 7600    | ( <i>Actinoplanetes</i> )            |     |
| <i>Streptoverticillium</i>                                                  | 250     | <i>Micromonospora</i>                | 740 |
| <i>Kitasatospora</i>                                                        | 39      | <i>Actinoplanes</i>                  | 248 |
| <i>Chainia</i>                                                              | 33      | <i>Dactylosporangium</i>             | 60  |
| <i>Microellobospora</i>                                                     | 11      | <i>Ampullariella</i>                 | 10  |
| <i>Nocardioides</i>                                                         | 10      | <i>Glycomyces</i>                    | 02  |
| <b>Thermomonosporaceae:</b>                                                 |         | <i>Catenuloplanes</i>                | 03  |
| <i>Actinomadura</i>                                                         | 350     | <i>Catellatospora</i>                | 03  |
| <i>Saccharothrix</i>                                                        | 67      | <b>Mycobacteriaceae:</b>             |     |
| <i>Microbispora</i>                                                         | 55      | ( <i>Actinobacteria</i> )            |     |
| <i>Actinosynnema</i>                                                        | 51      | <i>Nocardia</i>                      | 367 |
| <i>Nocardiosis</i>                                                          | 42      | <i>Mycobacterium</i>                 | 57  |
| <i>Microtetraspora/Nonomuria</i>                                            | 28/21   | <i>Arthrobacter</i>                  | 25  |
| <i>Thermomonospora</i>                                                      | 20      | <i>Brevibacterium</i>                | 17  |
| <i>Micropolyspora/Faenia</i>                                                | 15/3    | <i>Proactinomyces</i>                | 16  |
| <i>Thermoactinomyces</i>                                                    | 14      | <i>Rhodococcus</i>                   | 13  |
| <i>Thermopolyspora</i>                                                      | 01      | <b>Other (unclassified) species:</b> |     |
| <i>Thermoactinopolyspora</i>                                                | 02      | <i>Actinosporangium</i>              | 32  |
| <b>Pseudonocardiaceae:</b>                                                  |         | <i>Microellobospora</i>              | 12  |
| <i>Saccharopolyspora</i>                                                    | 141     | <i>Frankia 7</i>                     |     |
| <i>Amycalopsis/Nocardia</i>                                                 | 120/357 | <i>Westerdykella</i>                 | 06  |
| <i>Kibdellosporangium</i>                                                   | 34      | <i>Kitasatoa</i>                     | 05  |
| <i>Pseudonocardia</i>                                                       | 27      | <i>Synnemonyces</i>                  | 04  |
| <i>Amycolata</i>                                                            | 12      | <i>Sebekia</i>                       | 03  |
| <i>Saccharomonospora</i>                                                    | 02      | <i>Elaktomyces</i>                   | 03  |
| <i>Actinopolyspora</i>                                                      | 01      | <i>Excelsospora</i>                  | 03  |
| <b>Streptosporangiaceae:</b>                                                |         | <i>Waksmania</i>                     | 04  |
| ( <i>Maduromycetes</i> )                                                    |         | <i>Alkalomyces</i>                   | 02  |
| <i>Streptosporangium</i>                                                    | 79      | <i>Catellatospora</i>                | 02  |
| <i>Streptoalloteichus</i>                                                   | 39      | <i>Erythrosporangium</i>             | 02  |
| <i>Spirillospora</i>                                                        | 12      | <i>Streptoplanospora</i>             | 01  |
| <i>Planobispora</i>                                                         | 10      | <i>Microechinospora</i>              | 01  |
| <i>Kutzneria</i>                                                            | 04      | <i>Salinospora</i>                   | 01  |
| <i>Planomonospora</i>                                                       | 05      |                                      |     |

Table. 2

the numbers of actinomycetales species, including the all rare actinos, known to produce bioactive metabolites, are summarized These fastidious organisms, the rare actinos, produce perhaps the most diverse and most unique, unprecedented, sometimes very complicated compounds exhibiting excellent antibacterial potency and usually low toxicity. It is interesting that several chemical types, such as simple terpenoids or benzenoids are almost completely absent from these compounds. In this group of metabolites there are numerous practically very important compounds such as gentamicin, erythromycins, vancomycine, or rifamycin. Numerous recently introduced chemotherapeutic and

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agricultural agents (zircin, dalbavacin, spynosin), are also rare actino products. It is noteworthy that the vancomycinristocetin type complicated glycopeptides are produced almost exclusively by various rare actino species. The present relatively low occurrence of rare actinos, in contrast to *Streptomyces* species is derived from the facts that they are hard to isolate from the environment and difficult to cultivate and maintain under conventional conditions. These are reasons why these species are still regarded to be rare. Recently however advanced isolation techniques have been developed, and with these techniques from an environmental sample the overwhelming majority of these rare species could be isolated [15, 16]. Among fungal species, the various microscopic (filamentous) fungi (ascomycetes, fungi imperfecti, etc.) are the most frequent producers with about 6400 produced compounds. From the most common ascomycetes, namely from *Aspergillus*, *Penicillium* and *Fusarium* species 950, 900 and 350 compounds have been isolated, respectively. Besides them several other filamentous and endophytic species (*Trichoderma*, *Phoma*, *Alternaria*, *Acremonium* and *Stachybotrys*), are also good producers, each produces several hundreds of bioactive compounds. From higher fungal species – basidiomycetes or mushrooms – exemplified by *Ganoderma*, *Lactarius* or *Aureobasidium* species, altogether about 2000 active compounds have been derived. From yeasts, only 140 and from Myxomycetes (slime moulds) species 60 bioactive metabolites have been isolated.

| Microbial interactions                  |                                                                                                                                                 |
|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Microbe – Mammalians (humans)           | Antitumor antibiotics, pharmacologically active agents, enzyme inhibitors, (humans) immunoactive, CNS-active, etc. agents, feed additives, etc. |
| Microbe – Microbe                       | Antimicrobial antibiotics, microbial regulators, growth factors, signalling compounds, mating.                                                  |
| Microbe – Higher Plants.                | Herbicides, phytotoxins, plant growth regulators, chlorosis inducers, phytoalexins, etc.                                                        |
| Microbe – Lower Animals (invertebrates) | Insecticides, miticides, antiparasitic compounds, algicides, antifeedants, (invertebrates) repellents, molluscicides, anti-worm agents, etc.    |

Table. 3

There is no reason to suppose that the majority (if not all) of the natural products including microbial metabolites should not exhibit some kind of biological function. Do not forget that hundreds of presently known bioactive metabolites originally was discovered as “inactive”, natural product and their activity was only discovered later, investigating them with new more specific methods, or

Reinsulated them (sometimes from different species) using more sensitive methods, based on absolutely new principles. In our days, in fact, there would not be any reason to talk about “bioactive” or “inactive” secondary metabolites and treat them separately. They represent only temporary categories. The secondary metabolites may be evolved in nature as some kind of response to the effects of the environment, including living and physical environments. Nature being a sophisticated, versatile and energetic combinatorial chemist all over the times yields by infinite number of different and unpredictable ways, a series of exotic and effective structures, which have ever been made in laboratories. The natural product biosynthesis, driven enzymatically from a huge gene pool has evolved over billions of years. As a result of this evolutionary process the secondary metabolites certainly exists in coded form in the nucleic acid world as some kinds of regulators or effectors. I hope, that with the benefit of widening our knowledge about biosynthetic genes, in the future we will be able to clarify the role and functions of these latent activities and exactly prove my hypothesis, namely the necessity of the universal existence of the inherent biological activity of secondary metabolites.

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**BIOACTIVITIES OF SECONDARY METABOLITES** Although traditionally secondary products have played an important role in drug discovery, in the past few years most Big Pharmacy companies have either terminated or considerably scaled down their secondary product operations. This is despite a significant number of natural product-derived drugs being ranked worldwide secondary ethical drugs in the last years. The process in natural product drug discovery usually required several separation circles and structure elucidation and was thus time consuming. New approaches to improve and accelerate the joint drug discovery (secondary) and development process are expected to take place mainly from innovation in drug target elucidation and lead structure discovery. Powerful new technologies such as automated separation techniques, high throughput screening and combinatorial chemistry are revolutionizing drug discovery (secondary) [17]. Fungal and endophytes bacteria are receiving increasing attention by natural product chemists due to their diverse and structurally unprecedented compounds which make them interesting candidates for drug discovery. One fungal genus which is especially productive with regard to the accumulation of a diverse array of mostly bioactive compounds is *Pestalotiopsis*. This review demonstrates the unique chemical diversity of taxa from this genus and describes their reported bioactivities. To date, alkaloids, terpenoids, isocoumarin derivatives, coumarins, chromones, quinones, semiquinones, peptides, xanthenes, xanthone derivatives, phenols, phenolic acids, and lactones, have been reported from *Pestalotiopsis* species, making this genus a particularly rich source for bioprospecting when compared with other fungal genera. More than 130 different compounds that have been isolated from various species of *Pestalotiopsis* are covered in this review and their structures are reported within a biogenetic context. Bioassays disclosed that antifungal, antimicrobial, and antitumor activities are the most notable bioactivities of secondary metabolites isolated from this genus. Possible biogenetic pathways for monoterpenes, sesquiterpenes, quinones, and lactones that are covered in this review article are also illustrated. The presently known secondary microbial metabolites, exhibit a great numbers of diverse and versatile biological effects, first of all antimicrobial activities. In the scientific literature already hundreds of different pathogenic and other microbes (Gram-positive, Gram-negative bacteria, fungi, yeasts, etc.) are described as test organisms in the direct activity-based screenings. The most frequent test organisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus (Sarcina) lutea*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Candida albicans* and others. Antiviral tests, inhibition of viral enzymes, activities connecting with neoplastic diseases from simple cytotoxicity assay methods (P-388, KB, L-1210 cell lines) to angiogenesis inhibition, etc., are used most frequently for detection of other, non antimicrobial activities of metabolites. *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*. The list of non-antibiotic biological activities used in the new screening projects presently covers more than one thousand different types of bioactivities; cell based-, receptor binding- or enzymatic assay methods and many other specific tests and targets. In our days more and more newer, sophisticated and diverse assays are used worldwide in various screening protocols. The main types of bioactivities, the frequency of their occurrence in the scientific literature, according to historical and practical point of views, may be summarized in the Table 03.

## V. MAIN TYPES OF ACTIVITIES (STATISTICAL EVALUATIONS)

**ANTIBIOTIC ACTIVITIES** Antibiotics, also called antibacterial, are a type of antimicrobial. Drug used in the treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity. Antibiotics are not effective against viruses such as the common cold or influenza, and may be harmful when taken inappropriately. Additionally to antibiotic activities, some 11500 microbial metabolites, half of the all compounds, possess some kind of additional (or exclusive) “other”– non antibiotic– bioactivities. Among them there are about 6000 metabolites without any recognized antimicrobial activity exhibiting exclusively some kinds of “other” biological activities. They are the so called “other bioactive” metabolites. The numbers of antibiotics (“classical” antibiotics) and the “other bioactive” metabolites according to their origin, were summarized in the Tables 2 and 4. As derived from the numbers including in these Tables, in total 16500 compounds, 73% of all bioactive metabolites may be considered strictly as antibiotics. In the group of antibiotics the inhibitory activity against Gram-positive, Gram-negative and mycobacterium exist in 66%, 30% and 5%; (10900, 5000, and 350) of the compounds, respectively. Altogether 5600 compounds (34%) shows antifungal activity; for which 21% (3500) are active against yeasts, 11% (1800) against Phytopathogenic fungi and 24% (4000) are active against other fungal species. About 2000 compounds, e.g. the polyene antibiotics, exhibit exclusively antifungal/anti yeast activity. The combination of various antimicrobial and other in 1928, Alexander Fleming identified penicillin, the first chemical compound with antibiotic properties. Fleming was working on a culture of disease-causing bacteria when

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he noticed the spores of little green mold in one of his culture plates. He observed that the presence of the mold killed or prevented the growth of the bacteria. Antibacterial agents may be classified according to the class of target microorganism. Accordingly, antibacterial agents that inhibit only bacteria are described as narrower medium-spectrum, whereas those that also inhibit mycoplasma, Rickettsia, and chlamydia (so-called atypical bacteria) are described as broad-spectrum. The activity of antimicrobial drugs has also been described as being bacteriostatic or bactericidal, although this distinction depends on both the drug concentration at the site of infection and the microorganism involved. Bacteriostatic drugs (Tetracyclines, pencils, sulphonamides, lincosamides, and macrolides) inhibit the growth of organisms at the MIC but require a significantly higher concentration, the MBC, to kill the organisms (MIC and MBC are discussed further below). By comparison, bactericidal drugs (penicillin's, cephalosporin's, Aminoglycosides, Fluoroquinolones) cause death of the organism at a concentration near the same drug concentration that inhibits its growth. Bactericidal drugs are required for effectively treating infections in immune compromised patients and in immunoincompetent environments in the body.

**INCREASING THE BIOSYNTHETIC DIVERSITY** In the area of natural products there is no any other group where so wide range of specific and complex chemical structures, with fascinating array of diverse, unique functional groups occurs, than in the group of antibiotics and other bioactive microbial secondary metabolites. Compounds in this group cover all types of organic compounds from the simple acrylamidine (MW: 72) to the most complicated structures, such as the macro cyclic colubricidin (MW: 2154), which includes large glycosilated macrolactone ring, pyridine and pyrrole moieties. The polycyclic tetrapetalone-A [24] contains one of the most complicated heterocyclic ring systems with quinones and glycosidic functionalities or the macro cyclic versipelostatatin [23] also have fascinating new structures. The majority of the unique collection of molecular skeletons of microbial secondary metabolites never occurs in any chemical libraries. As it is estimated, this part is over 40% of the natural product diversity which means that at least half of the bioactive microbial metabolites had never been synthesized.

| Bioactivity types of microbial metabolites numbers of discovered bioactivities                                                                                                                      |                                     |               |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|---------------|
| Type of activity                                                                                                                                                                                    | Numbers of discovered bioactivities |               |
| ANTIBIOTIC ACTIVITIES:<br>(16500 compounds)<br><b>Antibacterial</b><br><br><b>Antifungal</b><br><br>Antiprotozoal:<br><b>Chemotherapeutic activity</b><br>Antitumor (cytotoxic)<br><b>Antiviral</b> | Gram-positive                       | 10000 – 12000 |
|                                                                                                                                                                                                     | Gram-negative                       |               |
|                                                                                                                                                                                                     | <i>Mycobacterium</i>                | 5000 – 6000   |
|                                                                                                                                                                                                     | Yeasts                              |               |
|                                                                                                                                                                                                     | Phytopathogenic fungi               | 800 – 1000    |
|                                                                                                                                                                                                     | Other fungi                         |               |
|                                                                                                                                                                                                     |                                     | 3000 – 3500   |
|                                                                                                                                                                                                     |                                     | 1500 – 1800   |
|                                                                                                                                                                                                     |                                     | 3500 – 4000   |
|                                                                                                                                                                                                     |                                     | 800- 1000     |
|                                                                                                                                                                                                     |                                     | 5000 – 6000   |
|                                                                                                                                                                                                     |                                     | 1000 - 1600   |

Table 4

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**SPECTRUM OF ACTIVITY** Antibacterial agents may be classified according to the class of target microorganism. Accordingly, antibacterial agents that inhibit only bacteria are described as narrower medium-spectrum, whereas those that also inhibit mycoplasma, Rickettsia, and chlamydia (so-called atypical bacteria) are described as broad-spectrum. The spectrum of activity of common antibacterial drugs is shown in table 05. The differential sensitivity of Gram-positive and Gram negative bacteria to many antimicrobials is due to differences in cell wall composition. Gram-positive bacteria have a thicker outer wall composed of a number of layers of peptidoglycan, while Gram-negative bacteria have a lipophilic outer membrane that protects a thin peptide glycan layer. Antibiotics that interfere with peptidoglycan syntheses more easily reach their site of action in Gram-positive bacteria. Gram-negative bacteria have protein channels (porins) in their outer membranes that allow the passage of small hydrophilic molecules. The outer membrane contains a lip polysaccharide component that can be shed from the wall on cell death. It contains a highly heat-resistant molecule known as endotoxin, which has a number of toxic effects on the host animal, including fever and shock [20].

| Spectrum of Activity of<br>Common Antibacterial Drugs | Class of Microorganism |            |            |           |          |
|-------------------------------------------------------|------------------------|------------|------------|-----------|----------|
|                                                       | Bacteria               | Mycoplasma | Rickettsia | Chlamydia | Protozoa |
| Aminoglycosides                                       | +                      | +          | -          | -         | -        |
| β-Lactams                                             | +                      | -          | -          | -         | -        |
| Chloramphenicol                                       | +                      | +          | +          | +         | -        |
| Fluoroquinolones                                      | +                      | +          | +          | +         | -        |
| Lincosamides                                          | +                      | +          | -          | -         | +/-      |
| Trimethoprim                                          | +                      | -          | -          | -         | +        |
| Sulfonamides                                          | +                      | +          | -          | +         | +        |
| Streptogramins                                        | +                      | +          | -          | +         | +/-      |
| Tetracyclines                                         | +                      | +          | +          | +         | -        |
| Pleuromutilins                                        | +                      | +          | -          | +         | -        |
| Oxazolidinones                                        | +                      | +          | -          | -         | -        |

Table 5

**TYPE OF KILLING ACTION** Growth inhibition–time curves are used to define the type of killing action and steepness of the concentration– effect curve. Typically, reduction of the initial bacterial count (response) is plotted against antimicrobial drug concentration. The killing action (time-, concentration-, or co-dependent) of an antibacterial drug is determined largely by the slope of the curve. Antibacterial drugs that demonstrate time-dependent killing activity include the β-Lactams, macrolides, tetracyclines, and Trimethoprim–sulphonamide combinations, chloramphenicol, and glycopeptides. A concentration-dependent killing action is demonstrated by the Aminoglycosides [21], Fluoroquinolones, and Metronidazole. The antibacterial response is less sensitive to increasing drug concentration when the slope is steep and vice versa.

**MECHANISMS OF ACTION** Inhibition of cell wall synthesis (β-lactam antibiotics, bacitracin, vancomycin). Damage to cell membrane function (polymyxins) Inhibition of nucleic acid synthesis or function (nitroimidazoles, nitrofurans, quinolones, Fluoroquinolones), Inhibition of protein synthesis (Aminoglycosides, phenicols, lincosamides, macrolides, Streptogramins, Pleuromutilins, tetracyclines), Inhibition of folic and folinic acid synthesis (sulphonamides, Trimethoprim).

**ANTIMICROBIAL DRUG COMBINATIONS** The use of antimicrobial combinations is indicated in some situations. For instance, mixed infections may respond better to the use of two or more antimicrobial agents. A separate example is fixed combinations such as the potentiated sulphonamides (comprising a sulphonamide and a diaminopyrimidine such as Trimethoprim) that display synergism of antimicrobial activity. Other examples include the sequential inhibition of cell wall synthesis; facilitation of one antibiotic's entry to a microbe by another; inhibition of inactivating enzymes; and the prevention of emergence of resistant populations. [18] Another potential advantage of using antimicrobial drugs in combination is that the dose, and therefore the toxicity, of drugs may be reduced when a

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particular drug is used in combination with another drug(s). Disadvantages from combining antimicrobial drugs in therapy also arise, and to address this possibility, combinations should be justified from both pharmacokinetic and pharmacodynamic perspectives. [19] For example, with a fixed combination of an amino glycoside and a  $\beta$ -Lactams, the former displays a concentration-dependent killing action and should be administered once daily, while the latter displays time-dependent killing and should be administered more frequently in order to ensure that the plasma concentration is maintained above the MIC of the organism for the majority of the dosing interval. One way to achieve this is to combine an amino glycoside and the procaine salt of benzyl penicillin. The former requires a high  $C_{max}$ : MIC ratio, while the procaine salt of benzyl penicillin gives prolonged absorption to maintain plasma concentrations above MIC for most of the interdose interval. Similarly, a bacteriostatic drug may prevent some classes of bactericidal drugs from being efficacious [19].

**FUTURE CHANCES** The total number of the microbial metabolites recognized until now, including both bioactive and inactive compounds, is around 50000 and the number of all known natural products is around one million. It is an obvious question, where is the border in the diversity of natural products? Where is the limit or is there any limit at all, in the continuous increase of the number of new microbial or natural compounds? Regarding the possible number of bioactive natural products do not forget that natural product chemists in the past rarely investigated the isolated compounds, especially not for a wide range of bioactivity. Also remember that in the past countless of compounds, including microbial metabolites formerly believed to be inactive, proved to be active in later investigations, or were rediscovered with screening of different stock of microbes or with specific screening methods. It is unpredictable how many “new” bioactive metabolites will be discovered in this way. The general needs of the human society are continuously increasing. We need every new compound which may be useful for the human society. More food, new drugs, and other goods are highly necessary for the benefit of humankind. The only question is the existence of sufficient natural and technical resources to fulfil these demands. The problem really is not whether we would be able to discover further new useful microbial compounds, but rather how can we optimize and quickly and effectively apply the chances derived from the new discoveries. How can we pick up and use effectively the proverbial needle found in the haystack. According to my opinion our ability to discover new types of microbial products with new types of activities and new lead compounds for the drug (and agricultural product) development, may be limited only by three factors: – the proper selection and certain luck to find and isolate the most promising new producers from our environment, to culture and maintain these producers, to modify the biosynthetic-genetic features of the strains isolated to obtain novel unnatural derivatives which cannot be obtained easily by chemical methods and finally the use of combinatorial chemical approaches to improve the natural leads, and – the intellectual power in developing new, effective screening protocols; the proper selection of our targets; test microbes, biochemical-enzymological-pharmacological systems and host organisms, the adequate (selective, sensitive, automated) detection techniques including effective identification, and dereplication and isolation methods, and the efficient scale up and finally the effective data management, the ingenuity and cooperation of scientists from various disciplines [22].

## NEW PRODUCERS

Natural resources (Maximizing the biodiversity): In the case of all approved therapeutic agents, the time frame has been extended to cover the 30 years from January 1<sup>st</sup> 1981 to December 31<sup>st</sup> 2010 for all diseases world-wide, and from 1950 (earliest so far identified) to December 2010 for all approved antitumor drugs world-wide. We have continued to utilize our secondary subdivision of a “natural product mimic” or “NM” to join the original primary divisions, and have added a new designation “natural product botanical” or “NB” to cover those botanical “defined mixtures” that have now been recognized as drug entities by the FDA and similar organizations. From the data presented, the utility of natural products as sources of novel structures, but not necessarily the final drug entity, is still alive and well. Thus, in the area of cancer, over the time frame from around the 1940s to date, of the 175 small molecules, 131 or 74.8% are other than “S” (synthetic), with 85 or 48.6% actually being either natural products or directly derived there from. In other areas, the influence of natural product structures is quite marked with, as expected from prior information, the anti-infective area being dependent on natural products and their structures. Although combinatorial chemistry techniques have succeeded as methods of optimizing structures, and have been used very successfully in the optimization of many recently approved agents, we are only able to identify only one de novo combinatorial compound approved as a drug in this 30-year time frame. Natural product resources, including the microbial world, are mainly unexplored both in its dimension and in the respect of geographic, ecological and environmental points of view. There surely exist, besides the presumed numbers of microorganisms, millions of microbes in the environment that are presently untouchable for the science. The DNA community analysis demonstrates the presence of much more novel organisms in the

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environmental samples, than those was originally believed, which are not detectable and cannot be isolated by “classical” methods. There is great potency in the isolation and cultivation of microbes which are difficult to isolate or cannot be isolated, less culturable or almost uncultivable or simply slowly growing both in the groups of the well known or less explored types of microbes.

DNA analysis methods proved that the number of microbes in the soil is much higher than formerly thought. It was estimated that about 1% of the aquatic and 10% of the terrestrial microorganisms (perhaps only 0.1% of soil microbes), are readily cultured or “culturable” by conventional methods. The application of new molecular techniques can greatly improve the affectivity of the exploration of uncommon, unreported groups of various microbes, first of all new actinomycetales strains, greatly increasing the chemical diversity. There is a big potential in the screening of the simply unknown species. The hardly detectable and almost uncultivable actinomycetales species, their genetic exploitation and metabolic expression give also almost unlimited possibilities. The *Cyanobacter* and *Myxobacter* groups (not included specifically in Table 9) are also very promising as new sources of additional new bioactive compounds. Besides the known types, certainly exist additional new genera and phyla of microbes, in the nature. By genetic methods there is the possibility to obtain totally new phyla of microbes (Archaea, Archacebacteria).

## GENETIC APPROACHES (GENETIC DIVERSITY)

Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary. Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals. The academic field of population genetics includes several hypotheses and theories regarding genetic diversity. The neutral theory of evolution proposes that diversity is the result of the accumulation of neutral substitutions. Diversifying selection is the hypothesis that two subpopulations of a species live in different environments that select for different alleles at a particular locus. This may occur, for instance, if a species has a large range relative to the mobility of individuals within it. Frequency-dependent selection is the hypothesis that as alleles become more common, they become more vulnerable [20]. This occurs in host–pathogen interactions, where a high frequency of a defensive allele among the host means that it is more likely that a pathogen will spread if it is able to overcome that allele. It is well known that small changes in culture conditions, or in the media, mixed- or co-cultivations, and similar simple methods all may result in big alteration in the biosynthesis of metabolites. The mutasynthesis or mutational biosynthesis, namely culturing microbes with unnatural precursors, also produced already hundreds of new hybrid molecules (Aminoglycosides, peptides, *etc.*). Microbes containing mutations in their biosynthetic genes can be fed by unnatural precursors, generating derivatives of complex natural products that are not easily obtainable by chemical methods. In the case of precursor directed biosynthesis when the producer is manipulated with “surgical” intervention to produce mainly the expected new hybrid compounds is also a promising approach. Manipulations of microbial physiology, the combinatorial biosynthesis are an additional possibility. Molecular biological approaches allow the identification and activation of genes that operate the biosynthetic machinery of the microbes. Microbial genes can be engineered to produce hybrid enzymes that are capable to synthesizing a series of new analogous molecules (*e.g.* erythromycin derivatives). The cloning of biosynthetic pathway genes of *e.g.* *Streptomyces* can be transferred to other producers to help the discovery of new series of compounds. This combinatorial biosynthetic approach led *e.g.* to the direct fermentative production of epirubicin, one of the best antitumor anthracycline antibiotics, or to some fluorinated vancomycine derivatives. In fact, the natural resources and their genetic manipulation are almost inexhaustible and certainly do not mean any limit in the increasing the number of further new “semi-natural” compounds. The combination of nucleic acid-sequencing techniques with molecular-phylogenetic analysis guarantees the increasing microbial diversity constituting an infinite pool of new potentially useful leads for the drugs and agrochemical compounds discovery [21].

**IDENTIFICATION (DETECTION AND DEREPLICATION)** The identification of active compounds from fermentation samples (extracts, whole broths) is one of the most complicated, labour intensive, time consuming step of the screening protocols. Recently the great progress in the robotic and automated methods in chromatographic isolation methods (LC-MS, LC-MS-ELSD, LC-NMR, HPLC-UV-VIS, HPLC-ELSD, HPLC-PDA (photodiode array detectors), HPLC-MS, CZE, *etc.*) and spectroscopic identification techniques (multi-dimensional NMR, X-Ray crystallography, NOESY, BMS, electrospray MS, HR-MS) are more or less satisfying the requirements of the modern HTS programs. For an effective dereplication and for selection the most promising new compounds a fully integrated bioinformatics

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system is the most critical factor. In the last decades using MS, NMR and X-Ray methods it became possible to solve the structure in minimum quantities of natural products. The structure determination of a new compound, isolated perhaps only on mg scale is a relatively easy task. Some aspects of this area, however, remain problematic *e.g.* in the case of highly water soluble and a practically insoluble compounds or those have low or no UV absorption.

## VI. CONCLUSIONS

Over viewing our present knowledge and the future perspectives it may be stated that there is no predictable limit in the further, perhaps not exponential but continuous growing of the number of new microbial metabolites, however, the qualitative improvement is much more important. Hopefully this increase will represent significant practical results both in the human therapy and agriculture. The tasks and possibilities are almost unlimited, only the economic and human effort invested, may be limiting factors. Rather, it requires that the experimental approach is undertaken with a global systems perspective that allows one to follow metabolic results to the point at which relevant connections become clear. Understanding microbial metabolism requires a long-term investment by the investigator and the scientific community. Perhaps this investment starts with the realization that technology is not likely to generate a magic bullet that results in a quick understanding of metabolic complexity and gene function. The proven success of classic logical thought combined with continuing technological advances makes this an era of great potential and excitement for understanding microbial metabolism. The philosophical discussion of various aspects of screening strategies and the discussion of the open scientific, practical and economic problems is far from the primary aim and topic of this review, but unquestionable these questions are continuously in the foreground of the interest of the scientific community working in this area. It has to say, I am somewhat anxious. Besides the tremendous technical improvements, achieved in the new screening projects, there are, however, some non-negligible practical and economic problems: Firstly. The problems of drug target host interactions. The hope that the new compounds derived from the HTS will fulfil the medical needs is constantly depending on three important requirements: – the newly discovered agents will be specific enough to the molecular targets (pathogen microbes or mammalian organs), and – the bioavailability (accessibility) of the molecular targets for the drug (uptake, transport) as well as – the interaction of the newly discovered drug with the animal/human host. (Toxic effect, pharmaco-kinetics). Against to the above and some other pessimistic thoughts I am sure, that the essence of “the laws of applied microbiology” and “microorganisms can do or will do anything” is more valid than ever. My motto is: microorganisms (perhaps with a small help of the scientists) can do produce any compounds desired.

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