Mohd Sayeed Akhtar
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# Natural Bio-active Compounds

## Volume 1: Production and Applications

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*This book is dedicated to*

*Abu Ali Ibn Sina (980–1037 AD)*

*A great physician, scholar, philosopher, astronomer, writer of medieval times, and father of early modern medicine.*

# Foreword

Bio-active compounds produced from natural sources, such as plants, fungi, lichens, etc., exhibit superior chemo-diversity and possess several pharmacological significances. Some of the major classes of bio-active compounds include phenolics, alkaloids, tannins, saponins, lignin, glycosides, and terpenoids. The discovery of such unique compounds has inspired many scientific communities to explore their potential applications in various fields including agriculture and biomedicine. For instance, plant metabolites are utilized to manufacture eco-friendly biopesticides and as drug sources in medicine. Due to numerous health-promoting properties, these phytocompounds are widely used by humans as a source of medication since from ancient times to the modern world. The assessment of natural bio-active compounds for their wide-ranging therapeutic potential has led to the discovery of many drug leads in recent times. Natural products research has become a trust area among scientists aimed toward understanding the chemistry, analytical methodologies, biosynthetic mechanisms, and pharmacological activities of several natural compounds. In recent times, the natural product-based medicine is considered as the most suitable and safe to be used as an alternative medicine. In this regard, there is an unprecedented task to fulfill the increasing demand for natural metabolites by flavor and fragrance, food, and pharmaceutical industries. Thus, many natural resources are being explored to produce and accomplish the demand for natural bio-active compounds.

The present book entitled *Natural Bio-active Compounds: Volume 1 – Production and Applications* includes 22 chapters contributed by academicians, scientists, and researchers from different parts of the globe. In Chap. 1, Brazilian author provides a holistic point of view to the current strategies adopted to screen and produce novel endophyte-derived bio-active metabolites, while Chap. 2 by Indian and German authors discusses the unmapped repository of endophytic natural products. In Chap. 3, Braga Adelaide et al., describe the production of polyphenols by microbes, while in Chap. 4, Malaysian authors have discussed on the progress and advances made in the research of endolichenic fungi. Also, they have highlighted on the emerging biotechnological approaches in exploring endolichenic fungi. Chapter 5, by Anand Shyamlal Gupta, mentions the chemistry, medicinal importance, isolation, and strategic approaches for the purification of glycosides from natural sources. Likewise, in Chap. 6, Sridhar et al. describe the treatment of obesity by natural products-based pancreatic lipase inhibitors, and Chap. 7 by Saboon et al. describes the applications

of natural compounds extracted from medicinal plants. Chapter 8, by Indian authors, narrates about the sources of seed oils, their methods of extractions, and bioactivity, while Chap. 9 by Desam Nagarjuna Reddy provides the comprehensive information on the specific chemical compounds occurring in essential oils and their medical applications and economic importance. In Chap. 10, an overview on the present scenario and future aspects of cellulose hydrogels and their applications is discussed by Pal et al., a group of Indian scientists. Chapter 11 by Mexican authors gives a detailed account on the current strategies for the production of plant secondary metabolites in a continuous and reliable manner, especially the influences of elicitors and eustressors on the production of plant secondary metabolites, while in Chap. 12, a collaborative work by Malaysian and Thailand researchers discusses the existing approaches in the management of colorectal cancer by targeting *KRAS* proto-oncogene. Similarly, Chap. 13 by Ansari and Akhtar explains the new insights on the recent progress of flavonoids as effective candidates in cancer therapeutics and prevention. Chapter 14 by Khairulmazmi and Tijjani highlights the uses and profiling of bio-active compounds of *Moringa oleifera,* their mode of action, and prospects in commercial biopesticides for agricultural applications. Subsequently, Chap. 15 focuses on the natural compound of genus *Brassica* and their therapeutic activities, while Chap. 16 by Kirubakari et al., entails the prospects of higher plants as antimicrobial agents. Chapters 17 and 18, by Indian authors, describe the phytochemistry and pharmacological properties of neem tree-derived bio-active compounds and the role of plant secondary metabolites acting on different targets for treating diabetes. In the next chapter, Javid et al., beautifully describe the pharmacological activities of *Leptadenia pyrotechnica*. Chapter 20 by Indian authors summarizes the therapeutic efficacy of garlic and its bio-active organosulfur compounds against risk factor-mediated atherosclerotic cardiovascular diseases, while Chap. 21, by Pakistani authors, provides comprehensive information on nutritive and pharmacological properties of *Physalis peruviana*. In the last chapter, Brazilian authors discuss on the content and chemical composition of the essential oil of *Baccharis milleflora* and their biological significances.

Understanding about various natural bio-active compounds is very much required in order to promote the drug discovery research and to complement the medical world by novel drug molecules with superior bioactivities. I believe this book surely provides updated information on the production and application of natural bio-active compounds to graduate and undergraduate students, teachers, industry persons, and healthcare professionals involved in natural product and therapeutic research areas. I congratulate the editorial board members, Dr. Mohd Sayeed Akhtar, Mallappa Kumara Swamy, and Uma Rani Sinniah, and all contributing authors for bringing the collection of their noble piece of work and also for the grand success of this book.

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Abdul Shukor Juraimi

# Preface

Secondary metabolites are the unique faction of compounds, produced by plants to protect itself against the various biotic and abiotic factors (diseases, pests, pathogens, herbivores, environmental stresses, etc.). Secondary metabolites do not influence the primary metabolic activities such as growth and reproduction of plants. The major classes include phenolics, alkaloids, tannins, saponins, lignin, glycosides, and terpenoids. Some of these compounds have become an integral part of plant-microbe interactions toward adapting to environmental irregularities. They regulate symbiosis, induce seed germination, and show allelopathic effect, i.e., inhibit other competing plant species in their environment. Moreover, these compounds induce adverse physiological activities, such as reduced digestive efficiency, reproductive failure, neurological problems, gangrene, goiter, and even death and also possess high toxicity. The discovery of such unique compounds has inspired many scientific communities to explore their potential applications in various fields including agriculture and biomedicine. For instance, plant secondary metabolites are utilized to manufacture eco-friendly biopesticides and as drug sources in medicine. Due to numerous health-promoting properties, these compounds are widely used as a source of medication since ancient times. The assessment of plant secondary metabolites for their wide-ranging therapeutic potential has led to the discovery of many drug leads in recent times. Therefore, this field of research has become a reliance area for researchers interested to explore the chemistry, analytical methodologies, biosynthetic mechanisms, and pharmacological activities of plant secondary metabolites.

The use of natural bio-active compounds and their products is considered as most suitable and safe to be used as an alternative medicine. Thus, there is an unprecedented task to fulfill the increasing demand for plant secondary metabolites by flavor and fragrance, food, and pharmaceutical industries. However, their supply has become one of the major constraints as their large-scale cultivation is very limited. Moreover, it is difficult to obtain a constant quantity of compounds from the cultivated plants as their yield fluctuates due to several factors including genotypic variations, geography, edaphic conditions, and harvesting and processing methods. In addition, medicinal plants have become endangered due to ruthless harvesting in nature. Alternatively, the plant tissue culture approaches can be well explored to produce secondary metabolites without practicing the conventional agriculture requiring more land space. In vitro cell and tissue cultures require less space and are

grown under a controlled lab conditions and hence offer advantages of producing the desired compounds continuously without affecting their biosynthesis and quality. Furthermore, these cultures can be scaled up to produce metabolites in very large bioreactors, and also, using genetically engineered cells/tissues, novel products can be obtained. The proper knowledge and exploration of these in vitro approaches could provide an optional source to produce plant secondary metabolites from many medicinal plants in large scale.

*Natural Bio-active Compounds: Volume 1 – Production and Applications* is a very timely effort in this direction. This book volume with 22 contributions from the authors of Australia, Brazil, India, Malaysia, Mexico, Nigeria, Pakistan, Portugal, Saudi Arabia, and Thailand discusses the production and applications of natural bioactive compounds isolated from plants as well as microbial endophytes. Moreover, chemistry, pharmacological properties, and biotechnological approaches against various human diseases are also well discussed. This book will be a valuable resource for researchers to work toward identifying and characterizing new bioactive agents from a diversified flora and to enable the discovery of novel therapeutic leads in the near future against various diseases, and also for the graduate and undergraduate students, teachers, industry persons, and healthcare professionals involved in natural product and therapeutic research areas.

We are highly grateful to all our contributors for readily accepting our invitation and for sharing their knowledge. Further, we greatly appreciate their commitment in composing the chapters and enduring editorial suggestions to finally produce this venture. We are also thankful to Professor Abdul Shukor Juraimi for his suggestion and writing the foreword for this volume. We also thank the team of Springer International, especially Dr. Mamta Kapila and Raagaipriya Chandrasekaran for their generous cooperation at every stage of the publication.

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# About the Book

This book has comprehensively reviewed latest information on various aspects of natural bioactive compounds produced from microbes, plants, and algae. It provides detailed information on several classes of phytocompounds including phenolics, alkaloids, tannins, glycosides, etc. and also discusses on their potential applications in various fields including agriculture and biomedicine. The health-promoting properties of these natural resources and their phytocompounds as detailed in the traditional medicine are detailed in this book with recent practical proofs and documentations with a special focus on their safety issues. Topics related to medicinal plants such as ethnopharmacology, phytochemistry, extraction methods, challenges in medicinal plants cultivation, toxicological effects, clinical studies, mode of action, potential biomolecular interactions, advancements in secondary metabolites production, targeted therapy, newly identified potential natural compounds, and novel drug discovery strategies including computational approaches are discussed in detail. Furthermore, various sources of natural products and their therapeutic applications will benefit to explore to overcome the current deficit in the supply of bioactive natural compounds. Overall, this book is a valuable resource for researchers to work toward identifying and characterizing new bioactive agents from a diversified flora, and to enable the discovery of novel therapeutic leads in the near future against various human ailments. This book is useful to industries, researchers, subject experts, and students working in multidisciplinary areas such as medicinal chemistry, pharmacology, biochemistry, and other topics related to drug discovery research.

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# About the Editors

**Dr. Mohd Sayeed Akhtar** (PhD) is an Assistant Professor at Gandhi Faiz-e-Aam College, Shahjahanpur, UP, India. He received his PhD from Aligarh Muslim University (AMU), India, in 2008, prior to conducting postdoctoral research at the Botanical Institute, University of Basel (BIB), Switzerland (2008–2010), and at Chonbuk National University (CBNU), Republic of Korea, in 2011. He was an Assistant Professor at Jimma University, Ethiopia (2011–2014), and a Fellow Researcher at the Institute of Tropical Agriculture, Universiti Putra Malaysia (UPM) (2014–2015). Dr. Akhtar has more than 15 years of research and 10 years of teaching experience in soil microbiology, applied microbiology, environmental microbiology, molecular biology, plant pathology, and plant nanobiotechnology. He has authored or coauthored more than 100 articles in peer-reviewed journals, conference proceedings, and book chapters and has edited 10 books with international publishers. Further, he serves as an Editorial Board Member and Reviewer for several high-impact international journals. His current research is focused on rhizospheric plant-microbe interactions and molecular biotechnology, bioremediation, biomineralization, nano-fertilizers, and nanobiotechnology.

**Dr. Mallappa Kumara Swamy** (PhD) is a Professor and Head of the Department of Biotechnology at East West First Grade College of Science, Bengaluru, Karnataka, India. He completed his postdoctoral research at the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. Before that, he had worked as an Associate Professor and Head of the Department of Biotechnology, Padmashree Institute of Management and Sciences, Bangalore University, Bengaluru, India. He received his PhD (Biotechnology) from Acharya Nagarjuna University, Guntur, India, in 2013. He has more than 15 years of teaching and research experience in the fields of plant biotechnology, secondary metabolite production, phytochemistry, and bio-active studies. Dr. Swamy has authored 80 research publications in peer-reviewed journals and 16 book chapters. Recently, he has edited four books published by Springer Nature Singapore Pte Ltd., Singapore, one book by CRC Press LCC, USA and one book by Studium Press Pvt. Ltd., India. He also serves as an Editorial Board Member and Reviewer for several high-impact factor international journals. His current research is focused on cell and tissue culture technology for bio-active compound production and on nanotechnology for medical applications.

**Dr. Uma Rani Sinniah** (PhD) is a Professor at the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM). She has more than 35 years of teaching and research experience in the fields of plant biology, seed technology, cryobiology, germplasm conservation, and plant secondary metabolites and has successfully completed several projects supported by various national and international funding bodies. She has published more than 95 research articles in peer-reviewed journals and 8 book chapters. Her current research is focused on seed science and technology, cryopreservation, plant cell and tissue culture, and secondary metabolite production.

# Production and Application of Novel Bio-active Compounds by Endophytic Microbes

1

Julio Alves Cardoso Filho

## Contents



**Abstract**

Traditionally, endophytes are microorganisms that inhabit plant tissues, establishing an association with their hosts for most or all of their life without causing any apparent damage. Recently, researchers have shown an increased interest in the potential of endophytes to produce bio-active compounds with activity against numerous human, animal, and plant diseases. The determination of these bio-active molecules and their modes of action are technically challenging. Thus,

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the aim of this chapter is to provide a holistic point of view to the current strategies adopted for screening and production of novel endophyte-derived metabolites, such as terpenoids, alkaloids, phenylpropanoids, aliphatic compounds, polyketides, and peptides, and to present their commercial applications in the medicinal, pharmaceutical, agricultural food, and cosmetic industries.

**Keywords**
Biotechnology industries · Pharmacology drugs · Polyketides · Nonribosomal peptides · Secondary metabolites

## 1.1 Introduction

An endophyte is defined as "any organism that forms latent asymptomatic infections within healthy tissue systems either in intercellular spaces (apoplasts) or inside the cells (symplasts) of plants" (Porras-Alfaro and Bayman 2011; Specian et al. 2012; Perotto et al. 2013; Chaparro et al. 2014; Dutta et al. 2014; Farrar et al. 2014; Akhtar et al. 2015; Hardoim et al. 2015; Kaul et al. 2016; Sengupta et al. 2017). The endophytic communities have been divided into different subgroups, including "mutualism, commensalism and parasitism", which are related to their host plants (Hardoim et al. 2012; Andreote et al. 2014; Nisa et al. 2015). The recent reviews highlight types of endophytic associations, such as algal endophytes (Sarasan et al. 2017), endophytic insect pathogenic fungi (Barelli et al. 2016; Moonjely et al. 2016; Behie et al. 2017), endophyte fungi (Vasundhara et al. 2016; Knapp et al. 2018; Akhtar and Panwar 2011; Akhtar et al. 2011; Akhtar et al. 2015; Swamy et al. 2016a, b), prokaryotic endophytes (Hollensteiner et al. 2018), endophytic actinobacteria (Álvarez-Pérez et al. 2017), plant growth-promoting bacteria (PGPB) (Akhtar and Siddiqui 2010; Akhtar and Azam 2014; Olanrewaju et al. 2017), plant growth-promoting fungi (PGPF) (Hossain et al. 2017), nematophagous endophytic fungi (Vidal-Diez de Ulzurrun and Hsueh 2018), mycorrhizas symbiosis (Berruti et al. 2016; Filho et al. 2017; Mills et al. 2018), actinorhizal symbiosis (Franche et al. 2016), and rhizobia symbiosis (Checcucci et al. 2017; Naveed et al. 2017). Most endophytes are unculturable (Liaqat and Eltem 2016); therefore, the analysis of their diversity and the molecular basis of their interactions with the plant are revealed by using molecular approaches (Kaul et al. 2016).

Endophytic microbes can drive the host plant demography (Saikkonen et al. 2016), shape plant communities (Yahr et al. 2016), guide the community structure and biodiversity of the aggregated organisms (Edwards et al. 2017), and have an impact on the phenotype and epigenome of their associated plants (Vannier et al. 2015). The field of drug discovery renewed our interest in endophytes microbes (Thatoi et al. 2013; Azevedo 2014; Lacava and Azevedo 2014; Kusari et al. 2015; Rukshana and Tamilselvi 2016; Sebastianes et al. 2017; Strobel 2018). Endophytes can synthesize homologous bio-active and structurally diverse secondary metabolites (SMs), such as alkaloids, benzopyranones, chinones, flavonoids, phenolic

acids, quinones, steroids, peptides, terpenoids, tetralones, cytochalasines, quinols, xathones, chinones, isocumarines, and benzopyranones, that mimic the structure and function of their host compounds (Cragg and Newman 2013; Higginbotham et al. 2013; Bhardwaj and Agrawal 2014; Zhang et al. 2014a, b; Stierle and Stierle 2015; Chen et al. 2016; Newman and Cragg 2016; Agrawal et al. 2017; Sarasan et al. 2017; Deshmukh et al. 2018). The SMs are small molecules, which act as a defense compound under abiotic (e.g., acidity, drought, and salinity) or biotic stress conditions (e.g., parasitic symbiosis), or act as a signaling molecule during biotic interactions between organisms in their ecological niches (Wisecaver et al. 2014; Knox and Keller 2015). SMs are usually synthesized by mevalonic acid (Bian et al. 2017), methylerythritol 4-phosphate (Banerjee and Sharkey 2014), shikimate-chorismate (Tohge et al. 2013), polyketide (PKs), and nonribosomal polyketide (Harvey et al. 2015; Amoutzias et al. 2016; Martinez-Klimova et al. 2017). The PKs are biosynthesized through modular polyketide synthases (PKSs) type I from acetate and propionate building blocks (Knox and Keller 2015; Ray and Moore 2016; Vesth et al. 2016). The PKSs are classified into type I (Gallo et al. 2013), type II, and type III based on their structure and biochemistry (Yuzawa et al. 2016, 2017; Parvez et al. 2018). The polyketide-derived drugs include several anticancer drugs (epothilone; taxol or paclitaxel), antibiotics (erythromycin), insecticides (spinosyn A), and antifungals (amphotericin B) (Miller et al. 2008; Cane 2010; Osswald et al. 2014; Finzel and Burkart 2016). Moreover, the hybrid PKS–NRPS (HPN) are involved in peptide toxins biosynthesis, such as pectenotoxin and enuazonic acid and destruxins (Wu et al. 2013; Zhao et al. 2015). NRPs are produced by nonribosomal peptide synthetases (NRPSs). NRPSs use proteinogenic and nonproteinogenic amino acids (DNA non-encoded amino acids) as building blocks for the peptide chain assembly (Felnagle et al. 2008). NRPS are modular enzymes with multiple domains, namely acetylation, condensation, and thioesterase (Ayuso-Sacido and Genilloud 2005). Some necrotrophic phytopathogenic fungi produce phytotoxins host-specific toxins (HSTs) and non-host-specific toxins (non-HSTs) (Pusztahelyi et al. 2015) by the activity of polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) (Scharf et al. 2014). These phytotoxins act as virulence factors (Vurro et al. 2018). Terpenoids are derived from the isomeric C-5 isoprenoid chain precursors isopentenyl and dimethylallyl diphosphate (IPP and DMAPP), which are synthesized from acetyl-CoA through the mevalonate pathway (MVA) that occurs among eukaryotes (Alberti et al. 2017) and in some prokaryotes (Lombard and Moreira 2011). The more common terpenoid fungi-derived products are mycotoxins, antibiotics, antitumor compounds, and phytohormones (Deka et al. 2017). The shikimate pathway occurs in microorganisms (e.g., oomycetes, ciliates, diatoms, ascomycetes, basidiomycetes and zygomycetes), plants, and green and red algae, but not in animals (Richards et al. 2006), and the end product is chorismate, the precursor for important SMs (e.g., flavors, fragrances, pharmaceuticals and food additives) (Tohge et al. 2013). MEP is also called the non-mevalonate or Rohmer pathway (Rohmer 1999). It occurs in algae, many eubacteria, and apicomplexan parasites, but not in archaea and animals (Eisenreich et al. 2004). MEP pathway enzymes (e.g., methyl erythritol phosphate) are effective targets for novel

biosynthesis of antimalarial, antibacterial, and herbicidal agents (Matsue et al. 2010). Undoubtedly, the database of drugs and others web tools (e.g., standardizing APIs) enable users to compare endophytes genomes and their resulting SMs for their utilities and make further contributions, and they allow the discovery and sustainable production of these desirable bio-active secondary metabolites (Khater et al. 2016; Vesth et al. 2016). Considering these facts, endophytes are assuredly considered as a source of novel chemically bio-active compounds and a promissory reservoir for drug discovery (Brakhage 2013; Weber and Kim 2016).

## 1.2 Natural Products in Drug Discovery: Current Status and Future Perspectives

Natural products (NPs) is a holistic term for low molecular weight compounds and their derivatives isolated from plants, animals, minerals, and microbes (Nicoletti and Fiorentino 2015; Newman and Cragg 2016; Zhang et al. 2016). Historically, empirical research into natural products dates back to 1550 BC, and the scientific period began in the 1950s (Dias et al. 2012; David et al. 2014). Approximately 29.5% of FDA (Drug and Device Information From the Food and Drug Administration–FDA) delivered drugs are the derivatives of NPs (Gu et al. 2013). Despite the importance of NPs, research aimed at exploiting NPs and their derivatives drastically decreased during the past 30 years (Newman and Cragg 2016; de La Torre and Albericio 2018). A possible explanation for this decline can be attributed to the shift in technology used for drug discovery (Chang and Kwon 2016). However, in recent years, low drug productivity has renewed the focus on natural products and their derivatives as drug-discovery sources (Cragg and Newman 2013; Pawar et al. 2017). NPs research still has a good approach as drug (e.g., cancer chemotherapeutic and chemopreventive agents) candidates with applications in agriculture, medicine, and the biopharmaceutical industry (Beutler 2013; Booker et al. 2015). The framework of any drug discovery from the concept or idea to market consists of some basic steps, including disease selection, drug discovery, target discovery, database mining, target validation, structure-based drug design, fragment-based lead discovery, quantitative structure–activity relationships, measuring pharmacological activity (in vitro and in vivo studies), target selection, lead optimization (e.g., compound identification, discovery toxicology), lead validation (e.g., considerations for optimizing absorption, distribution, metabolism, excretion, and toxicity), trial evaluation in preclinical stage for first in human studies, approved clinical trials (e.g., approved or cleared by FDA), drug manufacturing, and available for sale (Sarker and Nahar 2012a, b; Booker et al. 2015; Patridge et al. 2016). On average, only one of 5000–10,000 of the new synthetic molecules in development approved for clinical trials becomes a manufacturing and commercial pharmaceutical drug because their toxicity is discovered in the clinical phases, inducing their rejection, and they are subsequently discarded (Ruiz-Torres et al. 2017). In principle, the bioprospecting for novel compounds from natural products and their derivatives as drug discovery sources is a critical and expensive step (scientific framework

for drug development is ~$350 million) for current biopharmaceutical research (Mishra et al. 2017), and drug delivery requires a preparation and evaluation period of 12 years (Kesselheim et al. 2017). Natural crude extracts or fractions (e.g., plant, animal or microbe) usually occur as a complex mixture of unknown compounds with several types of polarities, and their separation processes are a painful challenge for their bioprospecting, isolation, structural identification, and biochemical characterization (Sacan et al. 2012; Sarker and Nahar 2012a, b). However, in the twenty-first century progress in virtual screening (Wright and Sieber 2016), combinatorial chemical techniques (Liu et al. 2017), high-throughput screening (HTS) platforms (Paytubi et al. 2017), rational drug design (Cozza 2017), high throughput chemistry (Shevlin 2017), and nanoprobes (Lyu and Pu 2017; Li et al. 2018) have provided useful tools for bioprospecting large compound libraries in a cost-effective manner (Owen et al. 2017) to discover drugs based on target-based screening (Lionta et al. 2014). These new innovative models of drug research and development for drug innovation (Shaw 2017; de La Torre and Albericio 2018) helped to build a new Golden Age of natural products drug discovery (Shen 2015; Liu and Wang 2017). We have briefly discussed the adoption and development of novel analytical technologies applied to the new drug discovery strategies and exploiting the NPs and their derivatives in the following sections.

### 1.2.1 High-Throughput Screening (HTS) for Unknown Natural Compound Detection

HTS technology, considered the "Rosetta stone" for drug design to the pharmaceutical industry, allows the identification of several hundred thousand synthetic compounds (e.g., in vitro assays) in several different types of libraries (e.g., combinatorial chemistry, genomics, protein, and peptide libraries). HTS technology can identify biological targets of interest (Paytubi et al. 2017), reduce the costs of drug development (Roy 2018), and has been conducted on microarrays cell-based assays (Nierode et al. 2016). In HTS libraries, the target molecules (e.g., small molecules, polymers, and antibodies) are arrayed on microarrays by robotic spotting technology (Kolluri et al. 2018) or soft lithography technology (Hong et al. 2017). A typical HTS system can screen 10,000 compounds per day, and ultra high-throughput screening (UHTS) can even conduct 100,000 assays per day in the commercial development of new drugs (Szymański et al. 2012). HTS technology has allowed (in vivo) the virtual screening of known molecules stored in public chemical databases [(PubChem (http://pubchem.ncbi.nlm.nih.gov), ChemSpider (http://www.chemspider.com), and ChEMBL (https://www.ebi.ac.uk/chembl). This technology has reduced animal test numbers and increased the biopharmaceutical industries drug discovery programs (Nowotka et al. 2017; Matsui et al. 2017). On the basis of products and services, the global HTS market is expected to reach USD 18.83 billion by 2021 (https://www.marketsandmarkets.com). The 3D cell-based HTS assays [e.g., microwell platform (Vrij et al. 2016), and microfluidic device (Edmondson et al. 2014; Chi et al. 2016)] provide a useful platform for discovery of NPs derivatives

(Ryan et al. 2016). These technologies improve in vitro the predictability and accuracy of drug screening (Sabhachandani et al. 2016). The National Institute of Health (NIH) Roadmap created the molecular Libraries Screening Center Network (MLSCN) (Huryn and Cosford 2007) as part of the Molecular Libraries Initiative (MLI) with the purpose of facilitating access to use of chemical probes and small-molecule tools for basic research that will interrogate novel biochemical pathways (Austin et al. 2004). The NIH National Center for Advancing Translational Sciences (NCATS) Chemical Genomics Center (NCGC) (www.ncgc.nih.gov), in the United States of America (USA), is another HTS center (Kaiser 2011) combining state-of-the-art technology with the best scientific minds in academia (Huggett 2016), government, and industry (Cox 2018). The NCGC focus is to translate the discoveries of the Human Genome Project into biology, specifically on new targets and untreatable diseases (Huang et al. 2011), by using industrial-scale HTS assays, informatics, and chemistry (Thomas et al. 2009). NCGC provides a more rapid development of research tools, better diagnostic methods, and disease treatments (Howe et al. 2015; Hu and Bajorath 2017) in the era of medicinal chemistry big data (Ekins et al. 2017). Recently, the HTS infrastructure, the European Lead Factory (ELF; https://www.europeanleadfactory.eu) project (Karawajczyk et al. 2017), some pharmaceutical companies (Bayer, AstraZeneca, UCB, Lundbeck, Sanofi, Merck), and their proprietary in-house compounds collections, joined to create a chemical space expansion for collaborative lead generation and drug discovery (Karawajczyk et al. 2015). The ELF project created the Joint European Compound Library (Besnard et al. 2015), an HTS library that is engaged in synthetic and or medicinal chemistry with 321,000 compounds (Karawajczyk et al. 2017) and linked to a cloud-based informatics system, the ELF Honest Data Broker (Paillard et al. 2016). Nowadays, some chemistry focused academic groups (Max-Planck Institute of Molecular Physiology, Germany; http://www.syncom.nl) and small and medium enterprises (Syncom, The Netherlands; http://www.mpi-dortmund.mpg.de/74682/Kumar) cooperate with ELF (Karawajczyk et al. 2015). Undoubtedly, academic and industrial ELF consortium partners offer a practical device to search for new synthetic and non-synthetic drugs discovery in chemical space (Shanks et al. 2015; Paillard et al. 2016), reducing the drug development framework, and helping the marketing of the drug (Bucci-Rechtweg 2017).

### 1.2.2 Hyphenated Techniques in Natural Products Analysis

NPs and their derivatives (e.g., crude extracts or fractions) are a mixture of unknown compounds with many types of chemical polarities, and their separation, screening, identification, and characterization are laborious processes (Sarker and Nahar 2012a, b). In order to obtain the structural information of the unknown compounds present in a crude sample, hyphenated systems usually create a multidimensional data set (e.g., chromatographic and spectroscopic data) for online identification and dereplication applications (Brusotti et al. 2014; Ibekwe and Ameh 2015). In these methods, the separation techniques [e.g., liquid chromatography (LC),

high-performance liquid chromatography (HPLC), capillary electrophoresis (EC) or gas chromatography (GC)] are coupled to an online spectroscopic detection technology [e.g., infrared (IR), Fourier-transform infrared (FTIR), photodiode array (PDA), ultraviolet-visible (UV-vis) absorbance, or fluorescence emission, mass (MS), or NMR spectroscopy] (Patel et al. 2014). As a result, innumerable modern hyphenated techniques (e.g., CE-MS, GC-MS, LC-MS, LC-PDA, and LC-NMR) were developed (Yu et al. 2016) that allowed combining the better aspects of chromatographic and spectral methods, to build more powerful integrated systems (e.g., LC-PDA-MS, LC-MS-MS, LC-NMR-MS, and LC-PDA-NMR-MS) for isolation and analysis of NPs and their derivatives (Patel et al. 2012).

### 1.2.3 Dereplication of Natural Products Analysis

Dereplication approaches combine the use of chromatographic (e.g., TLC and HPLC) and spectroscopic techniques (e.g., UV–vis and IR) with NP databases (DBs) bioprospecting (Chervin et al. 2017; Pérez-Victoria et al. 2016; Prabhu et al. 2015). Nowadays, LD-based ion sources are used for pretreated samples in MS analyses of NPs and untreated samples (native form) in secondary ion mass spectrometry (SIMS) (Bhardwaj and Hanley 2014). In microbial NPs screening, the re-isolation of known compounds (Pérez-Victoria et al. 2016) makes this phase more laborious (Nielsen and Larsen 2015; Chervin et al. 2017). An ultra-performance liquid chromatography photodiode array high- resolution in tandem mass spectrometric (UPLC-PDA-HRMS-MS/MS) technique can be used for dereplication of fungal secondary metabolites in crude culture extracts (Tawfike et al. 2013), which limits the occurrence of false positives (Kildgaard et al. 2014; Wolfender et al. 2015). The quadrupole-type system, ion trap, time of flight (TOF), and Orbitrap (as MS analyzers) are also satisfactorily used for dereplication systems (Kildgaard et al. 2014), increasing the selectively and accurately applied to the dereplication strategies. The use of a ChemSpider (ACD-Structure Elucidator) hosted by the Royal Society of Chemistry is another dereplication strategy applied for NPs and their derivatives (Elyashberg et al. 2009). The molecular networking (MN) and in silico fragmentation tools (Sacan et al. 2012) provide new product strategies for dereplication approaches of secondary metabolites applied in NPs research (Allard et al. 2016; Masimirembwa and Thelingwani 2012).

### 1.2.4 Chemical Derivatization Strategies in Natural Products Analysis

Chemical derivatization strategies represent a more recent approach for the separation of lipids from complex NPs mixtures, including isomeric lipids (Jiang et al. 2017). In LC-MS, chemical derivatizations are commonly used to increase the MS ionization efficiency and selectivity, facilitate structure elucidation, and improve the chromatographic separation (Qi et al. 2014). In liquid chromatography-mass

spectrometry (LC-MS), chemical derivatizations are required to reduce the polarities of the functional groups, improve their separation by chromatographic methods (TLC, LC, HPLC, and GC), and facilitate structure elucidation. Chemical derivatizations are applied in order to adapt the physicochemical properties of NPs derivatives products, generate derivatives, allow the synthesis of active molecular probes by conjugation of reporter tags (Robles and Romo 2014), and enable the structure-activity relationship (SAR), quantitative structure-activity relationships (QSAR), and molecular docking studies (Abdulfatai et al. 2017; Afifi et al. 2017).

## 1.3 Target-Based Drug Discovery (TBDD)

Nowadays, the pharmaceutical industry TBDD needs to select targets with reduced attrition rates in randomized trials (e.g., good laboratory practice stage of toxicology testing triggers) due to lack of differentiated efficacy (Chaparro et al. 2018). In this context, the traditional TBDD methods have been conducted by affinity chromatography, radiolabeling, and cell-based affinity tagging procedures (Azad and Wright 2012; Sakamoto et al. 2012). However, the current TBDD methods include both target-based (reverse pharmacology) and phenotypic target-based screening (target de-convolution) (Lee and Bogyo 2013; Vaidya 2014; Simoes-Pires et al. 2014; Jung and Kwon 2015; Nijman 2015; Arulsamy et al. 2016; Glenn and Croston 2017; Haasen et al. 2017). TBDD strategies are based on the interactions between a target and its phenotype (biological tractability) as well as an ability to modulate that phenotype using a chemical probe with a specific target (Garbaccio and Parmee 2016). The chemical probes are usually small-molecules used as molecular and biochemical modulators of a protein's function applied to TBDD to improve the target validation (Hajimahdi and Zarghi 2016). The current technologies applied to TBDD focusing on chemistry and phenotypic target-based screening and a summary of the potentiality, reliability, and limitations of these methods are discussed below.

### 1.3.1 Chemistry of Target-Based Screening

The chemistry-based screening target (e.g., cell-based target) presents four features for target validation based on the use of chemical probes, the exposure at the site of action, target engagement and selectivity, expression of functional pharmacology, and proof of phenotype perturbation (Bunnage et al. 2013; Garbaccio and Parmee 2016). These methods can help to optimize drugs use for pharmacogenomics-based personalized medicine (Mirsadeghi and Larijani 2017).

#### 1.3.1.1 Chemical Genomics

Chemogenomics is an emerging research field that combines genomics, chemistry, and computational sciences for the rapid validation of new targeted therapeutics compounds, where a specific molecular target has their biological function modulated by a small molecule (Jones and Bunnage 2017; Rakers et al. 2018). The

emergence of chemogenomics is due to the increasing number of in-house bioactivity databases (Lipinski et al. 2015) available both in commercial (GoSTAR: https://www.gostardb.com/) and public open databases (ChEMBL: https://www.ebi.ac.uk/chembl/) (Gaulton et al. 2017; Nowotka et al. 2017). The chemogenomics knowledge-based strategies provide a rational prediction of drug target gene interactions owing to the basic information of the computational design of target-directed combinatorial libraries found in the chemical space (Rakers et al. 2018). Nowadays, chemogenomics is considered as a viable alternative to some in silico approaches, such as docking, structure-based drug or ligand-based virtual screening strategies (Lionta et al. 2014). Currently, pharmacometabonomics is used to predict drug metabolism, pharmacokinetics, safety, and efficacy. It is complementary to pharmacogenomic and pharmacoproteomics or pharmacometabonomics (Shabaruddin et al. 2015; Everett 2016) and is therefore considered a new tool for personalized medicine (Everett 2015). However, the cost of pharmacogenomic assays continues to be very expensive to incorporate into standard health-care (Pink et al. 2014; Altar et al. 2015).

#### 1.3.1.2 Chemical Proteomics

Chemical proteomics emerged on the West Coast, notably in Cravatt's lab at the Skaggs Institute of Chemical Biology (Adam et al. 2002) and Bogyo's lab; then at Celera in South San Francisco (Zanders 2012). Chemical proteomics is a field of chemical biology focused on the interaction between engineered small molecular probes (chemical probes) and proteome (Medina-Cleghorn and Nomura 2014). Chemical proteomics strategies usually combine phenotypic screening with target identification screening for novel drug targets (Medina-Cleghorn et al. 2015; Counihan et al. 2017; Piazza et al. 2018). In other words, chemical proteomics uses the design of small molecule probes to understand protein function (Cravatt et al. 2008), based on their action mode on protein expression and posttranslational modifications on the proteome-level in target cells or tissues of interest (Yu et al. 2016), and can identify small molecule targets in complex biological samples (Futamura et al. 2013). Chemical proteomics can help with the selection and validation of targets (Bantscheff and Drewes 2012; Liu and Guo 2014). The most usual applications of chemical probes are biological tractability (establishes the interaction between a target and its phenotype) and chemical tractability (ability to modulate its phenotype by a small molecule or chemical probe) (Garbaccio and Parmee 2016). In this context, these chemical probes provide a better understanding of pharmacokinetic or pharmacodynamic models by maximizing target identification and avoiding biases during target validation (Bunnage et al. 2013) resulting in the delivery of novel therapeutics (Garbaccio and Parmee 2016). Nowadays, the use of quantitative proteomics methodologies, such as protein profiling (Chen et al. 2017), compound-centric chemical proteomics (Wright and Sieber 2016), and drug affinity responsive target stability (Pai et al. 2015), DNA, RNA, protein or cell microarrays (Li 2016; Rothbauer et al. 2016), and microfluidic cell-chips (Carey et al. 2018), provides new insights into chemical proteomics for developing therapeutic agents (Pan et al. 2016; Olivon et al. 2017).

### 1.3.2 Phenotypic-Based Screening

Phenotypic-based screening (PBS) is also known as the neoclassic pharma strategy (Lee and Berg 2013). It exhibited some advantages over chemical target-based methods for bio-active compounds identification owing to continuous negative results in the pharmaceutical industry (Priest and Erdemli 2014; Walker et al. 2015; Moffat et al. 2017). PBS uses unbiased phenotypic assays to find large molecules with the ability to alter a specific phenotype of cells (cell proliferation), tissues or animals into HTS chemical space libraries (Ayotte and La Plante 2017). Recently, PBS has gained renewed importance in discovering first-in-class or best-in-class medicines (Lexchin 2014a, b; Lexchin 2016; Moffat et al. 2017). PBS assays approach key aspects of the physiological process like cell-cell interactions and signal transduction pathways (Ayotte and La Plante 2017; Isgut et al. 2018). They usually depend on the cell-based phenotypic assay (e.g., RNAi, reporter gene assay, CRISPR/Cas9 system, Reverse Phase Protein Arrays (RPPAs), cell viability (e.g. MTS, alamar blue, Annexin V-FITC flow cytometry assay), signaling pathway (e.g., GPCR, nuclear receptor, MAPK/ERK), disease-related phenotypic assay (e.g., neurodegenerative diseases, such as Alzheimer's and Parkinson's disease), and more recently network-based phenotype mapping (Fang 2015; Moerke and Fallahi-Sichani 2016; Moffat et al. 2017). Nowadays, automated microscope-based screening [e.g., high content screening (HCS), high content imaging (HCI), or image cytometry (IC)] is ideally suited for screening multi-targeted agents and drug combinations (Dolman et al. 2018; Verjans et al. 2018). The methods combine the molecular information, biological relevance, and patient data to increase the productivity of discovering first-in-class (Drawnel et al. 2017). PBS methods enable a new challenge to screen and focus drug combinations based on polypharmacology strategies (Isgut et al. 2018). However, for a robust PBS implementation, it will be necessary to build more sophisticated systems biology databases, such as the Human Microbiome Project (Bauer and Thiele 2018), Genome-Scale Metabolic Models (GEMs) (Rejc et al. 2017), the Human Metabolic Atlas (HMA; http://www.metabolicatlas.org) (Bauer and Thiele 2018), and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) (Hadadi et al. 2016). As a result, the information acquired can be incorporated and used in standard health-care and clinical practice (Pink et al. 2014; Altar et al. 2015). In 2010, the BioAssay Ontology (BAO; http://bioassayontology.org) project was created (Visser et al. 2011). This Project was developed to standardize the Minimum Information About a Cellular Assay (MIACA; http://sourceforge.net/projects/miaca) (Brazma et al. 2001), or to set up the ontologies (Zander Balderud et al. 2015). In terms of ontology resources, some databases storing information about HTS for the human cell lines (e.g., genotype-to-phenotype relationships) and anticancer drugs can be localized in the Genomics of Drug Sensitivity (GDSC; www.cancerrxgene.org) (Yang et al. 2013), Cellular Microscopy Phenotype Ontology (CMPO; www.ebi.ac.uk/cmpo (Jupp et al. 2016), and Human Phenotype Ontology (HPO; www.human-phenotype-ontology.github.io) (Köhler et al. 2017). These approaches provide the generation/extraction of derived ontologies (or perspectives) and analyze the activities of compounds for identification of artifacts technology (Mandavilli et al. 2018; Wang et al. 2017).

## 1.4 Endophytic Microorganisms as a Source of Bio-active Compounds

Bio-active compounds are produced by soil-borne endophytes fungi (e.g., *Trichoderma* spp., *Talaromyces* spp.) (Pusztahelyi et al. 2016; Zhai et al. 2016), soil-borne endophytes bacteria (e.g., *Pseudomonas putida*) (Santoyo et al. 2016; Frank et al. 2017; Honeker et al. 2017), and plant–symbiont nonpathogenic associations (e.g., *Daldinia eschscholtzii*, *Hypoxylon rickii*, and *Pestalotiopsis fici*) (Macías-Rubalcava and Sánchez-Fernández 2017; Helaly et al. 2018). These compounds have a critical role in plant resistance to biotic (Chadha et al. 2015; Vesterlund et al. 2011) and abiotic stress factors (Choudhary 2012), which benefits the host survival in return (Maheshwari et al. 2017). However, this is a complex phenomenon, and the mechanisms of this protection (e.g., deter herbivores by producing toxic alkaloids) process are poorly understood (Arora and Ramawat 2017). Recently, Newman and Cragg (2016) disclosed a list of all FDA approved drugs from 1981 to 2014, and over 35% of these chemotherapeutic candidates are produced by microbes and/or endophytes; therefore, the field of natural product research should be significantly expanded (Martinez-Klimova et al. 2017; Mazzoli et al. 2017; Gao et al. 2016; Uzma et al. 2018). Mayer et al. (2013) reported 102 marine natural products of animals, algae, fungi, and bacterial origin having antibacterial, antifungal, antiprotozoal, antituberculosis, and antiviral activities. Out of these, 68 exhibited a huge biodiversity of receptors and their molecular targets. This study received global technical support research from chemists and pharmacologists based in several countries, including Australia, Belgium, Brazil, Canada, China, Colombia, Cuba, Egypt, Fiji, France, Germany, Indonesia, Israel, Italy, Japan, Luxemburg, Malaysia, Mexico, the Netherlands, New Caledonia, New Zealand, Norway, Panama, Papua New Guinea, Philippines, South Africa, South Korea, Singapore, Spain, and Switzerland. This study culminated FDA-approved pharmaceuticals and 11 compounds in phase I, II, and III of clinical development. A considerable amount of literature has been published on the microbial richness of marine endophytes, and it highlights the correlation with their antimicrobial bio-active compounds and habitat complexity (Rédou et al. 2015; Sarasan et al. 2017; Deshmukh et al. 2018). In this context, deep-sea fungi like *Aspergillus fumigatus* (48X3-P3-P1), *A. terreus* (1H3-S0-P1), *Eurotium herbariorum* CB-33, *Fusarium oxysporum* (1H3-P0-P1, 4H1-P0-P1, and 4H1-P3-P3), *Penicillium bialowiezense* (CB-5, CB-7, and CB-8), *P. chrysogenum* (2H5-M3-P2-(3), CB-11, CB-17, and CB-24), *Penicillium* sp. (CB-16), and *Oidiodendron griseum* (CB-36) were isolated from 2000 m below the seafloor, and their antimicrobial screening revealed 33% of bio-active compounds (using 16 microbial targets) against pathogenic bacteria and fungi (Rédou et al. 2015). The deep subseafloor, and the others marine ecosystems, currently represent the last frontier of an untapped reservoir of novel bio-active molecules. However, the knowledge about the endophytes microbes and their SMs is still limited, and several aspects (e.g., SMs ecological functions and the plant-endophyte relationship) need to be studied and understood (Jia et al. 2016; Negreiros de Carvalho et al. 2016).

### 1.4.1 Endophytic Bio-active Compounds Production by Synthetic Biology and Metabolic Engineering

The endophytes bio-active compounds extraction, purification, chemical analysis, and new chemical bio-active entities (NCBEs) identification (especially those of high added value), in minimum commercial quantity or to synthesize chemically in industrial amounts (Khatri et al. 2017; Singh et al. 2017;), are commonly very tedious and challenging processes (Kadir et al. 2013). Therefore, engineering microbes based on next-generation sequencing methodologies (NGS) for the production of SMs and NCBEs characterization are emerging as advantageous alternative methodologies (Turner et al. 2018). Nevertheless, the biggest global pharma companies, such as Pfizer Inc., Merck & Co, Jonnson & Jonnson, F. Hoffmann-La Roche AG, and Sanofi, drastically reduced their industrial R&D investment in NPs during the past 30 years due to high rediscovery rates of NCBEs and the lack of innovative screening approaches (Katz and Baltz 2016).

The biosynthesis of SMs are co-regulated by gene clusters (BGCs) on a single genetic locus (Wallwey and Li 2011; Brakhage 2013; Cacho et al. 2015; Smanski et al. 2016; Vesth et al. 2016), localized on subtelomeric regions in the chromosome (Knox and Keller 2015), and around a synthase gene (Andersen et al. 2013). The "silent" or "cryptic" gene clusters (Zn(II)2Cys6, Cys2His2, and basic region-leucine zipper (bZIP) family are transcriptional regulators) provide endophytes microbes species (endophyte fungi) precise temporal and spatial control over the SMs expression and probably helps the intra, and inter-kingdom, horizontal cluster transfer (Hong et al. 2013; Ortiz et al. 2013; Knox and Keller 2015). Moreover, one of the major challenges in NP discovery is that only a tiny fraction of those BGCs have been characterized to date, partially due to the fact that they are transcriptionally silenced or do not express in totality in their native hosts (due to tight regulation) under standard laboratory conditions (Bamisile et al. 2018). In order to activate those BGCs [e.g., *cadA, mttA, mfs*) of *A. Niger* itaconic acid (IA)], and their cryptic pathways, many innovative approaches are used, such as one strain many compounds (Blumhoff et al. 2013; Hewage et al. 2014), co-culture (Kamdem et al. 2018), and microbial biotransformation (Donova 2017). The most common approaches employed for gene clusters activation in fungal endophytes include gene deletions (Andersen et al. 2013), modulation in epigenetic mechanisms (Deepika et al. 2016), proteomic (Brakhage and Schroeckh 2011), genome mining (van der Voort et al. 2015), and heterologous hosts for cloning and expression of fungal metabolites (Anyaogu and Mortensen 2015). The identification of loss of *aflR* expression (LaeA), a global regulator of SMs in *Aspergillus* spp. (Zhao et al. 2017), and many bioinformatic algorithm tools, such as Secondary Metabolite Analysis Shell (antiSMASH) (Weber et al. 2015), the Secondary Metabolite Unknown Region Finder (SMURF) (Khaldi et al. 2010; www.jcvi.org/smurf), and motif-independent de novo detection algorithm (MIDDAS-M) (Umemura et al. 2013) for SMB (www.secondarymetabolites.org) gene clusters, allow the prediction of superclusters containing genes for more than one SM (Wiemann et al. 2013). More recently, synthetic biology and metabolic engineering were employed to activate

those "silent" gene clusters, provide the insertion of targeted mutations through biosynthetic metabolic pathways in heterologous hosts, express the target mutant enzyme catalysts, and aimed to produce larger amounts of commercially and industrially high-value bio-active compounds (Boruta and Bizukojc 2017), such as biopigments applied to many industrial activities, e.g., food, textile, cosmetic, and pharmaceuticals (Narsing et al. 2017). Synthetic biology (SynBio) is related to design rationalizing of biological systems by applying the key concepts of engineering (Fletcher et al. 2016). These engineering concepts include the abstraction, modularity, and use of standard interfaces (Bhatia and Densmore 2013), such as Pigeon, a graphical user interface, clearly valuable for any design enterprise (Chelliah et al. 2013). Metabolic engineering is directly linked to the use of the systems-level for design, optimization of cellular metabolism, and the gene regulatory metabolic networks on a genome scale (Khatri et al. 2017). In general, there are three major steps in metabolic engineering for production of small molecule drugs, including pathway discovery, pathway assembly in the recombinant host (homologous or heterologous recombination), and pathway optimization (Zhang et al. 2015). Metabolic engineering and synthetic biology (SynBio) approaches have revolutionized many fields of biotechnology (e.g., renewable biofuel production) making possible the insertion of non-native and de novo biochemical pathways to generate high-value drop-in bio-active chemical compounds (Chiarabelli et al. 2013; Salehi et al. 2017). Consolidation of synthetic biology and metabolic engineering approaches doubles the knowledge of the biosynthetic pathways and organisms for which molecular tools (CRISPR/Cas9 or genome-scale metabolic models (GSMM) are available and optimized to any extent (Zhou et al. 2016; Harrington et al. 2017). Model organisms, such as *Saccharomyces cerevisiae* and *Escherichia coli*, remain widely used host strains for industrial production due to their robust and desirable traits (Lee et al. 2012).

#### 1.4.1.1 Endophytic as Microbial Cell Biofactories of Bio-active Compounds

A brief overview of some endophytes as live platforms able to host and sustain the purposeful DNA designs as biofactory producers of bio-active metabolites obtained through synthetic biology and metabolic engineering is discussed below.

##### 1.4.1.1.1 *Aspergillus* Species

There is a large volume of published studies describing the role of *Aspergillus,* which consists of over 340 taxonomically species, such as *A. fumigatus*, *A. flavus*, *A. niger*, *A. parasiticus*, *A. nidulans,* and *A. terreus,* recognized as cell factories for human, agricultural, and biotechnological applications (Meyer et al. 2015; Park et al. 2017). Heterologous expression was used to access the genes cryptic clusters (GCC) in *A. nidulans.* It is a practical and effective system for amplifying GCC from a target fungus (*A. terreus*) by placing them under control of a regulatable promoter (Bhetariya et al. 2011; Netzker et al. 2015) based on *LaeA* a regulator gene for SMs that allows the transfer and expression of the asperfuranone biosynthetic pathway (Chiang et al. 2013) into *A. nidulans*, and eliminates unwanted toxins (e.g.,

carcinogenic mycotoxins aflatoxins from *A. flavus*, and sterigmatocystin from *A. nidulans*) and allergic agents (e.g., allergic bronchopulmonary aspergillosis from *A. fumigatus*) (Park et al. 2017). Some *Aspergillus* metabolites and their applications include citric acid as a food additive, fumagillin as an antimicrobial agent, lovastatin as a hypolipidemic agent, and succinic acid as a flavor additive and detergent (Tobert 2003; Fallon et al. 2011; Sorensen et al. 2011; Sanchez et al. 2012). The genomes of *Aspergillus* species have now been sequenced, and genome-editing techniques have been rapidly developed for filamentous fungi (Teotia et al. 2016; de Vries et al. 2017). A putative gene cluster (*gedC* and *gedR*) that encodes the geodin production in *A. terreus* was transferred by heterologous reconstitution (genetic toolbox; USER cloning and USER fusion protocols) into *A. nidulans,* and the penicillin cluster (*pcbAB*, *pcbC*, and *pende*) of *P. chrysogenum* was rewired and expressed from a polycistronic gene cluster under control of a single xylose-inducible promoter in *A. nidulans*. The recent strategies for heterologous expression of fungal biosynthetic pathways in *Aspergilli* were reviewed by Anyaogu and Mortensen (2015). This genus presents an extensive metabolic engineering toolbox (Richter et al. 2014; Gressler et al. 2015); hence, *Aspergillus* can be employed as a multi-purpose biofactory for large scale production of bio-active secondary compounds and in development of strategies for converting biomass to bioenergy (Raghavendra et al. 2016).

#### 1.4.1.1.2 *Penicillium chrysogenum*

*Penicillium chrysogenum* is a filamentous fungus used as an industrial producer of β-lactam antibiotics, such as penicillins and cephalosporins (van den Berg 2011). The penicillin biosynthesis is encoded by three genes [acvA (pcbAB), ipnA (pcbC), and aatA (pende)] (Terfehr et al. 2017). The biosynthesis of β-lactam in *P. chrysogenum* is regulated by a *LaeA* protein (a global regulator) and the Velvet complex proteins (VelA, VelB, VelC, and VosA) (Martín 2017). In 2008, the complete genome sequence of *P. chrysogenum* was elucidated (Martín 2017), unmasking genetic secrets of the industrial penicillin producer (van den Berg 2011). Recently, the metabolic engineering and synthetic biology approach resulted in the description of transcription factor *CreA* responsible for carbon repression (Cepeda-García et al. 2014). This finding opens the possibility of utilizing it to improve the industrial production of this antibiotic in others filamentous fungus (e.g., *Acremonium chrysogenum*) (Terfehr et al. 2017) and to use more sustainable methods for the fermentative production of unnatural antibiotics and related compounds (Salo et al. 2015). The classical strain *P. chrysogenum* has multiple copies of the penicillin biosynthesis cluster (pBC) encoded by three key enzymes: δ-(l-α-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACVS), isopenicillin N synthase (IPNS), and isopenicillin N acyltransferase (IAT) (Nijland et al. 2010). Much of the literature since the mid-1990s emphasizes the applications of the *P. chrysogenum* genetic engineered strain for β-lactam antibiotics production (Martinez-Klimova et al. 2017). In 2016, we celebrated a historical medical framework for the 75th anniversary of the first medical systemic administration of penicillin in humans (Lobanovska and Pilla 2017). In 2001, the European Surveillance of Antimicrobial Consumption (ESAC) reviewed the antimicrobial resistance (AMR) strains based on the inappropriate prescription

and administration of an antibiotic therapy (Fleming-Dutra et al. 2016; Nhung et al. 2017). On the other hand, the extensive livestock and agricultural use of antibiotics (about 63,000 to over 240,000 tons of annual global antibiotic use) contribute to antibiotic resistance (Osman et al. 2018). In this context, metabolic engineering and synthetic biology approaches were applied to *P. chrysogenum* (Weber et al. 2012). The overexpression of isopenicillin N Acyltransferase in *P. Chrysogenum* (Veiga et al. 2012), engineering of β-oxidation for improved semi-synthetic cephalosporin biosynthesis, can be used to overcome the current multidrug resistance (MDR) and extended spectrum beta lactamase (ESBL) or extended-spectrum cephalosporins (ESCs) caused by ESC-R *E. coli* and ESC-R *Salmonella* spp., resistant strains (Shrestha et al. 2017), due to plasmid-encoded AmpC β-lactamases (pAmpC) (mainly CMY-2) and CTX-M extended-spectrum β-lactamases (ESBLs) in Gram-negative (Trott 2013), which are considered major global health problems (Dandachi et al. 2018).

#### 1.4.1.1.3 *Saccharomyces* Species

Yeast has been continuously used for the production of high-value small and large molecules, such as alcohols, acids, hydrocarbons, and proteins (Shi and Zhao 2017). Several others yeast species have been used in biomass production (*Trichosporon* spp.), food processing (*Kluyveromyces* spp.), feed nutrition (*Ogataea polymorpha*), degreasing and bioremediation (*Geotrichum candidum*), therapeutic and detergent (*T. fermentum*), and pharmaceutical (*Rhodotorula* spp.) (Johnson 2013a, b) industries. Recent advances in synthetic biology and metabolic engineering for yeasts (e.g., *S. cerevisiae* and *S. pastorianus*) presented new tools, such as genetic engineering toolbox, libraries of synthetic promoters [(e.g., prototrophic markers (*ADE1, HIS2, LEU2, AURA3*) and drug resistance markers (*CUP1, SFA1, ble, kan*)], ribosome binding sites, degradation tags, transcription terminators, plasmids, riboregulators, riboswitches, and more limited CRISPR/Cas9 genome editing (Lian et al. 2018). *S. cerevisiae* was engineered by synthetic biology tools (11.3 kbp *NRPS* gene *pcbAB* and the *NRPS* activator gene *npgA*) associated with long-read DNA sequencing (cytosolic synthesis of amino-adipyl-cysteinyl-valine (ACV)) to produce and secrete a β-lactam NP (benzyl penicillin) against *Streptococcus pyogenes* (Awan et al. 2017). This work opened up the use of baker's yeast (as standard chassis organism) to the rational engineering of NPs derived antibiotics. Recently, SWITCH, a dynamic CRISPR/Cas9 tool, was used for genome engineering and metabolic pathway control of genomic loci (*bts1, ypl062W, yjI064w, rox1*, and *erg9*) for cell factory construction in *S. cerevisiae* to mevalonate production (Jakočiūnas et al. 2016). The production of fuels and chemicals from xylose by engineered *S. cerevisiae* under industrial fermentation conditions to improve the bioconversion of xylose to ethanol (pentose metabolism) was reported to consolidate the key platform for future biorefineries (d'Espaux et al. 2017). Two cytochrome P450 monooxygenases from *Fusarium oxysporum* (FoCYP), FoCYP539A7 and FoCYP655C2, were cloned and heterologously expressed in an engineered *S. cerevisiae* mutant (the acyl-CoA oxidase enzyme and the β-oxidation pathway were inactivated) to provide the production of industrially valuable ω-hydroxy fatty acids (Durairaj et al.