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Chapter 6

Bioprospecting of actinomycetes: Computational drug discovery approach

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Abstract

There is an urgent need for new drugs with increasing threat posed by multidrug resistant bacteria. Among the various sources of natural products actinomycetes hold prominent position due to their diversity and proven ability to produce bioactive metabolites. Generally, analytical instrumentation and chemical methods are widely used to identify and characterize potent compounds, however, of late genomic based approach and metabolomics tools are used for metabolite screening. This article deals with computer aided databases, genome based analysis and metabolomics tools to identify, mine and characterize natural products. A systematic approach including construction of natural product libraries and their crude extracts, dereplication, genome mining, bioinformatics, activation of silent gene clusters and increasing the active compound by precision engineering can lead to novel and potent drugs.

Keywords: marine ecosystem; actinomycetes; bioactive compounds; drug discovery; biodiversity

1. Introduction

The discovery of antibiotics in 19th century was a milestone in modern medicine and conferred one of the greatest benefits on mankind [1]. The availability of antibiotics from microbial products and industrial scale fermentation has allowed the successful treatment of many bacterial infections as well as the ability to perform invasive medical surgeries [2]. These “wonder drugs” categorized as antibiotics also have profound effect on livestock and

agricultural yield; as a result these antibiotics were referred as bioactive microbial metabolites. The continuous and overuse of antibiotics benefitted microorganisms to inherit genes from relative or can be acquired from nonrelatives on mobile genetic elements such as plasmids [3]. The horizontal gene transmission (HGT), spontaneous mutation, drug sensitive competitors' also benefitted bacteria to develop multiple mechanisms of resistance [4].

Another reason for the widespread resistance to antibiotics is the pervasive use of animal feeds to prevent infections. The extensive use of antibiotics in growth supplements for livestocks in both developing and developed countries contributes to increase risk of multiple drug resistant strains. The vicious cycle of antibiotic resistance keeps on repeating, by the use of antibiotics in food after ingestion by the animals it suppresses susceptible bacteria and allows antibiotic-resistant to flourish among the bacteria. The antibiotic resistant bacteria are transmitted to humans by consuming dairy and animal products which cause adverse health effects.

According to WHO (Fact Sheet, September 2016), each and every country contributes to antibiotic resistance. The infections caused by multiple drug resistant strains are at an increased risk of worse clinical outcomes and even death [5]. The centre for disease and control (CDC) has categorized various bacteria into urgent threat (*Clostridium difficile*, Carbepenem-resistant *Enterobacteriaceae* (CRE), Drug-resistant *Nisseria gonorrhoeae*), serious threat (Drug resistant *Campylobacter* sp., Fluconazole-resistant *Candida* sp.), Vancomycin resistant *Enterococci* (VRE), Drug resistant *Shigella*, Methicillin resistant *Staphylococcus aureus*, Drug-resistant *Streptococcus pneumoniae*, Drug-resistant *Mycobacterium* and concerning threat (Vancomycin-resitant *Staphylococcus aureus* (VRSA), Erythromycin-resistant Group A *Streptococcus*, Clindamycin-resistant Group B *Streptococcus*) of bacteria on the basis several factors including 10-year projection of incidence, transmissibility and availability of effective antibiotics, clinical, economic impact as well as barrier to prevention. The information and detailed report regarding alarming antibiotic resistant strains can be found at http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf. Looking at the present scenario of antibiotic resistance worldwide and incredible adaptability of microbes to adapt changes in environment due to their flexible metabolic power encourages researchers to develop therapeutic agents to combat antimicrobial resistance.

Historically, nature has been an enormous source of medicinal active compounds from plant and microbial sources for millennia. Microorganisms contribute majority of antibiotics which are clinically in use today. In golden era of microbial natural product screening, lots of efforts were put forward to screen potent microorganism from soil which has lead us to vast majority of microbial metabolites [6,7].

Actinomycetes are the most diverse microorganism which contributes to 45% of

antibiotics over fungi and unicellular bacteria. They are Gram-positive, spore forming and aerobic bacteria belong to the order Actinomycetales. The name "Actinomycetes" was derived from Greek "atkis" means ray and "mykes" means fungus, thus possessing the characteristics of both prokaryotes-bacteria and Eukaryotes-fungi [8]. This unique group can be characterized with aerial and substrate mycelium growth [9]. Actinomycetes have high GC content in their Deoxyribonucleic acid (DNA), free living microorganisms and are widely distributed in terrestrial and aquatic ecosystems, especially in soil forming as aerial mycelia [10].

The bioactive secondary metabolites produced by actinomycetes include antibiotics, antitumor agents, immunosuppressive agents and enzymes. These metabolites are known to possess antibacterial, antifungal, antioxidant, neuritogenic, anti-cancer, anti- algal, anti-helmintic, anti-malarial and anti-inflammatory [11,12]. Actinobacteria have made remarkable contributions to human life. The class Actinobacteria is especially notable for containing organisms producing diverse natural products, with members of the order Actinomycetales alone accounting for 10,000 such products [13].

2. Habitat of actinomycetes

Actinomycetes species are ubiquitous in nature and are most abundant in soil. They grow as hyphae like fungi responsible for the characteristically "earthy" smell of freshly turned healthy soil [14].They are primarily soil inhabitants [15] but have been found widely distributed in a diverse range of aquatic ecosystem, including sediments obtained from deep sea [16,17], even from greatest depth Mariana Trench [18,19]. Recently marine derived Actinomycetes are seeking attention as they produce novel antibiotic and anticancer agents with unusual structure and properties [20]. Maine Actinomycetes are the promising source for secondary metabolites [21]. It has been estimated that over past 10 years, 659 marine bacterial compounds have been isolated among which 256 compounds have been originated from actinomycetes [22,23]. Marine ecosystem harbours more than two third of the earth. In golden era of microbial natural product screening it was once thought that marine environment had unfavorable conditions for microbial natural products than terrestrial microbes but if we look at present scenario metabolites screening from terrestrial ecosystem leads to rediscovery of antibiotics whereas in the last 10 years research on unexplored marine ecosystem has accelerated in search of novel compounds to fight infectious diseases. Exploration of marine ecosystem attracts the interest of researchers based on four general aspects [24]:

- Biodiversity of microorganisms, especially isolated from unexplored or extreme environments;
- Structural diversity of secondary metabolites;
- Broad spectrum of active compounds; and

- Genetic engineering aimed at producing specific secondary metabolites and increasing the yields of important products.

Dr. Hans Peter- Fiedler Tubingen Germany, Professor Michael Goodfellow from the University of Newcastle Tyne and Professor Alan T. Bull from the University of Kent (UK) collaborated in year 2000-2013 to explore actinomycetes bioactive metabolites from unique terrestrial and marine habitats including sediments of deep-sea trenches located in the Pacific and Atlantic Oceans. All strains were taxonomically characterized to the genus level that permitted an individual submerged cultivation in the screening stage, applying genus-adapted cultivation media. The collaboration resulted in the discovery of various novel metabolites few of them are listed below:

3. Actinomycetes isolated from unique sources

- *Streptomyces sp.* $MECO_2$ was isolated from the stones of one of the Egyptian ancient tombs. Relatively high antitricophyton activity was attained with cultivation medium composed of (g/l): glucose, 5; casein, 0.0075; KNO_3, 0.05; NaCl, 2; K_2HPO_4, 6; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.02 and $FeSO_4.7H_20$, 0.01; pH 7 adjusted using phosphate buffer, inoculums size 2ml/50ml medium and agitation rate of 200 rpm at 30°C for 9 days incubation.

- A novel aerobic actinomycete, designated HA11110T, was isolated from a mangrove soil sample collected in Haikou, China. 16S rRNA gene sequence similarity showed that strain HA11110T belonged to the genus *Streptomyces*, most closely related to *Streptomyces fenghuangensis* GIMN4.003T (99.1 %), *Streptomyces nanhaiensis* SCSIO 01248T (98.8 %) and *Streptomyces radiopugnans* R97T (98.8 %). On the basis of phenotypic and genotypic data, strain HA11110T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces mangrovi* sp. nov. was proposed.

- Rare bioactive actinomycetes were isolated from unexplored regions of Sundarbans mangrove ecosystem and possess 93.57 % similarity with *Streptomyces albogriseolus* NRRL B-1305. The strain SMS_SU21, isolated from mangrove region possesses good antimicrobial and andantioxidant activity.

- Another research leads to discovery of a novel alkaloid, xinghaiamine A, from a marine-derived actinomycete *Streptomyces xinghaiensis* NRRL B24674T. Xinghaiamine A was identified to be a novel alkaloid with highly symmetric structure on the basis of sulfoxide functional group, and sulfoxide containing compound has so far never been reported in microorganisms. Biological assays revealed that xinghaiamine A exhibited broad-spectrum antibacterial activities to both Gram-negative persistent hospital pathogens (e.g. *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli*) and

Gram-positive ones, which include *Staphylococcus aureus* and *Bacillus subtilis*. In addition, xinghaiamine A also exhibited potent cytotoxic activity to human cancer cell lines of MCF-7 and U-937 with the IC50 of 0.6 and 0.5 mM, respectively.

- Jiao [34] and Baskaran [35] isolated 42 actinomycetes isolates from mangrove sediments of Aandaman islands, India. Among 42 isolates, 22 species were found to possess antibacterial property against pathogenic microorganisms.

- Caerulomycin A- antifungal potential was isolated from marine invertebrate-associated *Actinoalloteichus* sp. using optimized medium and fermentation conditions by Marine invertebrate sample was collected from deep sea (Anjuna Beach, Goa, India) [36]..

- Prudhomme [37] findings underline the potential of secondary metabolites, derived from marine microorganisms, to inhibit *Plasmodium* growth. Salinosporamide A, produced by the marine actinomycete, *Salinispora tropica,* shows strong inhibitory activity against the erythrocytic stages of the parasite cycle.

4. Rare actinomycetes

Rare actinomycetes (eg *Nocardia* sp.) are referred to those actinomycete strains whose frequency is much lower than *Streptomyces* strains isolated using conventional methods [38]. The low occurrence of rare actinomycetes in contrast to diverse *Streptomyces* sp. is derived from the facts that they are hard to isolate from the environment and difficult to cultivate and maintain under conventional conditions [6]. Rare actinomycetes demands pre treatment of sample, appropriate isolation procedures and variety of different selection media including enrichment media [39,40] which makes more difficult for their cultivation and maintenance when compared to *Streptomyces* species. The spores of some rare actinomycete genera including *Streptosporangium* and *Microbispora* can withstand pre treatment with various chemicals [40]. They are widely distributed in terrestrial and aquatic ecosystems. Environmental factors such as soil type, pH, humus content, and the characteristics of the humic acid content of the soil affect their distribution [41]. Rare actinomycetes include some of these genera *Actinomadura, Actinoplanes, Amycolatopsis, Actinokineospora, Acrocarpospora, Actinosynnema, Catenuloplanes, Cryptosporangium, Dactylosporangium, Kibdelosporangium, Kineosporia, Kutzneria, Microbiospora, Microtetraspora, Nocardia, Nonomuraea, Planomonospora, Planobispora, Pseudonocardia, Saccharomonospora, Saccharopolyspora, Saccharothrix, Streptosporangium, Spirilliplanes, Thermomonospora, Thermobifida,* and *Virgosporangium* [42].

For the isolation of rare actinomycetes, variety of selective and enrichment media, addition of different antibacterial and antifungal antibiotics to the isolation media [39,43,44] use of xylose, chloride, collidine, bromide and vanillin [41] which act as chemo-attractants for

accumulating spores, chloramine treatment [43], as chlorination is known to suppress growth of contaminant bacteria, use of humic acid-vitamin enriched media [43] and different kinds of radiation [45] favor selective isolation of different actinomycetes genera resulted in successful cultivation of diverse and rare actinomycetes. If we go through previous published work, only 11 rare actinomycetes species producing altogether 50 bioactive compounds were known in 19th century [6]. Today the number of taxonomically described rare actinomycetes is close to 100, and due to recently developed advanced isolation techniques. Hayakawa et al., 1988 [41] investigated the distribution of rare actinomycetes various locations throughout Japan using a special isolation medium, HV agar. The distribution of rare actinomycetes genera in cultivated field soils (154 samples) was remarkable. *Dactylosporangium* and *Microtetraspora, Saccharomonospora*, and*Micromonospora* were most frequently isolated from mountainous forest soils, level-land forest or cultivated field soils, and pasture soils, respectively. [41] has listed out various physical, chemical and enrichment methods to isolate, cultivate and maintain rare actinomycetes. [46] explored Egyptian habitats which could lead to various rare actinomycetes isolates including Micromonospora (23 isolate, 65.71%), less commonly Actinoplanes (11 isolates, 31.43%) and rarely Dactylosporangium (1 strain, 2.86%) genera. In 2008, Eccleston et al. [47] reported the occurrence of *Microsmonospora* from the Sunshine Coast in Australia [47,48,49] reported the isolation of a rifamycin-producing *Micromonospora* from mangrove in South China Sea. Different genera such as *Brevibacterium, Dermabacter, Kytococcus, Microbacterium, Nesterenkonia*, and *Rothia* were isolated from mangrove sediments in Brazil [50]. In China, a number of rare actinobacteria including *Actinomadura, Isoptericola, Microbispora, Nocardia, Nonomuraea*, and *Rhodococcus;* were isolated from mangrove soils and plants [43]. Results from Ara et al. [51] showed that 17 different genera of rare actinobacteria were identified from a total of 241 isolates.

Biopropspecting and understanding of rare actinobacteria diversity led to the discovery of novel genus and species strains, such as "*Microbacterium mangrovi* sp. nov." [52], "*Mumia flava* gen. nov., sp. nov." [53] and *Sinomonas humi* sp. nov." [54] isolated from a mangrove forest in Tanjung Lumpur, Malaysia. The conventional approach to understand biodiversity of actinomycete isolates has come down new approaches including construction of environmental genomic libraries [55] the use of selective isolation media and phylogenetic analysis [56,57] culture-independent methods [58] and digital image analysis [59] have been extensively used to explore novel strains.

5. Approaches to effective drug discovery

With the discovery of Penicillin, millions of microorganisms have been screened for their bioactive potential from various soil samples worldwide. The bioactive metabolites produced are widely used as antibacterial therapeutics, such as erythromycin, streptomycin, tetracycline, vancomycin and chemotherapeutic drugs such as doxorubicin. Actinobacteria

have made remarkable contributions to human. Presently, researchers are hunting for novel strains from unexplored area such as marine ecosystem due to exhaustive culture dependent screening of actinomycete isolates from terrestrial environment leading to rediscovery of bioactive metabolites. There are different approaches which have been employed for novel drug discovery strains:

- Optimization of nutritional requirements to obtain maximum yield of metabolites, crude extracts or purified compounds were screened for biological activity without knowing the drug target. Once the potent compound has been identified, efforts have been made to analyze the target and mode of action of compound including the metabolic pathway. This approach to drug discovery can be categorized as bioactive-guided screening [60].

- Another approach to drug discovery includes the chemical screening of effective metabolite with bioactive potential. It implies sophisticated analytical instrumentation to elucidate the structural properties of metabolites such as high-performance liquid chromatography, mass spectrometry or nuclear magnetic resonance. The substances used in this particular approach were obtained from microbial sources. Nowdays, various metabolomics tools such as Cycloquest, GNPS-Genome to Natural Product Platform, NRPquest (Nonribosomal Peptide), PEP2 path are available to characterize BCG to improve metabolic profile of actinomycetes whereas compound databases including (CHEBI-Chemical Entities of Biological Interest, Chemspider, Novel antibiotics, PubChem, Antibiticome) are extensively used to obtain complete biochemical profile of metabolite.

- The target oriented screening is useful in identifying compounds that hit a known and validated molecular target. These targets represent a cellular and molecular structure involved in pathology of interest that the drug in development is meant to act on. High-throughput metabolic modeling tools allows generation of genome-scale metabolic models enables linking between genotype, metabolic phenotype and biosynthetic gene cluster of secondary metabolite producing organism. The metabolic modeling of genome in metabolomics (secondary metabolites) is very useful in predicting intracellular flux distribution of actinomycetes in specific environmental or genetic condition and gene manipulation targets for overproduction of secondary metabolites. MODEL-SEED is only known high throughput modeling tool which has been deployed to reconstruct multiple actinomycetes species for large scale metabolic studies [61].

6. Genome mining and identification of BCG's

A huge database of mass spectra for known bioactive compounds is available from

chemical libraries and can be efficiently used for dereplication [62]. The major drawback of using these programs is rediscovery of known compounds. To overcome this problem, Genome mining can be the answer. It is an alternative strategy which has become increasingly popular during the last decades [63]. This approach detects and analyses the biosynthetic gene cluster of the chemical compounds and subsequently connect those genes to molecules. The actinomycete genome, for example, contains approximately 8,000 genes coding for 20–50 proteins from secondary metabolite synthetic gene clusters [64]. Bioinformatics technologies allow the rapid identification of known gene clusters encoding bioactive compounds and to make computer predictions of their chemical structure based on genetic sequence information [65,66]. The chemical classes and the structure of encoded compound can be predicted using biosynthetic gene cluster approach. This information can be used to either guide a more targeted drug discovery technique (such as reactivity-guided isolation; [67] or peptide and glycogenomic approaches [68,69] or allow the heterologous expression in an optimized expression host (activation of silent gene cluster). There are web based comprehensive suite such as BAGEL-Bayesian analysis of Gene essentiality which can be utilized to identify the genes encoding precursor peptides whereas identifying BCG's with BLAST and HMMeR provide us insight of polyketides synthesized by type I and type II PKS, ribosomally and post translationally modified peptides (RiPPS).

Another approach to this problem can be microbial natural product library. The primary approach to discovery of novel natural products from extract is bioassay guided fractionation. The combined use of LC, solid-phase extraction (SPE) and NMR spectroscopy (LC-SPE-NMR) has been developed and continues to undergo refinement via expansion of the types of NMR analysis one can perform on samples once they've been separated, removed from LC mobile phase and placed into deuterated solvents [70-74,75]. Sophisticated instrumentation permits the rapid identification of minor and major secondary metabolites in natural product. However, cost and effort requirements, for the foreseeable future, limit the availability of such techniques to many academic natural product laboratories. Once a crude extract library has been established it can be used for target-based and whole-cell highthroughput screens related to infectious disease for the identification of active natural products [24].

Recently, various bioinformatics approaches have been developed to organize or interpret large sets of MS/MS fragmentation data. For example, solutions such as MAGMa (MS annotation based on in silico generated metabolites) allow matching of multistage fragmentation data against candidate molecules substructures and were successfully applied on complex extracts [76-78]. Among these new approaches, molecular networking (MN) is a particularly effective one to organize MS/MS fragmentation spectra. MN compares all MS/MS spectra in a given extract and groups them according to their similarity [79-82] The applications of these tools lead to the identification of novel compounds by avoiding re-isolation of known compounds

which could flourish the idea of developing novel drugs.

7. Future prospects

With the increase in population worldwide it is very difficult to combat emerging antibiotic resistance in pathogenic microorganisms due to their adaptive metabolism. Natural products and their derivatives historically have been an inevitable source of therapeutic agents. However from past decade microbial natural product research in pharmaceutical industry has declined due to lack of compatibility of traditional natural-product extract libraries with high throughput screening. Lack of required purity, availability and productivity of novel strains and pure compounds are few reasons for progressively decline in identification bioactive natural product with HTS.

- For novel natural products, microbes that are morphologically distinctive, taxonomically new, or isolated from ecologically unique region or sources which have not been screened for natural products can be lead to new active compounds.

- By implying high throughput screening programs, cell and target based assays is capable of detecting most representative natural products with new chemotypes [24].

- Genome mining for drug targets and computational based screening programmes revealed much bigger potential to synthesize natural products than have been isolated from conventional approaches. For example, GlaxoSmithKline has conducted studies with the antibiotic GKS299423 acting on topoisomerase II, in order to prevent the bacteria from developing resistance [83].

- High performance liquid chromatography bioassays and Liquid Chromatography-Mass Spectrometry for active fractions can easily detect dereplication of known natural products from new ones leading to their structure elucidation.

- Molecular approaches also act as key source in search of novel bioactive metabolites. Culture-independent molecular approach studies employ direct extraction of nucleic acids from the samples [58]. It often involves the amplification of DNA or cDNA from RNA extracted from environmental samples by PCR and the subsequent analysis of the diversity of the amplified molecules (community fingerprinting) [13].

- Metagenomic and metaproteomic technologies enable powerful new approaches to gene, genome, protein and metabolic pathway discovery [84. This new source of metabolic and chemical diversity will lead to important new basic knowledge and also contribute to ongoing drug discovery efforts against many disease indications. For example, Sherman and colleagues recently reported a model system for developing a meta-genomic approach to identify and characterize the natural product pathways

from invertebrate-derived microbial consortia using the ET-743 (Yondelis) biosynthetic pathway[84].

- Another approach to overcome the problem of antibiotic resistance is the development of new drug combination or to develop the synergistic effect of drugs. Recently this approach has been proven to be successful in the treat of tuberculosis and HIV infected patients [63]

8. Table

Table 1: Novel metabolites produced by actinomycete isolates from unique sources

Antibiotic (isolated secondary metabolite)	Nature of antibiotic	Area explored	Name of the strain	Ref
Frigocyclinone	Angucyclinone	Antarctica	*Streptomyces griseus* NTK 97	[25]
Gephyromycin	Angucyclinone antibiotic	Antarctica	*Streptomyces griseus* NTK 14	[26]
Albidopyrone	Pyrone Antibiotic Inhibitory activity against protein-tyrosin phosphokinase 1B	North-Atlantic Ocean	*Streptomyces* strain NTK 227	[27]
Caboxamycin:	Benzoxazol antibiotic-Antibacterial, antitumor and phosphodiesterase inhibitory activities	Canary Basin at -3814 m	*Streptomyces* strain NTK 937	[27]
Abyssomicins B, C, D, G, H and atrop-Abyssomicin C	Polycyclic, Polyketide antibiotics Antibacterial activity against Gram-positive bacteria including MRSA and Vancomycin-resistant strains	Deep sea sediments	*Verrucosispora maris* AB-18-032	[28]
Atacamycins A–C	Macrolactone Antibioticsantitum and Phosphodiesterase inhibitoring activity	Hyper-arid soil from the Atacama Desert, North Chile	*Streptomyces* sp. C38	[29]
Dermacozines A–L	Phenazine antibiotics free radical scavenging activity, Antitumor and antiparasitic activities	Deep sea Sediments	*Dermacoccus abyssi* MT1.1 and MT1.2	[30
Proximicins A–C	Aminofuran antibiotics – strong antitumor activity	Deep sea Sediments	Marine strain *Verrucosispora fiedleri* MG-37	[31]
Pyrocoll	Diketopiperazine antibiotic Antibacterial, antiparasitic and antitumor active	Soil sample collected at Consett, County Durham, United Kingdom	Alkaliphilic *Streptomyces* strain AK 409,	[32]
Bendigoles A–C	Steroid metabolites	Activated sludge foam-Bendigo Biological Nutrient Removal Plant, Victoria, Australia	*Gordonia australis Acta* 2299	[31]
Langkocyclines A1, A2, A3, B1 and B2	Angucycline- Antibacterial and antitumor activities	Rhizosphere soil sample-Fine roots of the creeper *Clitorea* sp., growing on the sandy beach at Burau Bay, main Langkawi Island, Malaysia	*Streptomyces* sp. Acta 3034	[33]

9. Conclusions

By considering the present scenario of antibiotic resistance globally, there is no doubt that we will need antibiotics in future and we cannot decline the fact that natural products are most likely the best source. Among microorganisms, Actinomycetes are prolific producers of secondary metabolites and bioactive compounds contributing 70-80% of antibiotics commercially. However efforts have been made to cultivate rare and novel isolate from unexplored natural sources but steady decline in identifying novel compounds using conventional methods is nowhere leading us to discovery of pharmaceutical compounds. These challenges in microbial natural product discovery encourage researches to utilize computational, molecular and bioinformatics drug discovery programs to overcome the flaws in bioactive-guided screening, chemical screening, target oriented screening and high throughput screening. Genome mining approaches such as mining for biosynthetic genes, resistance genes, regulators, regulator guided activation, activation of silent gene clusters, target guided screening, bioactivity driven screening and chemical screening including real time mass-spectrometer, high-resolution mass-spectrometry can eliminate the rediscovery of known natural products from Actinomycetes isolates. These new approaches can lead us to successful novel compounds from unexplored regions or sources such as marine ecosystem.

10. References

1. Berdy, J. (2012): Thoughts and facts about antibiotics: where we are now and where we are heading. J Antibiot, 65: 385–395.

2. Hancock, R.E.W. (2001): Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infect. Dis, 1:156–164.

3. Read, A.F., Woods, R.J. (2014): Antibiotic resistance management. Evol Med Public Health, 28:147.

4. Davies, J., Davies, D. (2010): Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev, 74: 417-33.

5. The Review on Antimicrobial Resistance. Antimicrobial resistance: tackling a crisis for the health and wealth of nations London; The Review on Antimicrobial Resistance (2014) http://amrreview.org/sites/default/files/SECURING%20NEW%20DRUGS%20FOR%20FUTURE%20GENERATIONS%20FINAL%20WEB_0.pdf

6. Berdy, J. (2005): Bioactive microbial metabolites. J Antibiot, 58: 1–26.

7. Monciardini, P., Iorio, M., Maffioli, S., Sosio, M., Donadio, S. (2014): Discovering new bioactive molecules from microbial sources. Microb Biotechnol, 7: 209–220.

8. Das, S., Lyla, P.S., Khan, S.A. (2008): Distribution and generic composition of culturable marine actinomycetes from the sediments of Indian continental slope of Bay of Bengal. Chin J Oceanol Limnol, 26: 166- 77.

9. Lechevalier, H, Lechevalier, M.P. (1981): Introduction to the order Actinomycetales. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG,Editors. The Prokaryotes. Germany: Springer-Verlag Berlin, 2: 1915-22.

10. Yoshida, A., Seo, Y., Suzuki, S., Nishino, T., Kobayashi, T., Hamada-Sato, N., Kogure, K., Imada, C. (2008): Actinomycetal community structures in seawater and freshwater examined by DGGE analysis of 16S rRNA gene fragments. Mar. Biotechnol. 10: 554–563.

11. Kekuda, T.R.P., Shobha, K.S., Onkarappa, R. (2013): Studies on antioxidant and anthelmintic activity of two Streptomyces species isolated from Western Ghat soils of Agumbe, Karnataka. J Pharm Res, 3: 26-9.

12. Ravikumar, S., Inbaneson, S.J., Uthiraselvam, M., Priya, S.R., Ramu, A., Banerjee, M.B. (2011): Diversity of endophytic actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens. J Pharm Res, 4: 294-6.

13. Bull, A.T, Stach, J.E.M. (2007): Marine actinobacteria: new opportunities for natural product search and discovery. Trends Microbiol, 15: 491–499

14. Sprusansky, O., Stirrett, K., Skinner, D., Denoya, C., Westpheling, J. (2005): The bkdR gene of Streptomyces coelicolor is required for morphogenesis and antibiotic production and encodes a transcriptional regulator of a branched-chain amino acid dehydrogenase complex. J Bacteriol. 187: 664-71.

15. Kuster, E. (1968): Taxonomy of soil actinomycetes and related organisms. In: Gray S, Parkinson T,Editors. Ecology of soil bacteria. Liverpool: Liverpool University Press.

16. Walker, D., Colwell, RR. (1975): Factors affecting enumeration and isolation of actinomycetes from Chesapeake Bay and South eastern Atlantic Ocean sediments. Mar Biol, 30: 193-201.

17. Colquhoun, J.A., Mexson, J., Goodfellow, M., Ward, A.C., Horikoshi, K., Bull, A.T. (1998): Novel rhodococci and other mycolate actinomycetes from the deep sea. Antonie van Leeuwenhoek. 74: 27-40.

18. Takami, H., Inoue, A., Fuji, F., Horikoshi, K. (1997): Microbial flora in the deepest sea mud of the Mariana Trench. FEMS Microbiol Lett, 152: 279-85.

19. Pathomaree, W., Stach, J.E., Ward, A.C., Horikoshi, K., Bull, A.T., Goodfellow, M. (2016): Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. Extremophiles, 10: 181-9.

20. Jensen, P.R., Williams, P.G., Oh, D.C., Zeigler, L., Fenical, W. (2007): Species-specific secondary metabolite production in marine actinomycetes of the genus Salinispora. Appl Environ Microbiol, 73: 1146-52.

21. Lam, K.S. (2005): Discovery of novel metabolites from marine actinomycetes, Currt Opn Microbiol, 9: 245-251.

22. William, P.G. (2008): Panning for chemical gold marine bacteria as a new source of therapeutics trends in Biotechnol 27: 45-52.

23. Sarkar, S., Saha, M., Roy, D., Jaisankar, P., Das, S., Roy, S., Rattan Gucchi, L.J., Sen, T., Mukherjee, J. (2008): Enhanced production of antimicrobial compounds by three salt tolerant actinobacterial strains isolated from Sundarbans in niche mimic bioreactor, Marine Biotechnology, 10: 518-526.

24. Liu, X., Bolla, K., Ashforth, E.J., Zhuo, Y., Gao, H., Huang, P., Stanley, S.A., Hung, D.T., Zhang, T. (2012): Systematics-guided bioprospecting for bioactive microbial natural products Antonie van Leeuwenhoek, 101: 55–66.

25. Bruntner, C., Binder, T., Pathom-aree, W., Goodfellow, M., Bull, A.T. , Potterat, O., Puder, C., Hörer, S., Schmid, A., Bolek, W., Wagner, K., Mihm, G., Fiedler, H.P. (2005): Frigocyclinone, a Novel Angucyclinone Antibiotic Produced by a Streptomyces griseus Strain from Antarctica. J. Antibiot. 58: 346–349.

26. Bringmann, G., Lang, G., Maksimenka, K., Hamm, A., Gulder, T. A.M., Dieter, A., Bull, A.T., Stach, J.E.M., Kocher, N., Müller, W.E.G., Fiedler, H.P. (2005): Gephyromycin, the first bridged angucyclinone, from Streptomyces griseus strain NTK 14. Phytochem. 66: 1366-1373

27. Hohmann, C., Schneider, K., Bruntner, C., Brown, R., Jones, A.L., Goodfellow, M., Krämer, M., Imhoff, J.F., Nicholson, G., Fiedler, H.P., Süssmuth, R.D. (2009): Albidopyrone, a new alpha-pyrone-containing metabolite from marine-derived Streptomyces sp. NTK 227. J. Ant. 62: 75–79.

28. Keller, S., Nicholson, G., Drahl, C., Sorensen, E., Fiedler, H.P., Süssmuth, R.D. (2007): Abyssomicins G and H and

atrop-Abyssomicin C from the Marine Verrucosispora Strain AB-18-032. J. Antibiot. 60, 391–394.

29. Nachtigall, J., Kulik, A., Helaly, S., Bull, A.T., Goodfellow, M., Asenjo, J.A., Maier, A., Wiese, J., Imhoff, J.F., Süssmuth, R.D., Fiedler, H.P. (2011): Atacamycins A-C, 22-membered antitumor macrolactones produced by Streptomyces sp. C38. J Antibiot (Tokyo), 64: 775-80.

30. Abdel-Mageed, W.M., Milne, B.F., Wagner, M., Schumacher, M., Sandor, P., Pathom-aree, W., Goodfellow, M., Bull, A.T., Horikoshi, K., Ebel, R., Diederich, M., Fiedler, H.P., Jaspars, M. (2010): Dermacozines, a new phenazine family from deep-sea dermacocci isolated from a Mariana Trench sediment. Org. Biomol Chem., 21: 2352-62.

31. Schneider, K., Graf, E., Irran, E., Nicholson, G., Stainsby, F.M., Goodfellow, M., Borden, S.A., Keller, S., Süssmuth, R.D. (2008): Bendigoles A~C, New Steroids from Gordonia australis Acta 2299. J. Antibiot. 61 :356-64.

32. Dietera, A., Hamm, A., Fiedler, H.P., Goodfellow, M., Müller, W.E., Brun, R., Beil, W., Bringmann, G. (2003): Pyrocoll, an antibiotic, antiparasitic and antitumor compound produced by a novel alkaliphilic Streptomyces strain. J Antibiot (Tokyo). 56: 639-46.

33. Kalyon, B., Tan, G.Y., Pinto, J.M., Foo, C.Y., Wiese, J., Imhoff, J.F., Süssmuth, R.D., Sabaratnam, V., Fiedler, H.P. (2013): Langkocyclines: novel angucycline antibiotics from Streptomyces sp. Acta 3034. J. Antibiot. 66: 609-16.

34. Jiao, W., Zhang, F., Zhao, X., Hu, J., Suh, J.W. (2013): A Novel Alkaloid from Marine-Derived Actinomycete Streptomyces xinghaiensis with Broad-Spectrum Antibacterial and Cytotoxic Activities. PLoS ONE 8: 75994.

35. Baskaran, R., Vijayakumar, R., Mohan, P.M. (2011): Enrichment method for isolation of bioactive actinomycetes from mangrove sediments of Andaman islands, India. Mal J Microbiol, 7: 26-32.

36. Ambavane, V., Tokdar, P., Parab, R., Sreekumar, E.S., Mahajan. G., Mishra, P.D., Lisette D'Souza, L., Ranadive, P. (2014): Caerulomycin A—An Antifungal Compound Isolated from Marine Actinomycetes Advances in Microbiology, 4,: 567-578.

37. Prudhomme, J., McDaniel, E., Ponts, N., Bertani, S., Fenical, W., Jensen, P. (2008): Marine Actinomycetes: A New Source of Compounds against the Human Malaria Parasite. PLoS ONE, 3: 2335.

38. Tiwari, K., Rajinder, K. (2012): Gupta Rare actinomycetes: a potential storehouse for novel antibiotics Critical Review Biotech, 32: 108–132

39. Qiu, D., Ruan, J., Huang, Y. (2008 Selective isolation and rapid identification of members of the genus Micromonospora. Appl Environ Microbiol,74: 5593–7.

40. Khanna, M., Solanki, R., Lal, R. (2011): Selective isolation of rare actinomycetes producing novel antimicrobial compounds. Int J Adv Biotechnol Res, 2: 357–75.

41. Hayakawa, M. (2008): Studies on the isolation and distribution of rare actinomycetes in soil. Actinomycetologica, 22: 12–9

42. Lazzarini, A., Cavaletti, L., Toppo, G., Marinelli, F. (2001): Rare genera of actinomycetes as potential producers of new antibiotics. Antonie van Leeuwenhoek, 78: 399–405.

43. Hong, K., Gao, A.H., Xie, Q.Y., Gao, H., Zhuang, L., Lin, H.P. (2009): Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. Mar Drugs, 7: 24–44.

44. Zhang, J., Zhang, L. (2011): Improvement of an isolation medium for actinomycetes. Mod Appl Sci, 5: 124–7.

45. Bredholdt, H., Galatenko, O.A., Engelhardt, K., Fjaervik, E., Terekhova, L.P., Zotchev, S.B. (2007): Rare actinomycete bacteria from the shallow water sediments of the Trodheim fjord, Norway: isolation, diversity and biological activity. Environ Microbiol, 9: 2756–64

46. Rifaat, H.M., Nagieb, Z.A., Ahmed, Y.M. (2005):Production of xylanases by Streptomyces species and their bleaching

effect on rice straw pulp. Appl. Ecol. and Environment. Res.4: 151–160.

47. Eccleston, G.P., Brooks, P.R., Kurtböke, D.I. (2008): The occurrence of bioactive micromonosporae in aquatic habitats of the Sunshine Coast in Australia. Mar. Drugs, 6: 243–261.

48. Xie, X.C., Mei, W.L., Zhao, Y.X., Hong, K., Dai, H.F. (2006): A new degraded sesquiterpene from marine actinomycete Streptomyces sp. 0616208. Chin. Chem. Lett, 17:1463–1465.

49. Huang, H.Q., Lv, J.S., Hu, Y.H., Fang, Z., Zhang, K.S., Bao, S.X. (2008): Micromonospora rifamycinica sp. nov., a novel actinomycete from mangrove sediment. Int J Syst Evol Microbiol, 58: 17–20.

50. Dias, A.C.F., Andreote, F.D., Dini-Andreote, F., Lacava, P.T., Sá, A.L.B., Melo, I.S. (2009b): Diversity and biotechnological potential of culturable bacteria from Brazilian mangrove sediment. World J Microbiol Biotechnol, 25: 1305–1311.

51. Ara, I., Bakir, M.A., Hozzein, W.N., Kudo, T. (2013): Population, morphological and chemotaxonomical characterization of diverse rare actinomycetes in the mangrove and medicinal plant rhizosphere. Afr J Microbiol Res, 7: 1480–1488.

52. Lee, L.H., Azman, A.S., Zainal, N., Eng, S.K., Ab Mutalib, N.S., Yin, W.F. . (2014c): Microbacterium mangrovi sp. nov., an amylolytic actinobacterium isolated from Tanjung Lumpur mangrove forest. Int J Syst Evol Microbiol 64: 3513–3519.

53. Lee, L.H., Zainal, N., Azman, A.S., Eng, S.K., Ab Mutalib, N.S., Yin W.F. (2014b): Streptomyces pluripotens sp. nov., a bacteriocin-producing streptomycete that inhibits meticillin-resistant Staphylococcus aureus. Int J Syst Evol Microbiol 64: 3297–3306.

54. Lee, L.H., Azman, A.S., Zainal, N., Yin, W.F., Ab Mutalib, N.S., Chan, K.G. (2015): Sinomonas humi sp. nov., an amylolytic actinobacterium isolated from mangrove forest soil. Int J Syst Evol Microbiol 65: 996–1002.

55. Donadio, S., Monciardini, P., Alduina, R., Mazza, P., Chiocchini, C., Cavaletti, L., Sosio,M., Puglia, A.M. (2002): Microbial technologies for the discovery of novel bioactive metabolites. J Biotechnol, 99: 187–198.

56. Jensen, P.R., Mafnas, C. (2006): Biogeography of marine actinomycete Salinospora. Enviornt. Microbiol. 8: 1881-1888.

57. Hozzein, W.N, Ali, M.I.A., Rabie, W. (2008): A new preferential medium for enumeration and isolation of desert actinomycetes. World J Microbiol Biotechnol, 24: 1547–52.

58. Mincer, T.J., Fenical, W., Jensen, P.R. (2005): Culture-dependent and culture-independent diversity within the obligate marine actinomycete genus Salinispora. Appl Environ Microbiol, 71: 7019–28.

59. Velho-Pereira, S., Kamat, N. (2010): Digital image analysis of actinomycetes colonies as a potential aid for rapid taxonomic identification. Nat Precedings, http://dx.doi.org/10.1038/npre.2010.4209.1

60. Lee, J.A., Uhlik, M.T., Moxham, C.M., Tomand, D., Sall, D.J. (2012): Modern phenotypic drug discovery is a viable, neoclassic pharma strategy. J Med Chem, 55: 4527–4538.

61. Weber, T., Kim, H. (2016): The secondary metabolite bioinformatics portal: Computational tools to facilitate synthetic biology of secondary metabolite production. Sys. Synth. Biotech, 1: 69-79.

62. Wohlleben, W., Mast, Y., Stegmann, E., Ziemert, N. (2016): Antibiotic drug discovery. Microb. Biotech. 9: 541-548.

63. Ziemert, N., Alanjary, M., Weber, T. (2016): The evolution of genome mining in microbes - a review. Nat Prod Rep, doi:10.1039/C6NP00025H.

64. Galm, U., Shen, B. (2006): Expression of biosynthetic gene clusters in heterologous hosts for natural product

production and combinatorial biosynthesis. Expert Opin Drug Discov, 1: 409–437.

65. Zazopoulos, E., Huang, K., Staffa, A., Liu, W., Bachmann, B.O., Nonaka, K. (2003): A genomics guided approach for discovering and expressing cryptic metabolic pathways. Nat Biotechnol, 21:187–190.

66. Farnet, C.M., Zazopoulos, E. (2005): Improving drug discovery from microorganisms. In: Zhang L, Demain AL (eds) Natural products: drug discovery and therapeutics. Humana Press, New York, pp 95–106.

67. Castro-Falcon, G., Hahn, D., Reimer, D., Hughes, C.C. (2016): Thiol probes to detect electrophilic natural products based on their mechanism of action. Acs Chemical Biology. 11: 2328-2336.

68. Kersten, R.D., Yang, Y.L., Xu, Y., Cimermancic, P., Nam, S.J., Fenical, W. (2011): A mass spectrometry-guided genome mining approach for natural product peptidogenomics. Nat Chem Biol, 7: 794–802.

69. Kersten, R.D., Ziemert, N., Gonzalez, D.J., Duggan, B.M., Nizet, V., Dorrestein, P.C., Moore, B.S. (2013): Glycogenomics as a mass spectrometry-guided genome-mining method for microbial glycosylated molecules. PNAS 110: 4407–E4416.

70. Larsen, T.O., Smedsgaard, J., Nielsen, K.F., Hansen, M.E., Frisvad, J.C. (2005): Phenotypic taxonomy and metabolite profiling in microbial drug discovery. Nat. Prod. Rep., 22: 672-695

71. Bobzin, S.C., Yang, S., Kasten, T.P. (2000): LC–NMR: a new tool to expedite the dereplication and identification of natural products. J Ind Microbiol Biotechnol, 25: 342–345

72. Wolfender, J.L., Waridel, P., Ndjoko, K., Hobby, K.R., Major, H.J., Hostettmann, K. (2000): Evaluation of Q-TOF-MS/MS and multiple stage IT-MSn for the dereplication of flavonoids and related compounds in crude plant extracts. Analusis 28: 895-906.

73. Gu, J.Q., Wang, Y.H., Franzblau, S.G., Montenegro, G., Timmermann, B.N. (2006): Dereplication of pentacyclic triterpenoids in plants by GC-EI/MS. Phytochem Anal, 17: 102–106

74. Konishi, Y., Kiyota, T., Draghici, C., Gao, J.M., Yeboah, F., Acoca, S., Jarussophon, S., Purisima, E. (2007): Molecular formula analysis by an MS/MS/MS technique to expedite dereplication of natural products. Anal Chem, 79: 1187–1197.

75. Lambert, M., Staerk, D., Hansen, S.H., Sairafianpour, M., Jaroszewski, J.W. (2005): Rapid extract dereplication using HPLC-SPENMR: analysis of isoflavonoids from Smirnowia iranica.

76. Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G.F., Chater, K.F. (2007): Genomics of Actinobacteria: Tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev, 71: 495-548.

77. Ridder, L., Van der Hooft, J.J., Verhoeven, S., De Vos, R.C., Vervoort, J., Bino, R.J. (2014): In silico prediction and automatic LC-MS annotation of green tea metabolites in urine. Anal Chem, 86: 4767–4774.

78. Allard, P.M., Péresse, T., Bisson, J., Gindro, K., Marcourt, L., Pham, V.C. (2016): Integration of molecular networking and in-silico MS/MS fragmentation for natural products dereplication. Anal Chem, 88: 3317–3323.

79. Watrous, J., Roach, P., Alexandrov, T., Heath, B.S., Yang, J.Y., Kersten, R.D. (2012): Mass spectral molecular networking of living microbial colonies. PNAS, 109: 1743–E1752.

80. Bandeira, N. (2011): Protein identification by spectral networks analysis. Methods Mol Biol, 694: 151–168

81. Liu, W.T., Lamsa, A., Wong, W.R., Boudreau, P.D., Kersten, R., Peng, Y. (2013): MS/MS-based networking and peptidogenomics guided genome mining revealed the stenothricin gene cluster in Streptomyces roseosporus. J Antibiot, 67: 99–104.

82. Fang, J., Dorrestein P.C. (2014): Emerging mass spectrometry techniques for the direct analysis of microbial colonies. Curr Opin Microbiol, 19: 120–129.

83. Jones, D. (2010): The antibacterial lead discovery challenge. Nat Rev Drug Discov, 9: 751–2.

84. Rath, C.M., Janto, B., Earl, J., Ahmed, A., Hu, F.Z., Hiller, L. (2011): Metageomic characterization of the marine invertebrate microbial consortium that produces the chemotherapeutic natural product ET-743. ACS Chem Biol, 6:1244–56.