Annals of Phytomedicine 6(1): 13-19, 2017
DOI: 10.21276/ap.2017.6.1.3 ; Print ISSN : 2278-9839 and Online ISSN : 2393-9885
Ann. Phytomed., 6(1):13-19 (2017)

Review article

Reappraisal of actinomycetes for novel bioactive metabolites

Aabid Manzoor Shah, Shahid Rasool*, Aasif Majeed, Saleem Mushtaq, Mudasir Hafiz Khan, Aehtesham Hussain, Aiyatullah Shah and Qazi Parvaiz Hassan**

Microbial Biotechnology Division, CSIR-Indian Institute of Integrative Medicine, Sanatnagar, Srinagar-190005, Jammu and Kashmir, India
**Genetic Resources and Agrotechnology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, Jammu and Kashmir, India*
***Department of Agriculture Kashmir, Lal Mandi, Srinagar-190018, Jammu and Kashmir, India*

Received May 3, 2017: Revised June 7, 2017: Accepted June 12, 2017: Published online June 30, 2017

Abstract

The appearance of new deadly diseases like cancer and the burgeoning problem of drug resistance among common bacterial pathogens are a serious threat to available treatments. Since the channels of compounds under development are limited, this necessitates the discovery of new drugs. It is where actinomycetes can complement in the accomplishment of development of therapeutically new bioactive compounds, predominantly used in antibiotic production. Actinomycetes are diverse in their location and have proven ability to produce new bioactive compounds. By employing modern microbiological and molecular technologies, the target-directed search for detection and isolation of bioactive actinomycetes is gaining more strength. Therefore, the innovative isolation of actinomycetes from extreme ecosystems, their identification and cultivation using novel techniques are imperative to pursue for drug discovery.

Key words: Drug resistance, bioactive compounds, actinomycetes, extreme ecosystems, drug discovery

1. Introduction

Natural products are the chemical substances produced by living organisms of any source such as plants, animals or microorganisms. Some of the natural products have historically been proved to be the backbone of modern drug discovery efforts either from micro-organisms (*e.g*., doxorubicin from *Streptomyces peucetius*), plants (*e.g*., paclitaxel from *Taxus brevifolia*) or animals (*e.g*., vitamins A and D from cod-liver oil). The discovery of natural compounds as active principles was first described in the beginning of 19th century. Morphine produced and commercialized by E. Merck for the first-time in1826, was one among the first isolated compounds. Today in pharmacology drugs of diverse range which represent the keystone of modern healthcare are either natural products or their derivatives (Chin *et al*., 2006). In 2001, eight of the 30 top-selling drugs (*i.e*., simvastatin, pravastatin, amoxicillin, clavulanic acid, azithromycin, ceftriaxone, paclitaxel and cyclosporine), amounting US $16 billion in sales, were natural products or their derivatives (Sarker and Nahar, 2006). The most commonly known natural resources continue to provide molecules of greater structural diversity and offer major opportunities for the discovery of drug leads. Microbial diversity represents an important route to the discovery of new chemical entities and constitutes a prominent reservoir of novel chemistry and innovative biotechnology. The microbes are considered as miniatures of chemical factories because of their great potential to provide broad spectrum of structurally diverse secondary metabolites that in turn possess diverse biological activities and are therefore, prospective candidates for drug discovery. Of the total bioactive compounds isolated from microorganism, 45% are produced by actinomycetes, 38% by fungi, and 17% by other bacteria, thus among microbes, actinomycetes are economically and biotechnologically important (Berdy, 2005). Actinomycetes are group of gram-positive filamentous bacteria found in both terrestrial and aquatic ecosystems. After the isolation of actinomycin and streptomycin from actinomycetes, they have taken their prominent and permanent place in antibiotic research (Schatz *et al*., 1944). The initial work on actinomycetes was carried by Selman Waksman (1950) and this persuaded many others to carry research on them. From 1940s and 1950s, considered as the golden era of antibiotic research, most of the antibiotics were isolated and discovered mainly from streptomyces species. Of the total antibiotics discovered, about 80% of them were isolated from actinomycetes, mostly from genera, *Streptomyces* and *Micromonospora* (Pandey *et al*., 2004). A representative list of antibiotics with clinical significance produced by actinomycetes

Author for correspondence: Dr. Qazi Pervaiz Hassan
Senior Scientist, Microbial Biotechnology Division, CSIR-Indian Institute of Integrative Medicine, Sanatnagar, Srinagar-190005, Jammu and Kashmir, India
E-mail: qphassan@iiim.ac.in; pervaizqazi@yahoo.com
Tel.: +91-9596050105

are *Kanamycin, Gentamycin, Neomycin, Streptocin, Streptomycin, Chloramphenicol, Novobiocin, Spectinomycin, Thiostrepton, Lincomycin, Clindamycin, Thiostrepton, Ramoplanin, Vancomycin, Teicoplanin, Streptothricin, Daptomycin, Carbomycin, Erythromycin, Oleandomycin, Spiramycin, Abyssomicins, Rifamycin, Pyridomycin, Streptogramin A, Micromonosporin, Chlortetracycline, Oxy tetracycline, Thiolactomycin, Bonactin, etc.* (Mahajan and Balachandran, 2014). Actinomycetes are also recognised as potential source of antitumor compounds, majority of them have clinical importance such as peptides (*bleomycin* and *actinomycin* D), anthracyclines (*aclarubicin, daunomycin* and *doxorubicin*), enediynes (*neocarzinostatin*), aureolic acids (*mithramycin*), antimetabolites (*pentostatin*), carzinophilin, *mitomycins* (Olano *et al*., 2009). As actinomycetes have long been known as the excellent source of therapeutic compounds, the search for novel therapeutic agents is in progress and one of the major goals of microbiologists is to isolate from them, undetected new bioactive molecules. Selection of novel antibiotics from actinomycetes has intensely been carried by researchers through several years. Only a fraction of compounds isolated from Streptomyces are known. The isolation of remaining battery of compounds requires screening of unexplored environments and utilising novel techniques of study (Ramazani *et al*., 2013). It is thus, suggested to concentrate on those actinomycetes, inhabiting extreme ecosystems. Since these actinomycetes are not part of the previously isolated ones, there is high probability that these actinomycetes may be source of novel bioactives. Thus, taxonomists, biotechnologists, ecologists and several other scientists focus on those unexplored regions inhabited by novel actinomycetes and utilise novel selective isolation strategies (Baltz, 2008). Hence, this review covers the present scenario of diversity of actinomycetes, their bioactive compounds and even the possibilities to get novel actinomycetes that may have potential to produce novel compounds.

2. Actinomycetes and their biotechnological importance

The word "actinomycetes" is a combination of two Greek words "atkis" (a ray) and "mykes". These microorganisms resemble both fungi (to form mycelia of branching filaments) and bacteria (the structure of muramic acid containing bacterial cell wall). Actinomycetes are a heterogeneous group of high GC gram-positive bacteria. They are free living, filamentous, aerobic, saprophytic bacteria, cosmopolitan in their distribution, mostly found in terrestrial, aquatic environments and are also found colonizing plants. They are diverse in their chemical composition, morphology and show distinct evolutionary lineage. They are classified in the Kingdom: Bacteria, Phylum: Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae, eight diverse families such as Streptomyceteceae, Micromonosporaceae, Actinomycetaceae, Frankiaceae, Mycobacteriaceae, Actinoplanaceae, Dermatophilaceae and Nocardiaceae, which comprises sixty three genera. They show filamentous structure which can be branched to form a stable mycelium. The mycelium is not always intact or stable and may break into rod or coccus shaped fragments. When grown, the actinomycetes branch profusely both on the surface of agar and underneath it, forming a network of mycelia. The mycelia growing on the surface are called aerial mycelia, and those beneath the agar surface are called substrate mycelia (Balagurunathan and Radhakrishnan, 2010). Aerial mycelia bear spores or conidia which are reproductive parts employed in asexual reproduction. There is a great variation in cell wall composition among different groups of actinomycetes and this is considered as a characteristic feature for the identification and classification of novel genera of class actinobacteria.

Actinomycetes are considered as valuable repositories of bioactive metabolites. Isolation, characterization and screening of promising strains of actinomyceteshas seen to produce potential secondary metabolites and has attracted attention of major researchers worldwide. Among various genera of actinomycetes; *Saccharopolyspora, Micromonospora, Amycolatopsis, Streptomyces and Actinoplanes* are commercially important producers of biomolecules (Solanki *et al*., 2008). They are used as antibiotics against different microorganism, can kill cancerous cells, suppress immune system to protect against autoimmune diseases and are targeted as antiparasitic and antifungal agents. In plant biotechnology, these metabolites are used as pesticides and herbicides. About 70% of all known drugs have been isolated from actinomycetes. Of this percentage, 75% is used in medicine and 60% in agriculture (Atta, 2009). Actinomycetes are considered important not only because of potent therapeutic activities associated with their extracts but also for the fact that they often possess desired pharmacokinetic characteristics required for clinical advancement (Farnet and Zazopoulos, 2005). The actinomycetes are important not only in the field of pharmaceutical industries but also in the agriculture. The potential of actinomycetes to inhibit the growth of phytopathogens is of worth consideration, *e.g*., *Agrobacterium tumefaciens* that causes crown gall disease in plants, *Erwinia amylovora* that causes fire blight to apple, *etc*. (Jeffrey *et al*., 2007). Apart from this, actinomycetes have major contribution in biological buffering of soils, biodegradation of hydrocarbons of high molecular weight and biocontrol of terrestrial environments by processes like nitrogen fixation, *etc*. They are also capable to synthesize diverse range of biologically active secondary metabolites such as vitamins, herbicides, nutritional materials, cosmetics, *etc*. (Ogunmwonyi *et al*., 2010).

3. Role of actinomycetes as potential antibiotic producers

The antibiotics are the secondary metabolites produced by actinomycetes. Antibiotics are truly referred as the 'wonder drugs' for their virtual success against pathogenic microorganisms. These noteworthy compounds are heterogeneous collection of biologically active molecules and possess diverse structures and modes of action. Among its targets are microbial processes such as DNA, RNA and protein synthesis, electron transport chain, membrane function, germination, sporulation and many others (Kohanski *et al*., 2010). Thus, antibiotics serve as efficient targets for microbial infections. Before the antibiotics were discovered, people with simple wounds and infectious diseases could not be treated. The global demand for antibiotics is progressively increasing. The leading forms of infection, that are difficult to treat due to increased resistance of pathogenic organisms has further complicated the situation, as in the case of *Klebsiella pneumonia* resistant strains against carbapenem and other microorganisms (Procopioa *et al*., 2012). These resistant strains show limited or no response to treatment, thereby cause prolonged illness and pose serious threat to life. Resistance thus causes treatment failures, prolong phase of infectivity which increases the number of people infected within the community. The situation becomes more aggrieved when population contracts a multidrug-resistant (MDR) strain (Costelloe *et al*., 2010).

To improve healthcare, actinomycetes especially streptomyces species hold prominent position to produce a wide variety of compounds particularly once that are used as drugs of significant medicinal importance (Table 1) (Magarvey *et al*., 2004). Multidrug resistance by pathogens poses a challenge to search for new antibiotics which are effective against these pathogenic bacteria. Successful efforts are made to isolate natural products having novel structures that possess useful biological activities (Dancer, 2004). It is accepted that substantial progress is being made by biochemists and biotechnologists to synthesize chemical products used against pathogenic bacteria, but nature still possesses a reservoir of resources and an enormous potential to produce products of novelty to be used against these antimicrobial resistant strains (Bull and Stach, 2007). Of all the antibiotics produced, 80% alone come from actinomycetes. *Streptomyces* and *Micromonospora* are the two genera, producing most of the actinomycete antibiotics. The actinomycete antibiotics are classified into several major structural groups such as amino glycosides, ansamycins, anthracyclines, β-lactam, macrolides and tetracycline (Nanjawade *et al*., 2010). Streptomyces, the soil inhabitants compete with other soil microorganism and produce many of the compounds to kill its competitors (Laskaris *et al*., 2010). More than one antibiotics are produced by some actinomycetes (*e.g*., *Streptomyces griseus*) and different actinomycete species may produce the common antibiotic (*e.g*., streptothricin, actinomycin). Identical antibiotics may thus be produced from different actinomycetes as shown by its antibiotic spectrum and chemical composition.

Table 1: Clinically significant antibiotics produced by actinomycetes

Streptomyces sp.	Antibiotic
S. orchidaccus	Cycloserin
S.oriantalis	Vancomycin
S.fradiae	Neomycin
S.fradiae	Actinomycin
S.fradiae	Fosfomycin
S.fradiae	Dekamycin
S.nodosus	Amphotricin B
S.noursei	Nistatin
S.mediterranei	Rifampin
S.griseus	Streptomycin
S.knanamyceticus	Kanamycin
S.tenebrarius	Tobramycin
S.spectabilis	Spectinomycin
S.viridifaciens	Tetracycline
S.lincolensis	Lincomycin
S.lincolensis	Clindamycin
S.rimosus	Oxytetracyclin
S.erythraeus	Erythromycin
S.vensuella	Chloramphenicol
S.aureofaciens	Chlortetracycline
S.aureofaciens	Dimethylchlor
S.aureofaciens	Tetracycline
S.ambofaciens	Spiramycin
S.avermitilis	Avermicin
S.alboniger	Puromycin
S.niveus	Novobicin
S.platensis	Platenmycin
S.roseosporus	Daptomycin
S.ribosidificus	Ribostamycin
S.garyphalus	Cycloserine
S.vinaceus	Viomycin
S.clavuligerus	Cephalosporin

4. Role of actinomycetes as potential producers of anti-tumour agents

According to the World Health Organization, cancer is second killer after the cardiovascular disease and one of the top ten diseases affecting people worldwide. The reports are more specific when calculated in third world countries. In spite of recent investigations in cancer biology regarding its induction and progression and the success obtained thereafter, cancer still remains the most threatened disease affecting people worldwide. Although treatments for different types of cancers vary from surgery to several chemotherapies like immunotherapy, radiotherapy, hormonal therapies and anti-angiogenesis; more advanced treatments are still awaiting. In this regard, actinomycetes especially streptomyces provide a promising hope to be used as producers of clinically useful antitumour agent (Table 2). Emphasis is laid on the production of novel antitumour drugs that can be used against untreatable tumours and against those tumors resistant to multiple chemotherapeutic drugs. By employing novel drugs, undesirable side effects along with increased toxicity usually associated with conventional drugs is also thought to be eliminated. In addition, there is a demand for novel drugs with greater therapeutic efficiency (Olano *et al*., 2009).

Table 2: Clinically significant antitumour agents produced by actinomycetes

Streptomyces sp.	Antitumour agents
Streptomyces chrysomallus	Actinomycin D
Streptomyces verticillus	Bleomycin
Streptomyces parvulus Tu4055	Borrelidin
Streptomyces peucetius	Daunorubicin
Streptomyces peucetius	Doxorubicin
Streptomyces lavendulae NRRL2564	Mitomycin
Streptomyces carzinostaticus ATCC15944	Neocarzinostatin
Streptomyces hygroscopicus NRRL5491	Rapamycin
Streptomyces longisporoflavus	Staurosporine
Streptomyces galilaeus	Aclacinomycin
Streptomyces sahachiroi	Azinomycin B
Streptomyces sp. A2991200	Benastatin
Streptomyces bikiniensis	Chalcomycin
Streptomyces griseus ssp. Griseus	Chromomycin
Streptomyces neyagawaensis	Concanamycin
Streptomyces olivaceus	Elloramycin
Streptomyces griseoruber	Hedamycin
Streptomyces cinnamonensis	Monensin
Streptomyces carzinostaticus var. F-41	Neocarzilin
Streptomyces sp. DSM4137	Nigericin
Streptomyces sp. HK803	Phoslactomycin B
Streptomyces lavendulae NRRL1100	Saframycin A
Salinisporatropica CNB-440	Salinosporamide A
Streptomyces halstedii HC34	Vicenistatin

5. Ecology of actinomycetes

The fundamental concept of biogeography, “everything is everywhere, but the environment selects” has been widely propagated in ecological studies. Traditionally, the field encompasses studies of spatial distribution of plants and animals with relation

to time. This concept is undergoing rapid changes with the inclusion of microorganisms in this discipline. With reference to microbial community, the concept has been interpreted as, most bacteria are cosmopolitan (everything is everywhere) and bacteria thrive in those places where they adapt themselves to the surrounding environment and the later selects them (the environments selects). Several findings have supported the biogeographical patterns in microbial distribution (Wawrik *et al*., 2007; Hanson *et al*., 2012; Nemergut *et al*., 2011). Take the case of geographical distribution of *Roseobacter* cluster that grows in polar and temperate regions and does not grow in tropical and subtropical regions (Selje *et al*., 2004). Another marine bacterium, *Prochlorococcus marinus*, that grows in tropical and subtropical ocean water surfaces where it can be isolated but cannot be obtained from temperate and polar ocean surfaces. Some populations of actinomycetes adapt to both high and low light conditions and are found in different photic zones of oceans, *e.g*., *Prochlorococcus* species. Actinobacteria form one of the largest phylum of bacteria (Gao and Gupta, 2012) and an appropriate candidate for their biogeographical studies. Most of the actinomycetes are cosmopolitan in habitat and free living in different natural habitats such as soil, freshwater ecosystems, marine habitats and plant tissues. Of the total number of actinomycete genera, streptomyces species are found abundantly.

5.1 Actinomycetes in terrestrial environments

Actinomycetes constitute a large part of soil inhabitant microbes. The strong odour in the air when rain falls after a dry spell of weather is due to production of geosmin by the soil actinomycetes (Gust *et al*., 2003). Actinomycetes are abundant soil inhabitants, found in different types of soils such as desert soil, alkaline soil, salt soils and frost soils under the snow caps. The initial work on soil actinomycetes began in the last decades of the 19th century. But intensive studies on actinomycetes started only in 1943 with the discovery of streptomycin from *Streptomyces griseus*, the first successful drug against tuberculosis. Many actinomycetes have since then been isolated mostly with the objective of discovering new antibiotics. Majority of the actinomycetes have been isolated from soils (Abdulla, 2009). Rhizospheric soil forms a good host for many actinomycetes. However, the distribution of actinomycetes, especially the population density and diversity, seems to differ between rhizophere and other plant-free non-rhizospheric soil litter (Nimnoi *et al*., 2011). Rhizopheric soil seems to contain more diverse actinomycetes compared to non-rhizospheric soil. This high diversity may be due to symbiotic interactions with the plant and the edaphic conditions. The plant type may also influence the diversity of actinomycetes in the soil. Many researchers studied soil actinomycete community, using molecular biology tools and reported most of them to be unculturable (Priyadharsini and Dhanasekaran, 2015).

5.2 Actinomycetes in aquatic environments

Aquatic environments form the largest ecosystem on the earth. Marine habitats cover nearly two-thirds of the earth's surface. These large but unexplored habitats have been reported to contain diverse actinomycete communities (Valli *et al*., 2012). It is reported that *Streptomyces* forms a dominant genus in water bodies while *Micromonospora* flourishes under sediments (Sharma and David, 2012). The genera, *Marinispora, Salinibacterium, Salinispora, Serinicoccus and Solwaraspora* are unique to marine habitats. Freshwater ecosystems have been reported as promising source of bioactive actinomycetes (Hodges *et al*., 2012).

5.3 Actinomycetes in special habitats

Natural product resources have not been explored fully. They have been explored from the conventional environments, but their exploration from the extreme habitats is limited. Recently, they have been reported from wide variety of geographical ranges, different ecological conditions and extreme environments. It is presumed that millions of microbes await their discovery and their capacity to produce natural products of desirable medicinal value is unreported. Organisms growing in unusual habitats have unique physiological and biochemical characteristics, producing niche specific secondary metabolites which enable them to thrive in such extreme conditions of higher salinity, pH, pressure and temperature. The unique bioactive compounds which may be produced through this process may have commercial applications. Permeation of biotechnology into marine environment has ushered in unanticipated strategies for locating novel organisms and trapping their potential of producing bioactive compounds. Reservoirs of unidentified, untapped and unexplored natural products are buried under the ocean sediments, covering 70% of the earth's surface. The factories of these natural products are microorganisms, residing in deep ocean sediments. Among these, microorganisms actinomycetes are our focus of attention. Actinomycetes from these ocean sediments are said to be obligate marine inhabitants (Maldonado *et al*., 2005). It is expected that the genetic makeup and the metabolic composition of these actinomycetes is unknown and, hence, the possibility of getting novel metabolites of medicinal value are higher (Bull and Stach, 2007). Previous studies on the chemical structure and composition of marine *Actinomycetes* has revealed that they possess novel secondary metabolites, active against human tumours and other diseases (Blunt *et al*., 2007). The discovery of widespread populations of *Salinispora* (formerly *Salinospora*)- an obligate marine actinomycete genus from the ocean sediments by Fenical's research group was a breakthrough (Mincer *et al*., 2005). Later, *Salinispora* strains were isolated from sponges of some ocean reefs like the sponge, *Pseudo ceratinaclavata* inhabiting Great Barrier Reef. Novel actinomycetes have also been reported from other Great Barrier Reef sponges like *Rhopaloeides odorabile*, *Pseudo ceratinaclavata* and *Candida spongiaflabellate* and sponges inhabiting the Mediterranean sea like *Aplysina aerophoba* and *Theonella swinhoei* (Kim *et al*., 2002). Actinomycetes isolated from these sponges are uncommon; belong to Gordoniaceae, Micrococceae and Dermatophilaceae families. These sponge associated actinomycetes produce novel bioactive metabolites (Hill, 2004). Actinomycetes inhabit either in isolation under deep sea floor and marine snow or in association with marine invertebrates and form an exclusive group of these unique ecosystems. The actinomycetes adapted themselves to these high pressure and low temperature unique ecosystems and produced an enormous reservoir of secondary metabolites to be exploited for practical biomedical applications. Even, if the study on exploitation of actinomycetes from these extreme marine ecosystems is moving with snail's pace, the truth that several novel metabolites have been secluded in the past few years, makes it an advanced field of research to be dealt with due consideration .

The microorganisms also thrive in caves and mines and such environments are the hypogean environments. Like marine and

acidophilic environments, hypogean environments are unexploited for their microbial diversity. Light cannot reach in caves and because hypogean environments have high concentrations of inorganic minerals; such ecosystems provide inorganic energy sources rather than on organic sources (Peck, 1986). The microbes inhabiting these ecosystems, usually transport organic matter, if any from outside by seepage water. Such inputs of organic matter from outside the hypogean environments are called allochthonous (extrinsic) input of organic matter (Laiz *et al*., 1999). These dark environments with no light, limited organic matter and high mineral content form limiting ecosystems and provide ecological niches for highly specialized organisms. Hypogean ecosystems are further fragmented into various microhabitats which differ in their community structure. It was earlier reported that actinomycetes can be isolated from limiting ecosystems including these hypogean environments and these microorganisms can be used for screening of valuable bioactive metabolites. In Northern Spain, Italy, Korea and China, the studies of the biodiversity of caves and mines have revealed that actinomycetes form an abundant taxonomic group (Wang *et al*., 2009). Hence, actinomycete genera like *Fodinibacter, Beutenbergia, and Knoellia* as have been isolated from these hypogean environments. Among less isolated ones, isolated from Italian, Spanish and Korean caves, mines and catacombs are *Jiangella, Kribbella, Isoptericola, Nocardia, Myceligenerans, Actinocorallia, Amycolatopsis, Agromyces, Saccharothrix,* and *Pseudonocardia* (Jurado *et al*., 2008).

6. Identification and characterization of *actinomycetes*

The techniques like DNA-DNA hybridization, conserved region based PCR analysis and cloning of rRNA genes provides a more accurate and rapid method of comparing relatedness (Gentry, 2006). The 16S rRNA gene method has aided in finding phylogenetic relationships at species level, degree of similarity between the genomes of different species, and is therefore useful for defining proposed new species as well as the perfect assignment of a particular existing strain with ambiguous properties to the correct taxonomic group (Cho and Tiedje, 2001). In actinomycete classification, phenotypic methods like morphological, physiological and biochemical characterization and phylogenetic methods like molecular characterization are employed as a polyphasic taxonomic approach. Growth and morphology of streptomyces spp. are observed when cultures turned mature with heavy spore mass in order to determine diffusible pigments colour, substrate mycelium and aerial spore mass colour. Colour grouping is done to differentiate between large number of different isolates. However, this study only involves streptomyces spp. As such, morphological observation is considered stable and clearly defined feature for actinomycetes classification.

The polymorphism in DNA was studied using several genetic markers in plant breeding methods like RFLP, AFLP, microsatellites genomic sequencing, rRNA sequencing, isoenzymes, and in some cases, random amplified polymorphic DNA (RAPD) technique has been used for identification of individuals in different populations and for distinguishing isolates of various phytopathogens. The later method being simple, inexpensive and does not require any sequence information, has been widely used for estimating genetic diversity in natural populations. By employing the 16S rRNA gene sequence comparisons, it is presumed that a major fraction of microorganisms inhabiting different environments are still uncultured. Before the advent of molecular techniques, these uncultured organisms remained uncharacterized because a microorganism can only be cultivated after its physiological niche has been characterized and duplicated experimentally. Metagenomics-the culture independent genomic analysis of microbial communities provide the means to identify novel and industrially useful genes in the environment as well as understanding microbial diversity (Rodriguez-Valera, 2004).

7. Search for novel producer strains

Two techniques employed in metagenomic analysis of different microbial DNA are fluorescence *in situ* hybridization (FISH) and16S rRNA. Studies based on these techniques revealed that conventional techniques permit the growth of less than 1% microbes, the remaining 99% still unculturable. This phenomenon where a vast majority of microbes remained uncultured on culture plates is sometimes referred to as the great plate count anomaly (Amann *et al*., 1995). It is thus, proved that vast pools of microbes have not been cultured yet and research in these so called uncultured microbes is still in infancy. By employing conventional techniques, we have excluded a swathe of microbial world who's chemical and biological potentialities remain untapped. Conversely, the potential of these uncultured microbes to produce novel products of antibiotic or other medicinal value is set aside. Hence, two challenges are ahead-the first is to understand the biosynthetic pathways of unculturable microbes by interpreting their nuclear material, the second to interpret those biosynthetic pathways that are not expressed under the standard culture conditions of culturable microbes. It is presumed that by varying different growth conditions like the pH, temperature, culture medium and/or growth vessel, *via* the one strain-many compounds (OSMAC) approach, novel metabolites of these undiscovered biosynthetic pathways may be expressed (Bode *et al*., 2002). These challenges inform us only about a part of the story and it is difficult to predict the improvements. It is now obvious that most microorganisms need environmental signals for their growth and metabolism (Netzker *et al*., 2015). To mimic, the environments of complex microbial world, innovative approaches are employed by researcher's, *e.g.*, co-culture cultivation of microbes, but this technique is criticized since it does not account for adequate molecular complexity of the natural ecosystems. An alternative approach to override this criticism is to more fully simulate the natural environment (Ling *et al*., 2015). In this capacity, the iChip (isolation chip) technology is more rewarding; here an *in situ* procedure is followed. A soil sample, expected to contain numerous microbial strains is diluted, followed by placing it between a semi-permeable membrane and burying back in the soil. This procedure lead to the identification of a new class of antibiotics from a new species of gram-negative *Proteobacteria, Eleftheria terrae* that target lipid II, *e.g*., teixobactin. Another discovery is the isolation of closthioamide, the first secondary metabolite from *Clostridium cellulolyticum*a strictly anaerobic bacterium (Lincke *et al*., 2010). This technology also raises the tempting opportunity of isolation and cultivation of microorganisms from remote areas, *e.g*., in deep ocean trenchesor other planets. In addition, genes encoding secondary metabolic biosynthetic pathways that remain quiet and probably unexpressed under standard laboratory conditions may express under these secondary growth conditions (Rutledge and Challis, 2015). Genomic methods, *e.g*., heterologous gene expression

and cloning can target these orphan pathways and thus increase the possibility of producing non-natural analogs. Another method to access these orphan biosynthetic pathways is to omit a separate cultivation step and directly clone DNA from microbial communities *via* metagenomic strategies (Owen, 2013). These metagenomic approaches are normally either function or sequence based; the former screens and identifies cloned environmental DNA and evaluates the activity of their expression products, more specifically proteins as novel bioactive metabolites. The later concentrates on genes linked with production of specific natural products (Milshteyn *et al*., 2014).

8. Conclusion

The demand for improved tumour treatment and appearance of multidrug-resistant strains encourages a continuous exploration of natural bioactive metabolites. As the scope of naturally found bioactive compounds increases day-by-day, the search for novel active biomolecules increases. Diverse microbial world as a source of natural product resources, are mostly unexplored by the scientific world with respect of its ecological and environmental points of view. It is presumed that unique organisms may inhabit unique ecosystems with unique metabolic pathways. Screening of these unknown species from unexplored regions may yield enormous potential. The research on activities of bioactive molecules produced by microorganism inhabiting extreme environments has geared up. Among all microbes, actinomycetes have proved to have enormous potential to produce molecules of unique bioactivity. The effective exploration of uncommon and unreported groups of various microbes predominantly novel actinomycetes can greatly be increased with the application of new molecular techniques, which in turn may greatly increase the chemical diversity of bioactive compounds to be used as potent therapeutic agents. Attempts are on to discover novel actinomycetes and along with the existing ones, exploit their bioactive potential of producing molecules with practical applications.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgments

The first author (AMS) is grateful to Indian Council of Medical Research (ICMR), New Delhi for providing fellowship. The authors are also thankful to Dr. Ram A. Vishwakarma, Director IIIM (CSIR), for providing the necessary facilities.

References

Abdulla, H. (2009). Bioweathering and biotransformation of granitic rock minerals by actinomycetes. Microb. Ecol., **58**:753-761.

Amann, R.I.; Ludwig, W. and Schleifer, K.H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev., **59**:143-169.

Atta, H.M. (2009). An antifungal agent produced by streptomyces olivaceiscleroticus, AZ-SH514, World Applied Sci. J., **6**:1495-1505.

Balagurunathan, R. and Radhakrishnan, M. (2010). Biotechnological, genetic engineering and nanotechnological potential of actinomycetes. In: D.K. Maheshwari, R.C. Dubey, R. Saravanamurthu, Editors, Industrial Exploitation of Microorganisms, 1st edition. New Delhi: I.K. International Publishing House Pvt. Ltd., pp:302-436.

Baltz, R.H. (2008). Renaissance in antibacterial discovery from actinomycetes, Curr. Opin. Pharmacol., **8**:557-563.

Berdy, J. (2005). Bioactive Microbial Metabolites, J. Antibiot., **58**:1.

Blunt, J.W.; Copp, B.R.; Hu, W.P.; Munro, M.H.; Northcote, P.T. and Prinsep, M.R. (2007). Marine natural products. Nat. Prod. Rep., **24**:31-86.

Bode, H.B.; Bethe, B.; Hofs, R. and Zeeck, A. (2002). Big effects from small changes: Possible ways to explore nature's chemical diversity. Chem. Bio. Chem., **3**:619-627.

Bull, A.T. and Stach, J.E. (2007). Marine actinobacteria: New opportunities for natural product search and discovery. Trends Microbiol., **15**: 491-499.

Chin, Y.W.; Balunas, M.J.; Chai, H.B. and Kinghorn, A.D. (2006). Drug discovery from natural sources. The AAPS Journal, **8**:239-253.

Cho, J.C. and Tiedje, J.M. (2001). Bacterial species determination from DNA-DNA hybridization by using genome fragments and DNA microarrays. Appl. Environ. Microbiol., **67**:3677-3682.

Costelloe, C.; Metcalfe, C.; Lovering, A.; Mant, D. and Hay, A.D. (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: Systematic review and meta-analysis. BMJ, pp:340:c2096.

Dancer, S.J. (2004). How antibiotics can make us sick: The less obvious adverse effects of antimicrobial chemotherapy. Lancet. Infect. Dis., **4**:611-619.

Farnet, C.M. and Zazopoulos, E. (2005). Improving drug discovery from microorganisms, In: Natural Products. Humana Press, pp:95-106.

Gao, B. and Gupta, R.S. (2012). Phylogenetic framework and molecular signatures for the main clades of the phylum actinobacteria. Microbiol. Mol. Biol. Rev., **76**:66-112.

Gentry, C. (2006). Practical identity-based encryption without random oracles. In : Annual International Conference on the Theory and Applications of Cryptographic Techniques Springer Berlin Heidelberg, pp:445-464.

Gust, B.; Challis, G.L.; Fowler, K.; Kieser, T. and Chater, K.F. (2003). PCR-targeted Streptomyces gene replacement identifies a protein domain needed for biosynthesis of these squiterpene soil odorgeosmin. Proc. Natl. Acad. Sci., **100:**1541-1546.

Hanson, C.A.; Fuhrman, J.A.; Horner-Devine, M.C. and Martiny, J.B. (2012). Beyond biogeographic patterns: Processes shaping the microbial landscape. Nature Rev. Microbiol., **10**:497-506.

Hill, R.T. (2004). Microbes from marine sponges: A treasure trove of biodiversity for natural products discovery. AT Bull (ed.), Microbial diversity and bioprospecting. ASM Press, Washington, DC, pp:177-190.

Hodges, T.W.; Slattery, M. and Olson, J.B. (2012). Unique *Actinomycetes* from marine caves and coral reef sediments provide novel PKS and NRPS biosynthetic gene clusters. Mar. Biotechnol., **14**:270-280.

Jeffrey, L.S.H.; Sahilah, A.M.; Son, R. and Tosiah, S. (2007). Isolation and screening of *Actinomycetes* from Malaysian soil for their enzymatic and antimicrobial activities. J. Trop. Agric. and Food. Sci., **35**: 159.

Jurado, V.; Boiron, P.; Kroppenstedt, R.M.; Laurent, F.; Couble, A.; Laiz, L. and Bergeron, E. (2008). *Nocardia altamirensis* sp. nov., isolated from Altamira cave, Cantabria, Spain. Int. J. Syst. Evol. Microbiol., **58**: 2210-2214.

Kim, Y.H.; Cha, C.J. and Cerniglia, C.E. (2002). Purification and characterization of an erythromycin esterase from an erythromycin-resistant Pseudomonas sp. FEMS Microbial. Lett., **210**:239-244.

Kohanski, M.A.; Dwyer, D.J. and Collins, J.J. (2010). How antibiotics kill bacteria from targets to networks. Nature Rev. Microbiol., **8**:423-435.

Laiz, L.; Groth, I.; Gonzalez, I. and Saiz-Jimenez, C. (1999). Microbiological study of the dripping waters in Altamira cave (Santillanadel Mar, Spain). J. Microbiol. Methods, **36:**129-138.

Laskaris, P.; Tolba, S.; Calvo, L.; Bado, and Wellington, L. (2010). Coevolution of antibiotic production and counter resistance in soil bacteria. Environ. Microbiol., **12:**783-796.

Lincke, T.; Behnken, S.; Ishida, K.; Roth, M. and Hertweck, C. (2010). Closthioamide: An unprecedented polythioamide antibiotic from the strictly anaerobic bacterium, *Clostridium cellulolyticum*. Angewandte Chemie., **122:**2055-2057.

Ling, L.L.; Schneider, T.; Peoples, A.J.; Spoering, A.L.; Engels, I.; Conlon, B.P. and Jones, M. (2015). A new antibiotic kills pathogens without detectable resistance. Nature, **517**:455-459.

Magarvey, N.A.; Keller, J.M.; Bernan, V.; Dworkin, M. and Sherman, D.H. (2004). Isolation and characterization of novel marine derived actinomycete taxa rich in bioactive metabolites. Appl. Environ. Microbiol., **70**:7520-7529.

Mahajan, G. and Balachandran, L. (2014). Biodiversity in production of antibiotics and other bioactive compounds. In: Biotechnological Applications of Biodiversity. Springer Berlin Heidelberg, pp:37-58.

Maldonado, L.A.; Stach, J.E.; Pathom-Aree, W.; Ward, A.C.; Bull, A.T. and Goodfellow, M. (2005). Diversity of cultivable actinobacteria in geographically widespread marine sediments. Antonie Van Leeuwenhoek, **87**:11-18.

Milshteyn, A.; Schneider, J.S. and Brady, S.F. (2014). Mining the metabiome: Identifying novel natural products from microbial communities. Chem. Biol., **21**:1211-1223.

Mincer, T.J.; Fenical, W. and Jensen, P.R. (2005). Culture-dependent and culture-independent diversity within the obligate marine actinomycete genus Salinispora. Appl. Environ. Microbiol., **7**:7019-7028.

Nanjawade, B.K.; Chandrashekhara, S.; Ali, M.S.; Prakash, S.G. and Fakirappa, V.M. (2010). Isolation and morphological characterization of antibiotic producing actinomycetes. Trop. J. Pharm. Res., **9**:231-236.

Nemergut, D.R.; Costello, E.K.; Hamady, M.; Lozupone, C.; Jiang, L.; Schmidt, S.K. and Knight, R. (2011). Global patterns in the biogeography of bacterial taxa. Environ. Microbiol., **13**:135-144.

Netzker, T.; Fischer, J.; Weber, J.; Mattern, D.J.; König, C.C.; Valiante, V. and Brakhage, A.A. (2015). Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol., **6**:299.

Nimnoi, P.; Pongsilp, N. and Lumyong, S. (2011). Actinobacterial community and diversity in rhizosphere soils of *Aquilaria crassna pierre* ex Lecassessed by RT-PCR and PCR-DGGE. EýBiochem. Sys. Col., **39**:509-519.

Ogunmwonyi, I.H.; Mazomba, N.; Mabinya, L.; Ngwenya, E.; Green, E.; Akinpelu, D.A. and Okoh, A.I. (2010). Studies on the culturable marine *Actinomycetes* isolated from the Nahoon beach in the Eastern Cape Province of South Africa. Afr. J. Microbiol. Res., **4**:2223-2230.

Olano, C.; Méndez, C. and Salas, J. A. (2009). Antitumor compounds from actinomycetes from gene clusters to new derivatives by combinatorial biosynthesis. Nat. Prod. Rep., **26**:628-660.

Owen, J.G. (2013). Mapping gene clusters within arrayed metagenomic libraries to expand the structural diversity of biomedically relevant natural products. Proc. Natl. Acad. Sci. U.S.A., **110:**11797-11802.

Pandey, B.; Ghimire, P. and Agarawal, V.P. (2004). Studies on antibacterial activity of soil from Khumbu region of Mount everest, a paper presented in International Conference on The Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu, January, pp:12-15.

Peck, S.B. (1986). Bacterial deposition of iron and manganese oxides in North American caves. National Speleological Society Bulletin, **48**:26-30.

Priyadharsini, P. and Dhanasekaran, D. (2015). Diversity of soil allelopathic Actinobacteria in Tiruchirappalli district, Tamilnadu, India. Journal of the Saudi Society of Agricultural Sciences, **14**:54-60.

Procopioa, R.; Silvaa, I.R.; Martinsa, M.K.; Azevedoa, J.L. and Araujob, J.M. (2012). Antibiotics produced by streptomyces. Braz. J. Inf. Dis., **16**: 466-471.

Ramazani, A.; Moradi, S.; Sorouri, R.; Javani, S. and Garshasbi, M. (2013). Screening for antibacterial activity of streptomyces species isolated from Zanjan province, Iran. Int. J. Pharm. Chem. Biol. Sci., **33**:42-349.

Rodriguez-Valera, F. (2004). Environmental genomics, the big picture?, FEMS Microbiol. Lett., **231**:153-158.

Rutledge, P.J. and Challis, G.L. (2015). Discovery of microbial natural products by activation of silent biosynthetic gene clusters. Nature Rev. Microbiol., **13**:509-523.

Sarker, S.D. and Nahar, L. (2006). An Introduction to Natural Products Isolation,Series Editor John M. Walker School of Life Sciences, University of Hertfordshire Hatfield, Hertfordshire, AL10 9AB, UK, 1.

Schatz, A.; Bugle, E. and Waksman, S.A. (1944). Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria, Proc. Soc. Exp. Biol. Med., **55**:66-69.

Selje, N.; Simon, M. and Brinkhoff, T. (2004). A newly discovered Roseobacter cluster in temperate and polar oceans.Nature, **427**:445-448.

Selman Waksman, S.A. (1950). Actinomycetes. Soil Science, **70(2)**:161.

Sharma, S.C.V. and David, E. (2012). A comparative study on selected marine *Actinomycetes* from Pulicat, Muttukadu, and Ennore estuaries. Asian Pac. J. Trop. Biomed., **2**:S1827-S1834.

Solanki, R.; Khanna, M. and Lal, R. (2008). Bioactive compounds from marine *Actinomycetes*, Indian Journal of Microbiology, **48**:410-431.

Valli, S.; Suvathi, S.S.; Aysha, O.S.; Nirmala, P.; Vinoth, K.P. and Reena, A. (2012). Antimicrobial potential of *Actinomycetes* species isolated from marine environment. Asian Pac. J. Trop. Biomed., **2**:469-473.

Wang, Z.G.; Wang, Y.X.; Liu, J.H.; Chen, Y.G.; Zhang, X.X.; Wen, M.L. and Cui, X.L. (2009). *Fodinibacter luteus* gen. nov., sp. nov., an actinobacterium isolated from a salt mine. Int. J. Syst. Evol. Microbiol., **59**:2185-2190.

Wawrik, B.; Kutliev, D.; Abdivasievna, U.A.; Kukor, J.J.; Zylstra, G.J. and Kerkhof, L. (2007). Biogeography of actinomycete communities and type II polyketide synthase genes in soils collected in New Jersey and Central Asia. Appl. Environ. Microbiol., **73**:2982-2989.

Williams, S.T.; Locci, R.; Beswick, A.; Kurtböke, D.I.; Kuznetsov, V.D.; Le, F.J. and Quaroni, S. (1993). Detection and identification of novel *Actinomycetes*. Res. Microbiol., **144**:653-656.