JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

REVIEWING ANTAGONISTIC ACTINOMYCETES IN THE ERA OF DRUG RESISTANCE

Monsumee Alam[1], D.K Jha[2]*

Microbial Ecology Laboratory, Department of botany,
Gauhati University, Guwahati-781014, Assam, India
E-mail-id : monsumee.alam@gmail.com,*dkjhabot07@gmail.com

Received: March 2020 Revised and Accepted: May 2020

ABSTRACT:

Actinomycetes are the limitless source of newer chemical scaffolds and proved its effectiveness in diverse clinical applications. More than 10,000 structurally defined and experimentally proved antimicrobials are from the Actinomycetes. Among actinomycetes, approximately 55 % of the antimicrobial agents are obtained from the genus *Streptomyces*. Importance of Actinomycetes derived antibiotics is largely ignored because of its tedious process of isolation from various natural habitats leading to the discovery of previously explored genera. However, newer techniques are to be explored for effective isolation of Actinomycetes producing novel antibiotics. Enhanced cultivation methods proved beneficial in the detection and cultivation of novel bioactive Actinomycetes and modern molecular biology techniques proved beneficial in the target-specific search of antimicrobial Actinomycetes. Discovery and development of antibiotics form the Actinomycetes are of significant interest due to the mounting necessity of newer antibiotics. This review focuses on the diverse sources of antibiotics from Actinomycetes and its isolation.

INTRODUCTION:

The emergence of drug-resistant pathogens with the emergence of antibiotic era has increased many folds. The overuse and inappropriate consumption and application of antibiotics are the main driving factors for drug resistance amongst pathogens. There is an overall threat to the global health scenario due to the ever-increasing antimicrobial resistance of the pathogenic organisms (Levy and Marshall, 2004). Cross-resistance development with the newer antibiotics at the fast pace, is another cause of public health concern. Microorganism's resistance development can be occurred due to resistant gene and alteration in the phenotypic expression as a result of environmental and genetic factors. The difficulty of treating bacterial infections and reversing antimicrobial drug resistance to susceptibility in the modern era of pharmaceuticals has overburdened scientists and pharmaceutical companies to develop new antimicrobials effective against the untreatable multidrug-resistant pathogens. As majority of the antibacterial substances come from the well-known terrestrial or aquatic isolates of actinomycetes and the disease-causing bacterial agents are developing resistance to such drugs, the focus has shifted to rare genera of actinomycetes from unexplored or underexplored habitats as sources of novel bioactive secondary metabolites which can efficiently act against pathogenic bacterial strains. A variety of rare actinomycetes belonging to genera viz., *Actinoplanes*, *Catenuloplanes*, *Amycolatopsis*, *Kineospora*, *Micromonospora*, *Microbispora*, *Dactylosporangium*, *Nonomuraea*, *Nocardia* etc. are now being isolated worldwide from previously uninvestigated diverse natural habitats, using different selective isolation methods. These isolation strategies include methods to enhance growth of rare actinomycetes by enrichment, and eliminate unwanted microorganisms by pretreatment.

Streptomyces produces a large number of antibiotics along with clinically active anti-tumor agents like anthracyclines viz. aclarubicin, daunomycin, and doxorubicin, peptides viz. bleomycin and actinomycin D, aureolic acids viz. mithramycin, enediynes viz. neocarzinostatin, and antimetabolites such as pentostatin (Newman and Cragg, 2007). Other than *Streptomyces*, certain other genera of Actinomycetes like *Micromonospora*, *Nocardia*, *Actinomadura*, *Verrucosispora*, *Kocuria*, *Actinoplanes*, *Amycolatopsis*, and *Saccharopolyspora* exhibited biological importance (Sujatha et al. 2005). Non-Streptomyces actinomycetes are known to produce diverse antibiotics like abyssomicins, erythromycin, gentamicin, micromonosporin, PM181104, ramoplanin, telithromycin, teicoplanin, tigecycline, thiolactomycin, and vancomycin. Therefore the focus has now has shifted to the Non-Streptomyces actinomycetes from unexplored or underexplored habitats as source of novel bioactive secondary metabolites.

1.1 Actinobacteria:

Actinomycetes or Actinobacteria are Gram-positive aerobic bacteria that form branching filaments or hyphae and sexual pores. Actinomycetes exhibit characters on fungus and bacteria (Das *et al*., 2008); hence, its name derived from 'atkis' meaning a ray and 'mykes' meaning fungus. Unlike fungi, Actinomycetes possess delicate and thin hyphae (0.5 – 2 µm). Actinomycetes contain coiled gene derived particles inside the free DNA. Henceforth, these are considered as the prokaryotic organisms. Actinomycetes are high in Guanine-Cytosine (GC) (>55 mol %) base pairing content in DNA.

1.2 Occurrence and growth:

The Actinomycetes, particularly Streptomycetes are saprophytic, soil- inhibiting organisms that possess semi dormant spores which help them in surviving, especially under nutrient limited conditions (Mayfield et al., 1972). However, the phylum has been able to withstand to a wide range of ecological environments like soils, fresh and salt water and the air. They form an important part of microbial population and are abundant in alkaline soils, rich in organic matter. Actinomycetes can be found both on the soil surface and at depths of more than 2 m below the surface (Goodfellow and Williams, 1983). The thread like fine filaments formed by the actinomycetes is found abundant in soil. Their growth is quite similar to fungal hyphae and they produce the characteristically "earthy" smell of freshly turned healthy soil. The actinomycetes exist in various habitats in nature and represent a ubiquitous group of microbes widely distributed in natural ecosystems around the world (Srinivasan et al., 1991). They are known to be primarily soil dwellers but are found abundant in aquatic ecosystem as well. The population density of Actinomycetes depends on their habitat and the prevailing climate conditions. They are typically present at densities on the order of 10^6 to 10^9 cells per gram of soil. The dominance of the genus *Streptomyces* amongst soil populations is revealed and it accounts for over 95% of the Actinomycetes strains isolated from soil. Other factors, such as temperature, pH, and soil moisture, also influence the growth of Actinomycetes. Most of the Actinomycetes grows on common bacteriological media like trypticase agar, starch casein agar, nutrient agar, brain heart diffused agar along with blood agar. Actinobacteria in more numbers grow under aerobic conditions while very few like Actinomyces israelii grow under anaerobic conditions.

1.3 Biological uses of Streptomyces actinomycetes:

Streptomyces proved to be the biologically important actinomycetes and synthesize chemically diverse compounds. Approximately 75 % medicinally useful and numerous agriculturally significant compounds were produced from the Streptomyces. Streptomyces exhibited the potential to produce biologically active compounds like antibacterial, antifungal, insecticidal, antitumor, anti-inflammatory, anti-parasitic, antiviral, antifouling, anti-infective and plant-growth-promoting and herbicidal compounds. Streptomyces produces antibacterial compounds like AZ-AR-262, antimycin-A, abyssomicins, bonactin, chandrananimycins, frigocyclinone, glaciapyrroles, helquinoline and tunicamycins (Berdy 1980, 2005; Ramesh and Mathivanan 2009). Antifungal compounds like Streptomycin, blasticidin S, kasugamycin, polyoxins, validamycins, bafilomycin K, elaiomycins B, and reveromycins were obtained form the Streptomyces (Berdy 1980, 2005; Prabavathy et al. 2006; Prapagdee et al. 2008). Insecticidal compounds like indolocarbazole alkaloid staurosporine, cyclic depsipeptide valinomycin, and butanolide were obtained from the Streptomyces (Pimentel-Elardo et al. 2010). Anticancer compounds like caprolactones, chandrananimycins, chinikomycins, chloro-dihydroquinones, diazepinomicin (ECO-4601), 3,6-disubstituted indoles, IB-00208, mechercharmycins, salinosporamide A (NPI-0052) and trioxacarcins were isolated from Streptomyces (Lam 2006; Hong et al. 2009). Three new cyclic heptapeptides, cyclomarins A−C (1−3) were isolated as anti-inflammatory compounds from the Streptomyces species (Pimentel-Elardo et al. 2010). Fattiviracins were obtained as the antiviral compounds from the Streptomyces species (Sacramento et al. 2004). Compounds with straight alkyl side chains with 2-furanone ring isolated (Xu et al., 2010) from the Streptomyces species were proved to be effective as antifouling compounds.

1.4 Commercial application of Actinomycetes:

Actinomycetes are capable of degrading a wide variety of hydrocarbons, pesticides, aliphatic and aromatic compounds. Actinobacteria play a significant part in the microbial transformation of organic compounds which is having significant commercial value. Actinobacteria are useful in the bioconversion of underutilized urban and agricultural wastes into high quality and high-value useful products. Marine actinomycetes exhibit numerous enzyme activities and these catalyze diverse biochemical reactions through enzymes like amylase, lipase, deoxyribonuclease, lipase, and protease. Actinobacteria are important sources of enzymes that can be produced at an industrial scale. Actinobacterial systematics has been explored through chemotaxonomic,

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

molecular systematic and numerical taxonomic methods (Goodfellow and Cross 1984; Stackebrandt and Schumann 2006). Actinobacteria is being considered and concluded by the presence of branching (Ludwig and Klenk 2005) by observed insilico based 16srRNA. Phylogenetic relationships of the taxa in Actinobacteria above the genus level is merely depending on the taxon-specific 16S rRNA signatures (Zhi et al. 2009). Actinobacteria term is relevant to all while actinomycetes specifically designated to order Actinomycetales. Currently used classification of the actinobacteria exhibits significant improvement in comparison to the earlier classification of analysis (Zhi et al. 2009). Gram-positive bacteria are placed in the phylum Actinobacteria, Class Actinobacteria, subclass Actinobacteridae, order Actinomycetales. *Actinomycetes* comprise of 10 suborders, greater than 30 families, and over 160 genera.

Table 1: Taxonomy of Actinomycetes

Domain
Bacteria
Phylum
Actinobacteria
Class
Actinobacteria
Subclass
Actinonbacteridae
Order
Actinomycetales
Suborder
1. Actinomycineae
2. Micrococcineae
3. Catenulisporineae
4. Micromonosporineae
5. Propionibacterineae
6. Actinopolysporineae
7. Pesudonocardineae
8. Streptomycineae
9. Streptosporangineae
10. Frankineae
11. Kineosporiineae
12. Glycomycineae

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

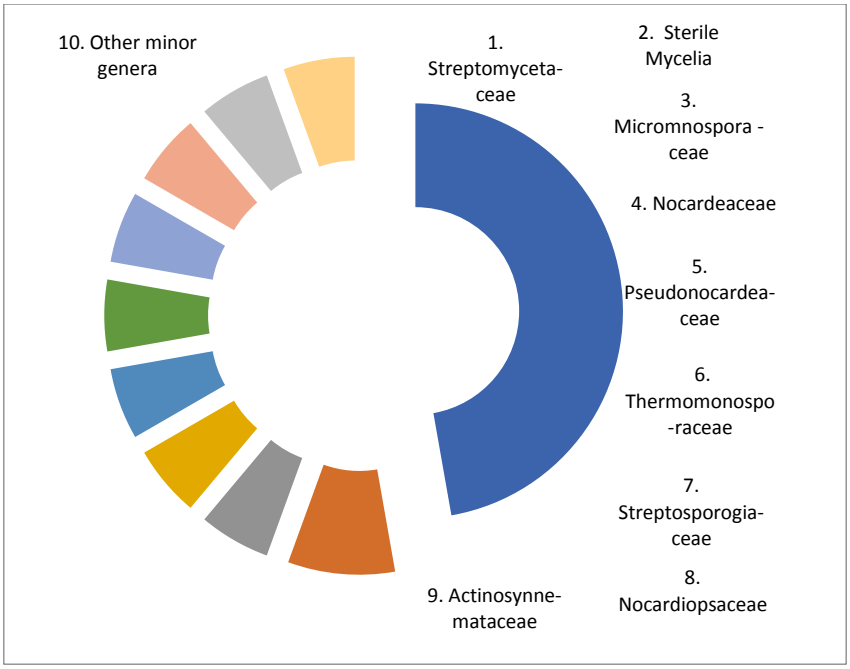

Figure 1: Taxonomic diversity of Actinomycetes

2.1 Exploiting Structural diversity and antagonistic activity:

Structural diversity is important in the case of antibiotics because most of current antibiotics are over used leading to drug-resistance which ultimately causes life-threatening infectious diseases. Actinomycetes comprise of the diverse chemical scaffolds; they have a huge possibility of synthesizing primary as well secondary metabolites. Actinobacteria can produce commercially useful and structurally diverse chemical compounds because of the extra-large DNA complement in these bacteria. Secondary metabolites with varied chemical scaffolds have the potential to act as antimicrobial compounds for diverse group of resistance causing microorganisms. As the new set of diverse chemical structures exhibits a newer mechanism of action against the infectious microorganism, the possibility to eliminate the problem of resistance thereby increases. Moreover advanced techniques like high-throughput fermentation and screening and combinatorial biosynthesis proved beneficial in generating diverse chemical scaffolds. It resulted in numerous antibiotic compounds with varied chemical diversity (Baltz 2008). Diverse chemical scaffolds produced by Actinobacteria are peptides, polyketides, polyesters, beta-lactams, anthracyclines, chloramphenicol, aminoglycosides, tetracyclines, nucleosides, polyenes, glycopeptides, and macrolides.

2.2 Isolation of *Actinobacteria:*

Several generalized and technically advanced methods are being developed and followed for the isolation of actinobacteria from diverse sources like soil, marine, and plant origin. Existing methods comprise of pre-treatment, enrichment, antibiotics, membrane filter, different media compositions, and integration for isolation of novel genera of the actinomycetes from soil. Conventional isolation techniques are not advantageous for the isolation of rare actinomycetes; hence, advanced isolation methods were being developed for these actinomycetes. The pre-treatment method facilitates isolation by promoting the growth of actinomycetes or removing unnecessary Gram-negative bacteria (Matsukawa et al., 2007 and Hong et al., 2009). Pre-treatment methods of isolation include physical, chemical, and a combination of physical and chemical pre-treatment. Physical pre-treatment methods like moist incubation using radiation, pollen baiting followed by drying, glycerol, centrifugation, air dry, dry heat, and cellulose infiltration are the most regularly used methods for the isolation of actinomycetes. Actinomycetes like *Streptomyces*, *Spirilliplanes*, *Actinomydura*, *Microbispora*, and *Spirilliplanes* are being isolated applying the physical-pre-treatment method (Hayakawa et al., 1991a, Tamura et al., 1997). Chemical pre-treatment methods like calcium carbonate and chitin treatment, Chloramine-T, SDS, yeast extract, calcium chloride, Phenol, Germicide, and Chemotactic agents are being applied for the isolation of actinomycetes. *Micromonospora*, *Streptomyces violaceusniger*, *Herbidospora*, *Microbispora*, *Microtetraspora*, *Nonomuraea*, and *Streptosporangium* are being isolated using chemical pre-treatment method e (Hayakawa et al., 2004, 1997, 1991a and 1989). It has been established that combined physical and chemical pretreatment

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

methods are the most appropriate methods for the isolation of actinomycetes. Combined dry heat treatment of soil with chemicals like phenol and benzethonium chloride proved beneficial in the isolation of actinomycetes. *Actinomadura viridis*, *Microbispora*, *Streptosporangium*, *Dectylosporangium*, and *Microtetraspora* are being isolated using combined physical and chemical pretreatment methods (Hayakawa et al., 1995a, 1991a, 1991b, and 1996b). Enrichment treatment like coal-vitamin medium, Rehydration, and centrifugation (RC) method and pre-treatment with peptone (6%) and lauryl sulfate (0.05%) are being applied for the isolation of actinomycetes. Actinomycetes like *Thermomonospora*, *Saccharopolyspora rectivirgula*, *Actinokineospora*, *Catenuloplanes*, *Kineosporia*, and thermophilic *Streptomyces* spp are being isolated using enrichment treatment method (Kurtboke et al., 1993, Hayakawa et al., 1991c). Membrane filter methods are being used for the specific isolation of the actinomycetes. It includes an overlap of nutrient agar medium on the 0.22 to 0.45 µm pore size cellulose ester membrane. Following inoculation and during incubation, actinomycetes penetrate the filter pores through its branched mycelial networks (Hirsch et al., 1983).

All these methods are generalized methods for the isolation of actinomycetes from the geographically diverse sediment samples. However, it is essential to implement taxon definite isolation procedures for the selective isolation of the actinomycetes from the geographically diverse habitats. Isolation of specific or selective actinomycete taxa is possible using glucose-yeast extract agar supplemented with rifampicin and streptomycin (Athalye et al. 1981), starch-casein-nitrate agar (Kuster and Williams 1964), humic acid-vitamin agar (Hayakawa and Nonomura 1987), SM3 agar (Tan et al. 2006), M3 agar (Rowbotham and Cross 1977) and raffinose-histidine agar (Vickers et al. 1984). However, the effectiveness of methods established for the selective isolation actinomycetes taxa was not completely established (Goodfellow et al. 2010).

2.3 Other approaches for the search of Actinomycetes*:*

It has been reported that < 1 % of the microorganisms were isolated and characterized through traditional culturing techniques and diverse groups of actinomycetes are inaccessible (Kennedy et al. 2010; Rath et al. 2011). Unculturable marine bacterial and fungal strains which produce biologically useful secondary metabolites have inadequate accessibility due to its wide genetic diversity to explore the chemical diversity for various therapeutic and industrial applications (Bull et al. 2005; Fenical and Jensen 2006). These strains include complex marine sources like a sponge, tunicates, dinoflagellates, and terrestrial sources like plant-microbe, biofilm, insect-gut, and the human gut. Exploration of these chemical and biological microbial resources through newer approaches would be helpful in the discovery of newer chemical scaffolds with diverse biological activities.

Direct extraction of nucleic acid from the actinomycetes proved to be the effective culture-independent method for obtaining actinomycetes (Monciardini et al. 2002; Das *et al*.2007; Mincer et al. 2005; Sun et al. 2010). It includes the amplification of DNA or cDNA from RNA isolated from the environmental samples using PCR (Bull et al. 2005). Subsequently, these samples can be analyzed, cloned, and sequenced to discover and count new actinomycetes (Stach et al. 2003; Riedlinger et al. 2004). Different selective primers were developed for PCR amplification of 16S rDNA from the varied Actinomycetales families like *Micromonosporaceae*, *Streptomycetaceae*, *Streptosporangiaceae*, and *Thermomonosporaceae*. Application of these primers on the different environmental samples exhibited recurrent existence of these Actinomycetes and divulged sequences were characterized as the new groups of actinomycetes. It was used as the promising prospectus for the discovery of secondary metabolites with the novel chemical scaffold (Monciardini et al. 2002). Other molecular techniques that were used for the identification of the actinomycetes were terminal restriction fragment polymorphism (T-RFLP) analysis, denaturing gradient gel electrophoresis (DGGE), and TGGE (thermal-GGE). T-RFLP is the combination of three techniques like comparative genomics/RFLP, PCR, and electrophoresis. T-RFLP is useful in the measurement of the size polymorphism of terminal restriction fragments from a PCR-amplified marker. It helps in electrophoretically resolving fragments of DNA with different sequences but with the same length (Muyzer 1999; Wawrik et al. 2005). PCR-DGGE was extensively implemented to estimate the diversity and community of actinomycetes in the environmental samples (Wawrik et al. 2005; Das et al. 2007; Nimnoi et al. 2010). Culture-dependent and metagenomic techniques proved to be promising techniques to explore complete uncultured microbe diversity and provided tools to explore the biochemical pathways of the uncultured microbes (Riesenfeld et al. 2004; Venter et al. 2004). Theoretical, technological and conceptual advances provided the basis for the application of the sequence directed metagenomic investigations of the marine actinomycetes. Random shotgun sequencing of the environmental DNA proved practicable due to recent advancements in the high-throughput sequencing technology and lower cost sequencing technologies (Kennedy et al. 2010). The most useful metagenomic investigation includes the whole metagenome shotgun sequencing method in the cloning and sequencing of the microbial DNA from marine environments (Kennedy et al. 2010). This technique is useful in providing full information on the detected gene and also exploring the individual metabolic pathways of the marine microorganisms (Rath et al. 2011). Metagenomic and metaproteomic techniques collectively proved promising newer approaches for the gene, genome, protein, and metabolic

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

pathways discovery of microorganisms (Rath et al. 2011). ET-743 (Yondelis) biosynthetic pathway was developed based on the meta-omic approach to explore the pathways from the invertebrate-derived microbial consortia (Rath et al. 2011). Moreover, the metaproteomic approach is useful in the expression of biosynthetic proteins. Hence, metabolic engineering which comprises of metagenomic and metaproteomic techniques would be useful in the discovery and development of new chemical entities and drugs (Rath et al. 2011). Pyrosequencing platforms are being considered as the next-generation sequencing methods for the *de novo* microbial genome sequencing (MacLean et al. 2009). Pyrosequencing is emerging as the cost-effective methodology which is applicable in the metagenomic analysis of marine microorganisms (Kennedy et al. 2010). Random shotgun sequencing and pyrosequencing don't require prior knowledge of gene sequencing; hence there is the possibility of the discovery of newer gene sequences (Kennedy et al. 2010). Molecular approaches related to the 16S rRNA analysis facilitate the exploration of marine microbial populations devoid of culturing and cultivation of microorganisms. It proved feasible for quantitative estimation of microbial diversity (Olsen et al. 1986; Amann et al. 1995; Monciardini et al. 2002). Diverse approaches of the 16S rRNA are available for exploring microbial diversity (Brinkhoff et al. 1998). The integration of different approaches is also proved useful in obtaining diverse microorganisms. Molecular approaches were being combined with the microbial approaches to the isolation of microbes (Großkopf et al. 1998). In a few of the studies, multiple techniques like molecular techniques, microbiological techniques, geochemical techniques, and microsensors were used for the exploration of novel microorganisms (Ramsing et al. 1996; Amann and Kuhl 1998; Teske et al. 1996). The integration of different technologies would be helpful in the exploration of the interaction between the microorganism and its environment. There is no technology available for the exploration of interaction among microorganisms and their environment. Exploration of this environmental interaction proved helpful in establishing an effective procedure for the isolation of microorganisms (Brinkhoff et al. 1998).

Application of genomic technologies is advantageous in the generation of the actinomycete metabolite gene clusters for the generation of known and predicted secondary metabolites (McLeod et al. 2006; Oliynyk et al. 2007; Goodfellow and Fiedler2010). Study of *Streptomyces avermitilis* revealed that secondary metabolite gene cluster is feasible through searching for homology to a polypeptide of known function which is responsible for secondary metabolism. It was evident that *S. avermitilis* comprises of 25 secondary metabolite clusters (Ōmuraetal. 2001; Ikedaetal.2003). Information about the secondary metabolites can be obtained from the genetic information of DNA libraries. It results in the discovery of gene clusters of both new and known secondary metabolites (Van Lanen and Shen 2006). Biochemical studies on the natural-product biosynthetic enzymes resulted in the exploration of new enzyme pathways and uncommon chemical conversions. The Discovery of novel enzymes is helpful in the implementation of full-fledged genomics-based natural-product discovery (Van Lanen and Shen 2006). Genome mining is also advantageous in the generation of secondary metabolites through the activation of silent clusters by the manipulation of the pathway-specific regulatory genes (Challis 2008). Gaudimycins were identified as two silent types II polyketide synthase systems in *Streptomyces* spp. (Palmu et al. 2007; Challis 2008). Genome mining also provides useful information to understand chemical or environmental signals which are necessary for the expression of tapped biological activity in the hidden natural-product diversity. It also provides information related to the new biosynthetic mechanisms and its genetic-hardware (Wilkinson and Micklefield 2007).

Other than molecular and microbial methods; optimization of fermentation technology also proved beneficial in increasing several-fold productions of secondary metabolites (Martin and Demain 1980; Sanchez and Demain 2002; Bode et al. 2002; Sujatha et al. 2005; Fourati et al. 2005). Secondary metabolites production from the actinomycetes depends on the various fermentation parameters like nutrients (Augustine et al. 2004; Fourati et al. 2005), metal ions (Gesheva et al. 2005), the partial pressure of oxygen (pO2) (Iwai and Omura 1982), inhibitors (Bibb 2005), pH (Sujatha et al. 2005), inducers (Martin and Demain 1980; Doull and Vining 1990; Cheng et al. 1995), temperature (Sujatha et al. 2005), precursors (Martin and Demain 1980; Omura and Tanaka 1986), mineral salts (Wang et al. 2003) and agitation (Bode et al. 2002). It has been established that alteration in these parameters proved advantageous in augmenting the quality and quantity of secondary metabolites (Bode et al. 2002). Most of the earlier studies were directed towards the alteration in the cultivation parameters and very few studies were conducted through alteration in the stress conditions like rheology and aeration. It has been established that alteration in the three or four conditions is optimum for improving the production of secondary metabolites. It indicates molecular biology and microbiological tools proved helpful in improving access to the new gene pool and fermentation technologies proved beneficial in producing newer chemical scaffolds. The integration of molecular biology and microbiology methods with fermentation technologies would be helpful in the identification of the biologically active druggable targets. Work in this direction is underway and it improved the rate of discovery from the microbial origin.

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

3.1 Analysis of the secondary metabolites:

Analysis of the isolated secondary metabolites using hyphenated techniques like HPLC-diode array screening and HPLC-UVvisual database (Huber and Fiedler 1991). Unknown metabolites were obtained by excluding the known metabolites from the database containing the primary and secondary metabolites. UV–Visible properties and retention times provided valuable information for the characterization of the new metabolites. Presumptive novel metabolites were confirmed using HPLC-MS analysis. Subsequently, scale-up fermentation of strains and isolation and structural elucidation of the secondary metabolites was carried out. This strategy has resulted in the isolation of a large number of secondary metabolites from the actinobacteria (Fiedler 2010). It is also beneficial in the selective separation of secondary metabolites and application of diode array proved advantages in the detailed analysis of the individual extract. This method is applicable in the analysis of the broad secondary metabolites in both culture filtrate and raw extracts of organisms. However, secondary metabolites with high molarity and metabolites without UV-Visible chromophores are unable to detect in culture filtrates and raw extracts. Integration of chemical and biological characteristics of the secondary metabolites are more valuable in the identification of secondary metabolites. These methods include target assays based on enzyme or receptor inhibition. It is useful in establishing a robust correlation between secondary metabolite, biological activity, and target (Baltz 2005). These methods are based on the advances in chemical biology which explore the biological relevance of the chemicals isolated from the actinobacteria.

3.2 Actinomycetes and approved drugs:

Tigecycline is derived product of chlortetracycline which is a 9-tert-butyl-glycylamido derivative of minocycline. Tigecycline exhibits more potent antibacterial activity against tetracycline-resistant organisms (Zhanel et al., 2004). Telithromycin is obtained from the *Saccharopolyspora erythraea* and it is a semi-synthetic derivative of the 14-members macrolide, erythromycin A. It affects respiratory tract resistant pathogens resistant to macrolides. It exhibits its action by inhibiting protein synthesis through interaction with the peptidyltransferase site of the bacterial 50S ribosomal subunit (Zhanel et al., 2002). Miglustat is isolated from the *Streptomyces lavendulae* which is an analog of nojirimycin. It exhibits action by reversibly inhibiting glucosylceramide synthase which is a ceramide-specific glucosyltransferase that catalyzes glucocerebrosid formation and subsequently decreases glucosylceramide (Pastores et al., 2005). Daptomycin is isolated from the *Streptomyces roseosporus* which is permitted for treatment and management of infections (cSSSIs). Daptomycin exhibits its action on the bacterial cell membranes which disrupt membrane potential. It results in the blockage of synthesis of proteins. Biapenem is thienamycin based carbapenem isolated from the *Streptomyces cattleya*. It is also active against β-lactamases producing microorganisms (Perry and Ibbotson, 2002). Ertapenem is a 1β-methylcarbapenem class compound based on thienamycin which is isolated from the *Streptomyces cattleya*. It exhibits broad-spectrum antibacterial activity against clinically relevant Enterobacteriaceae (Sader and Gales, 2001).

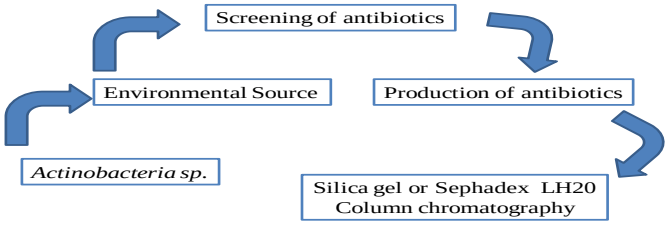

Figure 2: Scheme for the isolation of Actinobacteria antibiotic

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

Table 2: Number of *Actinomycetes sp.* producing bioactive microbial metabolites

Actinomycetes species	No.	Actinomycetes species	No.
Streptomycetaceae		**Streptosporangiaceae**	
Chainia	- 36	Kutzneria	- 5
Kitasatosporia	- 44	Planomonospora	- 3
Microellobosporia	- 21	Planobispora	- 12
Nocardioides	- 14	Spirillospora	- 14
Streptoverticillium	- 278	Streptoalloteichus	- 52
Streptomyces	- 8056	Streptodporangium	- 82
Micromonosporaceae		**Thermomonosporaceae**	
Actinoplanes	- 272	Actinomadura	- 357
Ampullariella	- 10	Actinosynnema	- 54
Catellatospora	- 2	Microbiospora	- 56
Catenuloplanes	- 5	Microtetraspora	- 28
Dactylosporangium	- 65	Micropolyspora	- 15
Glycomyes	- 6	Nocardiopsis	- 43
Micromonospora	- 832	Saccharothrix	- 72
Pseudonocardiaceae		Thermoactinopolyspora	- 2
Actinopolyspora	- 2	Thermopolyspora	- 1
Amycolata	- 14	Thermoactinomyces	- 16
Kibdellosporangium	- 37	Thermomonospora	- 21
Nocardia	- 365		
Pseudonocardia	- 31	**Mycobacteriaceae**	
Saccharomonospora	- 4	Arthrobacter	- 25
Saccharopolyspora	- 127	Brevibacterium	- 17
		Mycobacterium	- 57
		Nocardia	- 357
		Proactinomyces	- 14
		Rhodococcus	- 13

3.3 Antagonistic activity of *Actinobacteria:*

New drug discovery reports demonstrated that screening of plant extracts and microorganisms for the novel chemical skeletons for compounds (Shadomy, 1987; Lacey, 1978). Widely used antibiotics like macrolides and tetracyclines are obtained from various *Streptomyces aureofaciens* strains (White et al., 2001; Stryzhkova et al., 2002). Based on the source of the soil, these actinobacteria exhibit specificity to certain microorganisms. Actinobacteria obtained from the pepper-field soil proved effective against *Alternaria mali*, *Colletotrichumgleo sporioides*, *Fusarium oxysporum* and *Rhizoctonia solani*. Actinobacteria obtained from the radish – filed soil proved effective against *Magnaporthe grisea* and *Phytophthora capsica (*Lee and Hwang, 2002). Fifteen isolates exhibited effectiveness against Coagulase-negative Staphylococcus. Basilio et al. (2003) demonstrated phenotype diversity in Actinobacteria based on their studies. Basilio et al. (2003) screened 335 isolates for categorizing under salinity. 230 strains exhibited antimicrobial activity with 77 % Streptomyces species and 49 % actinomycetes from other taxa exhibited antimicrobial activity. This study supported the idea of isolating actinomycetes with alteration in the pH and salinity. Active secondary metabolites were produced from the actinomycetes which were isolated in the saline conditions. 563 different types of actinomycetes were evaluated for antimicrobial activity using susceptibility disk assay method. 286 actinomycetes produced compounds with antifungal activity. It was evident that soil rich in minerals produced compounds with maximum antifungal activity and most of the isolated antifungal compounds were polyene macrolide antibiotics. Nathan et al. (2004) reported 102 isolated actinomycetes from the subtidal marine sediments collected from the Bismarck Sea and the Solomon Sea off the coast of Papua New Guinea.These Actinobacteria were evaluated for antimicrobial activity against four pathogenic bacteria. Tested microorganisms were *Agrobacterium tumefaciens*, *Erwinia amylovora*, *Pseudomonas viridiflova*, *Clavibacter michiganensis* subsp. michiganensis, *Bacillus subtilis*, and *Sarcina lutea*. Isolated strains of Actinobacteria were *Streptomyces* (64), *Micromonospora* (8), *Nocardia* (5), *Streptoverticilium* (7), and *Saccharopolyspora* (4). Out of 64 strains of Streptomyces, 44 exhibited antibacterial activity. Out of 25 Actinobacteria, 56 % exhibited antimicrobial activity against bacteria and 8 % exhibited antimicrobial activity against fungi. Isolated Actinobacteria were identified using universal PCR. It was observed that 93 % were belonging to *the Streptomyces* genus and *Actinomadura* genus. Out of 94

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

Actinobacteria, 87 % were from *Streptomyces* genus and the remaining was from *Micromonospora* genus. 51 % Actinobacteria exhibited activity against pathogenic *Vibrio* spp. It was reported that *Streptomycetes* could be promising biocontrol agents in aquaculture. Augustine et al. (2005) isolated 312 Actinobacteria from soil and water samples. MAR01 was also active against Gram-positive and *Candida albicans*. It was designated as Meroparamycin. Srivibool and Sukchotiratana (2006) isolated 495 Actinobacteria from the 45 soil samples. Preliminary antimicrobial activity was performed on *Micrococcus luteus, Pseudomonas aeruginosa* and *Escherichia coli*. Out of 495 isolated Actinobacteria; 58 exhibited promising antimicrobial activity. Out of 21 Actinobacteria, 12 were active against microorganisms like *Mycobacterium phlei*, *Candida albicans* and *Fusarium moniliforme*.

Osaky et al. (2004) isolated 50 different strains of actinomyces form the soil. They demonstrated that 34 %, 16 %, 6 %, and 12 % isolated actinomycetes exhibited broad-spectrum, against Gram-positive, against Gram-negative and both Gram-positive and negative bacteria respectively. Rabah et al. (2007) isolated 19 strains of actinomycetes from the Egyptian soil and demonstrated that 68 % Streptomyces spp. demonstrated activity against Gram-positive and negative bacteria, fungi, and yeasts. The authors also optimized the method of production of antibiotics and reported that the use of the ISP-4 medium produces a maximum amount of antibiotics. Ceylan et al. (2008) isolated 15 different strains of Streptomyces spp. from different soil samples of Turkey. Five promising *Streptomyces* spp. were subjected for detailed study and it was demonstrated that *Streptomyces albovinaceus* was most effective against both bacteria and fungi. Awad et al. (2009) isolated 60 different *Streptomyces* species from the different regions of Egypt. Further characterization revealed that *Streptomyces* sp. NRC-35 exhibited prominent antimicrobial activity through ß-lactamase inhibition. Dehnad et al. (2010) isolated 150 actinomycetes from the different soil samples of Iran. Singh et al. (2012) demonstrated antimicrobial activity of 7 Actinomycetes strains against Methicillin-Resistant *Staph. aureus* (14 mm) and Vancomycin-resistant *Enterococci*. Bizuye et al. (2013) isolated 30 Actinomycetes spp. from samples soil of Ethiopia. Among them, three isolates exhibited promising antimicrobial activity against pathogenic microorganisms like *Klebsiella pneumonia* ATCC7000603, *E. coli* ATCC25922, methicillin-resistant *Staph. aureus* strains 2 (MRSA2) and MRSA4. Sripreechasak et al., (2014) isolated 31 Actinomycetes spp. from the 6 soil samples of Thailand. Among them 9, 8, 6, 2, and 5 Actinomycetes spp. exhibited activity against *B. subtilis*, *Kocuria rhizophila*, *Mucor racemosus*, *E. coli* and *Xanthomonas campestris* respectively.

Subramani and Narayanasamy (2009) showed approximately 40 species exhibited antimicrobial activity human pathogens and 55 % species exhibited activity against plant pathogens. Kumar et al. (2010) isolated 117 Actinomycetes spp. from India and demonstrated that 10 % Actinomycetes spp. exhibited activity against bacteria. Duraipandiyan et al (2010) demonstrated twelve isolated samples from the Himalayan soil. ERIH-44 isolated form the genus *Bacillus* exhibited promising antagonistic activity. MIC values of < 15.62 µg/ml, < 15.62 µg/ml, 125 µg/ml, 500 µg/ml, 500 µg/ml and 1000 µg/ml respectively against *B. subtilis*, *Staph. aureus*, *E.coli*, *P. aeruginosa*, *Botrytis cinereal*, and *Trichophyton mentagrophytes* respectively. Sharma et al. (2010) isolated 134 Actinomycetes from different soil samples of India. Isolated Actinomycetes acts antagonistic activities against a wide spectrum of microorganisms including multidrug-resistant methicillin-resistant *Staph. aureus* (MRSA) and *E. coli*. 38 % isolates exhibited growth of microorganisms while six isolates exhibited broad-spectrum antimicrobial activity.

Table 3: Diverse antibiotic compounds from Actinomycetes

Antibiotics	**Actinomycetes source**	**Chemical class**	**Mechanism of Action**
Abyssomicins	*Verrucosispora* AB-18-032	Natural polycyclic polyketide	PABA pathway inhibitor
Bekanamycin	*Streptomyces kanamyceticus*	Aminoglycoside	Inhibiting protein synthesis and increasing translation errors
Biapenem	*Streptomyces cattleya*	Carbapenem	It exhibits its action by inhibiting bacterial cell wall synthesis.
Bonactin	*Streptomyces* sp. BD21-2	Ester	
Carbomycin	*Streptomyces halstedii*	Macrolide	Inhibits bacterial protein synthesis
Chloramphenicol	*Streptomyces venezuelae*	Acetamide	Inhibits protein biosynthesis by impairing translation on the 50S ribosomal

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

			subunit at the peptidyl transferase step
Chlortetracycline	*Streptomyces aureofaciens*	Tetracyclines	Inhibits protein synthesis (elongation) by preventing binding of aminoacyl-tRNA to the 30S subunit.
Clindamycin	*Streptomyces* sp	Galactooctopyranoside	Inhibits bacterial protein synthesis
Daptomycin	*Streptomyces roseosporus*	Lipopeptide	Bactericidal activity by disrupting plasma membrane function without penetrating the cytoplasm
Erythromycin	*Saccharopolyspora erythrea* (*Streptomyces erythreus*)	Macrolide	Inhibits elongation at transpeptidation step of protein biosynthesis
Ertapenem	*Streptomyces cattleya*	Thienamycin	It exhibits its action by inhibiting cell wall synthesis and is mediated through ertapenem binding to penicillin-binding proteins (PBPs).
Framycetin	*Streptomyces fradiae*	Aminoglycoside	Inhibition of bacterial protein synthesis via binding to ribosomal subunits.
Gentamicin	*Micromonospora purpurea*	Aminoglycoside	Inhibits protein synthesis by binding to L6 protein of 50S ribosomal subunit
Grisein (Albomycin)	*Streptomyces griseus*	Cyclic hexapeptide (Sideromycin group)	Inhibits seryl-t-RNA synthetase and impairs protein biosynthesis
Kanamycin	*Streptomyces kanamyceticus*	Aminoglycoside	Inhibiting protein synthesis and increasing translation errors
Lincomycin	*Streptomyces lincolnensis*	Galactooctopyranoside	Inhibits bacterial protein synthesis
Micromonosporin	*Micromonospora* sp	Polyene lactam macrolide antibiotic	Inhibits bacterial protein synthesis.
Miglustat	*Streptomyces lavendulae*	Nojirimycin.	It exhibits action by reversibly inhibiting glucosylceramide synthase.
Neomycin	*Streptomyces fradiae*	Aminoglycoside	It binds the 30S and in some cases, the 50S subunit causing miscoding; inhibits initiation and elongation during protein synthesis
Novobiocin	*Streptomyces niveus*/ *S. spheroides*	Aminocoumarin	Inhibits DNA synthesis by inhibiting the DNA polymerization
Oleandomycin	*Streptomyces antibioticus*	Macrolide	Inhibits bacterial

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

			protein synthesis
Oxy tetracycline	*Streptomyces rimosus*	Tetracycline	Inhibits protein synthesis (elongation) by preventing binding of aminoacyl-tRNA to the 30S subunit
Pyridomycin	*Streptomyces pyridomyceticus*	Peptide	Inhibits bacterial protein synthesis
PM181104	*Kocuria* sp.	Peptide	Inhibits bacterial protein synthesis
Ramoplanin (INN)	*Actinoplanes* sp ATCC 33706	Glycolipodepsipeptide	Inhibits transglycosylation in peptidoglycan synthesis
Rifamycin	*Amycolatopsis rifamycinica*	Naphthalene containing subclass of ansamycins	Inhibits bacterial DNA-dependent RNA-polymerase
Spectinomycin	*Streptomyces spectabilis*	Aminocyclitol	Disrupts bacterial protein synthesis
Spiramycin	*Streptomyces ambofaciens*	Macrolide	Inhibits protein biosynthesis by the rapid breakdown of polyribosomes by binding 50S unit
Streptocin	*Streptomyces griseus*	Aminoglycoside	Inhibits bacterial protein synthesis
Streptogramin A	*Streptomyces virginiae*	Polyketide-Streptogramin	Inhibits protein biosynthesis by binding to the 50S ribosome unit.
Streptomycin	*Streptomyces griseus*	Aminoglycoside	Inhibits prokaryote protein synthesis by binding to S12 protein of the 30S ribosomal subunit, causing miscoding or inhibiting initiation.
Streptothricin	*Streptomyces lavendulae*, *Streptomyces noursei*	Imidazo pyridine-4one	Inhibits polypeptide synthesis via interaction with the ribosome.
Telithromycin	*Saccharopolyspora erythraea*	Macrolide	It exhibits its action by inhibiting protein synthesis through interaction with peptidyltransferase site of the bacterial 50S ribosomal subunit.
Teicoplanin	*Actinoplanin teichomyceticus*	Glycopeptide	Binds to the D-ALA-D-ALA terminal end of peptidoglycan precursors and inhibits cell-wall synthesis.
Tigecycline	*Saccharopolyspora erythraea*	Chlortetracycline	It acts as a protein synthesis inhibitor and exhibits action by binding to the 30S ribosomal subunit.

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

Thiolactomycin	*Nocardia* sp	Thiolactone	Inhibition of fatty acid synthesis
Thiostrepton	*Streptomyces azureus* and *Streptomyces laurentii*	Cyclic oligopeptide	Impairment of the coupling of the 30-S initiation complex to the 50-S ribosomal subunit.
Vancomycin	*Amycolatopsis orientalis*	Glycopeptide	Inhibits cell wall synthesis

CONCLUSION:

The microbes which are undiscovered and unusual or rare may contain possible cures for diseases demanding new antibiotics to combat the multidrug-resistant human pathogens and emerging deadly diseases. Application of selective isolation and enriched methods can lead to the discovery of new/novel and rare bioactive Actinomycetes from unexplored ecological niches having the potential to biosynthesize novel bioactive compounds. A combination of natural products chemistry along with modern genetics will successfully help in overcoming the demand and supply problems of the past, promote the production of natural secondary metabolites from the rare actinomycetes as well as identify various alternatives for future drug discovery programs. The microbial groups with proven track records such as the Actinomycetes t in drug discovery process form a strong entity in the future. Therefore we can conclude that targeting rare and novel actinomycetes will lead to discovery of novel chemical structures with strong bioactivity combating the problem of drug-resistance and other associated complexities.

REFERENCES:

[1] Das, S., Lyla, P. S and Khan, S. A. 2008. Distribution and generic composition of culturable marine actinomycetes from the sediments of Indian continental slope of Bay of Bengal. Chinese Journal of Oceanology and Limnology, 26 (2): 166 - 177.

[2] Lacey, J. 1978. Ecology of actinomycetes in fodder and related substrates, In: M. Mordarski, W. Kurylowicz and J. Jeljaszewicz (ed.) Nocardia and Streptomyces: Proceedings of the international symposium on Nocardia and Streptomyces. Warsaw. October 1976. Gustav fisher Verlag, Stuttgart: 161 - 168.

[3] Shadomy, S. 1987. Preclinical evaluation of antifungal agents in Recent trends in the discovery, development and evaluation of antifungal agents. New Jercy. Prous Science, 8 - 14.

[4] Stryzhkova, H. M., Kopeiko, O. P., Lavrinchuk, V. L., Bambura, O. L and Matscliukh, B. P. 2002. Spontaneous and induced variability of Streptomyces aurofaciensch lortetracycline producer. Microbial Zentable, 64: 19 - 23.

[5] White, J. D., Hanselman, R., Jackson, R.W., Porter, W. J., Ohba, Y., Tiller, T. and Wang, S. 2001. Total synthesis of Rutamycin B, amacrolide antibiotic from Streptomyces aurofacins. Journal of Organic Chemistry, 66: 5217 - 5231.

[6] Lee, J. Y and Hwang, B. K. 2002. Diversity of antifungal actinomycetes in various vegetative soils of Korea. Canadian Journal of Microbiology, 48 (5): 407 – 417.

[7] Sahin, N and Ugur, A. 2003. Investigation of the Antimicrobial activity of some Streptomyces isolates. Turkey Journal of Biology, 27: 79 – 84.

[8] Basilio, A., Gonzalez, I., Vicente, M.F., Gorrochategui, Cabello, Gonzalez, A. and Genilloud, O. 2003. Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. Journal of Applied Microbiology, 95 (5): 814 - 823.

[9] Iznaga, Y., Lemus, M., Gonzalez, L., Garmendia, L., Nadal, L and Vallin, C. 2004. Antifungal activity of Actinomycetes from Cuban soils. Biotechnology Department, 18: 494 – 496.

[10] Nathan A. Magarvey, 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. Applied Environmental Microbiology, 70 (12): 7520 – 7529.

[11] Oskay, M., Tamer, A. U and Azeri, C. 2004. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology, 3 (9): 441 – 446.

[12] Sujatha Peela, V. V. S. N., Bapiraju Kurada, and Ramana Terli. 2005. Studies on antagonistic marine actinomycetes from the Bay of Bengal. World Journal of Microbiology and Biotechnology, 21: 583 – 585.

[13] Kitouni, M., Boudemagha, A., Oulmi, L., Reghioua, S., Boughachiche, F., Zerizer, H., Handiken, H., Couble, A., Mouniee, D., Boulahrouf, A and Boiron, P. 2005. Isolation of actinomycetes producing

bioactive substances from water, soil and tree bark samples of North - East of Algeria. Journal of Medical Mycology, 15: 45 – 51.

[14] Dhanasekaran, D., Panneerselvam, A and Thajuddin, N. 2005. Antifungal actinomycetes in marine soils of Tamilnadu. Geobios, 32: 3740.

[15] You, J., Cao, L., Liu, G., Zhou, S., Tan, H and Lin, Y. 2005. Isolation and Characterization of actinomycetes antagonistic to pathogenic Vibrio spp. from near shore marine sediments. World Journal of Microbiology, 21 (5): 679 – 682.

[16] Augustine, S. K., Bhavsar, S. P and Kapadnis, B. P. 2005. A non-polyene antifungal antibiotic from Streptomyces albidoflavus PU23. Journal of Bioscience, 30: 201 – 211.

[17] Ilic, S. B., Konstantinovic, S. S and Todorovic, Z. B. 2005. UV/VIS Analysis and antimicrobial activity of Streptomyces isolates. Medicine and Biology, 12 (1): 44 - 46.

[18] Asha Devi, N. K., Lisa. Jeyarani and Balakrishnan, Z. K. 2006. Isolation and identification of marine actinomycetes and their potential in Antimicrobial Activity. Pakistan Journal of Biological Sciences, 9 (3): 470 - 472.

[19] Naggar, M. Y., El-Assar, S. A. and AbdulGaward, S. M. 2006. Meroparamycin production by newly isolated Streptomyces sp. Strains MAR01: Taxonomy, fermentation purification and structural elucidation. Journal of Microbiology, 7 (8): 432 - 438.

[20] Srivibool, R and Sukchotiratana, M. 2006. Bioperspectine of actinomycetes isolates from coastal soils. A new source of antimicrobial procedures. Journal of Science and Technology, 28 (3): 493 – 499.

[21] Singh, L. S., Baruah, I and Bora, T. C. 2006. Actinomycetes of Loktak Habitat: Isolation and screening for antimicrobial activities. Biotechnology, 5 (2): 217 – 221.

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

[23] Newman, D.J.; Cragg, G.M. 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70, 461-477.

[24] Baltz, R.H. Renaissance in antibacterial discovery from actinomycetes. Curr. Opin. Pharmacol. 2008, 8, 557-563.

[25] Zhanel, G.G., K. Homenuick, A. Noveddin, K. Nichol and L. Vercaigne et al., 2004. The glycyclines: A comparative review with the tetracyclines. Drugs, 64: 63-88.

[26] Zhanel, G.G., M. Walters, A. Noreddin, L.M. Vercaigne and A. Wierzbowski et al., 2002. ketolides: A critical review. Drugs, 62: 1771-1804.

[27] Pastores, G.M., N.L. Barnett and E.H. Kolodny, 2005. An open-label, noncomparative study of miglustat in type I Gaucher disease: Efficacy and tolerability over 24 months of treatment. Clin. Ther., 27: 1215-1227.

[28] Fenton, C., G.M. Keating and M.P. Curran, 2004. Daptomycin. Drugs, 64: 445-455. Perry, C.M. and T. Ibbotson, 2002. Biapenem. Drugs, 62: 2221-2234.

[29] Sader, H.S. and A.C. Gales, 2001. Emerging strategies in infectious diseases: New carbapenem and trinem antibacterial agents. Drugs, 61: 553-564.

[30] Hong, K., Gao, A,. Xie, Q., Gao, H., Zhuang, L., Lin, H., Yu, H., Li, J., Yao, X., Goodfellow, M., Ruan, J. 2009. Actinomycetes for Marine Drug Discovery Isolated from Mangrove Soils and Plants in China. Mar. Drugs 7:24-44

[31] Matsukawa, M., Nakagawa, Y., Limura, Y,. Hayakawa, M. 2007. A New Enrichment Method for the Selective Isolation of Streptomycetes from the Root Surfaces of Herbaceous Plants. Actinomycetologica, 21: 66-69.

[32] Hayakawa, M., Sadakata, T., Kajiura, T., Nonomura, H. 1991a. New methods for the highly selective isolation of Micromonospora and Microbispora from soil. J. Ferment Bioeng., 72: 320-326.

[33] Tamura, T., Hayakawa, M., Hatano, K. 1997. A new genus of the order Actinomycetales, Spirilliplanes gen. nov., with description of Spirilliplane syamanashiensis sp. nov. Int. J.Syst. Bacteriol., 47:97-102.

[34] Hayakawa, M., Nonomura. H. 1989. A new method for the intensive isolation of actinomycetes from soil. Actinomycetol., 3: 95-104.

[35] Hayakawa, M., Yoshida, Y., Iimura, Y. 2004. Selective isolation of bioactive soil actinomycetes belonging to the Streptomyces violaceusniger phenotypic cluster. J. Appl. Microbiol., 96: 973-981.

[36] Hayakawa, M., Iino, H., Takeuchi, S., Yamazaki, T. 1997. Application of a method incorporating treatment with Chloramine-T for the selective isolation of Streptosporangiaceae from soil. J. Ferment. Bioeng., 84:599-602.

[37] Hayakawa, M., Momose, Y., Kajiura, T., Yamazaki, T., Tamura, T., Hatano, K., Nonomura, H. 1995a. A selective isolation method for Actinomadura viridis in soil. J. Ferment. Bioeng., 79: 287-289.

[38] Hayakawa, M., Tamura, T., Iino, H., Nonomura, H. 1991b. New methods for the highly selective isolation of Streptosporangium and Dactylosporangium from soil. J. Ferment. Bioeng., 72: 327-333.

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

[39] Hayakawa, M., Momose, Y., Yamazaki, T., Nonomura, H. 1996b. A method for the selective isolation of Microtetrasporaglauca and related four-spored actinomycetes from soil. J. Appl. Bacteriol., 80: 375–386.
[40] Kurtboke, D., Wilson, C., Sivasithamparam, K. 1993. Occurrence of Actinomadura phage in organic mulches used for avo-cado plantations in Western Australia. Can. J. Microbiol., 39: 389- 394.
[41] Hayakawa, M., Tamura T, Nonomura, H. 1991c. Selective isolation of Actinoplanes and Dactylosporangium from soil by using collidine as the chemoattractant. J. Ferment. Bioeng., 72: 426-432.
[42] Hirsch, C., Christensen, D. 1983. Novel Method for Selective Isolation of Actinomycetes. Appl. Environ. Microbiol., 46: 925-929.
[43] Laidi RF, Kansoh AL, Elshafei AM, Cheikh B (2006) Taxonomy, identification and biological activities of a novel isolate of Streptomyces tendae. Arab J Biotechnol 9(3): 427-436.
[44] Singh V., Praveen V., Banga J., Tripathi C. K. M. (2009). Antimicrobial activities of microbial strains isolated from soil of stressed ecological niches of Eastern Uttar Pradesh, India. Indian J. Exp. Biol. 47, 298–303.
[45] Monciardini P, Sosio M, Cavaletti L, Chiocchini C, Donadio S. New PCR primers for the selective amplification of 16S rDNA from different groups of actinomycetes. FEMS Microbiol Ecol. 2002 Dec 1;42(3):419-29.
[46] Mincer TJ, Fenical W, Jensen PR. Culture-dependent and culture-independent diversity within the obligate marine actinomycete genus Salinispora. Appl Environ Microbiol. 2005 Nov;71(11):7019-28.
[47] Sun W, Dai S, Jiang S, Wang G, Liu G, Wu H, Li X. Culture-dependent and culture-independent diversity of Actinobacteria associated with the marine sponge Hymeniacidon perleve from the South China Sea. Antonie Van Leeuwenhoek. 2010 Jun;98(1):65-75.
[48] Das M, Royer TV, Leff LG. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Appl Environ Microbiol. 2007 Feb;73(3):756-67.
[49] Bull AT, Stach JE, Ward AC, Goodfellow M. Marine actinobacteria: perspectives, challenges, future directions. Antonie Van Leeuwenhoek. 2005 Apr;87(3):65-79.
[50] Kennedy J, Flemer B, Jackson SA, Lejon DP, Morrissey JP, O'Gara F, Dobson AD. Marine metagenomics: new tools for the study and exploitation of marine microbial metabolism. Mar Drugs. 2010 Mar 15;8(3):608-28.
[51] Rath CM, Janto B, Earl J, Ahmed A, Hu FZ, Hiller L, Dahlgren M, Kreft R, Yu F, Wolff JJ, Kweon HK, Christiansen MA, Håkansson K, Williams RM, Ehrlich GD, Sherman DH. Meta-omic characterization of the marine invertebrate microbial consortium that produces the chemotherapeutic natural product ET-743. ACS Chem Biol. 2011 Nov 18;6(11):1244-56.
[52] Fenical W, Jensen PR. Developing a new resource for drug discovery: marine actinomycete bacteria. Nat Chem Biol. 2006 Dec;2(12):666-73.
[53] Stach JE, Maldonado LA, Ward AC, Goodfellow M, Bull AT. New primers for the class Actinobacteria: application to marine and terrestrial environments. Environ Microbiol. 2003 Oct;5(10):828-41.
[54] Riedlinger J, Reicke A, Zähner H, Krismer B, Bull AT, Maldonado LA, Ward AC, Goodfellow M, Bister B, Bischoff D, Süssmuth RD, Fiedler HP. Abyssomicins, inhibitors of the para-aminobenzoic acid pathway produced by the marine Verrucosispora strain AB-18-032. J Antibiot (Tokyo). 2004 Apr;57(4):271-9.
[55] Wawrik B, Kerkhof L, Zylstra GJ, Kukor JJ. Identification of unique type II polyketide synthase genes in soil. Appl Environ Microbiol. 2005 May;71(5):2232-8.
[56] Muyzer G. DGGE/TGGE a method for identifying genes from natural ecosystems. Curr Opin Microbiol. 1999 Jun;2(3):317-22.
[57] Pongrawee Nimnoi, Neelawan Pongsilp, Saisamorn Lumyong. Genetic diversity and community of endophytic actinomycetes within the roots of Aquilaria crassna Pierre ex Lec assessed by Actinomycetes-specific PCR and PCR-DGGE of 16S rRNA gene. Biochemical Systematics and Ecology. Volume 38, Issue 4, August 2010, Pages 595-601.
[58] Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: genomic analysis of microbial communities. Annu Rev Genet. 2004;38:525-52.
[59] Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, et al. Environmental genome shotgun sequencing of the Sargasso sea. Science 2004;304:66–74.
[60] MacLean D, Jones JDG, Studholme DJ. Application of 'next-generation' sequencing technologies to microbial genetics. Nat Rev Microbiol 2009;7:287–96.
[61] Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR. Microbial ecology and evolution: a ribo somal RNA approach. Annu Rev Microbiol 1986;40:337–65.

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

[62] Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 1995;59:143–69.
[63] Brinkhoff T, Santegoeds CM, Sahm K, Kuever J, Muyzer G. A polyphasic approach to study the diversity and vertical distribution of sulfur-oxidizing Thiomicrospira species in coastal sediments of the German Wadden sea. Appl Environ Microbiol 1998;64:4650–7.
[64] Großkopf R, Janssen PH, Liesack W. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultiva tion and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol 1998;64:960–9.
[65] Ramsing NB, Fossing H, Ferdelman TG, Anderson F, Thamdrup B. Distribution of bacterial populations in a stratified fjord (Mariager Fjord, Denmark) quantified by in situ hybridization and related to chemical gradients in the water column. Appl Environ Microbiol 1996;62:1391–404
[66] Amann RI, Kuhl M. In situ methods for assessment of microorganisms and their activities. Curr Opin Microbiol 1998;1:352–8.
[67] Teske A, Wawer C, Muyzer G, Ramsing NB. Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. Appl Environ Microbiol 1996;62:1405-15.
[68] Berdy J. Recent advances in and prospects for antibiotic research. Process Biochem 1980;15:28–35
[69] Berdy J. Bioactive microbial metabolites. J Antibiot (Tokyo) 2005;58:1–26.
[70] Ramesh S, Mathivanan N. Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes. World J Microbiol Biotechnol 2009;25:2103–11
[71] Prabavathy VR, Mathivanan N, Murugesan K. Control of blast and sheath blight dis eases of rice using antifungal metabolites produced by Streptomyces sp. PM5. Biol Control 2006;39:313–9.
[72] Prapagdee B, Kuekulvong C, Mongkolsuk S. Antifungal potential of extracellular metabolites produced by Streptomyces hygroscopicus against phytopathogenic fungi. Int J Biol Sci 2008;4:330–7.
[73] Ebrahimi Zarandi M, Shahidi Bonjar GH, Padasht Dehkaei F, Ayatollahi Moosavi SA, Rashid Farokhi P, Aghighi S. Biological control of rice blast (Magnaporthe oryzae) by use of Streptomyces sindeneusis isolate 263 in greenhouse. Am J Appl Sci 2009;6:194–9.
[74] Pimentel-Elardo SM, Kozytska S, Bugni TS, Ireland CM, Moll H, Hentschel U. Anti parasitic compounds from Streptomyces sp. strains isolated from Mediterranean sponges. Mar Drugs 2010;8:373–80.
[75] Lam KS. Discovery of novel metabolites from marine actinomycetes. Curr Opin Microbiol 2006;9:245–51.
[76] Hong K, Gao AH, Xie QY, Gao H, Zhuang L, Lin HP, et al. Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. Mar Drugs 2009;7:24–44.
[77] Sacramento DR, Coelho RRR, Wigg MD, Linhares LFTL, Santos MGM, Semedo LTAS, et al. Antimicrobial and antiviral activities of an actinomycete (Streptomyces sp.) isolated from a Brazilian tropical forest soil. World J Microbiol Biotechnol 2004;20:225–9.
[78] Xu Y, He H, Schulz S, Liu X, Fusetani N, Xiong H, et al. Potent antifouling compounds produced by marine Streptomyces. Bioresour Technol 2010;101:1331-6.
[79] Martin JF, Demain AL. Control of antibiotic biosynthesis. Microbiol Rev 1980;44:230–51.
[80] Sanchez S, Demain AL. Metabolic regulation of fermentation processes. Enzyme Microb Technol 2002;31:895–906.
[81] Bode HB, Bethe B, Höfs R, Zeeck A. Big effects from small changes: possible ways to explore nature's chemical diversity. Chem Biol Chem 2002;3:619–27.
[82] Sujatha P, Bapi Raju KVVSN, Ramana T. Studies on a new marine Streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant Staphylococcus aureus. Microbiol Res 2005;160:119–26.
[83] Fourati BFL, Fotso S, Ben Ameur-Mehdi R, Mellouli L, Laatsch H. Purification and structure elucidation of antifungal and antibacterial activities of newly isolated Streptomyces sp. strain US80. Res Microbiol 2005;156:341–7.
[84] Augustine SK, Bhavsar SP, Baserisalehi M, Kapadnis BP. Isolation and characteriza tion of antifungal activity of an actinomycete of soil origin. Indian J Exp Biol 2004;42:928–32.

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

[85] Gesheva V, Ivanova V, Gesheva R. Effects of nutrients on the production of AK-111-81 macrolide antibiotic by Streptomyces hygroscopicus. Microbiol Res 2005;160:243–8.
[86] Iwai Y, Omura S. Culture conditions for screening of new antibiotics. J Antibiot 1982;35:123–41.
[87] Bibb MJ. Regulation of secondary metabolism in Streptomycetes. Curr Opin Microbiol 2005;8:208–15.
[88] Doull J, Vining L. Nutritional control of actinorhodin production by Streptomyces coelicolor A (3) 2: suppressive effects of nitrogen and phosphate. Appl Microbiol Biotechnol 1990;32:449–54.
[89] Cheng JR, Fang A, Demain AL. Effect of amino acids on rapamycin biosynthesis in Streptomyces hygroscopicus. Appl Microbiol Biotechnol 1995;43:1096–8.
[90] Omura S, Tanaka Y. Macrolide antibiotics. In: Pape H, Rehn HJ, editors. Biotechnology, IV. Weinheim: VCH Verlagsgesellschaft; 1986. p. 360–91.
[91] Wang YH, Yang B, Ren J, Dong ML, Liang D, Xu AL. Optimization of medium compo sition for the production of clavulanic acid by Streptomyces clavuligerus. Process Biochem 2003;40:1116–66
[92] Athalye M, Lacey J, Goodfellow M (1981) Selective isolation and enumeration of actinomycetes using rifampicin. J Appl Microbiol 51:289–291
[93] Kuster E, Williams ST (1964) Selective media for isolation of streptomycetes. Nature 202:928–929.
[94] Hayakawa M, Nonomura H (1987) Humic acid-vitamins agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol 65:501–509.
[95] Tan GYA, Robinson S, Lacey E, Goodfellow M (2006) Exploration of Amycolatopsis diversity in soil using genus-specific primers and novel selective media. Syst Appl Microbiol 29:557–569.
[96] Rowbotham TJ, Cross T (1977) Ecology of Rhodococcus coprophilus and associated actinomycetes in freshwater and agricultural habitats. J Gen Microbiol 100:231–240
[97] Vickers JC, Williams ST, Ross GW (1984) A taxonomic approach to selective isolation of streptomycetes from soil. In: Ortiz-Ortiz L, Bojalil LF, Yakoleff V (eds) Biological, biochemical and biomedical of actinomycetes. Academic Press, London, pp 553–561
[98] Goodfellow M, Maldonado LA, Jones AL, Mexson J, Fiedler H-P, Stach JEM, Bull AT (2010) Verrucosispora maris sp. nov., a novel actinomycete isolated from a marine sediment which produces abyssomicins. Int J Syst Evol Microbiol 101(1):185-93.
[99] Goodfellow M, Cross T (1984) Classification. In: Goodfellow M, Mordarski M, Williams ST (eds) The biology of the actinomycetes. Academic Press, London, pp 7–164.
[100] Stackebrandt E, Schumann P (2006) Introduction to the taxonomy of Actinobacteria. In: Dworkin M, Falkow S, Schleifer RH, Stackebrandt RM (eds) The prokaryotes, 3rd edn, vol 3, Archaea and Bacteria: Firmicutes, Actinomycetes. Springer, New York, pp 297–321.
[101] Ludwig W, Klenk H-P (2005) overview: a phylogenetic backbone and taxonomic framework for prokaryotic systematics. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) Bergey's manual of systematic bacteriology, 2nd edn, vol 2, the proteobacteria, part A, introductory essays. Springer, USA, pp 49–65
[102] Zhi X-Y, Li W-J, Stackebrandt E (2009) An update on the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int J Syst Evol Microbiol 59:589–608
[103] Levy, S.B. and Marshall, B. (2004). Antibacterial Resistance Worldwide: Causes, Challenges and Responses. Nature Medicine, 10: S122–S129
[104] Mayfield, C.I., Williams, S.T., Ruddick, S.M. and Hatfield, H.L. (1972). Studies on the ecology of actinomycetes in soil. IV. Observations on the form and growth of streptomycetes in soil. Soil Biology Biochemistry, 4: 79-91.
[105] Goodfellow, M. and Williams, S.T. (1983). Ecology of Actinomycetes. *Annual Review of Microbiology*, **37**: 189-216
[106] Srinivasan, M.C., Laxman, R.S. and Deshpande, M.V. (1991). Physiology nutritional aspects of actinomycetes: an overview. World Journal of Microbiology and Biotechnology, 7 (2): 171-184.