2.1 Historical aspects

The antibiotic research from the discovery of Fleming to our days has been a fascinating, exciting, continuously changing and developing adventure. As a result of the frenzied research of the past more than 50 years, ten thousands of natural products derived from microbial sources are known. Interest towards the field is generally increasing, although sometimes declining, interest and the whole story shows some cyclic features with successes and failures which evolved around changing clinical needs and new enabling technology.

After the revolution in the “heroic” or “golden era”, in the forties and early fifties, when almost all groups of important antibacterial antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered, the success story had continued. It seemed in the fifties and sixties that the main problems of chemotherapy had been solved. Antibiotics discovered in this period were mainly isolated from species of *Streptomyces*, representing some 70 to 80% of the all isolated compounds. They are primarily active against bacteria and fungi. In this period the discovery of antitumor, antiviral and non antibiotic enzyme inhibitory metabolites had just started.

In the next period, between the seventies and nineties the efficiency of research had decreased. The costs of research had increased, and although the number of discovered new compounds still increased, they were mainly analogues of known compounds. The scope of search for various bioactive microbial products had, however, broadened. The exploration and wide utilization of the antitumor (doxorubicin) and agricultural antibiotics,
(antiparasitic, avermectin, feed additive monensin and herbicide glufosinate), the early discoveries of utilization of microbial metabolites in the pharmacological fields (cyclosporin, statins), were important new features. The problems of chemotherapy (emerging new pathogens and escalation of multi-resistant strains) had become serious. In this period, besides the leading role of actinomycetes products (65 to 70%), considerable increase in the discovery of “actinomycetes” products (up to 30%) were also noticeable. The chemical structures of almost all discovered compounds had been elucidated.

After these years, from the nineties, the exponential increase of the number of new metabolites (mainly non-antibiotic compounds, analogous and minor compounds), still continued but the occurrence of new chemical types had diminished. Due to urgent clinical needs, the increasing serious problems of chemotherapy (multi-resistant strains, reappearing mycobacteria, HIV, etc.), new challenges in the therapy of physiological diseases and in the agriculture, the renovation of the classical screening methods, allowed by the new technologies, were highly required. Methodological innovation accompanied by changes in conception. The cost effective high throughput screening (HTS) methods (robotics, instrumentation) based on mainly biochemical rationale and the wide application of diverse genetic manipulations, became more and more widespread. The rapid progress of the human genome project provided access to a wide range of new molecular targets implicated in diverse non-infectious diseases. The screenings have become more efficient than ever. The dramatic increase of the isolation of non-antibiotic compounds with pharmacological and agricultural activities, (up to 60 to 65% of all isolated compounds), the increasing share of various
fungal metabolites (up to 50%), and the chemical synthesis of more and more complicated structures, were the most characteristic features of this present period. It seems, with the opening of the 21st Century, a new era of antibiotic research had opened.

Notwithstanding the failures, the almost exponential increasing of the total number of discovered compounds in the last decades surprisingly became constant. In 1940 only 10 to 20, in 1950 300 to 400, in 1960 approximately 800 to 1000 and in 1970 already 2500 antibiotics were known. From that time the total number of known bioactive microbial metabolites has doubled in every ten years. In 1980 about 5000, in 1990 about 10000 and in 2000 already almost 20000 antibiotic compounds were known. By the end of 2012 over 42000 bioactive secondary metabolites (including antibiotics) were reported in the scientific and patent literature.

Unfortunately, these quantitative improvements do not mean similar qualitative, practical results. The expected corresponding spectacular successes, in spite of the great scientific and technical developments, are still waiting to be fulfilled.

Because of the fascinating technical improvements in the separation and isolation techniques, it is likely that over the antibiotics close to one million naturally occurring compounds are known. However, it is supposed, that today due to the intensive use of genetic methods and high throughput screening techniques (HTS), the number of existing and detected compounds may be definitely higher. The majority of natural products were derived besides the microbial products isolated from prokaryotic bacteria and eukaryotic microorganisms (protists), where almost all of the antibiotic
producing microorganisms (except the animal protozoa) belonged to higher plants and various animal organisms. Higher plant metabolites represent at least 500000 to 600000 compounds, covering a great number of common plant compounds, such as alkaloids, flavonoids, terpenoids, steroids, carbohydrates etc.

The natural products may exhibit various effects or no discovered interaction with other living organisms. In other words, they may show some kind of biological activity. The activity may be highly specific, exhibiting usually in low concentration (representing the usual bioactivities), or may be very unspecific (toxic) action. The exact number of bioactive natural products (compounds with discovered bioactivity or toxicity), was almost undeterminable, but this figure by all means is at least 200000 to 250000, including more than 20000 microbial metabolites.

The usual definition of natural products in the widest sense emphasizes that they “are chemical (carbon) compounds isolated from diverse living things”. These compounds may be derived by primary or rather secondary metabolism of living organisms. The primary metabolites (polysaccharides, proteins, nucleic and fatty acids) were common in all biological systems. The secondary metabolites were, however, low molecular (MW 3000), chemically and taxonomically extremely diverse compounds with obscure function, characteristic mainly to some specific, distinct types of organisms.

The exact definition and the real position and functions of secondary metabolites for long time is the most disputed and most obscure field in the whole area of microbiology. They seem to be absolutely needless for the
Review of Literature

producers without any apparent function in their life cycle. Most characteristic features were their incredible array of unique chemical structures and their very frequent occurrence and versatile bioactivities (Demain and Fang, 2000).

“Bioactivity” or “biological activity” may appear at the in vitro molecular level or may be studied at the in vivo level, the activity on the whole organism. Taking into consideration the possible differences and specific features surely manifesting in these different levels, this term will be used generally for any type of interaction between chemicals and any kind of molecular targets or living organisms.

Secondary metabolites were known from the ancient times, and they were mainly botanicals. The first crystalline fungal product from *Penicillium glaucoma* considered as microbial secondary metabolite was mycophenolic acid, discovered in 1896 by Gosio. The secondary metabolites isolated from microorganisms that exhibit either antimicrobial (antibacterial, antifungal, antiprotozoal), antitumor and/or antiviral activities, are called as antibiotics. In the light of our recent knowledge, however, the term “antibiotic” is more or less an outworn conception, but a similar, simple, expressive, inclusive term is still waiting to be invented. We use it because we have not found better one. Today its original definition actually should be extended to all of those (microbial) secondary metabolites which regulates growth processes, replications, and/or exhibits some kind of responding (regulating, inhibiting, stimulating) action to the prokaryotic or eukaryotic cells at the biochemical level, in minimal concentration. This broadest definition should cover besides the so-called “classical antibiotics” (microbial compounds exhibiting antimicrobial and/or antitumor and/or antiviral activities) practically all
bioactive compounds obtained either from microorganisms or from any other living things. It was a never returning opportunity that led to the wonderful “golden era” of the antibiotics research. The practical importance of antibiotics and other secondary metabolites is tremendous. They are widely used in the human therapy, veterinary, agriculture, scientific research and in countless other areas.

The concept of secondary metabolism as proposed initially (Bulock, 1961; Bulock, 1967; Weinberg, 1970; Weinberg, 1971) was oversimplified, but it was still useful for understanding the biosynthesis of microbial metabolites. According to Martin (1978) primary and secondary metabolites might be better termed "general" and "special" metabolites, respectively. Primary metabolism involves an interconnected series of enzyme mediated catabolic, amphibolic, and anabolic pathways, which provide biosynthetic intermediates and energy and convert biosynthetic precursors into essential macromolecules, such as deoxyribonucleic acid, ribonucleic acid, protein, lipids, and polysaccharides. Primary metabolism was essentially identical for all living things. The metabolic reactions of primary metabolism are finely balanced, and metabolic intermediates other than those necessary for cell survival rarely accumulate. In addition to this general type of metabolism, certain taxonomic groups are capable of synthesizing special types of metabolites by using either the same general enzymes or special synthetases produced by specific cells under specific nutritional conditions. Thus, fatty acid synthesis was common to all living cells, whereas polypeptide synthesis, in which the same precursors and similar biosynthetic enzymes were used, was restricted to certain taxonomic groups of microorganisms and plants.
Secondary metabolites were also termed "idiolites" (Walker, 1974) because they are formed during the idiophase (production phase) of batch cultures. These special metabolites usually possess chemical structures and are not essential for growth of the producing organism, although they probably have survival functions in nature. Secondary metabolites are produced only by some species of a genus, usually as families of closely related components.

The chemical diversity and unusual structures of idiolites were illustrated by the many classes of organic compounds to which they belong. These include, among many others, amino sugars, quinones, coumarins, epoxides, ergot alkaloids, glutarimides, glycosides, indole derivatives, lactones, macrolides, naphthalenes, nucleosides, peptides, phenazines, polyacetylenes, polynenes, pyrroles, quinolines, terpenoids, and tetracyclines (Weinberg, 1970; Berdy, 1974). Secondary metabolites include unusual chemical linkages, such as, β-lactam rings, cyclic peptides made of normal and modified amino acids, unsaturated bonds of polyacetylenes and polynenes, and large rings of macrolides. Idiolites were produced typically as members of a particular chemical family. There were at least 10 natural penicillins, 3 neomycins, 4 tyrocidines, 5 mitomycins, 10 bacitracins, 10 polymyxins, 20 actinomycins, 4 levorins, 4 polyfungins, and 13 bleomycins. One *Micromonospora* strain produced no fewer than 48 aminocycitol antibiotics (Berdy, 1974). The recent development of rapid separation and analytical techniques has increased markedly the number of minor components isolated.
The proportion of each component in a mixture depends on genetic and environmental factors, apparently because of the low specificity of the enzymes involved in secondary metabolism. In contrast, in primary metabolism the biosynthetic processes were always carried out with great specificity; generally, only one substrate was accepted and only one product was formed. Primary metabolism has narrow specificity because errors in the biosynthesis of essential cell components were usually lethal, whereas errors in secondary metabolism were usually of no consequence to the producing cells, especially since the modified metabolite often retains biological activity.

Secondary metabolites were synthesized by a greater variety of pathways than primary metabolites. Despite this variety of pathways and final products, most idiolites were assembled from a few key intermediary metabolites. An important characteristic of secondary metabolism was that idiolites were usually produced only at low specific growth rates of the producing cultures. This type of regulation affects a whole range of biosynthetic processes. Individual biosynthetic pathways were also affected by regulatory mechanisms, such as induction, catabolite regulation, and end product regulation. In other words, secondary metabolism seems to be governed by (i) overall regulatory controls which operate as functions of growth rate and (ii) specific regulatory effects on individual pathways (Martin, 1978).

In general, natural products including the microbial metabolites may be practically utilized in three different ways:

- Applying the natural/fermentation product directly in the medicine, agriculture, or in any other fields;
• Using as starting material for subsequent chemical or microbiological modification (derivatization);

• They can be used as lead compounds for chemical synthesis of new analogs or as templates in the rational drug design (RDD) studies.

2.2 The Producers of Microbial Metabolites

2.2.1 Distribution of Bioactive Natural Products

In the last decade, screening of microbial natural products (NPs) as sources of novel lead candidates for the development of new drugs has been vanishing from industrial laboratories to find refuge in academic and small biotechnology companies. It was well known that strong competition from synthetic and combinatorial libraries associated with ultrahigh-throughput screening platforms has posed a serious challenge to the natural products drug discovery area. This challenge has not been addressed in terms of technology innovation, compatibility with new screening technologies, productivity, and development times as required by large pharmaceutical companies (Pelaez and Genilloud, 2003; Koehn and Carter, 2005; Singh and Pelaez, 2008). Nevertheless, in spite of this loss of expertise, new microbial natural products have continued to be described at a low rate by smaller biotechnology companies and academic research groups (Newman and Cragg, 2007), and today NPs remain a continuing source of inspiration for synthetic chemists, with some signs of renewed interest in their potential (Harvey, 2007; Baltz, 2008; Li and Vederas, 2009).

Drug discovery from natural products in industrial and academic laboratories has traditionally been focused on empirical exploitation of the most prolific microbial groups viz., actinomycetes and filamentous fungi.
Historically, actinomycetes have been the origin of the largest number of new antibiotic drug candidates and lead molecules with applications in many other therapeutic areas (Berdy, 2005; Singh et al., 2010). Today, traditional approaches based exclusively on screening of a large number of strains have been exhausted and proven to be totally inefficient for delivery of novel molecules in a cost effective manner. New approaches have had to be developed to respond to the lack of new molecules as sources of novel privileged scaffolds and to improve the possibility of success of finding new chemical entities as leads for the development of drug candidates.

Novel genomic approaches aimed at exploring the biosynthetic potential of actinomycetes revealed in molecular genomics studies and focused on novel bioengineering and metagenomic tools have seen extraordinary development by many research groups (Banik and Brady, 2008; Brady et al., 2009; Corre and Challis, 2009; Craig et al., 2009; Nett et al., 2009; Scherlach and Hertweck, 2009), who have shown from different perspectives the cryptic biosynthetic capabilities of these filamentous bacteria and other microorganisms in the environment, and the possibilities of starting to learn how to harness the bacterial genome. In spite of the success of these approaches in revealing examples of novel chemistry, most of these techniques have not yet been tested on an industrial scale. Nevertheless, they demonstrate the challenge that lies in developing these tools to produce new drugs from microbial NPs.

Success in drug discovery from microbial products still relies on the proper combination of some key factors that need to be met at some point in the screening process and that have been valid for decades. From the
microbiology point of view these include the selection of appropriate individual strains, which can also be translated into genomic approaches able to access new gene pools involved in production of potential novel metabolites, and second, identification of adequate cultivation conditions or use of recombinant clones in appropriate surrogate hosts, switching on expression and ensuring production of novel chemistry. Both factors, in conjunction with selection of druggable biological targets, are still valid elements today in the drug discovery equation, but they need to be approached from a different perspective and with the help of the new analytical technology that has rapidly evolved during the last decade.

The research in natural products took advantage of some of these technology developments to explore from a different perspective how to improve the process and bring novelty to NP drug discovery. In recent years, the researchers benefited among others from developments in high throughput DNA sequencing that has ensured rapid and cheap identification of producing organisms, commercially available miniaturized microfermentation devices that enabled a change of scale in exploring new nutritional conditions for production of novel compounds, or improved analytical and chemical isolation methods that ensured rapid detection and characterization of compounds. Together, these have yielded new insights into microbial diversity and production of bioactive molecules that would have been inaccessible in such a short period of time with classical screening programs.
2.2.2 Access to biodiversity: Isolation of novel actinomycetes species in the environment

Access to the microbial diversity of actinomycetes in the environment has traditionally been focused on intensive sampling from a wide diversity of geographical locations and habitats. Large numbers of samples were processed empirically by general isolation methods, ending in most cases with the recurrent isolation of the predominant species in these habitats in spite of the biogeography of the samples used. Millions of strains have been isolated and screened over the decades in industrial laboratories and the probability of isolating novel compounds from common and frequently found related species were today too low to be worth the effort. In spite of the estimates regarding the potential production of unknown novel molecules by *Streptomyces* (Watve *et al*., 2001), a large number of species were widespread across many different environments, and they frequently produce well known and structurally related molecules that have to be identified and discarded as early as possible in drug discovery programs. Contrary to the theory that proposes that everything was everywhere, specific environmental conditions were strong selecting factors for specific microbial assemblages, and the distribution of some microbial species, even in widely occurring taxa, exhibits biogeographic patterns mostly determined by these microenvironmental conditions that may translate into novel chemistry (Fiedler *et al*., 2005; Bull, 2004; Martiny *et al*., 2006; Thornburg *et al*., 2010). The evidence strongly suggests that minor species or genetically distinct strains of actinomycetes that have not yet been cultured under laboratory conditions still occur in most environments, and new species were being systematically described that might be the source of novel chemistry with potential new
activities. On the basis of this working hypothesis, explorations were directed towards a large variety of sources, including specific terrestrial niches, plant host associations with unique characteristics, and marine environments. These approaches laid emphasis on exploration of untapped actinomycetes communities that might be associated with rhizosphere, plant endophytes, lichens, and endolithic bacteria (Gonzalez et al., 2005; Salazar et al., 2006), as well as marine sediments and invertebrate associated actinomycetes, which might guide isolation of novel microbial communities potentially producing novel chemical compounds. (Montalvo et al., 2005; Pathom-aree et al., 2006; Bredholt et al., 2008; Gontang et al., 2010; Janso and Carter, 2010; Trujillo et al., 2010).

This sourcing approach, combined with novel isolation methods developed in house or by different groups targeting cultivation of species underrepresented or previously not cultivated under laboratory conditions (Suzuki, 2001; Janssen et al., 2002; Taechowisan et al., 2003; Davis et al., 2005; Hamaki et al., 2005; Tamaki et al., 2005), ensured the isolation of novel or less frequently occurring species within the actinomycetales that are today common members of our screening collections. Most of these methods were focused on selective isolation of members of these taxa through use of poor nutritional media devoid of carbon sources, and including in some cases sub inhibitory concentrations of antibiotics that might favor development of slow growing representatives of Streptomyces species selected from a wide diversity of habitats and geographical origins. More than 45% of the strains in the collection belong to the major lineages of the so called rare actinomycetes or non Streptomyces. Whereas the majority of the strains are of terrestrial
origin, a marine program initiated in the last decade rapidly contributed to the diversification of the collection with a small group of 5,000 marine isolates obtained from sediments and marine invertebrates sampled at different depths at tropical and temperate Atlantic latitudes.

Antibiotics and similar natural products, being secondary metabolites can be produced by almost all types of living things. They were produced by prokaryotic (Prokaryote, Monera) and eukaryotic organisms belonging to the plant and animal Kingdom, alike. The secondary metabolite producing ability, however, was very uneven in the species of living world. In the Prokaryote and Plant Kingdom there were distinct groups of organisms, namely unicellular bacteria, eukaryotic fungi and first of all filamentous actinomycetes being the most frequent and most versatile producers.

In the group of prokaryotic, unicellular bacteria viz., Bacillus and Pseudomonas species were the most frequent producers. In the recent years species of myxo and cyanobacteria seem to join these distinguished organisms as prolific species. Mycobacteria, mycoplasmatales and spirotheces were far less frequent producers. The total number of known bioactive compounds in this group was about 3800; 17% of all microbial metabolites. The filamentous actinomycetales species produces over 10000 bioactive compounds, 7600 derived from Streptomyces and 2500, represent the largest group (45%) of bioactive microbial metabolites.

Microbial natural products were an important source of both existing and new drugs. Among the producers of commercially important metabolites, bacteria have proven to be a prolific source with a surprisingly small group of taxa accounting for the vast majority of compounds discovered (Bull, 2004).
Among these, Actinomycetes were the most economically and biotechnologically priceless prokaryotes. Representative genera of actinomycetes include *Streptomyces*, *Actinomyces*, *Arthrobacter*, *Corynebacterium*, *Frankia*, *Micrococcus*, *Micromonospora* and several others. Secondary metabolites produced by actinomycetes possess a wide range of biological activities (Pecznska Czoch and Mordaski, 1988; Mann, 2001; Bull, 2004; Berdy, 2005). The genus *Streptomyces* alone produces a large number of bioactive molecules. It has an enormous biosynthetic potential that remains unchallenged without a potential competitor among other microbial groups. A large number of *Streptomyces* spp. have been isolated and screened from soil in the past several decades (Williams *et al*., 1989; Watve *et al*., 2001). Consequently the chances of isolating a novel *Streptomyces* strain from terrestrial habitats have diminished. Above 500 species of *Streptomyces* account for 70 to 80% of relevant secondary metabolites as shown in (Table 1) with small contributions from other genera, such as *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora* and *Actinoplanes*. An important reason for discovering novel secondary metabolites was to circumvent the problem of resistant pathogens, which were no longer susceptible to the currently used drugs (Carlos, 2003; Ekwenye and Kazi, 2007). The number of deaths due to these clever pathogenic organisms is on the rise. Secondary metabolites from marine actinomycetes may form the basis for the synthesis of novel therapeutic drugs, which may be efficient to combat a range of resistant microbes (Lam, 2006; Fenical and Jensen, 2006).
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Source</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Erythromycin</td>
<td><em>Saccharopolyspora erythrae</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Essential component of insect control agent</td>
</tr>
<tr>
<td>2.</td>
<td>Rhamnose</td>
<td><em>Saccharopolyspora spinosa</em></td>
<td>Essential component of insect control compound spinosad</td>
</tr>
<tr>
<td>3.</td>
<td>Zorbamycin</td>
<td><em>Streptomyces flavovirdis</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>4.</td>
<td>Kanamycin</td>
<td><em>Streptomyces kanamycticus</em> 12-6</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>5.</td>
<td>Kanglemycin C (K-C)</td>
<td><em>Nocardia mediterranei var. kangiensis</em> 1747-64</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Rapamycin</td>
<td><em>Streptomyces hygroscopicus</em></td>
<td>Antifungal</td>
</tr>
<tr>
<td>7.</td>
<td>Pandavir (nigericin)</td>
<td><em>Streptomyces hygroscopicus</em></td>
<td>Affects ion transport and ATPase activity</td>
</tr>
<tr>
<td>8.</td>
<td>FK520 Ascomycin</td>
<td><em>Streptomyces hygroscopicus var. ascomyceticus</em></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Himastatin</td>
<td><em>Streptomyces hygroscopicus</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>10.</td>
<td>Jinggangmycin</td>
<td><em>Streptomyces hygroscopicus</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>11.</td>
<td>Oxytetracycline</td>
<td><em>Streptomyces rimosus</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>12.</td>
<td>Amphoterinic B</td>
<td><em>Streptomyces nodosus</em></td>
<td>Antifungal</td>
</tr>
<tr>
<td>13.</td>
<td>Asukamycin</td>
<td><em>Streptomyces nodosus subsp. asukaensis</em></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Tylosin</td>
<td><em>Streptomyces fradiae</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>15.</td>
<td>Urdamycin A</td>
<td><em>Streptomyces fradiae</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>16.</td>
<td>Fosfomycin</td>
<td><em>Streptomyces fradiae</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>17.</td>
<td>CE-108</td>
<td><em>Streptomyces diastaticus</em></td>
<td>Antifungal</td>
</tr>
<tr>
<td>18.</td>
<td>Rimocidin</td>
<td>*Streptomyces diastaticus var. 108</td>
<td>Antifungal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antibacterial, antifungal</td>
</tr>
<tr>
<td>19.</td>
<td>Shurimycins A and B</td>
<td><em>Streptomyces hygroscopicus</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>20.</td>
<td>Chloramphenicol</td>
<td><em>Streptomyces venezuelae</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>21.</td>
<td>Rifamycin</td>
<td><em>Amycolatopsis mediterranei</em> U-32</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>22.</td>
<td>Amythiamicins</td>
<td><em>Amycolatopsis</em> sp.</td>
<td>Antibacterial, Antileukemic, anti-VRE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(vancomycin-resistant enterococci)</td>
</tr>
<tr>
<td>23.</td>
<td>Cyclo (L-leucyl-L-prolyl)</td>
<td><em>Streptomyces sp. KH614</em></td>
<td>Antibacterial, antifungal</td>
</tr>
<tr>
<td>24.</td>
<td>Ipomicin</td>
<td><em>Streptomyces ipomoeae</em> group III</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>25.</td>
<td>Streptomycin</td>
<td><em>Streptomyces griseus</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>26.</td>
<td>Valinomycin</td>
<td><em>Streptomyces griseus</em></td>
<td>Mitochondrial toxin</td>
</tr>
<tr>
<td>27.</td>
<td>Griseorhodin</td>
<td><em>Streptomyces griseus</em> FCRC-57</td>
<td>Telomerase inhibitor</td>
</tr>
<tr>
<td>28.</td>
<td>Fredericamycin A</td>
<td><em>Streptomyces griseus</em> FCRC-48</td>
<td>Antitumor</td>
</tr>
<tr>
<td>29.</td>
<td>Capuramycin</td>
<td><em>Streptomyces griseus</em> SANK 60196</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>30.</td>
<td>Frigocyclinone</td>
<td><em>Streptomyces griseus</em> strain NTK 97</td>
<td>Antibacterial, Inhibitor of bacterial gyrase</td>
</tr>
<tr>
<td>31.</td>
<td>Clorobiocin</td>
<td><em>Streptomyces coelicolor</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>32.</td>
<td>Meilingmycin</td>
<td><em>Streptomyces nanchangensis</em></td>
<td>Antiparasitic</td>
</tr>
<tr>
<td>33.</td>
<td>Nanchangmycin</td>
<td><em>Streptomyces nanchangensis</em></td>
<td>Insecticidal</td>
</tr>
<tr>
<td>34.</td>
<td>Eremomycin</td>
<td><em>Amycolatopsis orientalis</em> subsp. <em>Eremomycini</em></td>
<td></td>
</tr>
<tr>
<td>35.</td>
<td>Nikkomycins</td>
<td><em>Streptomyces ansochromogenes</em></td>
<td>Antifungal</td>
</tr>
</tbody>
</table>
36. Avilamycin A  Streptomyces viridochromogenes Tu57  Antibacterial
37. Tubulactomicin A  Nocardia sp.  Antibacterial
38. Benzantrhins A and B  Nocardia lurida  Antibacterial
39. Azureomycins A and B  Pseudonocardia azurea nov. sp.  Antibacterial
40. Nogalamycin  Streptomyces nogalater  Antibacterial
41. Aclacinomycin A  Streptomyces galilaeus  Antitumor
(aclarubicin)
42. Cinerubin R  Streptomyces eurythermus  Antibacterial
43. Scopafungin  Streptomyces hygroscopicus var. enhygrus  antibacterial
var. nova UC-2397
44. Spiramycin  Streptomyces ambofaciens  Antibacterial
45. Pristinamycin I  Streptomyces pristinaespiralis  Antibacterial
46. Lankacidin  Streptomyces rochei  Antibacterial
47. Lankamycin  Streptomyces rochei  Antibacterial
48. Actinomycin C  Streptomyces chrysomallus  Antitumor
49. Duanomycin  Streptomyces sp.  Antitumor
50. Midecamycin  Streptomyces mycarofaciens  Antibacterial
51. Avermectin  Streptomyces avermitilis  Anthelminthic
Cell growth
inhibitor
52. Oligomycin  Streptomyces avermitilis  Antibacterial
53. Resormycin  Streptomyces platensis  Antibacterial
54. Ileumycin  Streptomyces lavendulae  Antifungal
55. Mitomycin C  Streptomyces lavendulae  Antibacterial
56. Lomofungin  Streptomyces lomodensis  Antibacterial
57. Kalafungin  Streptomyces tanashiensis strain Kala UC5063  antiprotozoal
58. Thiamycins  Streptomyces michiganensis var. amylyticus var. nova  Anthelminthic, antiprotozoal
59. Axenomycins  Streptomyces lisandri nov. sp.  Anthelminthic, antiprotozoal, antifungal
60. Neihumicin  Micromonospora neihuensis  Cytotoxic
61. Fortimicin A (Astromicin)  Micromonospora olivasterospora  Antibacterial
62. Gentamicin  Micromonospora purpurea var. violaceae  Antibacterial
63. Tetracycline  Streptomyces aureofaciens  Antibacterial
64. Monomycin  Actinomycetes circulatus var. monomycini  Antibacterial
65. PC-766 B  Nocardia brasiliensis  Antioxidant
66. Medecamycin  Streptomyces mycarofaciens  Antibacterial
67. Dunaimycins  Streptomyces diastatochromogenes  Immunosuppressi
<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Source</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>68.</td>
<td>Novobiocin</td>
<td><em>Streptomyces niveus</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>69.</td>
<td>Carminomycin</td>
<td><em>Actinomadura carminata</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>70.</td>
<td>Maduramycins</td>
<td><em>Actinomadura rubra</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>71.</td>
<td>MM461156</td>
<td><em>Actinomadura pelletieri</em></td>
<td>Antitumor, antibacterial</td>
</tr>
<tr>
<td>72.</td>
<td>Verucopeptin</td>
<td><em>Actinomadura verrucosporae</em></td>
<td>Antitumor, Antimicrobial</td>
</tr>
<tr>
<td>73.</td>
<td>Saptomycins</td>
<td><em>Streptomyces sp. HP 530</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>74.</td>
<td>Oxaprapalines B, D, G</td>
<td><em>Streptomyces sp. G324</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>75.</td>
<td>Lavendamycin</td>
<td><em>Streptomyces lavendulae</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>76.</td>
<td>Chlorocarcins A, B, C</td>
<td><em>Streptomyces lavendulae No. 314</em></td>
<td>Antitumor, antibacterial</td>
</tr>
<tr>
<td>77.</td>
<td>Mimosamycins</td>
<td><em>Streptomyces lavendulae No. 314</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>78.</td>
<td>Lavendomycin</td>
<td><em>Streptomyces lavendulae</em></td>
<td>Antitumor, antibacterial</td>
</tr>
<tr>
<td>79.</td>
<td>Sohbumycin</td>
<td><em>Streptomyces sp. 82-85</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>80.</td>
<td>Furaquinocins C, D, E, F, G, H</td>
<td><em>Streptomyces sp. KO 3988</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>81.</td>
<td>Arizona A1 and B1</td>
<td><em>Actinoplanes arizonaensis</em> sp. nov.</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>82.</td>
<td>Coloradocin</td>
<td><em>Actinoplanes coloradoensis</em> sp. nov.</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>83.</td>
<td>Teichomyccins</td>
<td><em>Actinoplanes teichomyceticus</em> nov. sp.</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>84.</td>
<td>Lipiarmycin</td>
<td><em>Actinoplanes deccanensis</em> nov. sp.</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>85.</td>
<td>Candiplaneacin</td>
<td><em>Ampullariella reguralis subsp. mannitophila subsp. nov.</em></td>
<td>Antifungal</td>
</tr>
<tr>
<td>86.</td>
<td>Victomycin</td>
<td><em>Streptosporangium violaceochromogenes</em> nov. sp.</td>
<td>Antitumor, antibacterial</td>
</tr>
<tr>
<td>87.</td>
<td>Maggiemycin and anhydromaggiemycin</td>
<td><em>Streptomyces sp.</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>88.</td>
<td>Gilvusmycin</td>
<td><em>Streptomyces sp.</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>89.</td>
<td>Kazusamycin</td>
<td><em>Streptomyces sp.</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>90.</td>
<td>Okicenone</td>
<td><em>Streptomyces sp.</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>91.</td>
<td>Hydramycin</td>
<td><em>Streptomyces violaceus</em></td>
<td>Antitumor, Anthelmintic, antiviral</td>
</tr>
<tr>
<td>92.</td>
<td>Musacin C</td>
<td><em>Streptomyces griseovirdis</em></td>
<td>Antitumor, antibacterial</td>
</tr>
<tr>
<td>93.</td>
<td>Kanchanamycins</td>
<td><em>Streptomyces olivaceus</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>94.</td>
<td>Elloramyacin</td>
<td><em>Streptomyces olivaceus</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>95.</td>
<td>Fattiviracin A1</td>
<td><em>Streptomyces microfl avus</em></td>
<td>Antiviral</td>
</tr>
<tr>
<td>96.</td>
<td>FK 506</td>
<td><em>Streptomyces tsukubaensis</em></td>
<td>Antiviral</td>
</tr>
<tr>
<td>97.</td>
<td>Retamycin</td>
<td><em>Streptomyces olindensis</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>98.</td>
<td>Manumycin</td>
<td><em>Streptomyces parvalus</em></td>
<td>Antitumor, enzyme inhibitory</td>
</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Studied Organism</td>
<td>Activity</td>
</tr>
<tr>
<td>-----</td>
<td>---------------------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>99.</td>
<td>Granaticin</td>
<td><em>Streptomyces thermoviolaceus</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>100.</td>
<td>Pimaricin</td>
<td><em>Streptomyces natalensis</em></td>
<td>Antifungal</td>
</tr>
<tr>
<td>101.</td>
<td>Virginiamycin M</td>
<td><em>Streptomyces virginae</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>102.</td>
<td>Daptomycin (commercialized as Cubicin)</td>
<td><em>Streptomyces roseosporus</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>103.</td>
<td>Enduracidin</td>
<td><em>Streptomyces fungicidicus</em> B5477</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>104.</td>
<td>Apramycin</td>
<td><em>Streptomyces tenebrabi</em>a* UD2</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>105.</td>
<td>Mithramycin</td>
<td><em>Streptomyces argillaceus</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>106.</td>
<td>Blasticidin S</td>
<td><em>Streptomyces griseochromogenes</em></td>
<td>Antifungal, antitumor</td>
</tr>
<tr>
<td>107.</td>
<td>Leptomycin</td>
<td><em>Streptomyces lividans</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>108.</td>
<td>Landomycin E</td>
<td><em>Streptomyces globisporus</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>109.</td>
<td>Phenalinolactones A–D</td>
<td><em>Streptomyces sp.</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>110.</td>
<td>Pipalamycin</td>
<td><em>Streptomyces sp.</em></td>
<td>Apoptosis inducer, antibacterial</td>
</tr>
<tr>
<td>111.</td>
<td>Biphenomycin A and B</td>
<td><em>Streptomyces griseorubiginosus</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>112.</td>
<td>Streptocidins A–D</td>
<td><em>Streptomyces sp. Tu6071</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>113.</td>
<td>Zelkovamycin</td>
<td><em>Streptomyces sp. K96-0670</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>114.</td>
<td>Methylsulfomycin I</td>
<td><em>Streptomyces sp. RSP9</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>115.</td>
<td>YM-216391</td>
<td><em>Streptomyces nobilis</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibit binding of platelet derived growth factor to its receptor</td>
</tr>
<tr>
<td>116.</td>
<td>RP-1776</td>
<td><em>Streptomyces sp.</em></td>
<td></td>
</tr>
<tr>
<td>117.</td>
<td>RS-22 A, B and C</td>
<td><em>Streptomyces violaceusniger</em></td>
<td>Antifungal, antibacterial</td>
</tr>
<tr>
<td>118.</td>
<td>Vicenistatin</td>
<td><em>Streptomyces sp. Tu6239</em></td>
<td>Antitumor, Antibacterial, antitumor</td>
</tr>
<tr>
<td>119.</td>
<td>Ripromycin</td>
<td><em>Streptomyces sp.</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>120.</td>
<td>Vinylamycin</td>
<td><em>Streptomyces sp.</em></td>
<td>Antibacterial</td>
</tr>
<tr>
<td>121.</td>
<td>Cephamycin C</td>
<td><em>Streptomyces lactamurans</em></td>
<td>Antibacterial</td>
</tr>
</tbody>
</table>

Existence of terrestrial actinomycetes has been reported in the relatively untapped marine ecosystem. The immense diversity of this habitat along with its under exploitation was the fundamental reason for attracting researchers towards it for discovering novel metabolite producers. Actinomycetes comprise about 10% of the bacteria colonizing marine aggregates and can be isolated from marine sediments (Ward and Bora,
2006). Many actinomycete isolates from deep oceans contain non-ribosomal polyketide synthetase (NRPS) and polyketide synthetase (PKS) pathways, the hallmarks of secondary metabolite production (Salmon et al., 2003). There was an occurrence of distinct rare genera in the marine ecosystem as evidenced by the taxonomic description of the first marine actinomycete *Rhodococcus marinonascens* (Helmke and Weyland, 1983). Actinomycetes have also been isolated from free swimming as well as sessile marine vertebrates and invertebrates (Ward and Bora, 2006). Unusual actinomycetes belonging to Micrococcaceae, Dermatophilaceae and Gordoniaceae have been isolated from sponges (Lam, 2006). Tetrodotoxin producing actinomycete has been isolated from puffer fish ovaries (Wu et al., 2005), the organism was found to be most closely related to *Nocardiopsis dassonvillei*.

Researchers were finding new genera from different environments on a regular basis and discovering new metabolite producers never reported earlier. Actinomycete genera identified by cultural and molecular techniques from different marine ecological niches include *Actinomadura, Actinosynnema, Amycolatopsis, Arthrobacter, Blastococcus, Brachybacterium, Corynebacterium, Dietzia, Frankia, Frigoribacterium, Geodermatophilus, Gordonia, Kitasatospora, Micromonospora, Micrococcus, Microbacterium, Mycobacterium, Nocardioides, Nocardiosis, Nonomurea, Psuedonocardia, Rhodococcus, Saccharopolyspora, Salinispora, Serinicoccus, Solwaraspora, Streptomyces, Streptosporangium, Tsukamurella, Turicella, Verrucosispora and Williamsia* (Ward and Bora, 2006). In spite of improvements being made in the cultural methods for the isolation of rare marine actinomycetes, many of these organisms still remain
unculturable and have to be detected by using molecular techniques (Stach et al., 2003; Mincer et al., 2005).

Among the eukaryotic microscopic fungi the producing capability of imperfect fungi, the ascomycetes and several other filamentous and endophytic fungal species were the most significant. The basidiomycetes were also frequently reported producers while yeasts, phycymycetes, slime moulds rarely produce bioactive metabolites. The total number of bioactive fungal product was approximately 8600, representing 38% of all microbial products. Notwithstanding the recent drop, the predominant part, 45% of the presently known bioactive microbial metabolites, over 10000 compounds were still isolated from various actinomycetales species, 34% from *Streptomyces* and 11% from the rare actinomycetes. The most frequent producers, the *Streptomyces* species produces 7600 compounds (74% of all actinomycetales), while the rare actinomycetes represent 26%, altogether 2500 compounds. The representation of rare actinomycetes products in 1970 was only 5%. In this group *Micromonospora*, *Actinomadura*, *Streptoverticillium*, *Actinoplanes*, *Nocardia*, *Saccharopolyspora* and *Streptosporangium* species were the most frequent producers, each produces several hundreds of antibiotics.

### 2.3 Antibiotics produced by the streptomycetes

Some examples produced by streptomycetes in Figure 1 and the related actinomycetes are described as below. Represented as antimicrobial compound against tuberculosis are streptomycin (*Streptomyces griseus*), rifamycin (*Amycolatopsis mediterranei*), erythromycin (*Saccharopolyspora erythraea*), and oleandomycin (*Streptomyces antibioticus*). There were more
bioactive compounds such as avermectin (*Streptomyces avermitilis*), bleomycin (*Streptomyces verticillus*) and daunomycin (*Streptomyces peuceticus*) as antitumor compounds, FK506 (*Streptomyces tsukubaensis*) as an immunosuppressant, and validamycin (*Streptomyces hygroscopicus* var. *limoneus*) as a treatment of rice sheath blight disease.

**Figure 1. Antibiotics produced by Streptomyces**

New drugs, especially antibiotics, were urgently needed to counter and reverse the spread of antibiotic resistant pathogens (Talbot *et al.*, 2006; Payne *et al.*, 2007) and to combat life threatening diseases such as cancer (Olano *et al.*, 2009a). It was widely acknowledged that the most promising source of new drugs remain natural products (Bull *et al.*, 2000; Fenical and Jensen, 2006; Bull and Stach, 2007), especially given the inconvenient truth
that alternative strategies, such as combinatorial chemistry and fragment based drug design, have been relatively unproductive with only one *de novo* combinatorial New Chemical Entity approved anywhere in the world (Newman, 2008). Experience has shown that previously unknown, important natural products were found when new screening systems were introduced or when high quality biological materials from new sources are examined in existing screens. It is, therefore, essential in drug discovery programmes to foster these two aspects of novelty by building upon scientific and technological developments in these areas.

The choice of bacteria for pharmacological screening programmes was a daunting one given the taxonomic diversity of cultivable prokaryotes (Bull, 2004a; De Vos et al., 2009). However, this diversity was but a tiny fraction of the uncultivated prokaryotic diversity present in natural habitats (Bull et al., 2000; Bull, 2004a, b; Sogin et al., 2006), a silent majority of prokaryotes which encompasses enormous genetic diversity for exploitable biotechnology (Whitman et al., 1998; Bull, 2004a, b). This extensive gene pool was being sampled by the application of innovative procedures for the selective isolation of previously unknown bacteria (Fry, 2004; Epstein et al., 2010), including actinomycetes (Goodfellow, 2010), thereby compounding the problem outlined above.

Amongst prokaryotes, members of the order Actinomycetales, notably the genus *Streptomyces*, remain the richest source of natural products, including clinically useful antibiotics, antimetabolites and antitumour agents (Berdy, 2005; Newman and Cragg, 2007; Olano et al., 2009a, b). Actinomycete sources account for about 45% of all microbial bioactive
secondary metabolites with 7,600 of these compounds (80%) being produced by *Streptomyces* (Berdy, 2005). Despite this astonishing productivity, it has been predicted that only about 10% of the total number of natural products that can be synthesized by these organisms have been discovered (Watve *et al.*, 2001). However, the key to a resurgence of interest in actinomycetes as a source of new chemical entities came from the application of genomic technologies which showed that the whole genomes of *Rhodococcus* sp. RHA1 (McLeod *et al.*, 2006), *Salinispora tropica* CNB-440 (Udwary *et al.*, 2007), *Streptomyces avermitilis* MA-4680 (Omura *et al.*, 2001; Ikeda *et al.*, 2003) and *Streptomyces coelicolor* A(3)2 (Bentley *et al.*, 2002) each contained around 20 or more natural product biosynthetic gene clusters for the production of known or predicted secondary metabolites. In contrast, few, if any, such gene clusters have been detected in the genomes of other bacteria, as shown by the presence of three in *Bacillus subtilis* 168 (Kunst *et al.*, 1997), four in *Pseudomonas aeruginosa* PA01 (Stover *et al.*, 2000) and two in *Ralstonia solanacearum* GMI 1000 (Salanoubat *et al.*, 2002).

Hence, the focus was on actinomycetes as a source of novel, clinically significant natural products. However, it was becoming increasingly difficult to find such metabolites from common actinomycetes as screening ‘old favourites’ leads to the costly rediscovery of known compounds (Williams, 2008). This problem can be met by using standard procedures for the selective isolation of novel actinomycetes from poorly studied habitats (Sembiring *et al.*, 2000; Goodfellow *et al.*, 2007; Okoro *et al.*, 2009), by applying new methods for the selective isolation of rare and uncommon actinomycetes (Suzuki *et al.*, 2001a, b; Tan *et al.*, 2006) and by devising
innovative procedures for the cultivation of specific components of previously uncultivated actinomycetes known to be present in natural habitats (Stach et al., 2003a, b; Giovanonni and Stingl, 2005; Allgaier and Grossart, 2006).

2.4 Prokaryotic systematics

Prokaryotic systematics is the scientific study of the kinds, diversity, and relationships within and between Archaea and Bacteria. The subject is usually divided into three separate, sequential, but interrelated sub disciplines, namely classification, nomenclature and identification. The initial step, classification, is the process of ordering organisms into taxonomic groups (taxa) on the basis of similarities and differences. The outcome is an orderly arrangement or system that was designed to show natural relationships between taxa and to serve as an information storage and retrieval system. The term classification encompasses both processes and the outcomes of the exercise are often referred to as taxonomies. Sound classification of prokaryotes is a prerequisite for stable nomenclature and reliable identification procedures.

Classifications based on large suites of genotypic and phenotypic properties are termed as phenetic. This approach encompasses measurable features of prokaryotes (e.g. biochemical, chemical, morphological and physiological properties), including genetic relationships (e.g. DNA:DNA homology values). Phenetic classifications show relationships between organisms as they exist now, without reference to evolutionary pathways or ancestry. In contrast, phylogenetic classifications express inferred evolutionary relatedness between organisms and thereby reflect the extent of change over time. In practice, phylogenetic classifications were usually found
to be phenetically coherent. Current approaches to prokaryotic classification based on 16S rRNA gene sequences purport to be phylogenetic, but many are in fact phenetic measures of affinity with homologous nucleotide sequences as characters.

The second step, nomenclature, deals with the terms used to denote ranks in the taxonomic hierarchy (e.g. species, genera, families) and with the practice of assigning the correct, internationally recognized names to taxonomic groups according to rules laid out in successive editions of the International Code of Nomenclature of Bacteria (Lapage et al., 1975, 1992). Two reforms in the ‘Bacteriological Code’ edited by Lapage and his colleagues in 1975 have had far reaching impacts on the nomenclature of prokaryotes.

• A definitive document and starting date for the recognition of names introduced with the publication of the Approved Lists of Bacterial Names on January 1, 1980 (Skerman et al., 1980). Names published prior to this date and omitted from the Approved Lists lost their standing in nomenclature, a development that cleared away thousands of meaningless names. Old names can be resurrected if the system for doing so was followed.

• Names of new taxa can only be validly published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM; formerly the International Journal of Systematic Bacteriology), but can be effectively published in appropriate international journals and then cited in Validation Lists published in the IJSEM.

These changes mean that the IJSEM serves as a convenient ‘one-stop-shop’ for the recognition of validly described new names of species, genera and
A principle of paramount importance in nomenclature and identification is the nomenclatural type concept. A taxon in the taxonomic hierarchy up to class may contain a number of elements. The elements of species are strains and those of a genus are species and so on. The nomenclatural type of a taxon is that element with which the name of a taxon was permanently associated. The type species of a genus, for instance, must be retained in the genus even if all other species are removed from it.

The type of a taxon does not have any physical existence above the rank of species, it is merely a name. In contrast, at the species and subspecies level the nomenclatural type is represented by a particular strain, the type strain, which does have a physical existence, as any number of subcultures. Type strains are designated by taxonomists who describe new species. They are the permanent living embodiments of validly described species and have to be deposited in two service culture collections in different countries, so that they are readily available for study. Type strains are of the greatest importance for taxonomic work as they are reference points when attempting to identify unknown microorganisms. The knowledge that type strains may not be entirely typical of a species is outweighed by the fact that by definition they are authentic.

The correct use of names is central to all aspects of the microbial sciences as microbiologists need to know which organisms they are studying before they can pass on information about them within and out with the scientific community. In other words, an organism's name is a key to its literature, an entry to what was known about it. Comprehensive accounts on
the nomenclature of prokaryotes can be found elsewhere (Bousfield, 1993; Sneath, 2005), as can practical guidelines for the recognition of new prokaryotic taxa (Truper, 1999, 2005). Once prokaryotes have been rigorously characterized and classified it is a relatively easy matter to name them.

Identification, the final stage of the taxonomic trinity, is sometimes seen as the raison d'être of prokaryotic systematics due to the importance of accurately identifying unknown organisms, not least pathogenic bacteria. It is both the act and the result of determining whether unknown organisms belong to established and validly named taxa (Krieg, 2005). It involves determining the key characteristics of unknown organisms and matching them against databases containing corresponding information on established taxa (Priest, 2004). Organisms found to fall outside known groups should be described and classified as new taxa. Classifications of prokaryotes are not only markedly data dependent but are in a continuous state of development as high quality information becomes available from the application of both new and improved taxonomic methods. Such taxonomies are essentially pragmatic as they were driven by practical imperatives not by theoretical considerations akin to the biological species concept (Goodfellow et al., 1997; Schleifer, 2010). Current approaches to the classification of prokaryotes are based upon the integrated use of genotypic and phenotypic features acquired through the application of chemotaxonomic, molecular systematic and numerical and non-numerical phenotypic methods. This practice, known as polyphasic taxonomy, was introduced by Colwell (1970) to signify successive or simultaneous studies on groups of prokaryotes using methods chosen to yield high quality data. The polyphasic approach provides a sound basis for stable nomenclature and
reliable identification, essential factors for a practical or utilitarian taxonomy
designed to serve diverse end users. Detailed accounts of the polyphasic
approach to the classification of prokaryotes are available (Vandamme et al.,
1996; Goodfellow et al., 1997; Gillis et al., 2005).

The widespread application of polyphasic taxonomy led to significant
improvements in the classification of prokaryotes, notably in groups like the
Actinobacteria and Cyanobacteria where traditional approaches based on
form and function proved unreliable (Goodfellow and Maldonado, 2006;
Kroppenstedt and Goodfellow, 2006; Gupta, 2009). It has not been possible to
assemble a recommended set of methods for polyphasic studies as
taxonomic toolkits are influenced by the biological properties and ranks of the
taxa under study and by the equipment available to investigators. However,
sequencing highly conserved macromolecules, notably 16S rRNA genes, has
provided valuable data for constructing phylogenies at and above the genus
level (Woese, 1987; Ludwig and Klenk, 2005) whereas DNA:DNA
relatedness, molecular fingerprinting and phenotypic techniques are methods
of choice for delineating taxa at and below the rank of species (RosselloMora
and Amann, 2001). It is important to remember that distinguishing phenotypic
features are required for the formal description of new species (Wayne et al.,
1987). Procedures used to characterize and circumscribe prokaryotic taxa
have been considered in detail (Felis et al., 2010; Tindall et al., 2010) and the
strengths and weaknesses of genomic methods have been highlighted by
Schleifer (2010).

2.4.1 Actinobacteria

Actinobacterial systematics has been revolutionized by the application
of chemotaxonomic, molecular systematic and numerical taxonomic methods (Goodfellow and Cross, 1984; Stackebrandt and Schumann, 2006). The class Actinobacteria is now seen to be one of the major phyla in the domain Bacteria, as inferred from its branching position in the 16S rRNA gene tree (Ludwig and Klenk, 2005). The separation of this taxon from other bacterial groups is supported by conserved indels in protein (e.g. cytochrome cooxidase subunit 1, CTP synthase and glutamyl-tRNA synthase) and 23S rRNA sequences (Gao and Gupta, 2005; Gao et al., 2006) and by characteristic gene arrangements (Kunisawa, 2007) though it is still not possible to identify the phylogenetically closest neighbours to the actinobacteria with any confidence (Ventura et al., 2007).

The current hierarchical classification of the phylum Actinobacteria is outlined in Figure 2. The phylogenetic relationships of taxa above the genus level was based solely on taxon specific 16S rRNA signatures, as spelt out by (Zhi et al., 2009). In contrast, classification at generic and species ranks also take into account the discontinuous distribution of chemotaxonomic, morphological and physiological properties, as exemplified by the circumscription of novel genera, such as Actinovallomurus (Tamura et al., 2009) and Plantactinospora (Qin et al., 2009) and by new species of Dactylosporangium and Streptomyces (Kumar and Goodfellow, 2010). It should be noted that the term actinobacteria refers to all members of the phylum whereas the designation actinomycetes only refers to strains belonging to the order Actinomycetales.

The current classification of actinobacteria is a marked improvement on earlier taxonomies of the group but needs to be seen as a staging post
leading towards better classifications in the future. It can, for instance, be
anticipated that the suprageneric relationships between taxa based on
16S rRNA signatures will need to be adjusted as sequences of novel taxa
were added to the 16S rRNA Actinobacteria gene tree (Zhi et al., 2009). In
addition, the somewhat opaque evolutionary history of the phylum should be
clarified by the generation of trees based on whole genome sequences
(Ventura et al., 2007), especially ones taken to represent diverse taxa, not
least those lying towards the root of the 16S rRNA tree. It is evident that such
a phylogeny driven approach provides invaluable data for the reconstruction
of prokaryotic phylogenetic history and for the discovery of new protein
families and biological properties (Wu et al., 2009).

The phylum actinobacteria, one of the largest groups in the domain
Bacteria largely consists of environmental bacteria and the denizens of many
varied habitats: soils, the rhizosphere, marine and extreme arid environments.
A number live in close association with higher organisms; for example, as
components of different microbiomes they constitute more than a third of the
healthy human microbiota. Members of the genus Frankia, on the other hand,
can form symbiotic nodules in certain species of trees and shrubs, and fix
atmospheric nitrogen to allow their hosts to survive in nutrient limiting
environments. Actinobacteria typically have elevated guanosine-cytosine
contents (65-75% G + C) and their genome sizes range from the 2.5-Mb skin
commensal Micrococcus luteus to the 9.7-Mb environmental strain
Rhodococcus jostii. Since the discovery of antibiotics in the 1940s, the
actinomycetes have received a great deal of attention, and Streptomyces
species in particular have become renowned as the principal sources of
therapeutic pharmaceuticals. There have been several good reviews on actinobacteria of late, notably that by Ventura et al. (2007) on evolutionary and genomic aspects, as well as occasional articles focusing on specific genera. Interest in the phylum in recent years is evidenced by the increasing number of citations.

Figure: 2 Classiﬁcation of Actinobacteria, at supragenic level

Streptomyces were demonstrably a rich source of compounds, but no more so than other members of the actinobacteria, also the bacilli and bacterial genera such as the myxobacteria and pseudomonads (Wenzel and Muller, 2009). Among the eukaryotes, fungal genomes are replete with
biosynthetic gene clusters for encoding small molecule production. The ability
to make bioactive small molecules is not exclusive to microorganisms. Plants
are rich sources of a great variety of compounds that have been used as
pharmaceuticals for millennia; this resource remains poorly understood and
still largely untapped.

There is a global crisis in the treatment of infectious diseases; people
were dying of infections that were previously treatable. Microorganisms are
the source and the solution for the crisis, and for this reason it is imperative
that the search for novel therapeutic agents be intensified. The constant moan
of the pharmaceutical industry, that the natural reservoir of molecules with
antibiotic activity is close to being exhausted and that they can no longer find
useful bioactive compounds, is due in part to Waksman’s focus on the
streptomycetes. It can also be explained by the inability to detect bioactive
compounds when they are present only in low concentrations; the industry
has found all the easily accessible bioactives, the so-called “low hanging fruit”
(Baltz, 2006). Presumably it is not considered essential to develop the
technology to find compounds that were missed. In addition, actinobacteria as
a whole have been ignored, even though they too possess the capacity to
produce a huge number of bioactive small molecules; to date, only a small
proportion have been examined for therapeutic purposes. We are now in the
“genomic era” and in the case of streptomycetes, exciting new information
coming from complete genome sequencing efforts reveals that most of these
bacteria have the genetic capacity to produce many more structurally different
bioactive compounds than suspected. Some of the beneficial actinobacteria
and their action are tabulated in Table 2. As such, they represent an
inexhaustible collection of hidden chemical and biochemical diversity. Moreover creative techniques for generating some of these compounds are being developed and exploited (Baltz, 2008; Challis, 2008).

2.4.2 The “GOOD” about Actinomycetes

This characteristic refers mainly to the discovery and production of microbial small molecules with antibiotic activity that began with Waksman’s work on actinomycetes in the early 1940s. These seminal studies, together with the discovery of the fungal product penicillin by Fleming and coworkers and its characterization around the same time, are responsible for momentous and radical changes in medicine (Demain and Sanchez, 2009). The availability of antimicrobial agents made possible, for the first time, the successful treatment of most types of infectious diseases. The discovery of antibiotics also presaged many other uses for microbial compounds in human and animal therapy and in agriculture. Recently these microbial sources have provided treatments for many non-infectious diseases including cancer and heart disease. Another role, often overlooked, is their use in prophylaxis and in immunosuppression prior to invasive surgery, which has been one of the most important factors in the development and success of organ transplantation. Hundreds of millions of dollars have been invested by pharmaceutical companies in saving the lives of millions of people with profits of many billions of dollars. In addition, many actinobacterial strains have been developed for industrial applications such as bioremediation, the destruction of toxic xenobiotics, vitamins, fine chemical transformation and production and, more recently, for the development of biofuel conversions. Novel uses of the extensive biosynthetic capacities of the Rhodococci are being discovered
and one can predict their increasing importance as industrial microorganisms (Martinkova et al., 2009).

Table 2. Some beneficial actinobacteria and their compound and application

<table>
<thead>
<tr>
<th>Producing organism</th>
<th>Compound</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces aureofaciens</td>
<td>Tetracycline</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Streptomyces griseus</td>
<td>Streptomycin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Streptomyces kanamyceticus</td>
<td>Kanamycin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Streptomyces lactamurans</td>
<td>Cefoxitin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Streptomyces mediterranei</td>
<td>Rifamycin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Amycolatopsis orientalis</td>
<td>Pristinamycin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Micromonospora purpurea</td>
<td>Gentamicin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Saccharopolyspora erythraea</td>
<td>Erythromycin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Streptomyces avermitilis</td>
<td>Ivermectin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Streptomyces clavuligerus</td>
<td>Clavulanic acid</td>
<td>inhibitor</td>
</tr>
<tr>
<td>Streptomyces hygroscopicus</td>
<td>Bialaphos</td>
<td>Herbicide</td>
</tr>
<tr>
<td>Streptomyces hygroscopicus</td>
<td>Rapamycin</td>
<td>Immunosuppressive</td>
</tr>
<tr>
<td>Streptomyces noursei</td>
<td>Nystatin</td>
<td>Antifungal</td>
</tr>
<tr>
<td>Streptomyces verticillus</td>
<td>Bleomycin</td>
<td>Anticancer</td>
</tr>
</tbody>
</table>

In spite of the numerous benefits accruing from these seemingly inexhaustible sources, the ecology and biology of actinobacteria and their roles in environmental communities are poorly understood and the functions of their myriad low molecular weight products in the environment are even less well studied. The development of these products as antibiotics led to the assumption that their primary function in nature was for use as molecular weaponry by their producers. The field was driven by the concept of antagonism: during the past half century, their discovery and the proof of their biological activity relied solely on tests of their inhibition of the growth of other microbes under laboratory conditions. Given the number of bacterial genera
and the inestimable number of compounds involved, this implies that the microbial world is nothing less than a constant theatre of war (Hibbing et al., 2009).

2.5 Complex Life Cycle of Streptomyces

A major feature of streptomycetes is their ability to carry out a complex developmental life cycle that, phylogenetically, can be considered as one of the probably several evolutionary attempts at multicellularity (Chater and Losick, 1997). They form highly structured multicellular colonies composed of physiologically distinct hyphae. Besides, they have evolved separated somatic and germ line functions through two distinct cell lineages: the substrate and the aerial mycelium. As in higher multicellular systems, the final architecture of the streptomycetes colony is the integrated result of two major physiological processes: those promoting growth and differentiation and those causing senescence and hyphal death. Much of what is currently known on the life cycle of streptomycetes comes largely from studies addressed to identify the mechanisms responsible for growth and hyphal differentiation. By contrast, there has been little or no exploration of the mechanisms underlying cell degeneration and hyphal death.

There are many excellent reviews on the development of colony in streptomycetes (Chater, 1993; Chater and Losick, 1997; Chater, 1998; Hodgson, 1992; Locci and Sharples, 1984). The focus is on *Streptomyces* colonies considered as multicellular organisms and some of the key features involved in their development such as the coexistence of different cellular types are described and how the cells communicate with each other by emitting extracellular signals and responding to them. Finally, the similarities
between cell death in streptomyces and in higher eukaryotic systems and will speculate on the possibility that they could have a common evolutionary origin.

2.5.1 The developmental cycle of *Streptomyces*

The colony growth cycle of the streptomycetes initiates when a spore germinates and produces one or more long multinucleoid filaments (Hardisson *et al.*, 1978). These filaments, which elongate by apical growth (Brana *et al.*, 1982; Gray *et al.*, 1990) and branch repeatedly, originate a vegetative mycelium (substrate mycelium) that develops both on the culture medium and into it. This mycelium grows attached to its substrate, forming an intricate network of hyphae that penetrates the medium solubilizing organic molecules by the action of extracellular hydrolytic enzymes. Such a filamentous morphology allows full utilization of the solid materials in the soil, and enables the streptomycetes to colonize solid substrates more efficiently than non-motile, unicellular microorganisms. As the colony ages, and the nutrients become exhausted, specialized branches emerge from the surface of the colony, originating the reproductive aerial mycelium that grows upwards, vertically into the air (Miguelez *et al.*, 1994).

While the substrate mycelium has a primarily vegetative function, the role of the aerial mycelium appears to be mainly reproductive, forming spores and placing them in a favourable position to be dispersed, thus solving the problem raised by the immobility of the substrate mycelium. By the end of the colony growth cycle, the aerial hyphae undergo multiple septation which originates chains of uninucleoid compartments which, finally, metamorphose into thick walled spores (Hardisson and Manzanal, 1976; Manzanal and
Hardisson, 1978). Then the spores on suitable medium undergo their life cycle within seven steps shown in Figure 3. A spore germinates with spreading the filaments into such a solid medium ("vegetative mycelium", step 0-2). Then the developed vegetative mycelium forms sporophores vertically toward the surface ("aerial mycelium", step 3-4). The aerial mycelium spirals (step 5), and then the polynucleated aerial filaments are partitioned (step 6). The resulting sheaths will become spores (step 7), and another cycle continues. The secondary metabolites including antibiotics are secreted during the generation of aerial hyphae from the vegetative mycelium.

**Figure 3. Life cycle of streptomycetes**
2.6 Actinomycetes and its importance

Actinomycetes are a group of organisms that morphologically resemble fungi and physiologically resemble bacteria (Sultan et al., 2002) and they exhibit a wide range of morphological forms extending from cocci through fragmenting hyphal to permanent and highly differentiated branched mycelium (Sultan et al., 2002; Mustafa et al., 2004; You et al., 2005). Actinomycetes can utilize a variety of organic nutrients but special media are often preferable (Sathi et al., 2001). Their growth is characterized by small compact, soft to leathery colonies tenaciously adhering to the medium, the surface being either flat or elevated (Sathi et al., 2001).

The outer zones of the colonies are smooth but fringes of minute hyphae are observed. Colonies of some actinomycetes have smooth surfaces while others their surfaces are much folded (Mutitu et al., 2008). The colony surface is usually dry with conical or elevated appearance and it is either free from mycelium or covered with mycelium. The color of the mycelia ranges from nearly colorless to white, chalky red, or grey or olive or pink (Mustafa et al., 2004). The color is due to the pigment produced by phenazines, phenoxygeninones and prodiginines (Rahman et al., 2000). Some species forms fairly rings consisting of concentric spore bearing and spore free rings disposed in zones (Sathi et al., 2001).

Many characteristics of actinomycetes have been employed for the purpose of classification and ideally, these should be constant under the same cultural conditions (Sathi et al., 2001). These include shape of the spores, branching manner of the spore chains, nature of the spore chain, form or structure of sporophores (You et al., 2005), cultural characteristics and
spore color on various media and carbon utilization (Rahman et al., 2000; Sultan et al., 2002), whole cell sugar and lipid composition (Rahman et al., 2000) and antibiotic production.

Actinomycetes are well known for the production of antibiotics with the group producing over half of the known antibiotics (Hongjuan et al., 2006). Use of antibiotics and other antimicrobial products in plant disease management has been gaining popularity due to the realization of the adverse effect to the human health and environment occasioned by use of the synthetic chemical pesticides (Agrios, 2005). The antagonistic metabolites are normally composed of heterogenous and biologically active compounds (Mustafa et al., 2004). To fully make use of these metabolites, it is necessary to characterize them by separating them in solvent systems, conducting bioassays and ascertaining their stability under different physical and chemical conditions. Enhancement of antimicrobial activity can be achieved through partial purification and characterization methods (Mutitu et al., 2008).

Though the actinomycetes are the largest group of actinobacteria with the group producing over 70% of all naturally occurring antibiotics (Hongjuan et al., 2006), their identification is still short of the universal standard and there is a need to undertake more studies on this.

2.6.1 Isolation of actinomycetes

Several methods have been proposed to reduce the presence of bacteria and fungi during the isolation of actinomycetes from marine soil sample. Porter et al. (1960) recommended the use of antifungal antibiotic with glycerol arginine agar medium for the selective isolation of aerobic actinomycetes from soil samples. With CaCO₃ treatment, soil samples
recorded high counts of actinomycetes as compared to other bacteria and fungi (Tsao et al., 1960). Williams et al. (1972) found that the soil samples heated at 40-45°C for 2-16 hr reduced the bacterial population considerably without affecting the colony counts of *Streptomyces*.

Soil dilution plate technique was described by Johnson and Cuel (1972) for the isolation of *Streptomyces* species. For selective isolation of actinomycetes from natural mixed microbial populations, Hirsch and Christersen (1983) developed a novel method in which nutrient agar medium was overlaid with a 0.22–0.45 µm pore cellulose ester membrane filter and the surface of the filter was inoculated.

Pisano et al. (1989) isolated actinomycetes from the marine sediments by amending rifampicin in the culture medium. *Pseudonocardia* species and *Nocardia* species were isolated from the marine soil samples by using UV irradiation (Galenko et al., 1990). Selective soil treatment methods such as phenol (1.5%, 30 min at 30°C), dry heat as well as phenol, dry heat and benzene thonium chloride (0.02 %, 30 min at 30°C) and pollen baiting for the isolation of action planes species were described by Hayakawa et al. (2000). Pirouz et al. (1999) recommended the use of yeast extract–malt extract dextrose agar medium for the maintenance and preservation of *Streptomyces* species.

Wang et al. (1999) reported the use of agents like gentamycin, tunicamycin and novobiocin for the isolation of specific genera of soil actinomycetes. High frequency radiation was proposed by Li et al. (2002) for the selective isolation of actinomycetes from soils. Takahashi et al. (2003) recommended the use of antibiotics like novobiocin, chlorotetracyclin and
gellan gum as solidifying agent instead of agar in the growth media for the isolation of actinomycetes. Pretreatment of soil samples with CaCO$_3$ followed by drying at 45°C for 1 hr was forced to reduce the incidence of bacteria and molds and favor the isolation of *Streptomyces* species (Narayana *et al*., 2004).

Soil extract agar medium supports the growth of novel bacterial and actinomycete strains (Hamaki *et al*., 2005). Vargas *et al*. (2008) enumerated the actinomycetes population on Kuster's medium supplemented with cycloheximide and sodium propionate. (Hayakawa, 2008) reported the use of luvenic acids for rapid isolation of actinomycetes from soil compounds.

After isolation the actinomycetes are subjected to the screening against test organisms, production, extraction and purification of antimicrobial compounds by different methods. Screening of the antimicrobial metabolites producing isolates are tested for antimicrobial activity against the test organisms like Gram-positive and Gram-negative organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Enterobacter aerogenes*. Production of antimicrobial metabolites was described by Liu *et al*. (1992) and extraction of bioactive compounds was described by Westley *et al*. (1979). Different solvents were used for the production of antimicrobial compounds like chloroform, n-hexane, ethanol, n-butanol, ethyl acetate, methanol, etc. Meroparamycin, by *Streptomyces* sp. MAROI, Sparsomycin, by *Streptomyces* sp. AZ-NIOFDI, Himastatin, by *Streptomyces hygrosopicus* X-14547A, by *Streptomyces antibioticus* NRRL8167 and IB-00208, by *Actinomadura* were some of the antibiotics, isolated by these methods. Activity of antimicrobial compounds was detected
by the UV region (200–400nm) by using a Perkin-Elmer lambda 15UV/VIS spectrophotometer. Purification of all the antibiotics were purified by thin layer chromatography, column chromatography by using different solvents chloroform, methanol (4:1), n-butanol, glacial acetic acid, water (4:1:1), isopropanol, water (8:2). Characterization of the all the bioactive compounds were determined by IR, Mass and NMR spectroscopy.

2.7 Taxonomy and Identification of Actinomycetes

In actinomycetes taxonomy, the concept of genus has been gradually established with the advent of chemotaxonomy and molecular evolution in addition to morphological and physiological properties. However, concept of species has been confused and even now there are many problems in the recognition and definition of species. In particular, the genus *Streptomyces*, has more than 400 valid names of species which include many names that were considered synonyms, consequently, correct identification of unknown strains may be impossible. Traditional classification of *Streptomyces* and its problems were described by a taxonomic system for the description of *Streptomyces* at the specific level proposed by International Streptomyces Project (ISP) has been applied almost world-wide.

Traditionally, identification of bacteria, including actinomycetes, has been very time consuming and laborious, except for some pathogenic species for which simple identification methods have been established. With most animals and plants, real-time identification of many individuals is possible with the naked eye or optical-microscopic observation. For bacterial identification, incubation prior to morphological observation and various tests for phenotypic characteristics are needed. These difficulties have affected both basic and
applied research on bacteria. Bacteria have few morphological characteristics that can be used to estimate phylogenetic relationships. Molecular phylogenetic methods were applied to bacterial taxonomy earlier than for other organisms (Olsen et al., 1994; Larsen et al., 1993). Nucleotide sequences for the 16S rRNA gene have been published for almost all actinomycete species, and reference to these sequences allows for easy identification of a wild strain. However, DNA sequencing and analysis involves many steps, require expensive equipment and chemical reagents, and was particularly difficult when many strains are to be analyzed. Fortunately, advances in DNA sequencing technology and high-through-put analytic methods are achieved every year. Furthermore, the potential for ‘DNA barcoding’ of animals and plants has gained popularity in recent years (Ratnasingham and Hebert, 2007). Thus, methods based on DNA sequencing have the potential to be the most generally applicable for identification of not only bacteria but also plants, animals, and other taxa in the near future.

*Streptomyces* species are Gram-positive soil inhabiting bacteria possessing genomes with a very high G+C content (ca. 72%) (Chater, 1993). The most striking feature of these species was their production of a wide variety of antimicrobial compounds with critical applications as drugs of medical, agricultural and veterinary importance. The *Streptomyces* species characterized so far have relatively large linear chromosomes (ca. 8 Mb) (Lin et al., 1993) with terminal inverted repeats ranging from 44 bp to 550 kb (Chen et al., 1993) and terminally bound proteins (Bao and Cohen, 2001).

Members of the genus *Streptomyces* are common in natural habitats (Strap and Crawford, 2006) and were well known as the primary source of
commercially significant secondary metabolites (Berdy, 1995; Demain, 1998, 1999; Demain and Elander, 1999; Lazzarini et al., 2000; Wateve et al., 2001; Strohl, 2004). Because of their ecological and biotechnological significance, many streptomycetes have been isolated from natural habitats and characterized. The genus currently contains 559 validly described species though it is evident that the taxon was still underspeciated (Sembiring et al., 2000; Manfio et al., 2003; Goodfellow et al., 2007). It is important to isolate and detect novel Streptomyces species for pharmaceutical search and discovery programmes as screening of known streptomycetes tends to lead to the costly rediscovery of previously characterized bioactive compounds (Bull et al., 2000; Bull, 2004a, b).

It is an accepted practice to circumscribe Streptomyces species using a combination of genotypic and phenotypic properties, as exemplified by the delineation of species in the Streptomyces griseus, Streptomyces violaceusniger and Streptomyces violaceoruber 16S rRNA gene clades (Duangmal et al., 2005; Liu et al., 2005; Goodfellow et al., 2007). However, it is not practical in either bioprospecting or ecological studies to characterize large numbers of unknown streptomycetes isolated from natural habitats using polyphasic taxonomic approaches. Thus, while taxonomic studies on streptomycetes require a thorough examination of relatively few isolates, bioprospecting and ecological investigations need to be based on the isolation of many strains in order to determine the occurrence, frequency and distribution of taxa present in microbial communities.

The need to determine the taxonomic diversity of streptomycetes in natural habitats was emphasized by Williams et al. (1969). These workers
assigned a large number of soil streptomycetes to groups based on aerial spore mass, colony reverse and diffusible pigment colors formed on oatmeal agar, and on their capacity to produce melanin pigments on peptone-yeast extract-iron agar. They found that isolates assigned to such color groups clustered together when overall similarities between them were calculated based on morphological and physiological features. It was subsequently shown that color groups are a valuable index of the taxonomic diversity of streptomycetes in natural habitats (Goodfellow and Haynes, 1984; Atalan et al., 2000; Sembiring et al., 2000) as isolates taken to represent such taxa key out to either established or to novel *Streptomyces* species, or to *Streptomyces* species groups based on computer-assisted identification (Goodfellow and Haynes, 1984; Williams and Vickers, 1988; Atalan et al., 2000) and on Curie-point mass pyrolysis spectrometric and polyphasic taxonomic procedures (Manfio et al., 2003; Goodfellow et al., 2007).

Biosystematic studies based on the color grouping procedure of Williams et al. (1969) show that streptomycetes communities in natural habitats are taxon rich, as exemplified by the assignment of over 550 streptomycetes isolated from rhizosphere and non-rhizosphere soils to 37 multimembered and 105 single membered color groups (Sembiring et al., 2000). However, this approach to establishing the taxon richness of streptomycetes in natural habitats was limited by the need to assign isolates to color groups simultaneously given the subjective nature of the procedure used to distinguish between different colored pigments. This limitation also means that it is not possible to generate cumulative databases thereby making it impossible to compare the results of independent studies on
indigenous populations of streptomycetes.

*Streptomyces* are aerobic, filamentous soil bacteria (Stackebrandt *et al*., 1991) known for the production of secondary metabolites and biologically active materials. The vast majority of natural antibiotics in clinical usage were produced by *Streptomyces* and fungi (Keiser *et al*., 2004). The identification and classification of *Streptomyces* has relied on phenotypic characteristics and, more recently, on genetic approaches. The phenotypic methods included fatty acid analysis, whole cell analysis, biochemical tests, antisera specificity test, phage typing, and protein profiling (Anderson and Wellington, 2001; Goodfellow *et al*., 1987; Korn-Wendisch and Schneider, 1992; Saddler *et al*., 1987; Taguchi *et al*., 1996; Wipat *et al*., 1994). The genotypic methods are based on molecular data such as DNA–DNA hybridization, restriction digestions of total chromosomal DNA, randomly amplified polymorphic DNA, PCR assays, and nucleic acid sequence comparison (Gharaibeh *et al*., 2003; Huddleston *et al*., 1997; Labeda, 1992; Rauland *et al*., 1995; Wenner *et al*., 2002). The detection and classification of *Streptomyces* in both environmental and pure culture samples is now most commonly performed by molecular approaches based on oligonucleotide probing or selective PCR amplification (Locatelli *et al*., 2002). While some attempts to develop *Streptomyces* specific detection methods target streptomycin biosynthesis genes (Huddleston *et al*., 1997; Izumikawa *et al*., 2003; Kong *et al*., 2001; Malkawi *et al*., 1999; Rintala *et al*., 2004; Stackbrandt *et al*., 1997; Williamson *et al*., 2000), the majority of genetic methods were developed to achieve more precise knowledge of the occurrence of *Streptomyces* in different environments targeted the 16S rRNA gene (Donadio
et al., 2002; Rintala et al., 2001). Group-specific probes targeting *Streptomyces* specific regions of 16S rRNA genes have been developed (Stackbrandt et al., 1997) and the comparison of PCR amplified rRNA sequences is a prevailing tool in *Streptomyces* taxonomy. To examine sequence variations within the *Streptomyces* genus, three regions (a, b, and c) in the 16S rRNA gene corresponding to nucleotides 982–998, 1102–1122, and 158–203, respectively, of *Streptomyces coelicolor* were generally investigated (Anderson and Wellington, 2001). Although the study of these 16S rRNA variable regions, especially the c region, has been used to resolve inter-species relationships within the *Streptomyces*, detecting strain level differences has not been possible with this approach (Hain et al., 1997).

Several studies have tried to use sequence data from variable regions of 16S rRNA to set up taxonomic structure within the genus, but the variation was regarded as too limited to help resolve problems of species differentiation (Anderson and Wellington, 2001; Keiser et al., 2004; Rintala et al., 2004).

Ribosomal intergenic spacer (RIS) regions show faster rates of evolution than 16S or 23S rRNA genes, and the analysis of intergenic 16S-23S rRNA spacer regions has been shown to be more useful for detecting species and strain level relationships of *Streptomyces* cultures than the analysis of 16S rRNA gene sequences (Hain et al., 1997). The RIS comprises conserved regions as well as regions highly varied in length and sequence in *Streptomyces* (Song et al., 2004). Therefore, RIS regions can be expected to show clear differences between closely related organisms, especially at the species/strain level. The corresponding DNA fragments can be amplified, using conserved nucleotide regions in the flanking 16S and 23S
rRNA gene sequences. Moreover, the target region may include some part of the 16S rRNA gene so that DNA sequence comparisons can enable traditional genetic taxonomic classification (Locatelli et al., 2002; Yu and Mohn, 2001). The combined use of denaturing gradient gel electrophoresis (DGGE) and the analysis of RIS-region DNA fragments to characterize bacterial cultures are rare (Blaiotta et al., 2003; De oliveira et al., 1999; Janse et al., 2003), and this technique has not been employed for the analysis of *Streptomyces* cultures (Song et al., 2004).

### 2.7.1 Development of a simple-identification method for actinomycetes based on partial 16S rDNA sequences

A simple-identification method was described for actinomycetes based on direct polymerase chain reaction (PCR) amplification, direct sequencing, and BLAST homology search (Muramatsu et al., 2003; Altschul et al., 1997).

Species of *Streptomyces* are among the best studied and best characterized bacteria due to their significant role for medical science, ecology and the biotech industry (Lechevalier and Lechevalier, 1967; Embley and Stackebrandt, 1994; Bentley et al., 2002). They have a particularly complex secondary metabolism, which produces a large collection of biologically useful compounds. Most importantly, they were employed at large industrial scale in the production of most of the available antibiotics applied in human and veterinary medicine, as well as a large number of anti-parasitic agents, herbicides, immunosuppressants and several enzymes important in the food and other industries (Bentley et al., 2002; Cerdeno-Tarraga et al., 2003; Hopwood, 2007).

The genus *Streptomyces* was taxonomically located in the diverse bacterial order Actinomycetales. This group was characterized by an
astonishing diversity in terms of morphology, ecology, pathogenicity, genome size, genomic G+C content, and the number of coding sequences in the genome (Embley and Stackebrandt, 1994; Hopwood, 2007; Ventura et al., 2007). Morphologically, some species were rod-shaped or coccoid, while others form fragmenting hyphae or a branched mycelium (Ventura et al., 2007). Spore formation was also very common in Actinomycetales but it is not ubiquitous (Ventura et al., 2007). Ecologically, some actinomycetes were soil living bacteria, some were marine, were colonizing thermal springs (Barabote et al., 2009) or growing on gamma-irradiated surfaces (Phillips et al., 2002) or as plant root symbionts (Normand et al., 2007), and some were important pathogens of humans, animals and plants (Goodfellow and Williams, 1983; Castillo et al., 2002; Tokala et al., 2002). For instance, *Mycobacterium tuberculosis* causes tuberculosis (Cole et al., 1998), *Corynebacterium diptheriae* infection results in diptheria (Cerdeno-Tarraga et al., 2003), *Propionibacterium acnes* is the agent of acnes (Leyden, 2001) and *Streptomyces scabies* causes potato scab (Takeuchi et al., 1996).

*Streptomyces* species were found mostly in the soil where they live as saprophytes (Hopwood, 2007), but recently some species have been described in the rhizosphere of plant roots and in other plant tissue (Castillo et al., 2002; Tokala et al., 2002), isolated from leaf cutting ants (Kost et al., 2007) and also associated with marine sponge species (Zhang et al., 2008).

Considering the importance of *Streptomyces* and its relatives in terms of both biological behavior and metabolic products, it becomes essential to understand its evolutionary relationships to other species in the diverse Actinomycetales order. On the one hand, an evolutionary study may help to
explain how *Streptomyces* emerged and adapted to the soil environment. On the other hand, information obtained from a well resolved phylogeny can be used for the comparison of genome sequences, comparative genome reannotation, and genome visualization. A robust phylogeny is central for ongoing efforts in many groups to reconstruct system wide metabolic models of *Streptomyces* and related species (Borodina *et al*., 2005), which were used for systematic strain engineering in biotechnology.

Currently available phylogenies of the group were based on 16S rRNA, individual genes, or comparative genomic approaches (Embley and Stackebrandt, 1994; Takeuchi *et al*., 1996; Stackebrandt *et al*., 1997; Anderson and Wellington, 2001; Egan *et al*., 2001; Gao and Gupta, 2005; Chater and Chandra, 2006; Manteca *et al*., 2006). Such reconstructions tend to be relatively unstable and were not guaranteed to reflect the overall evolutionary history in a complex group with widespread horizontal gene transfer. To address this issue and to determine potential problematic areas in the single gene phylogenies made the use of whole-genome information. Not only reconstructed phylogenetic trees based on eight highly conserved single genes, including three rRNA sequences (5S, 16S, 23S rRNA) and five ubiquitous protein sequences (isoleucyl tRNA synthetase, ribosomal protein S1, SecY, GTPase, DNA topoisomerase), but also integrated three different phylogenetic reconstructions based on complete genome sequences, using gene content, gene order and gene concatenation analysis.

Partial 16S rDNA sequencing has been carried out to confirm the taxonomic affiliation of a large fraction of this screening collection and to assess molecular data on its microbial diversity and individual phylogenetic
relationships. In spite of the high value of this data, this method was rapidly shown to be inefficient to be applied upfront as a selection criterion. The intraspecies heterogeneity existing in actinomycetes taxa could not be resolved in phylogenetic inner branches, requiring alternative dereplication tools to assess the uniqueness of potential new strains. Levels of intraspecies variability among strains from the same species obtained from the same environment have frequently been observed (Anderson and Wellington, 2001; Guo et al., 2008) and make it necessary to introduce additional molecular fingerprinting tools for selection. From the different molecular fingerprinting methods available [Amplified Fragment Length Polymorphism (AFLPs), Random Amplified Polymorphic DNA (RAPDS), Repetitive sequence-based PCR (REP-PCR) fingerprinting], random BOX-PCR amplification (PCR based on primers targeting the highly conserved repetitive DNA sequences of the BOX element of *Streptococcus pneumoniae*) was shown to be one of the most useful tools to detect variability within actinomycetes species and to establish the relatedness of isolates (Lanoot et al., 2005; Versalovic et al., 1994; Vos et al., 1995; Welsh and McClelland, 1990). Other complementary tools were developed in the laboratory, focusing on detection of Polyketide Synthase (PKS) and Non Ribosomal Peptide Synthetase (NRPS) biosynthetic genes. They were also used to develop rapid strain fingerprints on the basis of biosynthetic genes restriction pattern that might provide additional information on their diversity and uniqueness from the biosynthetic perspective (Ayuso et al., 2005; Ayuso-Sacido and Genilloud, 2005). The systematic 16S rDNA sequence analysis applied on strains producing biological activities was extremely useful to reveal the high rate of strains from
a given niche and associated with different taxonomic affiliations that produced the same or related compounds.

2.8 Optimization studies for the production of antimicrobial compounds by actinomycetes.

The study of the formation of antibiotics usually involves a search for optimal media for production. The approach was made by a systematic study of the suitability of large number of carbon sources and nitrogen sources for antibiotic formation. Early reports showed that *Streptomyces* species could utilize sugars, sugar alcohols, and some organic acids. On the basis of the utilization of different carbon sources, Pridham and Gottlieb (1948) tried to characterize actinomycetes. Umezawa *et al.* (1957) studied the carbon nutrition of *S. kanamyceticus* for kanamycin production in complex media and reported that glucose, maltose, dextrin, starch, lactose, and sucrose were better carbon sources than glycerol. (Romano and Nickerson, 1958) studied the utilization of amino acids as carbon sources by *Streptomyces* species. The effect of carbon and nitrogen sources on the growth of *S. griseus* and streptomycin production was studied Dulaney (1948). Majumdar and Majumdar (1967) studied the utilization of carbon and nitrogen compounds for neomycin production by *S. fradiae*.

Intermediate metabolites from primary metabolism serve as precursors for the biosynthesis of antibiotics. The composition of the culture medium, closely connected with the metabolic capacities of the producing organism, greatly influences antibiotic biosynthesis. In fact, changes in the nature and concentration of carbon and nitrogen sources, phosphorus concentration, and trace elements have been reported to affect antibiotic biosynthesis in different organisms (Vilches, 1990).
Margalith and Pagani (1961) studied the impact of several features such as age of the culture, mycelia growth, pH and composition of the media on the production of rifamycin complex by *Streptomyces mediterranei*. Chandrasekhar and Seshadri (1964) reported that the incorporation of pulses and cereals in the medium containing glucose, NaCl and K$_2$HPO$_4$ gave best results for the production of antimicrobial metabolites by *Streptomyces*. The growth pattern of six strains of *Streptomyces* and their antibiotic production were examined by Sahay and Srivastava (1977) using different carbon and nitrogen sources in the culture media. Mandal *et al.* (1981) studied the effect of carbon and nitrogen sources on the production of oxytetracycline by *Streptomyces rimosus*. Larsen (1993) observed that moderate halophiles growing at optimal NaCl concentrations viz., 5-20% showed antimicrobial activity against bacteria and fungi.

Bhattacharya *et al.* (1998) noticed the effect of nutrients on the production of antimicrobial metabolites by *Streptomyces hygroscopicus* D15. The optimum temperature for the production of bioactive compounds like quinolones, macrocyclic peptide antibiotic compound 41, 403 and boxazomycin A and B by *Pseudonocardia* sp. CL38489 (Dekker *et al*., 1998), *Pseudonocardia* G495-11 (Saitou *et al*., 1987) and *Pseudonocardia fastidiosa* (Clemer *et al*., 1977) respectively was recorded as 28°C. Sun *et al.* (2002) determined the combined effects of pH and nutrients on the growth and sporulation of some strains of *Streptomyces*.

Stapley *et al.* (1972) isolated a number of actinomycetes from soil which produced one or more number of a new family of antibiotics, the cephamycins, which were structurally related to cephalosporin C. The
cephamycin were produced in submerged fermentation in a wide variety of medium by one of more or eight different species of *Streptomyces*, including a newly described species, *S. lactamdurang*. These antibiotics exhibit antibacterial activity against a broad spectrum of bacteria which includes many that were resistant to the cephalosporins and penicillins.

Richard *et al.* (1986) isolated a microorganism designated as Rv-79-9-101 and now identified as *Micromonospora purpurea chromogenes* sub species *halotolerans*, from a mud sample in the Philippines, which produced a complex of antibiotics called crisamicins. Thin layer chromatography and bioautography, employing solvent extracts of whole fermentation broths, revealed a minimum of five antimicrobial components. The major biologically active component of the antibiotic complex-crisamicin A, was obtained in pure form after preparative silica gel column chromatography followed by crystallization. Based on physicochemical data crisamicin A has been identified as a novel member of the isochromanequinone group of antibiotic. It exhibits *in vitro* activity against gram-positive bacteria but little or no activity towards Gram-negative bacteria or fungi. Soliveri *et al.* (1988) described the effect of different nutrients on the production of the macrolide polyene antibiotics. PA-5 and PA-7, produced by *Streptoverticillium* sp. 43/16. Optimal production yields of PA-5 and P-7 have been achieved with L-proline and glycine as nitrogen sources, respectively.

James *et al.* (1989) isolated *Actinomadura melliaura* culture, SCC 1655 from a soil sample collected in Bristol Cove, California by plating soil suspensions on water agar containing 75 mg/ml Cephalosporin C. The compounds AT2433-A1 (A1), AT2433-A2 (A2), AT2433-B1 (B1) and AT2433
B2 (B2) were isolated from the culture broth of *A. melliaura*, SSC 1655. Structurally these compounds are closely related to rebeccamycin (L), an indolocarbazole antitumor antibiotic. A1, A2, B1 and B2 were active against *Staphylococcus aureus* A9537, *Streptococcus faecalis* A20688, *Streptococcus faecium* (ATCC 9790), *Micrococcus lutea* (ACC 9341) and *Bacillus subtilis* (ATCC 6633). A1 and B1 were active against p388 leukemia in mice. James *et al.* (1991) reported that *Streptomyces thermoviolaceus* was grown in a chemostat under conditions of glutamate limitation.

Ahmed *et al.* (1992) studied the effect of ammonium on growth and spiramycin biosynthesis in *Streptomyces ambofaciens* on a chemically defined medium. Spiramycin biosynthesis was better in the presence of valine and isoleucine than in the presence of ammonium. This production was reduced in the presence of excess ammonium (100 mM). The addition of catabolic intermediates of valine and isoleucine reserved the negative effect of ammonium. Valine dehydrogenase (VDH), the enzyme responsible for valine, bencine and isobencine catabolism, was repressed when excess ammonium was present in the medium. This repression was approximately 25% when the ammonium concentration was increased from 50 to 100 mM.

Li, *et al.* (1992) mutagenized natural non-antibiotic producing *Streptomyces* sp. 1254 by UV irradiation and two active mutants were isolated. Mutant 113 produced novel anthracycline compounds designed mutactimycins. Mutactimycin A was active against the bacteriophage of *B. subtilis* and some viruses in tissue culture.

New methods for the detection and identification of novel actinomycetes from a range of environment were described by Williams *et al.*
(1993) and also their approach for the detection of actinomycetes ranged from investigation of neglected habitats and extreme environment (e.g. alkali soils and oil drills) to the analysis of DNA extracted from the environment and use of specific phages. The continuing problems of the identification of actinomycete were also considered.

Ensign et al. (1993) investigated the physiology of some actinomycete genera. Actinomycetes were widespread in the environment and were mainly organotrophic. Studies of their ecology have been primarily focused on their detection and isolation, with comparatively little attention to the control mechanisms that determine their occurrence and behavior in their natural environments. Several actinomycete genera produce motile spores. The significance of flagella proteins and factors influencing spore motility and germination were considered. The genus Frankia forms nitrogen fixing associations with non-leguminous plants. Molecular techniques have been used to clarify the endophyte host relationship. Micromonospora species were common in the environment. The growth and physiology of gentamicin-producing strains were described. Thermophilic actinomycetes in the genus Thermoactinomyces are common in composts and other self heating environments.

Shigetoshi and Sinroku, (1997) described the scale-up of Streptomyces hygroscopicus sub species aureolacrimosus in submerged culture and the production of milbemycin, a 16-membered macrolide pesticidal antibiotic. Ogata et al. (1997) reported a brownish-orange crystalline antibiotic having acid-base indicating property which was isolated from the culture filtrate of a strain of Streptomyces grown on MgSO₄ rich medium. The mature aerial
mycelium of this strain was gray and bore long straight chains of spores with smooth surface. Several taxonomic characteristics were investigated. The antibiotic, named A-60 had strong activity against Gram-positive bacteria and weak activity against Gram-negative bacteria. The effect of MgSO₄ and other metal salts on production of the antibiotic was investigated. The productivity was accelerated by the addition of MgSO₄, MgCl₂, MnCl₂, FeSO₄, FeCl₂ or BaCl₂.

Yoshiko et al. (1998) observed that physiological differentiation (including antibiotic production) in microorganisms usually starts when cells encounter adverse environmental conditions. Judith et al. (2000) reported two novel angucyclinone type antibiotics, simocyclinones D4 and D8, in the mycelium extract of *Streptomyces antibioticus* Tii 6040 by HPLC-diode-array and HPLC-electrospray-mass-spectrometry screening. The compounds showed antibiotic activities against Gram-positive bacteria and cytostatic effects on various tumor cell lines.

Tamotsu et al. (2000) showed that Aristostatins A and B; new members of tellocarcin class of antibiotics were isolated from the culture broth of an actinomycete strain. The producing strain, TP-A 0316 was identified as *Micromonospora* sp. Aristostatins were obtained from the culture fluid by solvent extraction and chromatographic purification. They showed antibiotic activity against Gram-positive bacteria and antitumor activity. Klaus et al. (2001) detected four novel cyclic homodecapetide antibiotics, stretocidin A-D in the mycelium extract of *Streptomyces* Sp. Tii 6071 by HPLC-diode-array and HPLC electrospray mass-spectrometry screening. The peptides which
were closely related in structure to the tyrocidines and gramicidins of \textit{Bacillus brevis} show antibiotic activities against Gram-positive bacteria.

Marcelo \textit{et al.} (2003) isolated strain Tii 6239 from a soil sample collected in Brazil and assigned as a new species of the genus \textit{Streptomyces}. In the course of HPLC-diode assay screening program, three metabolites were detected in the culture filtrate and mycelium extract of strain Tii 6239. They were characterized as members of macrolactum group, the new compound ripromycin, previously described ikarugamycin and a new derivative of it, ikarugamycin epoxide. They exhibited antibiotic activity against gram positive bacteria and cytostatic effects to various human tumor cell lines. Zhi-Hua and Pei-Lin (2004) reported that strain improvement and medium optimization to increase the productivity of spiramycin were carried out.

Lintskarova \textit{et al.} (2005) used conventional mutagenesis (UV irradiation and exposure to nitrosoguanidine) to produce and regenerate protoplasts, aiming at increasing the antibiotic activity of a \textit{Streptomyces fradiae} strain producing tylosin. Variants exceeding the activity of the initial procedure strain by 0.5–28.3% were obtained. The most active variants were produced by a combined exposure to UV and nitrosoguanidine, as well as upon regeneration of protoplasts formed from the cells of clones produced by UV irradiation. Unstable inheritance of the trait of increased tylosin production was demonstrated.

Gesheva \textit{et al.} (2005) reported the influence of different carbon and nitrogen sources on the production of AK-111-81 nonpolyenic macrolide antibiotic by \textit{Streptomyces hygroscopicus} 111-81. Substitution of glucose with lactose or glycerol significantly affected maximal antibiotic AK-111-81
productivity as the growth rate was close to that of the basal fermentation medium. Addition of ammonium succinate to the fermentation medium markedly increased the antibiotic productivity as the growth rate was low. Divalent ions as Mn$^{2+}$, Cu$^{2+}$, Fe$^{2+}$ stimulated AK-111-81 antibiotic biosynthesis. These results allowed developing a new fermentation medium showing 6-fold increase of AK-111-81 antibiotic formation compared with the basal fermentation medium.

2.9 Geosmin produced by actinomycetes

Freshly plowed soil has a typical odor which was undoubtedly detected even by primeval men and extolled in all tongues by bucolic poets. In the 19th century, the odor of the soil was subjected, for the first time, to scientific scrutiny. Berthelot and Andre (1891) noted that the substance responsible for the typical earthy odor of the soil could be extracted from soil by steam and was probably neutral. As microorganisms began to be grown in pure culture, it became obvious that some of them, mainly actinomycetes, produced an earthy odor. In addition, these organisms were suspected of imparting taints to bodies of water and their inhabitants. Thaysen (1936) and Thaysen and Pentelow (1936) obtained ether-soluble extracts of actinomycetes which had a manurial odor at high concentrations, but which, upon dilution, became earthy-smelling.

The production of important biologically active compounds by actinomycetes was usually followed by the formation of volatile substances with strong odor (Behal, 2000). Among the number of soil microorganisms, actinomycetes growing in a pure surface or submerged culture were characterized by the production of a strong and earthy odor. Gerber and
Lechevalier (1965) and Gerber (1968) obtained diethyl ether-soluble extracts from actinomycetes that contained a highly concentrated odor, which and after dilution, became earthy-smelling. Rosen et al. (1968) described odor production by the odoriferous strain *Streptomyces griseoluteus*. Dougherty et al. (1966) studied the volatile metabolites of actinomycetes by NMR and MS spectrometry.

Streptomyces were currently among the microorganisms most intensely used for industrial production of antibiotics and various other biologically active substances. The production of an antibiotic in a fermentor was often accompanied by the biosynthesis of volatile odorous substances. Odorous substances accompanying the activation of streptomyces were first mentioned in the last century. These volatile substances were isolated from cultivation medium and their chemical structure was determined (Gerber and Lechevalier, 1965; Gerber, 1968; Dougherty et al., 1966). Some of the streptomycetes species were used to produce the volatile compounds like geosmin (trans-1,10-dimethyl-trans-9-decalol). They are *Streptomyces avermitilis*, *Streptomyces halstedii*, *Streptomyces albiflavus*, *Streptomyces griseus*, and *Streptomyces griseus* LP-16. (Rezanka et al., 1994; Rezanka and Votruba, 1998a; Rezanka and Singler, 2006).

The most common off flavor compound and the main source of soil odor produced by microorganisms is geosmin. It was a well known and widely studied compound (Rosen et al., 1968) whose presence in water was recognized by the characteristic earthy smell even at concentrations of 1.3 nmol/L; the smelling water was difficult to drink (e.g., fish living in geosmin contaminated water taste also of mud). Reports on this topic (Aoyama et al.,
1991; Bowmer et al., 1992) emphasized that geosmin was a main odor component produced by Streptomyces. Enantioselective multidimensional gas chromatography identified a single geosmin enantiomer in pure cultures of Streptomyces sp. strain isolated from rotten grapes and revealed that the chiral compound geosmin was in the (−) form much more odoriferous than in the (+) one. Geosmin production was also studied in terms of its genetic determinants (Saadoun and El-Migdadi, 1998) and it was found to inhibit plant seed germination (Ogura et al., 2000).

### 2.10 Melanin Production

When 3,4-dihydroxyphenylalanine was oxidized in the presence of phenol oxidases or by silver oxide, a red pigment was formed. Enzymatically, this pigment was converted to a melanin. In the absence of enzyme, it has been shown that the pigment may rearrange to either 2-carboxy-5,6-dihydroxyindole or to 5,6-dihydroxyindole. The structure of the red pigment has therefore been inferred to be either 2-carboxy-2,3-dihydroindole-5,6-quinone or the tautomeric 2-carboxy-2,3-dihydro-6-hydroxyindole-1,5-quinonimine, and on this basis accompanying hypothesis for the enzymatic conversion of 3,4-dihydroxy-phenylalanine to synthetic DOPA melanin. This study was undertaken to determine whether or not the postulated reactions beyond the formation of the red pigment do take place in the presence of oxygen and enzyme. The enzymic oxidation of 3,4-dihydroxyphenylalanine was followed spectrophotometrically. The process was observed to proceed in three chromophoric phases, the first corresponding to the formation of the red pigment, the second to an intermediate purple pigment, and the third to the formation of melanin. By comparison of the observed spectra with those of
known substances, it was possible to show that a rearrangement of the red pigment does occur during the enzymic oxidation, that synthetic dihydroxyphenylalanine melanin is probably a polymer of indole, 6-quinone, and that the inferred o-quinonoid formulation of the red pigment is correct (Manson, 1948)

Pigment synthesis by streptomycetes has aroused considerable interest for many years. The brown-black pigment has been referred to as 'melanin', and as 'melanin like because of demonstrations of an involvement of tyrosine in its synthesis. In this regard Skinner (1938) observed that pigmentation resulted from the addition of tyrosine to a defined medium upon which he was growing some actinomycetes while Douglas and San Clemente (1956) observed a dark brown pigment in flasks in which mycelial homogenates of *Streptomyces scabies* were mixed with tyrosine, dihydroxyphenylalanine, or both. In attempting to correlate pigment formation and antibiotic synthesis by *S. antibioticus*. Sevcik (1957) manometrically determined the presence of phenol oxidase activity, with tyrosine and several other substrates. While these studies suggest that the pigment was melanin, apparently no attempts have been made to isolate and characterize the pigment.

The actinomycetes also synthesize and excrete dark pigments, melanin or melanoid which were considered to be a useful criterion for taxonomical studies. Melanin compounds were regular, dark brown polymers that were produced by various microorganisms by the fermentative oxidation, and have the radio protective and antioxidant properties that can effectively protect the living organisms from ultraviolet radiation. Melanins were
frequently used in medicine, pharmacology and cosmetic preparations. Biosynthesis of melanin with tyrosinase transform the tyrosine into L-DOPA (3,4-dihydroxy phenyl-L-alanine), which was further converted into dopachrome and autooxidised to indol-5,6-quinone. The later was polymerized spontaneously into DOPA-melanin which gives dark brown pigment until the further examination (Arai and Mikami, 1972; Peczynska-Czoch and Mordarski, 1988).

A blue color pigment was produced by *Streptomyces coelicolor* strain (Sanchez-Marroquin and Zapata, 1953). *Streptomyces hygroscopicus* sub sp. *ossamyceticus* isolated from Thar Desert Soil; Rajasthan during the year 2006 produced a yellow color pigment with antibiotic activity (Selvameenal *et al*., 2009). The Brown-black pigment was produced by *Streptomyces lavendulae*, when tyrosine was added to the defined medium (Mencher and Heim, 1962).