

RAPID DECOMPOSITION OF SOLID WASTE BY ACTINOMYCETE

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Abstract

The taxonomic and ecological positions of antibiotic producing actinomycetes are well recognized for their metabolic flexibility, commonly accompanied by the production of primary and secondary metabolites of economic significance. Various approaches including classical, chemo taxonomical, numerical taxonomic and molecular have been routinely employed for the identification of actinomycetes. The metabolic perspective of actinomycetes not only provides an interesting area for research but also offers the possibility of commercialization of the metabolites generated in the process. Enzymes such as amylase, lipase, and cellulases produced from actinomycetes play an important role in food, fermentation, textile and paper industries. Certain enzymes used as therapeutic agents in human cancer, mostly in acute lymphoblastic leukemia. Actinomycetes are useful in cancer treatment, bioremediation and it produces some valuable antibiotics such as novobiocin, amphotericin, vancomycin, neomycin, gentamycin, chloramphenicol, tetracycline, erythromycin, nystatin, etc. Actinomycetes are also used as plant growth promoting agents (help to produce plant growth hormone Indole-3-acetic acid), biocontrol tools, biopesticide agents, antifungal compounds, and biocorrosion and as a source of agroactive compounds. Therefore, actinomycetes play a significant role in the production of various antimicrobial agents and other industrially important substances such as enzymes. The potential of actinomycetes in the discovery of novel compounds with activity against microorganisms has been realized, and hence opens exciting avenues in the field of biotechnology and biomedical research.

Keywords: Actinomycetes; antibiotic; bioremediation enzymes; metabolic.

INTRODUCTION

During 1914 to 1939, Selman A. Waksman had been consistently systematically screening soil bacteria and fungi to find an antibiotic for tuberculosis. In 1939, he discovers the effect of certain fungi specially actinomycetes on bacterial growth. In 1940, he was able to isolate an effective T.B. antibiotic, actinomycin and for this he got success in 1944, with the discovery of Spectromycin. For all this work in 1952, he got the Noble prize in physiological & medicine.

Actinomycetes are filamentous Gram- positive bacteria, characterized by a complex life cycle belonging to the phylum Actinobacteria, which represents one of the largest taxonomic units among the 18 major lineages currently recognized within the Domain Bacteria (Ventura et al. 2007). *Actinobacteria* are widely distributed in both terrestrial and aquatic ecosystems, mainly in soil, where they play an essential role in recycling refractory biomaterials by decomposing complex mixtures of polymers in dead plants, animals and fungal materials. They are also important in soil biodegradation and humus formation as they recycle the nutrients associated with recalcitrant polymers, such as chitin, keratin, and lignocelluloses, (Goodfellow and Williams 1983, McCarthy and Williams 1992, Stach and Bull 2005) this produces several volatile substances like geosmin responsible of the characteristic "wet earth odor" (Wilkins 1996) and exhibit diverse physiological and metabolic properties, for example the manufacture of extracellular enzymes (McCarthy and Williams 1992, Schrempf 2001).

The bioactive secondary metabolites produced by microorganisms is reported to be around 23,000 of which 10,000 are produced by actinomycetes, thus representing 45% of all bioactive microbial metabolites discovered (Berdy 2005). Among actinomycetes, approximately 7,600 compounds are produced by Streptomyces species (Berdy 2005). Several of these secondary metabolites are potent antibiotics. As a result of which streptomycetes have become the primary antibiotic producing organisms exploited by the pharmaceutical industry (Berdy 2005). Members of this group are producers of clinically useful antitumor drugs such as anthracyclines (aclarubicin, daunomycin and doxorubicin), peptides (bleomycin and actinomycin D), aureolic acids (mithramycin), enedignes (neocarzinostatin), antimetabolites



(pentostatin), carzinophilin, mitomycins, etc (Newman and Cragg 2007; Olano et al., 2009). However, the search for novel drugs is still a priority goal for cancer therapy. The rapid development of resistance to multiple chemotherapeutic drugs and their undesirable side effects has increased demand for novel antitumor drugs that are active against fewer side effects with untreatable tumors, and with the greater therapeutic efficiency (Demain and Sanchez 2009).

Progress has been made recently on drug discovery from actinomycetes by using high-throughput fermentation and screening, combinatorial biosynthesis and mining genomes for cryptic pathways, to generate new secondary metabolites related to existing pharmacophores (Baltz

2008). The isolation of marine actinomycetes has been a great source of new compounds and their isolation all around the world from deepest sediments to the shallow costal sediments from the Mariana Trench, demonstrates that actinomycetes are ever-present in marine sediments, but at lower numbers than in soil (Ghanem et al. 2000, Zheng et al. 2000, Fiedler et al. 2005, Maldonado et al. 2009). Marine microorganisms encompass a complex and diverse assemblage of microscopic life forms, of which it is estimated that only 1% has been cultured or identified (Bernan et al. 2004). In addition, marine actinomycetes have been found in symbiosis with different marine invertebrates, especially sponges (Piel 2004, Kim and Fuerst 2006). Marine actinomycetes have attracted great attention since they have developed unique metabolic and physiological capabilities that not only ensure survival in extreme habitats, but also offer the prospective to produce compounds with antitumor and other interesting pharmacological activities that would not be observed in terrestrial microorganisms (Blunt et al. 2006, Mayer et al. 2007, Williams 2009, Blunt et al. 2009, Fenical et al. 2002), perhaps because of their close relationships with marine eukaryotic organisms including mammals (Baltz 2008, Piel 2004).

However, one of the main problems associated with marine actinomycetes is the difficulty often found in their culture, because of specific necessities like sea salt while in some cases these microorganisms are obligate halophiles (Tsueng et al. 2008). There are a number of reports on techniques and approaches for accessing previously uncultured soil actinomycetes and the biosynthesis gene clusters they harbor (Janssen et al. 2002, donadio et al. 2002). In the case of marine actinomycetes these studies are only beginning, several attempts to optimize their isolation and growth from several sources (Piel 2004, Bull and Stach 2007, Bull et al. 2005) as well as the improvement of the fermentation process for the production of specific compounds (Tsueng et al. 2008, Lam et al. 2007, Selvin et al. 2009) and the development of tools to facilitate the genetic manipulation of the isolated biosynthesis gene clusters (Moore et al. 2005).

Structure of Actinomycetes

The actinomycetes (sing. actinomycete) are a large group of aerobic, high G-C percentage gram-positive bacteria that form branching filaments or hyphae and asexual spores. These bacteria closely resemble fungi in overall morphology. Presumably this resemblance results partly from adaptation to the same habitat. Studies of the fine structure of actinomycetes spores during germination have been confined to the genera *Streptomyces* (Kalakoutswl and Agre 1973). The latter genus forms endospores which behave in a similar way to those of *Bacillus*, a new wall layer being synthesized inside the cortex of the spore and extending to form the germ-tube wall.

In the Streptomyces species studied, the spores had a two-layered wall and the inner one extended to form the germ-tube wall. It is not clear if this layer is newly synthesized during germination or if it is formed by reorganization of wall material existing in the dormant spore. Ultra structural changes during the germination of fungal spores have been studied more extensively. Most fungi fall into one of two groups: (i) those in which the germ- tube wall is formed from a layer which is synthesized *de now* within the existing spore wall; (ii) those in which the germ- tube wall is formed by the extension of a wall layer already present in the dormant spore (Bartnicki-Garcsi 1968). Some conflicting results have been obtained and closely related species have been reported to fall into different groups (Khan 1975). This may be partly due to the use of different fixatives, potassium permanganate giving inferior results to those obtained with osmium tetroxide or aldehydes (Borderd and Trincia 1970). Marked changes in spore wall layers can also be induced by hydration during specimen preparation (Florancee et al. 1972).

When grown on an agar-surface, the actinomycetes branch forming a network of hyphae growing both on the surface and under-surface of the agar. The on-the- surface hyphae are called aerial hyphae and the under-surface hyphae are called substrate hyphae.

Septa normally divide the hyphae into long cells (20 mm and longer) possessing many bacterial chromosomes (nucleoids). These are the aerial hyphae that extend above the substratum and reproduce asexually. Most actinomycetes are non-motile. When motality is present, it is confined to flagellated spores.



Cell Wall Composition

The composition of cell wall in actinomycetes varies greatly among different groups and is of considerable taxonomic significance. Four major cell wall types are distinguished in these filamentous bacteria on the basis of the three features of peptidoglycan composition and structure. These features are (i) diaminopimelic acid isomer on tetrapeptide side chain position 3, (ii) sugar content of peptidoglycan, and (iii) the presence of glycine in interpeptide bridges. As is evident in, characteristic sugar patterns are present only in cell wall types II-IV of those actinomycetes with meso-diaminopimelic acid.

Isolation of actinomycetes

For the isolation of actinomycetes, various methods can be performed on the basis of different sources and media. Samples were collected from different ecological habitats. Further characterization can be performed to study the different strains of actinomycetes.

Identification

Various approaches for the identification of actinomycetes square measure given in brief below:

Molecular Approach

The most influential approaches to taxonomy are through the study of nucleic acids as a result of this square measure either direct cistron merchandise or the genes themselves and comparisons of nucleic acids yield goodly data true connexion.

Molecular science, which has each classification and identification, has its origin within the early supermolecule crossbreeding studies, however has achieved a new standing following the introduction of supermolecule sequencing techniques (O"Donnell et al. 1993). Importance of phyletic studies supported 16S rDNA sequences is increasing within the science of bacterium and actinomycetes (Yokota 1997). Sequences of 16S rDNA have provided actinomycetologists with a phyletic tree that enables the investigation of evolution of actinomycetes and conjointly provides the premise for identification.

Analysis of the 16S rDNA begins by analytic DNA (Hapwood et al. 1985) and amplifying the gene coding for 16S rRNA exploitation the enzyme chain reaction (Siva Kumar 2001. The refined DNA fragments are directly sequenced. The sequencing reactions are performed exploitation DNA sequencer so as to work out the order during which the bases are organized at intervals the length of sample (Xu et al. 1999) and a computer is then used for finding out the sequence for identification exploitationphyletic analysis procedures. Though, analysis of 16S rDNA generally allows us to identify the organism support the genus level only.

Table.1 Different sources and media for isolation of actinomycetes.

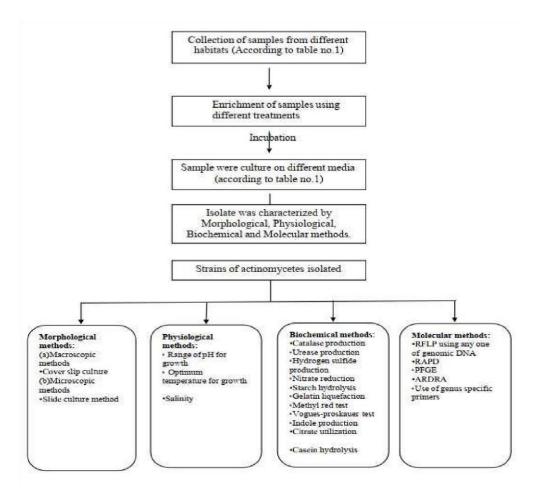
SOURCE	MEDIA	REFERENCES	
FROM SOIL:			
Forest Soil	Starch-casein medium	Kuster & Williams(1964)	
Humus Layer of Forest Soil	(a)Humic acid-vitamin agar	Cho et al, 1994	
	(b)Starch casein nitrate agar(SCS)	Hayakawa et al, 1987a	
	(c)Hair hydrolysate vitamin agar(HHVA)	Hayakawa et al, 1987b	
	(d)Bennet"s agar(BA)	Seong C.N., 1992	
Corn Field, Cow Barn yard, Forest	(a)Arginine-glycerol salt(AGS)medium	Porter et al, 1960	
	(b)Chitin medium	Lingappa & Lockwood, 1961	
	(c)Modified Benedict"s medium	Porter, Wilhelm & Tresner, 1960	
	(d)Soybean meal-glucose medium	Tsao,Leben & Keitt, 1960	
	(e)Gauze"s agar medium	Rehacek, 1959	
	(f)Czapek"s agar medium	Waksman, 1961	
	(g)Egg albumen medium	Waksman, 1961	
	(h)Glucose-asparagine medium	Waksman, 1961	
	(i)Glycerol-asparaginate agar 2	Waksman, 1961	



Lake Soil	Chitin agar	S.C. HSU & J.L. Lockwood, 1975	
Soil	Coal-vitamin agar	Wakisaka et al, 1982	
Antartic Soil	Mineral salt(MS) medium	Kosmachev (1954)	
Mitidja plain (Algeria)	Yeast extract-malt extract agar	Shirling & Gottlieb, 1966	
Marine Soil	Starch casein nitrate(SCN) agar medium	Ravel J, Amorso (1998)	
FROM WATER:			
Stream Sediments & Lake muds	(a)Chitin agar media	Lingappa & Lockwood (1961,1962)	
	(b)M3 agar medium	Jones, 1949	
	(c)Benett"s medium	Jones, 1949	
Marine Sediments	(a)Starch-casein agar	A.Grein & S.P. Meyers, 1958	
	(b)Asparagine agar	A.Grein & S.P. Meyers, 1958	
	(c)Glycerol-glycine agar	Lindenbein, 1952	
Marine Sediments(South China)	(a)AIM medium	J.L. You et al	
FROM OTHER SOURCES:			
FROM ROOT & STEM SAMPLES OF FOUR PLANTS:			
Cinnamomum zeylanicum,Zingiber spectabile,Elettariopsis curtisii, Labisia pumila	Starch yeast casein agar(SYCA),Actinomycetes Isolation agar (AIA), Humic Acid vitamin gellan gum (HVG),Tap water yeast extract agar (TWYE), Coal –vitamin agar (CVA)	Zin et al, 2007	
Mangroove Sediments	Asparagine-glucose agar medium	Shirling & Gottlieb, 1966	

Procedure: Various steps for the Isolation and characterization of actinomycetes were performed which are mentioned below:





Chemotaxonomical Approach

Chemotaxonomy is that the study of chemical variation in organisms and also the use of chemical characters within the classification and identification. It so one in all the precious strategies to spot the genera of actinomycetes. Studies of Cummins and Harris (Cummins and Harris 1956) established that actinomycetes have a cytomembrane composition comparable to that of gram-positive bacterium, and conjointly indicated that the chemical composition of the cytomembrane may furnish sensible strategies of differentiating numerous varieties of actinomycetes. This can be due to the actual fact that chemical components of the organisms that satisfy the subsequent conditions have important which means in science.

They must be distributed universally among the microorganisms studied; and, The parts ought to be homologous among the strains at intervals a taxonomic group, whereas important variations exist between the taxa to be differentiated. Presence of Diaminopimelic Acid (DAP) isomers is one in all the foremost necessary cell-wall properties of gram-positive bacterium and actinomycetes. Most bacterium has a characteristic wall envelope, composed of peptidoglycan. The 2, 6-Diaminopimelic Acid (DAP) is cosmopolitan as a key amino acid and its optical isomers. The systematic significance lies largely within the key amino acid with two amino bases, and determination of the key amino acid is typically adequate for characterization. If DAP is present, bacterium typically contain one of the isomers, the LL-form or the *meso*-form, largely settled within the peptidoglycan.

Classical Approach

Classical approaches for classification build use of physiological, morphological, and biochemical characters. The classical methodology delineated within the identification key by Nonomura

(Nonomura 1974) and Bergey"s Manual of Determinative Bacteriology (Buchanan and Gibbons 1974) is very much useful in the identification of streptomycetes. These characteristics are normally utilized in employed in taxonomy of streptomycetes for several years. They are quite helpful in routine identification. These are discussed below:

1. Aerial Mass Color

The colour of the mature sporulating aerial mycelium is recorded in an exceedingly straightforward method (White, grey, red, green, blue and violet). Once the aerial mass color falls between two colors series, both the colors are recorded. If the aerial mass color of a strain to be studied shows intermediate tints, then also, both the colors series are noted.



2. Melanoid Pigments

The grouping is formed on the assembly of melanoid pigments (*i.e.* light-green brown, brown black or distinct brown, pigment changed by alternative colours) on the medium. The strains are grouped as melanoid pigment created (+) and not created (-).

3. Reverse Side Pigments

The strains were divided into two groups, consistent with their ability to provide characteristic pigments on the reverse aspect of the colony, namely, distinctive (+) and not distinctive or none (-). In case, a color with low saturation like yellowness, olive or yellowish brown occurs, it is included in the latter group (-).

4. Soluble Pigments

The strains are divided into two groups by their ability to provide soluble pigments apart from melanin: particularly, produced (+) and not produced (-). The color is recorded (orange, red, green, violet, blue and yellow).

5. Spore Chain Morphology

With relevancy to spore chains, the strains are sorted into "sections". The species belonging to the genus Streptomyces are divided into three sections (Shirling and Gottlieb 1966), particularly rectiflexibiles

(RF), retinaculiaperti (RA) and Spirales (S). Once a strain forms two types of spore chains, both are noted (e.g. SRA).

6. Reproductive Structure Surface

Spore morphology and its surface options ought to be determined under the scanning electron microscope. The cross hatched cultures arranged for observation under the light microscope can be used for this purpose.

The electron grid ought to be cleaned and adhesive tape should be placed on the surface of the grid. The mature spores of the strain ought to be rigorously placed on the surface of the adhesive tape and gold coating should be applied for half an hour and also the specimen is examined under the electron microscope at completely different magnifications. The reproductive structure silhouettes are characterized as spiny, smooth, warty and hairy.

Numerical Taxonomic Approach

Numerical taxonomy involves examining several strains for a large number of characters prior to assigning the test organism to a cluster based on shared options. The numerically defined taxa are polythetic; therefore, no single property is either indispensable or adequate to entitle an organism for membership of a group. Once classification has been achieved, cluster-specific or predictive characters is chosen for identification (Williams et al. 1983). Numerical taxonomy was initially applied to *Streptomyces* (Silvestri et al. 1962). The numerical taxonomic study of the genus *Streptomyces* by Williams et al. (1983) involves determination of 139 unit characters for 394 type cultures of *Streptomyces*; clusters were outlined at 77.5% or 81% Ssm and 63% Sj similarity levels, and also the former co-efficient is being employed to outline the clusters. His study includes 23 major, 20 minor and 25 single member clusters.

The numerical classification of the genus *Streptomyces* by Kampfer *et al.* (Kampfer et al. 1991) involves determination of 329 physiological tests. His study includes 15 major clusters, 34 minor clusters and 40 single member clusters which are defined at 81.5% similarity level Ssm using the simple matching coefficient (Sokal and Michener 1958) and 59.6 to 64.6% similarity level Sj using Jaccard coefficient (Sneath 1957). Thus, numerical taxonomy provides us with a useful framework for *Streptomyces* taxonomy, as well as identification of species. **Role of Streptomycetes**

Streptomyces is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae (Kampfer et al. 1991). Over 500 species of Streptomyces bacteria have been described by Euzeby (Euzeby 2008). Streptomycetes have genomes with high GC-content and these are gram-positive (Madigan and Martinko 2005). Found predominantly in soil and decaying vegetation, mainly streptomycetes produce spores and are noted for their distinct "earthy" odor which results from production of a volatile metabolite, geosmin. Streptomycetes are characterized by a complex secondary metabolism. They make over two-thirds of the clinically useful antibiotics of natural origin (e.g. neomycin, chloramphenicol) (Kieser et al. 2000).

Streptomyces-derived antifungals tend to be macrolide polyenes (large ring structure with lots of conjugated carboncarbon double bonds) and include such illustrious members as: nystatin (the first

Actinobacteria-sourced human antifungal, made by S. noursei), amphotericin B (made by S. nodosus, originally isolated from a sample of Venezuelan soil) and natamycin (made by S. natalensis). There are a friggin" tonne of Steptomyces-derived antibiotics used specifically as antibacterial agents. These include Streptomycin by S. griseus, neomycin and kanamycin, respectively produced by S. fradiae and S. kanamyceticus. Other antibacterial antibiotics of note include: erythromycin (a macrolide that often subs for penicillin when people be allergic to it, made by S. erythraea), tetracycline (a longstanding acne drug that makes you light-sensitive, made by S. rimosus), chloramphenicol (cheap, effective, but can cause aplastic anemia, made by S. venezuelae), vancomycin (a relatively ginormous glycopeptide that can turn people red,



made by S. orientalis) and thienamycin (made by S. cattleya, modified by us to make imipenem, the first carbapenem beta-actam antibiotic). A number of the antibiotics produced by Streptomyces have proven to be too toxic for use as antibiotics in humans, other than because of their toxicity towards cells (specifically dividing cells) they have been reinvented as chemotherapy drugs. We are talking drugs like: actinomycin-D (the original), bleomycin (glycopeptide made by S. verticullus), mitomycin (aziridine made by S. lavendulae) and plicamycin (made by S.plicatus) (Birnbaum et al. 1985).

The Research of microbial metabolites

A variety of actinomycetales, first of all the filamentous fungi and Streptomyces species, and to a lesser extent several bacterial species are the most important producers both in respect of versatility, diversity, and numbers of structures of the produced metabolites. The frequency and significance of these major types of microbes as producers of bioactive metabolites had varied significantly during the last decades. At the start of the antibiotic era the fungal (penicillin, Griseofulvin) and bacterial (Gramicidin) species were in the forefront of the interest, but after the detection of streptomycin and afterward cholramphenicol, tetracyclines and macrolides the attention turned to the species of Streptomyces.

In the fifties and sixties the majority (70%) of antibiotics was discovered from these species. The most characteristic and a little bit surprising feature of the recent years just is this declining representation of the formerly exhaustively investigated Actinomycetes. They contribute to among all microbial products present in only 30-35%, in contrast to the 75-80% share from the sixties to the eighties. The most frequent producers, the Streptomyces

Table.2 In Table the numbers of actinomycetales species, including the all rare actinos, known to produce bioactive metabolites, are summarized.

11	netabolites, are su	IIIIIaiizeu.	
Actinomycetales species	No.	Actinomycetales species	No.
Streptomycetaceae:		Thermomonosporaceae:	
Streptomyces	8000	Actinomadura	345
Streptoverlicillium	258	Saccharothrix	68
Kitasatosporia	37	Microbispora	54
Chainia	30	Actinosynnema	51
Microellobosporia	11	Nocardiopsis	41
Nocardioides	9	Microtetraspora/Nonomuria	26/21
Micromonosporaceae: (Actinoplanetes))	Thermomonospora	19
Micromonospora	740	Micropolyspora/Faenia	13/3
Actinoplanes	248	Thermoactinomyces	14
Dactylosporangium	58	Thermopolyspora	1
Ampullariella	9	Thermoactinopolyspora	1
Glycomyces	2	Mycobacteriaceae: (Actinobacteria)	
Catenuloplanes	3	Nocardia	357
Catellatospora	1	Mycobacterium	57
Pseudonocardiaceae:		Arthrobacter	25
Saccharopolyspora	131	Brevibacterium	17
Amycalotopsis/Nocardia	120/357	Proactinomyces	14
Kibdellosporangium	34	Rhodococcus	13
Pseudonocardia	27	Other (unclassified) species:	
Amycolata	12	Actinosporangium	30
Saccharomonospora	2	Microellobosporia	11
Actinopolyspora	1	Frankia	7
Streptosporangiaceae: (Maduromycete	es)	Westerdykella	6
Streptosporangium	79	Kitasatoa	5
Streptoalloteichus	48	Synnenomyces	4



Spirillospora	11	Sebekia	3
Planobispora	10	Elaktomyces	3
Kutzneria	4	Excelsospora	3
Planomonospora	2	Waksmania	3
		Alkalomyces	1
		Catellatospora	1
		Erythrosporangium	1
		Streptoplanospora	1
		Microechinospora	1
		Salinospora	1

species produce 7600 compounds (74% of all actinomycetales), although the atypical Actinomycetes represent 26%, altogether 2500 compounds. The representation of rare action products in 1970 was only 5%. In the group Nocardia, Streptoverticillium, Micromonospora, Streptosporangium, Actinoplanes, Saccharopolyspora and Actinomadura, species are the most frequent producers; each produces several hundreds of antibiotics.

Enzyme production from Actinomycetes

Marine actinomycetes physiological, biochemical and molecular characteristics such as 16SrRNA and terrestrial actinomycetes a great difference, followed by metabolic pathway is also different from terrestrial actinomycetes, which produced a variety of biologically active enzymes.

Actinomycetes secrete amylases to the outside of the cells to carry out extracellular digestion. α amylase starch degrading amylolytic enzymes is of great significance—in biotechnological applications such as food industry, fermentation and textile to paper industries (Pandey et al. 2000). Actinomycetes are one of the known cellulose producers (Jang and Chenks 2003, Arunachalam et al. 2010). Cellulases are a collection of hydrolytic enzymes which hydrolyze the glucosidic bonds of cellulose and related cello-digosaccharide derivatives (Ito 1997). Lipase is produced from a variety of actinomycetes, bacteria, and fungi (Kulkarni and Gadre 2002). Lipases have broad applications in the detergent industries, foodstuff, oleochemical, diagnostic settings and also in industries of pharmaceutical fields (Schmid et al. 1998).

Actinomycetes are of enormous importance since they possess a capacity to produce and secrete a variety of extracellular hydrolytic enzymes (Saadoun et al. 2007, Tan et al. 2009). Many actinomycetes have been isolated from various natural sources, as well as in plant tissues and rhizospheric soil. Biological functions of actinomycetes mainly depend on sources from which the bacteria are isolated. Microbial alkaline proteases for manufacturing uses are produced mostly from Streptomyces and Bacillus. Actinomycetes, particularly

Streptomycetes are known to secrete multiple proteases in culture medium (Sharmin 2005).

Actinomycetes have been revealed to be an excellent resource for L-asparaginase. A range of actinomycetes, mainly those isolated from soils such as *Streptomyces griseus*, *S. karnatakensis*, *S. albidoflavus* and *Nocardia* sp. have abilities to produce L-asparaginase enzyme (DeJong 1972, Narayana et al. 2007, Mostafa and Salama 1979). The production of Lasparaginase has been studied in *Serratia marcescens* (Khan et al. 1970), *Erwinia carotovora* (Maladkar et al. 2000), *Enterobacter aerogenes* (Mukherjee et al. 2000), *Pseudomonas aeruginosa* (Abdel-Fattah and Olama 2002), *Bacillus subtilis* (Fisher and Wray 2002) and *Saccharomyces cerevisiae* (Ferrara et al. 2006). Microbial L-asparaginase has been generally used as a therapeutic agent in the cure of certain human cancers, mostly in acute lymphoblastic leukemia (Gallagher et al. 1989, Verma et al. 2007). The roots and rhizomes of several Thai medicinal plants such as lemon grass (*Cymbopogon citratus*), ginger (*Zingiber officinale*) have long been used in Thai traditional medicine for stomach ache and asthma treatment (Wutthithamavet 1997).

Rhizosphere soil of these plants may be an



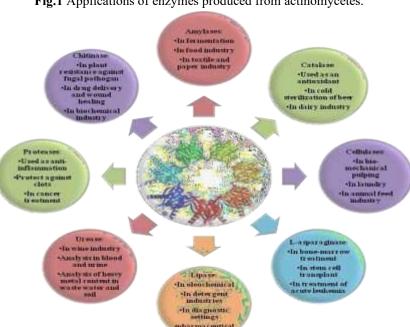


Fig.1 Applications of enzymes produced from actinomycetes.

attractive actinomycetes source, able of producing novel secondary metabolites. Catalase, Chitinase and Urease enzymes also produce from actinomycetes.

Applications of Actinomycetes Ecological Importance

Actinomycetes are abundant in soil, and are responsible for much of the digestion of resistant carbohydrates such as chitin and cellulose. They are liable for the pleasant odor of freshly turned soil. Several actinomycetes and other actinobacteria are renowned as degraders of toxic materials and are used in bioremediation. They are significantly well adapted to survival in harsh environments. Some are able to grow at elevated temperatures (>50°C) and are essential to the composting method.

Human Health Importance

Actinomycetes are produced many antibiotics, that are best recognized and most valuable. These antibiotics include amphotericin, nystatin, chloramphenicol, gentamycin, erythromycin, vancomycin, tetracycline, novobiocin, neomycin, etc.

Fig no.2 Applications of Actinomycetes





In these antibiotics some are targeted bacterial ribosome"s and are used in treating respiratory infections, for example in treating the Legionnaires" disease used tetracycline and erythromycin. Vancomycin antibiotic are attacks on deadly organisms such as methicillin- resistant *staphylococcus aureus* (MRSA) (multiply drug resistant) and bacterial cell walls. Rifamycins are useful for treating leprosy and tuberculosis, these targets bacterial RNA polymerase. Amphotericin is one of the minority antibiotics that attack fugal membranes. These antibiotics usually do not influence human cells and for that reason have fewer side effects. On the other hand actinomycetes metabolites for example adriamycin, prevent DNA replication, because of this it is used in treating the cancer, although rapamymycin is used to repress the immune system to facilitate organ transplants.

Infections

Members of the actinomycetes genus are usual commensal members of oral cavities in human. They can be able to cause serious infections when they attack on tissue through breaks in the oral mucosa. The disease is becoming less universal, but in the USA it is still present, mainly in inner immunity. Additionally Nocardia species may also be involved.

Hypersensitivity pneumonitis (HP)

Thermophilic actinomycetes are the most common cause of HP. Farmer's lung disease is HP resulting from exposure to hay that has become colonized with thermophilic actinomycetes, which produce an abundance of airborne spores. Clouds of these spores are released when farmers (especially dairy farmers) handle stored hay in winter and early spring. The same fungi that cause molding of hay are common inhabitants of soil, and have additionally been documented to colonize ventilation systems, garments dryers, refrigerator drip pans, and any other site that combines heat, cellulosic or other carbohydrate material, and water.

Common species include *Thermoactinomyces vulgaris Saccharopolyspora rectivirgula Thermoactinomyces viridis* and others. *Volatile Organic Compounds* (VOCs)

The odor of freshly turned soil is that the results of geosmin, a volatile organic compound made by actinomycetes. Geosmin is additionally made by some cyanobacteria and produces an earthy taste in drinking water. Some fungi also produce geosmin, which might impart an equivalent earthy taste to wine made of moldy grapes. In general, folks realize the geosmin odor pleasant in soil. However, one indoor air research group is investigating the possibility that exposure to geosmin is related to building-related symptoms. The data at present is too restricted for conclusions. However, in the future, assortment of samples which will reveal these organisms could be suggested. *Actinomycetes as Antifungals* Urauchimycins be a Member of antimycin class, a set of well-identified antifungals. Antimycins act by inhibiting the electron flow in the mitochondrial respiratory chain (Barrow et al. 1997). Antimycins have been identified in *Streptomyces* isolated from the integument of attine ants

(Schoenian et al. 2011, Seipke et al. 2011, Seipke et al. 2012). Schoenian and colleagues (Schoenian et al. 2011) Identify the well-know antimycins A1–A4 in 50% of the actinobacteria identified as *Streptomyces* isolated from workers of several *Acromyrmex* species. Compounds of this class may have an effective role in the attine ant-microbe association. Another antifungal compound broadly Disperse in *Streptomyces* related to attine ants is candicidin (Haeder et al. 2009, Schoenian



et al. 2011, Seipke et al. 2011, Seipke et al. 2012). Urauchimycins A and B were isolated from *Streptomyces* sp. From a marine sponge Ni-80 was isolated.

In 2006, two new urauchimycins were represented: urauchimycin C, isolated from Streptomyces sp. B1751 from marine sediment, and urauchimycin D, isolated from Streptomyces sp. AdM21 from soil (Yao et al. 2006). In the study by Imamura and coworkers (Imamura et al. 1993), the urauchimycins A and B repressed the morphological differentiation of C. albicans equal to a concentration of 10 µg mL-1. Urauchimycins C and D showed no inhibitory action against C. albicans, Mucor miehei, and bacteria (Yao et al. 2006).

Urauchimycin B showed inhibitory activity against all *Candida* strains evaluated, showing MIC like to those provided by nystatin. Urauchimycin B showed a wide spectrum of activity against *Candida* spp. with MIC values equal to the nystatin antifungal, which indicates the potential for medical use. Antimycins were used from many years for the cure of human infections, but due to its mechanism of action and allied side effects, its use in the treatment of human disease was discontinued (Barrow et al. 1997). However, with the urgent need for new antifungal agents that complement or alternate for the insufficient products obtainable in the marketplace, it is appealing and essential to establish the toxicity obtainable by urauchimycin B, to evaluate whether it can be used as an antifungal agent for humans and animals. In addition, assessment of the isolated compound against *Candida* species as opposed to commercially offer antifungal agents should be performed to prove the potential of this relatively unexplored antifungal. Actinobacteria of attine ants are capable to create antifungal compounds active against other fungal species and not only against the specific

fungal parasite *Escovopsis*. The few current studies that focused on the chemical characterization of bioactive compounds formed by Actinobacteria associated with attine ants support the potential isolation of novel molecules with biological activity (Oh et al. 2009, Barke et al. 2010, Carr et al. 2012, Haeder et al. 2009, Schoenian et al. 2011). Thus, an exploration program of isolation of bioactive molecules from actinobacteria from attine ants definitely will result in the discovery of novel compounds with activity against microorganisms that are potentially pathogenic to humans.

Actinomycetes for Extracellular Peroxidase Activity

Peroxidases catalyze the peroxide- dependent oxidation of a range of inorganic and organic compounds and are widely distributed throughout plants, animals, and microorganisms. They are primarily intracellular enzymes with vital roles in cellular processes (Everse et al. 1990), but extracellular peroxidases concerned within the degradation of complex organic compounds have conjointly been described. The white-rot basidiomycete *Phanerochaete chrysosporium* secretes a complex array of peroxidases throughout secondary metabolism, and their characterization and role in lignocellulose degradation are well documented (Gold and Alic 1993). The production of extracellular peroxidases by actinomycete bacteria has been described (Godden et al. 1992, Ramachandra et al. 1988, Winter et al. 1991), but evidence for the involvement of this peroxidase activity within the degradation of lignin is ill defined (Godden et al 1992, Spiker et al. 1992). The intracellular peroxidases of streptomycetes have conjointly been specifically studied in relevance their role within the biosynthesis of halogenated antibiotics (Van Pee et al. 1987).

Extracellular peroxidases would be expected to possess improved stability over their intracellular counterparts, significantly those from thermophiles (Iqbal et al. 1994), and so have potential for applications in, as an example, diagnostic kits. Actinomycetes are a potentially rich source of peroxidases for introduction into a market that substantial and almost totally dominated by horseradish peroxidase (HRP), which is both well characterized and extremely active. Peroxide-dependent oxidation of luminal by HRP is that the basis of variety of diagnostic immunoassays (Thorpe et al. 1985) and therefore the enhanced chemiluminescence system for nonradioactive detection of nucleic acid hybridization (Stone and Durrant 1991). Screening actinomycetes for extracellular peroxidases is hampered by the necessity to preconcentrate culture supernatants, but in this paper researchers describe a unique use of the chemiluminescent assay with the Amerlite analyzer (Amersham PLC, Bucks, and United Kingdom) to examine a taxonomic range of actinomycetes for extracellular peroxidase activity. This enabled the identification of a group of high-level extracellular peroxidase producers, and in these strains peroxidase activity was conjointly determined with alternative assay systems. The objective was to evaluate the reliability and applicability of a range of available assays for the determination of actinomycete peroxidase activity.

Actinomycetes as source of Agroactive compounds

Actinomycetes have the most fruitful source of microorganisms for all types of bioactive metabolites, including agroactive type. Over one thousand secondary metabolites from actinomycetes were discovered during 1988-1992. Most of these compounds are produced by various species of the genus Streptomyces. In fact, about 60% of the new insecticides and



herbicides reported in the past 5 yr originate from Streptomyces (Tanaka and Omura 1993). It is also estimated that as many as three-quarters of ail Streptomycetes species are capable of antibiotic production (Alexander 1977). Actinomycetes produce a variety of antibiotics with diverse chemical structures such as polyketides, b-lactams and peptides in addition to a variety of other secondary metabolites that have antifungal, anti-tumor and immunosuppressive activities (Behal 2000). Kasugamycin is a bactericidal and fungicidal metabolite discovered in *Streptomyces kasugaensis* (Umezawa et al. 1965). This antibiotic acts as an inhibitor of protein biosynthesis in microorganisms but not in mammals, and its toxicological properties are excellent. Hokko Chemical Industries developed a production process to market the systemically active kasugamycin for control of rice blast *Pyricularia oryzae* Cavara and bacterial *Pseudomonas* diseases in several crops.

Polyoxin B and D were isolated as metabolites of *Streptomyces cacaoi*var. *Asoensis* in 1965 by (Isono et al. 1965) as a new class of natural fungicides. The mode of action of the polyoxins makes them very acceptable with regard to environmental considerations. They interfere with the fungal cell wall synthesis by specifically inhibiting chitin syntheses (Endo and Misato 1969). Polyoxin B found application against a number of fungal pathogens in fruits, vegetables and ornamentals. Polyoxin D is marketed by several companies to control rice sheath blight caused by *Rhizoctonia solani* Kùhn.

The validamycin family was detected by researchers in 1968 in a greenhouse assay when screening Streptomycetes extracts for activity against rice sheath blight. Validamycin A was found to be a prodrug which is converted within the fungal cell to validoxylamine A, an extremely strong inhibitor of trehalase (Kameda et al. 1987). This mode of action gives validamycin a favorable biological selectivity because vertebrates do not depend on the hydrolysis of the disaccharide trehalase for their metabolism.

The isolation of the antifungal metabolite mildiomycin from a culture of *Streptoverticillium rimofaciens* Niida was reported in 1978, also by Takeda scientists (Iwasa et al. 1978). Mildiomycin is strongly active against several powdery mildews on various crops (Harada and Kishi 1978), acting as an inhibitor of the fungal protein biosynthesis (Feduchi et al. 1985). Its low toxicity in vertebrates would make it an environmentally sound crop protection agent (Harada and Kishi 1978), but the fact that this product never appeared in recent publications would indicate, however, that Takeda's efforts to develop mildiomycin might not be successful yet.

The compounds mentioned above are a few examples of agroactive compounds isolated from actinomycetes. Microbial screening and chemistry procedures have been until recently the main tools to discover new agroactive compounds. However, genomic technologies that allow the rapid characterization of microbial genomes will certainly become the method of choice for the discovery of new bioactive molecules in the coming years. Furthermore, molecular techniques such as combinatorial biosynthesis (Hutchinson 1999) may lead to the discovery of drugs that cannot be found in nature. Indeed, genetic domains, modules and clusters involved in the microbial biosynthesis of known secondary metabolites can be interchanged and modified to produce bioactive products with unique properties.

Actinomyccetes as plant growth promoting Agents

In attempts to develop commercial biocontrol and plant growth promoting products using rhizobacteria, it is important to recognize the specific challenges they present. To begin with, the interaction between PGPR species and their plant symbionts appears to be specific, even within a crop or cultivar (Chanway et al. 1988, Glick 1995, Kloepper 1996, Lazarovits and Nowak 1997). While a rhizobacterium screened for growth promotion may reveal the positive effects on one crop, it may have no effect, or even retard growth of another crop (Gardner et al. 1984, O'Neill et al. 1992). Although rhizobacteria may present unique challenges to our attempts to harness their beneficial attributes, the prospects for improved agriculture by the use of biocontrol-PGPR seem excellent. Advances in our understanding of the PGPR Systems responsible for plant growth improvement is a first logical step in opening the way to improving these bacterial strains through genetic engineering, and creating more interest in their progress for widespread commercial use for both biocontrol and plant growth promotion.

Despite the well-documented history of *Streptomyces* in biocontrol and preliminary evidence of their capacity to enhance plant growth (Aldesuquy et al. 1998), *Streptomyces* species have been poorly investigated specifically for their potential as PGPR. These are amazing as streptomycetes, usually accounting for an abundant proportion of the soil microflora, are particularly effective colonizers of plant root Systems and are able to endure unfavorable growth conditions by forming spores (Alexander 1977). While the beneficial effect of some strains of PGPR on particular crops is certain, the mechanisms employed by PGPR are unclear (Glick 1995). PGPR can affect plant growth in two general ways, either directly or indirectly. Indirect promotion occurs when PGPR lessen or prevent the harmful effects of one or more deleterious



microorganisms. This is mainly attained through biocontrol, or the antagonism of pathogens of soil plant. Specifically, colonization or the biosynthesis of antibiotics (Fenton et al. 1992) and other secondary metabolites can prevent pathogen establishment and invasion. Direct promotion of plant growth by PGPR occurs when the plant is supplied with a compound that is synthesized by the bacteria, or when PGPR or else facilitates plant uptake of soil nutrients. Possibilities include siderophore synthesis, nitrogen fixation, solubilization and phytohormone synthesis, of minerals to make them available for plant uptake and use (Glick 1995).

Some researchers isolate as a seed treatment of oat, barley, carrot and wheat, in order to increase their growth. The isolate was originally selected for the biological control of *Rhizoctonia solani*. Although the S. *griseus* isolate did enhance the average dry foliage weight, tiller number, grain yield, and advanced head emergence for wheat and oat over the controls, the differences were not statistically significant. As a seed treatment for carrot, the isolate was more successful. Marketable yields were increased over controls by 17% and 15% in two separate field trials. Specifically, both trials also indicated an increased yield of large and very large grade carrots over the controls (Merriman et al. 1974). Nearly 20 yr later, El-Abyad et al. (El- Abyad et al. 1993) described the use of three *Streptomyces* spp. in the control of bacterial, *Fusa rium* and *Verticillium* wilts, early blight, and bacterial canker of tomato. The isolâtes used were S. *pulcher, S. canescens*, and S. *citreofluorescens*. As a seed-coating, all three of the strains were effective at variable levels in controlling the test pathogens. In addition, tomato growth was observed to be significantly improved with the antagonistic *Streptomyces* spp. as a seed-coating.

The culture filtrates alone of two different *Streptomyces* spp. (S. olivaceoviridis (Preobrazhenskaya and Ryabova) Pridham et al. and S. rochei Berger et al.) was found to significantly increase the shoot length and shoot fresh mass, respectively, of wheat plants. Hormone extraction, purification, and bioassay showed that both species produced substantial amounts of growth-regulating substances, including auxins, gibberellins, and cytokinins (Aldesuquy et al. 1998). This demonstrated that selected *Streptomyces* spp. produces at least one class of compounds that directly influence plant growth.

Direct and indirect interactions between actinomycetes and other nonpathogenic soil microorganisms also influence plant growth. For example, some researchers (Mohammadi and Lahdenpera 1992), reported that actinomycetes stimulated the intensity of mycorrhizal formation and that resulted in improved plant growth.

Actinomycetes as Biocontrol tools

A prime example of *Streptomyces* biocontrol agent is *Streptomyces griseoviridis* Anderson *et al.* strain K61. This strain, originally isolated from light coloured *Sphagnum* peat (Tahvonen 1982a, Tahvonen 1982b), has been reported to be antagonistic to a variety of plant pathogens together with *Alternaria brassicola* (Schw.) Wiltsh., *Botrytis cinerea* Pers.:Fr., *Fusarium avenaceum* (Fr.:Fr.) Sacc, *F. culmorum* (Wm. G. Smith) (Tahvonen 1982a, Tahvonen 1982b ,Tahvonen and Avikainen 1987).

Streptomyces griseoviridis strain K61 is used in root dipping or growth nutrient treatment of eut flowers, potted plants, greenhouse cucumbers, and varied alternative vegetables (Mohammadi and

Lahdenpera 1992). MycostopTM (developed by Kemira Oy) is a biofungicide that contains *S. griseoviridis* as the active ingrédient. This product is offered in United States (Cross and Polonenko 1996) and Europe (Tahvonen 1982a). Many properties related to actinomycetes may justify the ability of sereval of them to act as biocontrol tools. Those properties are the ability to colonize plant surface, the antibiosis against plant pathogens, the synthesis of particular extracellular proteins, and also the degradation of phytotoxins. **Plant colonization and biocontrol**

Evidence indicates that actinomycetes are quantitatively and qualitatively vital within the rhizosphere (Barakate et al. 2002, Crawford et al. 1993, Doumbou et al. 2001, Miller et al. 1990), where they may influence plant growth and defend plant roots against invasion by root pathogenic fungi (Lechevalier 1988). However root microorganism interactions have been extensively studied just for the nitrogenfixing *Frankia* species (Sardi et al. 1992) and a small number of species of the genus *Streptomyces* that are phytopathogens (Loria et al. 1997).

It is usually assumed that root colonization by introduced bacteria is important for the biocontrol of root pathogens which increasing the population of such an introduced bacteria on roots should enhance disease control (Suslow and Schroth 1982]. The key to the idea of root colonization is that rootcolonizing bacteria grow on roots in the présence of the indigenous microflora (Schroth and Hancock 1982) and therefore root colonists are compétitive with soil bacteria and fungi. Whereas the tendency to use the term root colonization, alternative terms have been proposed. Rhizosphere compétence was used (Schmidt 1979) in relation to rhizobia, to explain soil microorganisms that show better growth in response to developing plant roots. In this context, rhizosphere compétent microorganisms are those that show the classical rhizosphere impact. The term rhizosphere compétence has been employed in relation to biological control agents, and Baker (Baker 1991) redefined it because the ability of a microorganism, applied by seed treatment, to colonize the



rhizosphere of developing roots, a définition that does not differ considerably from that proposed by Schmidt (Schmidt 1979). Many reports hâve used rhizosphere ability and root colonization interchangeably as synonyms (Hozore and Alexander 1991, Suslow and Schroth 1982). Whereas every définition differs from the others, there is gênerai agreement that root colonization is a vigorous method involving growth of the introduced bacteria on or around roots and isn't merely a passive chance encounter between a soil bacterium and a root. Researchers consider true colonists to be those bacteria that colonize plant surfaces in compétitive conditions, in natural field soils.

A microorganism that colonizes roots is idéal to be used as a biocontrol agent against soil-borne diseases (Weller 1988). Streptomyces griseoviridis may be a example for colonization of plant rhizosphere by actinomycetes. S. griseoviridis is an antagonistic microorganism effective in biocontrol of plant diseases like the the Fusarium wilt of carnation, the dampingoff of Brassica and also the root rot disease of cucumber (Tahvonen and Lahdenpera. 1988). The active rootcolonization ability of the biocontrol agent S. griseoviridis was tested on turnip râpe and carrot in nonsterile soil and by plate test (Kortemaa et al. 1994). Plate test and successful root-colonization, root-colonization frequencies and population densities for sand-tube technique ail indicate that S. griseoviridis colonizes, at least at the seedling stage, turnip râpe better than carrot root. Since the responses of S. griseoviridis to root colonization of two plant species in standard conditions were clearly différent, the mechanism of root colonization should be affected by some property that varies between different plant species. Plant species are known to produce various types and quantities of root exudates (Curl and Truelove 1986), which influence root colonization (Weller 1988). It's potential that the root exudates of carrot lack some characteristics necessary for the prolifération of S. griseoviridis. The value of S. griseoviridis seed dressing on barley and spring wheat against foot rot disease was investigated by (Tahvonen et al. 1994) health organization verified that wheat yields is exaggerated by seed dressirigs more efficiently than those of barley.

Proteins involved in biocontrol

Actinomycetes have the capability to produce a wide variety of extracellular enzymes that permit them to degrade varied biopolymers in soil. The capability of actinomycetes to produce extracellular enzymes gained revived attention because of their vital role in biocontrol. Especially, various correlations between fungal antagonism and bacterial production of chitinases or glucanases have been noted (Fayad et al. 2001, Lim et al. 1991, Valois et al. 1996). Chitin and b-1,3-glucans are major constituents of many fungal cell walls (Sietsma and Wessels 1979), and various workers have confirmed *in vitro* lysis of fungal cell walls either by bacterial chitinases or glucanases alone or by a mixture of each enzymes (Fiske et al. 1990, Ordentlich et al. 1988). These studies have lent support to the hypothesis that these hydrolytic enzymes might contribute to biocontrol efficacy. Several attempts to test this hypothesis by means of both genetic and molecular approaches hâve been undertaken recently.

Many *Streptomyces* species are lignocellulose decomposers (Chamberlain and Crawford 2000) and are sources of antibiotics (Tanaka and Omura 1993). Strains with both the abilities to degrade lignocellulose and antagonize fungal root pathogens should have sensible potential for development into a biocontrol product, which could be useful to turf grass growers or managers, to regulate each thatch accumulation and fungal diseases of turf (Chamberlain and Crawford 2000). The ability to degrade complex substrates could also be an asset in biocontrol. Doumbou *et al.* (Doumbou *et al.* 1998) showed that actinomycetes degrading thaxtomin A, a phytotoxin created by the plant pathogenic *S. scabies*, protected growing potato plants against common scab.

Actinomycetal proteins apart from hydrolases may additionally be concerned in biocontrol. For example, Vernekar *et al.* (Vernekar et al. 1999) discovered an alkaline protease inhibitor (API) as a unique category of antifungal proteins against phytopathogenic fungi like *Altemaria, Fusarium,* and *Rhizoctonia*. The activity of API seems to be related to its ability to inhibit the fungal serine alkaline protease, which is indispensable for their growth.

Antibiosis and biocontrol

Over one thousand secondary metabolites from actinomycetes were discovered throughout the years 1988-1992. Actinomycetes produce a variety of antibiotics with various chemical structures like polyketides, p-lactams and peptides in addition to a variety of alternative secondary metabolites that have antifungal, anti-tumor and immunosuppressive activities (Behal 2000). Antibiotics are usually considered to be organic compounds of low molecular weight produced by microbes. It is proposed that the production of antibiotics increases an organism aptitude for survival in the former case by acting as an alternative (chemical) défense mechanism (Maplestone et al. 1992). Many studies have reported antagonism between actinomycetes and a diversity of phytopathogens like *Altemaria, Fusarium, Macrophomina, Phytophthora*,



Pythium, Rhizoctonia, Verticillium (Chattopadhyay and Nandi 1982, Hussain et al. 1990, Merriman et al. 1974, Valois et al. 1996).

Antibiosis as a mechanism of biological control of plant disease has been studied in many Systems (Chamberlain and Crawford 2000, El-Abyad et al. 1993, Kortemaa et al. 1994). Gottlieb (Gottlieb 1976) has reviewed the proof that antibiotics may be produced by members of soil microflora in their natural environment, and function there in an antagonistic capacity. Various experiments have verified the difficulty in introducing a new organism into normal soil which already has an established indigenous population and, in contrast, the benefit with that organism is introduced into sterile soil (Gottlieb 1976). Such experiments clearly demonstrate that microorganisms in soil are in direct competition, so that, any issue which kills other organisms would certainly be advantageous to the producer. The evidence does not prove that antibiotics are responsible for the competitive antagonism between species since, as yet, antibiotics haven"t been physically isolated from soil. However, there"s proof for the analytical detection of antibiotics in soil;especially, (Zviagintsev et al. 1976) antibiotic heliomycin is created by *Actinomyces olivocinereus* Vinogradova in unsterilised, unsupplemented soil. They used the method of fluorescent microscopy, utilizing the intrinsic fluorescence of the actinomycete, to examine the development of precursor and synthesis of the antibiotic directly in soil.

Actinomycetes as Biopesticide Agents

As the environmental contamination by toxic chemicals increases, different approaches for controlling pest populations became analysis priorities. These have enclosed biological or ecological management strategies for limiting the harmful impacts of pest populations, particularly in agriculture (Nakas and Hagedorn 1990, Canaday 1995, Hokkanen and Lynch 1995).

Several sorts of microorganisms including fungi, bacteria, nematodes and viruses that are antagonistic to insects are reported as methods to biologically control them. Actinomycetes play a significant role in the biological control of insects through the production of insecticidally active compounds against the house fly *Musca domestica* (Hussain et al. 2002). The mortality of larval and pupal stages, were terribly high reaching up to 90% after actinomycetes treatments (Hussain et al. 2002). Actinomycetes were effectively used against *Culex quinquefasciatus* (Sundarapandian et al. 2002). Actinomycetes are a vital cluster of microorganisms, not only as degraders of organic matter within the natural environment, but also as producers of antibiotics and other valuable compounds of commercial interest (Saugar et al. 2002, Bentley et al. 2002, Basilio et al. 2003).

Additionally, actinomycetes are important for the production of enzymes, like chitinase (eg. *Streptomyces viridificans*), cellulases (eg. *Thermonospora* spp.), peptidases, proteases (*Nocardia* spp.), Xylanases (*Microbispora* spp.), ligninases (*Nocardia autotrophica*), amylases (*Thermomonospora curvata*), sugar isomerases (*Actinoplanes missouriensis*), pectinase, hemicellulase and keratinase (Solans and Vobis 2003).

To select non-streptomycete actinomycetes by reducing the numbers of streptomycete actinomycetes on isolation plates, *Streptomyces* phages was applied (Kurtböke et al. 1992, Long and Amphlett 1996). The isolation of *Streptomyces* phages are of sensible importance for a range of reasons such as the evils they cause to fermentation industries (Chater

1986), their value for typing streptomycetes in taxonomic studies (Korn–Wendish and Schneider 1992), their use for the detection and understanding of host controlled restriction-modification systems (Diaz et al. 1989), their utilization as tools for genetic exchange and analysis in

Streptomyces spp. (Herron and Wellington 1990), the study of their general and molecular biology (Lomovskaya et al. 1980) and ecology (Williams et al. 1987).

Chitinase is originally an enzyme used by insects to degrade the structural polysaccharide "chitin" during the molting process (Zhang et al. 2002). The largest chitinase activity among bacteria has been determined in species of *Streptomyces*, *Serratia, Vibrio* and *Bacillus* (Reguera and Leschine 2001). Chitinase enzyme is extremely necessary within the biological control of insects (Reguera and Leschine 2001) and plant pathogenic fungi (El- Tarabily et al. 2000, El-Tarabily 2003). Species of *Streptomyces* showed high multiplicity of chitinase genes

(Williamson et al. 2000, Saito et al. 2003), as in the case of *Streptomyces coelicolor* and *Streptomyces griseus* (Itoh et al. 2003). However, screens for antagonism have focused mostly on bacteria, fungi, viruses and nematodes (Collier et al. 2001). There's scarcity of published information with respect to the utilization of actinomycetes significantly, rare non-

Actinomycetes as production of plant growth hormone (indole-3-acetic acid)

streptomycete actinomycetes, as biocontrol agents of insect pests.

Actinomycetes have an extended tradition in the analysis of bioactive compounds. Several species manufacture a large form of secondary metabolites, including anti- helminthic compounds, anti-tumour agents and majority of identified



antibiotics. Free-living actinomycetes have additionally been concerned in the improvement of plant growth by production of plant growth-producing substances like auxins and gibberellin-like compounds (Persello-Cartieaux et al. 2003, Bloemberg et al. 2001).

Indole-3-acetic acid (IAA) is the principal form of auxin, which regulates many basic cellular processes including cell division, elongation and differentiation.

It also leads to decrease in root length and increase in root hair formation, so enhancing the potential of the plant to absorb soil nutrients. Besides, there are several developmental processes in which auxin plays a role, together with embryo and fruit development, organogenesis, vascular tissue differentiation, root patterning, elongation and tropistic growth, apical hook formation and apical dominance (Paciorek et al. 2006, Dobbelaere et al. 1999, Bennett et al. 1998).

Manulis6 observed induced synthesis of IAA by six diverse *Streptomyces* species in the presence of tryptophan and recommended indole-3-acetamide as the main pathway, as *S. violaceus* and *S. exfolitus* catabolized indole-3- acetamide (IAM), indole-3-lactic acid (ILA), indole-3- ethanol (IEt) and indole-3-acetaldehyde (IAAld) into IAA, besides attainable presence of different pathways for IAA biosynthesis.

In recent years the tactic of immobilizing living cells has gained a large variety of applications (D"Souza et al. 1999, Baianu et al. 2004). Encapsulation of microbial cells for soil application provides a variety of benefits like application to the soil, reduced off-site drifting, and protection of cells from environmental stress (Leung et al. 1997, Bashan 1986). Additionally, they possess high cellloading capability, high retention of cell viability, increased rate of production of microbial products and also act as a reservoir, which releases the bacteria at a slow and constant rate (Mahmoud and Rehm 1987).

Actinomycetes are known to be durable organisms and thus appropriate for soil applications. The spores of most actinomycetes endure desiccation and show slightly higher resistance to dry or wet heat than vegetative cells. Actinomycetes will colonize dry soil owing to their filamentous nature and exist in soil for extended periods as resting arthrospores that germinate in the occasional presence of exogenous substrates4. So far, the potential of filamentous actinomycetes in encapsulated state for the assembly of IAA has neither been completely examined nor used in field conditions to any noticeable extent.

Abiotic soil factors have an effect on the population dynamics of the inoculant, imposing stresses of varied natures on the cells. They'll additionally act indirectly, by affecting the activity of the indigenous soil microflora (Van Veen et al. 1997). Typical environmental stresses faced by the organisms in the soil may include salinity, unfavourable soil pH, extremes in temperature, inadequate or excessive soil moisture, significant metal toxicity and biocides (Slonczewski 2000, McGrath et al. 1997, Manna et al.2001).

Actinomycetes in Biocorrosion

Corrosion is a principal reason of pipe failure and high preservation costs in gas pipelines (Zhu et al. 2003). Biocorrosion is defined as a caustic harm initiated or aggravated by the direct or indirect activities of microorganisms (Zuo 2007). A broad range of bacteria is present in most if not all areas of oil production and have been described from water injection plants, drilling mud, and live reservoir cores (Feio et al. 2000, Magot et al. 2000, Korenblum et al. 2005, Von Der Weid et al. 2008).

Antimicrobial substance (AMS) formed by a Streptomycetes strain having its activity against an aerobic bacterium *B. pumilus* LF-4, and sulfate-reducing bacterium *D. alaskensis* NCIMB 13491 known to be involved in biofilm formation and biocorrosion. Strain 235 was identified as belonging to *S. lunalinharesii* species cluster, was initially isolated from a Brazilian soil. This strain was previously recognized as producer of bioactive compounds against phytopathogenic bacteria and fungi. The antimicrobial activity was seen over a broad range of pH, and after treatment with several chemicals and heat but not with Proteinase K and trypsin. The AMS, of proteic nature, has publicized to be promising for use in oil making plants, given its stability in the presence of several chemicals and solvents, and over a broad range of temperature and pH values.



Actinomycetes in Bioremediation

Petroleum hydrocarbons are widely used in our daily life as chemical compounds and fuel. Greater use of result, petroleum has become one of the most common contaminants of large soil surfaces and eventually is considered as a major environmental problem (Sanscartier et al. 2009).

There are several ways in which hydrocarbons degraded in the environment. One mechanism through which they can be removed from the environment is Bioremediation.

Bioremediation is the use of soil microbes to degrade pollutants to harmless substances (Collin 2001).

Actinomycetes possess many properties that make them good candidates for application in bioremediation of soils contaminated with organic pollutants. They play an important role in the recycling of organic carbon and are able to degrade complex polymers (Goodfellow and Williams 1983). Some reports indicated that Streptomyces flora could play a very important role in degradation of hydrocarbons (Radwan et al. 1998, Barabas et al. 2001). Many strains have the ability to solubilise lignin and degrade lignin-related compounds by producing cellulose- and hemicellulose-degrading enzymes and extracellular peroxidase (Mason et al. 2001). In some contaminated sites Actinomycetes represent the dominant group among the degraders (Johnsen et al. 2002). Actinomycetes species have the capability to live in an oily environment. So we can apply these microorganisms in Bioremediation to deduct oil pollutants.

Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by actinomycetes. Several pharmaceutical companies used microbial natural products as one of the major source of novel drugs. Researchers have been going on to discover more novel molecules with potential therapeutic application especially from actinomycetes. A wide range of antibiotics in the market are obtained from actinomycetes. They are capable to degrade a wide range of hydrocarbons, pesticides, and aliphatic and aromatic compounds and also have a property to perform microbial transformations of organic compounds which are of great commercial value. They re a promising tool use in bioconversion of agriculture and urban waste into chemically vital products. Their metabolic potential offers a strong area for research. For novel drug delivery, scientists still exploit the chemical and biological diversity from diverse actinomycetes group to maximize the possibility of successful discovery of novel strain in cost effective manner. However further characterization of actinomycetes and their product for utilization in plant biotechnology, environmental biotechnology, urban waste management and some other applications yet to be done. The potential numbers of metabolites from actinomycetes may be discovered in the future.

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