CHAPTER II
LITERATURE REVIEW

1. Characteristics of the genus *Streptomyces*

The *Streptomyces* strains are gram-positive bacteria in the family Streptomycetaceae, order Actinomycetales (Table 1). Outgrowth from spores or fragments of mycelium, (colony forming unit, CFUs) develop into hyphae (branching filaments) that penetrate the agar (substrate mycelium) and hyphae that branch repeatedly and become cemented together on the surface of the agar to form a tough, leathery colony. In strains of *Streptomyces*, the colony becomes covered with aerial mycelium (free, erect hyphae surrounded by a hydrophobic sheath that grow into the air away from the colony). These hyphae are initially white but assume a range of colors when spore formation begins. Colonies then appear powdery or velvety and can then be readily distinguished from the more typical bacteria colonies. *Streptomyces* strains produce a wide variety of pigments responsible for the color of the vegetative and aerial mycelia. Colored diffusible pigments may also be formed. Many strains produce one or more antibiotics. *Streptomyces* strains use a wide range of organic compounds as sole sources of carbon for energy and growth (Goodfellow, 1989; Cross, 1994).

Cultural characteristics of the genus *Streptomyces* on various culture media are such characters as the colors of the soluble pigment, the color of the vegetative growth, the aerial mycelium and spores, and the micromorphology of the sporulation structures. Mycelia pigments and pigments that are produced in the substrate mycelium and diffuse out into the medium have been used as criteria for descriptions the species of *Streptomyces*.

Table 1 Classification and morphological characteristics of Streptomycetaceae[a] (Holt, 1989; Cross, 1994).

Genus	Morphological characeristics
Streptomyces[b]	Aerial mycelium with chain (usually long) of non-motile conidia.
Actinomadura	Short chains of conidia on aerial mycelium, often curled into a crozier.
Streptoverticillium	Whorls of straight chains of conidia formed.
Kineosporia	No aerial mycelium; club-shaped sporangia formed terminally on the vegetative mycelium.
Sporichthya	No substrate mycelium is formed, aerial mycelium only, motile elements formed.
Micromonospora	No sporangia. Single conidia formed on substrate mycelia, often in large black mucoid masses.
Microbispora	Chains of conidia with only two spores.
Nocardioides	Both aerial and substrate mycelia breaking up into fragments.
Intrasporangium	Only substrate mycelium formed bearing terminal or subterminal vesicles.

[a] Streptomycetaceae, All are aerobic sporoactinomycetes with cell wall type I (containing of L-diaminopimelic acid, L-DAP).

[b] *Streptomyces*, This genus includes the most common soil forms and most of the important producers of antibiotics.

Physiological and biochemical properties such as reaction on starch, gelatin or milk, nitrate reduction and melanin formation and utilization of carbon sources have been used extensively to characterize *Streptomyces* strains and species.(Goodfellow, and William, 1983; Jensen, Dwight, and Fenical, 1991).

Streptomycetes strains are widely distributed in terrestrial and aquatic habitats. Most are strict saprophytes, but some form parasitic associations with plants or animals. Surprisingly little is know about the role of streptomycetes in natural environments,

although evidence of their occurrence and numbers in habitats is extensive. Several recent reviews on streptomycete ecology are available (Cross, 1981; Kutzner, 1981; Goodfellow and Williams, 1983; Williams *et al.*, 1984; Goodfellow and Simpson, 1987).

The survival capacity of streptomycete spores appears to be greater than that of the hyphae. Streptomycete spores have a net negative surface charge except at low pH levels and a relatively low endogenous metabolism and are generally more resistant to heat than the corresponding hyphae (Goodfellow and Simpson,1987). Spores are released above soil when particles are disturbed by wind or rain, whereas dispersal within soil is assisted by movement of water and arthropods (Ruddick and Williams, 1972). In dry soil, streptomycete counts decrease markedly at moisture tensions above pH 4.0, but their proportion to other bacteria may be higher because their spores are more resistant to desiccation than are the vegetative cells of bacteria. Optimum counts from neutral soil and optimum radial growth of streptomycetes inoculated into sterile occur at moisture tensions between pH 1.5 and 2.5. Some streptomycetes from acid soil are able to grow on media at high osmotic potentials (Wong and Griffin, 1974)

Soil, fodder and composts appear to be the primary reservoirs for streptomycetes. Specific growth rates and doubling times for streptomycetes in laboratory culture are approximately intermediate between those of bacteria and fungi (Flowers and Williams, 1977). Because many soil are acidic, pH is clearly an important factor determining the distribution and activity of streptomycetes. Acidophilic and neutrotolerant streptomycetes, the latter growing between pH 3.5 and 7.5 but optimally around pH 5.5, are common in acid soils (Khan and Williams, 1975).

1.1 Criteria used for the classification and identification of *Streptomyces* species

All of 15 criteria are summarized in Table 2 (Holt, 1989).

Table 2 Criteria used for the classification and identification of *Streptomyces* species

Character	Character state
1. Spore chain morphology	*Rectiflexibiles, Rectinaculiaperti* or *Spirales*.
2. Spore surface ornamentation	Smooth, warty, spiny, hairy or rugose.
3. Other morphological features	Fragmentation of substrate mycelium, sclerotia formation, sporulation on substrate mycelium.
4. Color of spore mass	Blue, gray, green, red, violet, white or yellow.
5. Pigmentation of substrate mycelium (colony reverse)	Yellow-brown (no distinctive pigment), blue, green, violet or red-orange. pH sensitivity of pigments.
6. Diffusible pigments	Yellow-brown, blue, green, red-orange or violet. pH sensitivity of pigments.
7. Melanin pigment production	On peptone-yeast extract-iron agar and tyrosine agar.
8. Antimicrobial activity	Activity against *Aspergillus niger, Bacillus subtilis, Candida albicans, Micrococcus luteus, Pseudomonas fluorescens, Escherichia coli, Saccharomyces cerevisiae* and *Streptococcus murinus*.
9. Enzyme activity	Lecithinase, lipolysis and proteolysis (on egg-yolk medium). Hydrolysis of chitin, hippurate and pectin. Nitrate reduction. Hydrogen sulfide production. β-Lactamase and β-lactamase inhibitor production.
10. Degradation activity	Adenine, allantoin, arbutin, casein, DNA, elastin, esculin, gelatin, guanine, hypoxanthine, RNA, starch, testosterone, Tween 80, L-tyrosine, urea, xanthine and xylan.
11. Resistance to antibiotics (μl/ml)	Cephaloridine (100), dimethylchlotetracycline (500), gentamicin (100), lincomycin (100), neomycin (50), oleandomycin (100), penicillin G (10 i.u.), rifampicin (50), streptomycin (100), tobramycin (50) and vancomycin (50).
12. Growth temperatures and pH	4°C, 10°C, 37°C and 45°C. pH 4.3.

Table 2 (continued)

Character	Character state
13. Growth in the presence of inhibitory compounds (% w/v)	Crystal violet (0.0001), phenol (0.1), phenylethanol (0.1, 0.3), potasium tellurite (0.001, 0.01), sodium azide (0.01-0.02), sodium chloride (4, 7, 10, 13) and thallous acetate (0.001, 0.01).
14. Use of nitrogen sources (0.1% w/v)	DL-α-amino-*n*-butyric acid, L-arginine, L-cysteine, L-histidine, L-hydroxyproline, L-methionine, potassium nitrate, Lp-Phenylanine, L-serine, L-threonine and L-valine.
15. Use of carbon sources (0.1% w/v)	Adonitol, L-arabinose, cellobiose, dextran, D-fructose, D-galactose, *meso*-inositol, inulin, D-lactose, manitol, D-mannose, D-melezitose, D-melibiose, raffinose, L-rhamnose, salicin, sucrose, trehalose, xylitol and D-xylose, sodium acetate, sodium malonate, sodium propionate and sodium pyruvate.

2. Characteristics of the genus *Micromonospora*

Micromonospora is well-developed, branched, septate mycelium averaging 0.5 μm in diameter. Spores are borne single, sessile or short long sporophores, which often occur in branch clusters. The formation of single spore on substrate mycelium is one of the well-defined criteria in the genus *Micromonospora.* Spore surface ornamentation of the strains and the species of *Micromonospora* have been characterized by the terms smooth, rough, warty, or blunt spiny of sporophore development monopodial or in some case sympodial. Aerial mycelium is absent or in some culture appears irregularly as a restricted white or grayish bloom, gram-positive, not acid fast. Walls contain *meso*-diaminopimelic acid. Xylose and arabinose are present in cell hydrolysates. Colonies on agar media are initially pale yellow or light orange, becoming orange, red, brown, blue-green, or purple. Mature colonies take on

a progressively dark hue along with the production of brown-black, green-gray or black spores, and become mucoid. They are sensitive to pH below 6.0. Growth occurs normally between 20°C and 40°C but not above 50°C (Holt, 1989). The genera *Streptomyces* and *Micromonospora* are differentiated from each others by characteristics as shown in Table 3.

Table 3 Differential characteristics of the genera *Streptomyces* and *Micromonospora*

Characteristics	*Streptomyces*	*Micromonospora*
Colony appearance	powdery	mucoid
Substrate mycelium	+	+
Aerial mycelium	+	-
Sporangia	-	-
Spore motility	-	-
Amino acid	L-DAP	*meso*-DAP
Sugar in whole-cell hydrolysate	Glucose, glycerol, mannitol, fructose and sucrose	Xylose and arabinose

3. Antibiotics from *Streptomyces* species

The word " antibiotics" used to describe a type of association in which one living creature was destroying another in order to sustain its own life. Waksman lays published the following definition of the word: "Antibiotic is a chemical substance, produced by microorganisms, which has the capacity to inhibit the growth and even to destroy bacteria and other microorganism" (Waksman, 1953), Florey *et al.*, would seem to agree with Waksman, as they report that he proposed a definition in 1945 (Florey, 1949). Benedict and Langlyke modified this definition to comprise substances which act upon certain organisms at least in very dilute solutions. Abraham and Newton described the word "Antibiotics" as natural compounds derived from organisms which

themselves or chemical modification are able at low concentration to inhibit or kill other microorganisms and abnormal cells in higher animals.

Additional examples are *Streptomyces olivaceus* producing (*E*)-4-Oxonon-2-enoic acid (Ballini and Boscica, 1998), *Streptomyces* sp. producing mathemycin B (Mukhopadhyay *et al.*, 1999), *Streptomyces* sp. GK 9244 producing tetrin C, a new substance in glycosylated polyene macrolide group (Ryu *et al.*, 1999), *Streptomyces* sp. HILY-9420704 producing methylsulfomycin I, a new substance in cyclic peptide group (Vijaya *et al.*, 1999), *Streptomyces fradiae* producing actinomycin Z (Lackner *et al.*, 2000). Recently, antibacterials and antifungals were produced by *Streptomyces* strains (Table 4).

Table 4 Antimicrobial compounds from *Streptomyces*

Compounds	Strains	Activity	References
Actamycin	*Streptomyces* sp. E/784	Antibacterial activity	Hooper and Rickards, 1998
Actinomycin Z	*S. fradiae*	Growth inhibition of *B. subtilis* ATCC 6051	Lackner *et al.*, 2000
Bagremycins A and B	*Streptomyces* sp. Tu 4128	Moderate activity against gram-positive bacteria and some fungi	Bertasso *et al.*, 2002
Benzanthraquinone YM-181741	*Streptomyces* sp. Q 57219	Selective activity against *Helicobacter pylori*	Taniguchi *et al.*, 2002
Cedarmycins A and B	*Streptomyces* sp. TP-AO 456	Antibiotic activity against gram-positive and negative bacteria and yeasts	Sasaki *et al.*, 2002 a
Cremimycin	*Streptomyces* sp. MJ 635-86F5	Broad antimicrobial activity against gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA)	Igarashi *et al.*, 1998

Table 4 (continued)

Compounds	Strains	Activity	References
Cyclomarins A, B and C	*Streptomyces* sp. CNB-982	Potent antiinflammatory activity	Renner *et al.*, 1999
Demethyl mutactimycins	*Streptomyces* sp. GW 60/1571	Moderate antimicrobial activity against gram-positive bacteria	Speitling *et al.*, 1998
5'- and 7'-demethylnovobiocins	*Streptomyces* sp. TP-A0556	Antibiotic activity against gram-positive and negative bacteria	Sasaki *et al.*, 2001
Dihydrophencomycin	*Streptomyces* sp. B8251	Weakly antimicrobial activity	Puseker *et al.*, 1997
(*E*)-4-Oxonon-2-enoic acid	*S. olivaceus*	Growth inhibition of gram-positive and negative bacteria	Ballini and Bosica, 1998
Enterocin	*Streptomyces* sp. BD-26T	Bacteriostatic against gram-positive and gram-negative bacteria	Sitachitta, Gadepalli and Davidson, 1996
Feigrisolide B	*Streptomyces griseus*	Strong antibacterial activity, as well as medium cyctotoxic and antiviral activity	Tang *et al.*, 2000
Lactonamycin	*Streptomyces rishiriensis* MJ 773-88K4	Antimicrobial activity against gram-positive bacteria including MRSA and vancomycin-resistant *Enterococcus* (VRE)	Matsumoto *et al.*, 1999
Mathemycin B	*Streptomyces* sp.	Inhibition of *Fusarium culmorum* 100	Mukhopadhyay 1999
2-methyl-heptyl isonicotinate	*Streptomyces* sp. 201	Antimicrobial activity against *B. subtilis, Shigella* sp., *Klebsiella* sp., *E. coli, Proteus mirabilis* and the pathogenic fungi	Bordoloi *et al.*, 2002

Table 4 (continued)

Compounds	Strains	Activity	References
Methylsulfomycin I	*Streptomyces* sp. HLI Y-9420704	Antimicrobial activity against a wide range of gram-positive bacteria including MRSA and vancomycin and teicoplanin resistant strain	Vijaya-Kumar, 1999
Radamycin	*Streptomyces* sp. RSP9	Potent antibiotic activity against several gram-positive bacteria	Gonzalez-Holgado *et al.*, 2002
Rubiginones D_2, H and I	*Streptomyces* sp. Go N1/5	Growth inhibition of gram-positive bacteria and cytostatically active against different tumor cell lines	Puder, Zeeck and Beil, 1999
Spirofungin	*Streptomyces violaceusniger* Tu 4113	Various antifungal activity and particularly against yeasts	Holtzel *et al.*, 1998
Streptocidins A-D	*Streptomyces* sp. Tu 6071	Antibiotic against gram-positive bacteria	Gebhardt, Pukall and Fiedler, 1999
Tetrin C	*Streptomyces* sp. GK 9244	Antifungal activity against *Mortierella ramannianus*	Ryu *et al.*, 1999
Vinylamycin	*Streptomyces* sp. MI 982-63F1	Antimicrobial activity against gram-positive bacteria including MRSA	Igarashi *et al.*, 1999
Watasemycins A and B	*Streptomyces* sp. TP-A0597	Antibiotic activity against gram-positive and negative bacteria and yeasts	Sasaki *et al.*, 2002 b
Zelkovamycin	*Streptomyces* sp. K 96-0670	Antimicrobial activity against *Xanthomonas oryzae*, *Acholeplasma laidlawii* and *Staphylococcus aureus*	Zhang *et al.*, 1999

The ansamycins are a class of macrocyclic compounds. Their structures consist of an aromatic nucleus in which two non-adjacent positions are linked by a long aliphatic chain of up to twenty-four atoms (Thomson, 1987). Most of the ansamycins are obtained from bacteria but one of benzenoid group, the maytansinoids, occurs in higher plants (Kupchan *et al.*, 1972). Ansamycins displayed many biological activity such as antibacterial activity, antifungal activity, antitumor activity and herbicidal activity (Rinehart, Jr. and Shield, 1976). Source and biological activity of ansamycins are displayed in Table 5.

Table 5 **Sources and biological activity of ansamycins** (Glasby, 1993)

Compounds	Sources	Activity
Halomicins A, B and C	*Micromonospora halophytica*	Antibacterial activity against gram positive bacteria
Protostreptovaricins I-V	*S. spectabils*	Antiviral activity against Rauscher leukemia virus RNA-dependent DNA polymerase
Rifamycin B	*S. mediterranei*	Antibacterial activity against gram-negative bacteria
Rifamycin O	*S. mediterranei* and *S. tolypophorus*	Antibacterial activity against gram-negative bacteria
Rifamycin W	*Nocardia. mediterranei*	Antibacterial activity against gram-positive bacteria
Rifamycin X	*S. mediterranei*	Antibacterial activity against gram-positive bacteria
Rifampicin	Semi-synthetic produced from rifamycin B	Anti-TB and Antibacterial activity
Rifocin	*S. mediterranei* and *Micromonospora chalcea*	Therapeutically effective in the treatment of pneumococcal, staphylococcal and streptococcal infections

4. Antimicrobial screening

4.1 Primary screening

There are several criteria to be met for a useful front-line screening bioassay. It must be rapid, convenient, reliable, and inexpensive require little material and be able to identify a broad spectrum of bioactivity.

The classical agar diffusion method has been used to isolate and identify antibiotic producing microorganisms. It was these screening methods that helped to discover the principal antibiotics against gram-positive bacteria and to some against gram-negative pathogens and pathogenic fungi. In screening for antibiotics the primary screen can be used not only for bioactivity detection, but also for fermentation control aimed at production of larger amounts of biomaterial (Shiring and Gottlieb, 1966).

Waskman and Starkey, (1987), using the plate method for counting, have observed that some of the colonies of actinomycetes on the plate are surrounded by clear zone, free from the growth of bacteria and fungi. By far the most successful method in the search for antibiotics has consisted of testing the antagonistic properties of large numbers of microorganisms.

There is no ideal medium, which permits the plating out of natural substance with the resulting growth of all the actinomycetes present in the substrate, and which inhibitors the growth of all other microorganisms. Addition of selective inhibitors permits reduction of the number of fungi and true bacteria and helps in the isolation of actinomycetes in pure cultures (Corks and Chase, 1952). This has been used with success for the antifungal antibiotic cycloheximide to eliminate fungal growth.

Demonstration that an antagonist can produce a diffusible substance effective upon the test organism chosen in a given screening program must be followed by

demonstration that this substance can also be produced in liquid media (Waskman, 1967). This is of prime importance, since antibiotics must be obtained in liquid media for large-scale production.

4.2 Secondary screening

Primary screening allows the detection and isolation of microorganisms that possess potentially interesting industrial applications. This screening is usually followed by a secondary screening to further test the capabilities of and gain information about these organisms. Primary screening can be qualitative or quantitative in its approach. The qualitative approach tells us the spectrum or range of microorganisms, which is sensitive to antibiotic. The quantitative approach tells us the yield of antibiotic. Secondary screening should reveal whether there is pH, aeration of other critical requirements associated with particular microorganism, both for the growth of the organism and for the formation of antibiotics.

The preceding discussion emphasizes the fact that secondary screening can provide a broad range of information which helps in deciding which of various microbial isolates posses possible usefulness as an industrial organisms.

5. Fermentation of *Streptomyces*

The fermentation development for biologically active compounds production is an interdisciplinary task. Process depends on the specific contributions of both biologist and engineer. An understanding of physiology of an interesting microorganism including optimization of growth and fermentation conditions such as temperature, aeration and nutrient concentration will be important in bringing out the potential. In general, biologically active compounds are produced in batch fermentation or in fed-batch process. Continuous cultivation is seldom introduced at the production scale.

However, the satisfactory batch process should be worked out before establishing another process.

Fermentation processes used for screening of biologically active compound differs from processes used for biomass production or production of primary metabolites in a number of aspects. From the standpoint of production control for biologically active compound, the important thing is that conditions, which are optimal for rapid growth, are seldom optimal for the production phase. The opportunity to find a position for biologically active compound producer depends on several factors. One of these is the number of media used for cultivation. It is suggested to use at least 3 different types of media in each microorganism during cultivation. The fermentation time and incubation temperature varies optimally around 30°C. The nutritional ingredients used for screening are important. The enriched media with organic nitrogen and organic carbon such as casein, arginine, peptone, yeast extract, starch, corn meal and/or corn steep liquor are usually added into the screening medium. Nitrate or ammonia may serve as nitrogen sources. (Luria, 1960).

5.1 Nutrient effects on cell growth and antibiotic production

Certain nutritional requirements are common to all bacterial species, including members of the genus *Streptomyces*. These include requirements for energy and for assimilable sources of carbon, oxygen, nitrogen, hydrogen, phosphorus, sulfur, and a number of metals (Table 6). In addition, many species need a variety of complex molecules, including vitamins and amino acids.

5.1.1 Carbon and energy

Nearly all species of *Streptomyces* are chemoheterotrophs. As such, they depend on the oxidation of organic molecules for both energy and to provide carbon for the production of cellular components. These organisms are usually found naturally in

the soil and have the ability to utilize a variety of organic carbon sources. The most common substrates used for antibiotic production are starch, oils, and various types of simple sugars. It must be emphasized that other nutrients are also present, such as phosphate, minerals, and nitrogenous compounds, when crude preparations of these substrates are used.

Because the cellular content of carbon is so high, and also because of carbon's role in energy production, the carbon/energy substrate is usually the major medium component and, frequently, is the major cost item in the mix. Therefore, a careful screening of acceptable substrates should be done to determine the optimal carbon source. When evaluating a new carbon source, not only must the yield of antibiotic on supplied substrate be considered, but also the substrate cost.

5.1.2 Nitrogen

The streptomycetes is capable of assimilating nitrogen in a number of forms. Those usually employed in industrial fermentations include proteins, ammonia and ammonium salts, urea, and nitrate salts (Waksman. 1967). The organisms generally utilize nitrogen in the order presented above. When proteins are employed, they must first be hydrolyzed by extracellular proteolytic enzymes before they can be assimilated. Ammonia assimilation has been extensively studied in bacteria (Dalton, 1979). Proteins can serve both as a source of nitrogen and as a supplement to the carbon energy supply. When other carbon sources are depleted, the streptomycetes will generally start utilizing proteins for energy and will release ammonia to the medium. This causes a rise in the pH of the broth. Excessive ammonia levels can also inhibit antibiotic production (Young, 1981; Masuma *et al.*, 1983).

5.1.3 Phosphorus

Phosphorus is supplied to the fermentation medium in the form of inorganic phosphate (Waksman, 1967) and/or in the form of complex organic phosphates. The complex phosphates are generally present in the natural products used to supply protein to the medium. Phosphorus is a conserved substrate in that it can be recycled within the cell and is not lost from the medium. Phosphorus also differs from nitrogen and carbon in that the requirement for phosphorus and the yield coefficient for phosphorus (grams of cells per gram of P) can vary widely between cultures and between different fermentation conditions. Cellular phosphorus levels can vary between 0.2 and 10%. Excess phosphate is also a strong inhibitor or repressor of antibiotic production.

5.1.4 Sulfur

Sulfur requirements are usually met by the supplementation of the medium with sulfate. Some sulfur is also supplied with proteins containing cysteine and methionine. Most streptomycete media contain large excesses of sulfur, as it is not generally inhibitory to either growth or product formation. Under aerobic conditions, sulfur is generally considered to be a conserved substrate.

5.1.5 Major cations

The major cations include sodium, potassium, calcium and magnesium. These are added to the medium as counter ions of sulfate, phosphates, chlorides, etc., as naturally occurring minerals in water, and as naturally occurring minerals in other complex substrates. The effect of the major cations on the fermentation process can be rather complex. In some cases, high levels of a particular cation can stimulate or inhibit product formation. Many organisms require a proper balance between sodium and

potassium levels. Calcium and magnesium can also form insoluble salts and in some cases can remove other nutrients from solution.

5.1.6 Trace minerals

Numerous enzymes require certain metal ions as cofactors. The ions that are often required include calcium, magnesium, manganese, iron, cobalt, copper, zinc, and molybdenum. These ions are generally required in very low concentrations but may be essential in achieving high production rates. Excessive levels can also be inhibitory. Many of these metal ions are readily available in water and complex nutrients. However, the concentration of a particular metal ion can change dramatically with seasonal variations in agricultural products and water sources. Yeast products are frequently used to supplement trace minerals, as are specially designed mineral mixtures.

		120 rpm, 2 days		120 rpm, 6 days	and Fie
Deacetylravidomycin M	Starch, glucose, peptone, meat extract, yeast extract, $CaCO_3$	pH 7.0, 27°C, 3 days	Soluble starch, solvent extracted toasted soy bean meal, $Na_2S_2O_3$, $FeSO_4.7H_2O$, K_2HPO_4, KCl	pH 6.5, 27°C, 6 days	Arai *et*
Neuroprotectins A and B	Soluble starch, glucose, soybean meal, NaCl, $CaCO_3$, beef extract, yeast extract, K_2HPO_4	pH 7.2, 28°C, 2 days	Soluble starch, glucose, soybean meal, NaCl, $CaCO_3$, beef extract, yeast extract, K_2HPO_4	pH 7.2, 28°C, 6 days	Kobaya 2001
Goadsporin	Soluble starch, glucose,	pH 7.0, 30°C,	Soluble starch, glucose,	pH 7.0, 30°C,	Onaka

Radamycin	Yeast extract, sucose, xylose, $MgCl_2$	pH 7.2, 28°C, 200 rpm, 1 day	Yeast extract, sucose, xylose, $MgCl_2$	pH 7.2, 28°C, 200 rpm, 4-7 days	Gonzale Holgad 2002
Indocarbazostatin A and Indocarbazostatin B	Soluble starch, glucose, NZ-case, yeast extract, fish meal, $CaCO_3$	pH 6.5, 30°C, 2 days	Soluble starch, glucose, pharmamedia, soybean meal, corn steep liquor, yeast extract, NaCl, $MgSO_4.7H_2O$, $CaCO_3$	pH 7.0, 30°C, 3 days	Matsuu 2002

6. Isolation, purification and structure elucidation of antibiotics

6.1 Identification of antibiotics by thin layer chromatography

Over the years many investigators have devised numerous procedures for classification and identification of antibiotics by chromatographic techniques. In earlier years, these various chromatographic systems were quite usual because of the relatively small number of antibiotics compared to the present. In 1959, Miyazaki *et al.* described a method of grouping antibiotics examined by means of ascending chromatography. Zuidweg *et al.*, 1969, have used Sephadex in thin layer chromatography for the identification of antibiotics. The solvents selected by the thin layer chromatography can be applied to column chromatography for preparative separation.

6.2 Autobiographic detection of antibiotics in preparation chromatogram

Autobiography is a technique that has been used to screen for antimicrobial activity. The most common methods are based on the agar diffusion technique. The developed thin layer chromatography (TLC) plate is placed in contact with an agar plate that has been inoculated with the test organism. The compound diffuse from the chromatographic layer to the agar plate, and after an inoculation period, zones of inhibition are made visible with appropriate stains. The procedure requires the use of microbiologic equipment and suffers from the problem of differential diffusion exhibited by various classes of compounds. An improved technique, developed by Hamberger and Cordell, involves application of a suspension of the test bacteria or conidia of the fungi in a nutritive broth on the TLC plate. The plate is then incubated in a humid atmosphere (overnight), and zones of inhibition are detected by spraying with a reagent (the colorless p-iodonitrotetrazolium chloride) specific for dehydrogenase activity. The tetrazolium salt is converted into an intensely pink-colored formazan over a period of 4 hrs. A clear spot against a colored background indicates the presence of

antibacterial compounds. The assay, using *Bacillus subtilis* and *Escherichia coli*, proved insensitive to a number of cytotoxic compounds belonging to the camptothecin, quassinoid and lignan series. Containing the assay system in a glove bag can reduce dispersal of bacteria. While these techniques are useful as a front-line screen, selective search strategies have been devised and have identified a variety of new antibiotics (Masuma, R., Tunaka, Y., and Omura, S. 1983).