# CHAPTER II

# LITERATURE REVIEW

There was a description by Coyne (1999) on how the actinomycetes are close friends to human and they can influence our daily life style. "Take a handful of garden or field soil, hold it close to your nose, and breathe deeply. What do you smell? It's not obvious that you should smell anything based simply on the composition of soil, because most soils are primarily made up of inert materials such as sand, silt, and clay. But you probably do smell something: an earthy, musty, smell. Maybe it's smell that brings back old memories of cutting grass in spring or burning leaves in fall. The smell is real even if the images it evokes are just memories. What your sense of smell detects are microbial products called geosmins (1,10-dimethyl-9-decalols). Geosmins produce the smell of freshly plowed soils and musty cellars-the smells that remind city folk of country life. Geosmins are produced by the group of microorganisms—THE ACTINOMYCETES" (Coyne, 1999)

## 1. Characteristics of actinomycetes

Actinomycetes are prokaryote that form filamentous mycelia and spores like fungi and were originally called ray fungi. There were two important characteristics that distinguish actinomycetes from fungi: one, actinomycetes have no cell nucleus and form hyphae 0.5 to 1.0 $\mu$m in diameter, which are smaller than fungal hyphae. Two, actinomycetes are not photosynthetic. Most of them are saprophyte, growing by decomposing organic matter. Some actinomycetes are human or plant pathogens, for example potato scab disease by *Streptomyces scabies* and *S. turgidiscabies* (Lehtonen and *et. al.*, 2004) but some strains are particularly beneficial, genus *Frankia* form associations with woody shrubs (non-leguminous plants) and fix nitrogen. They are found in soil, sediment and composts. These microorganisms require $O_2$ for growth. Their spores can tolerant desiccation, and the spores of thermophilic actinomycetes were found to be resistant to high temperatures at maximum resistance to 100° C (Charles, 2005). Furthermore, actinomycetes are tolerant of alkaline conditions. In alkaline soils, 95% of the microbial isolates may be actinomycetes. At pH of less than 5, actinomycetes make up less than 1% of the microbial population (Coyne, 1999).

## 2. Characteristics of the genus *Streptomyces*

*Streptomyces* are gram-positive bacteria in family Streptomycetaceae, order Actinomycetales (Table 2.1). Germination of spores or fragmentation of vegetative mycelium, develops into hyphae (branching filament) that penetrate the agar (substrate mycelium), and the hyphae that branch repeatedly and become cemented to the surface of the agar forming a tough, leathery colony. *Streptomyces* colony is 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 turn to a range of colors when begin spore formation. Colonies then appear powdery or velvety and can then be readily distinguished from the typical bacterial 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, 198; Cross, 1994).

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

Table 2.1 Key to morphological characteristics of family Streptomycetaceae[a] (Holt, 1989; Cross, 1994)

| Genus | Morphological characteristics |
|---|---|
| *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 isolated from soil 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). The cell wall peptidoglycan contains major amount of L-diaminopimelic acid (L-DAP). The lack of mycolic acid but contain major amounts of saturated iso- and anteiso- fatty acids; possess either hexa- or actahydrogenated menaquinones with nine isoprene units as the predominant isoprenolog, and have complex polar lipid patterns that typically contain diphosphatidylinositol and phosphatidylinositolmanosides. The predominant menaquinone component is one group, MK-9 group. The range of G+C contents of the DNA was 69-78 mol%.

Streptomycetes strains are widely distributed in terrestrial and aquatic habitats. Most are strict saprophytes, but some form parasitic associations with plants or animals. But little is know about the role of streptomycetes in natural environments, although evidence of their occurrences and numbers in habitats is extensive. Recent reviews on streptomycete ecology are Cross, 1981; Kutzner, 1981; Goodfellow and Williams, 1983; Williams *et al.*, 1984; Goodfellow and Simpson, 1987.

The survival capacities of streptomycete spores are greater than that of the hyphae. Streptomycete spores have a net negative surface charge except at low pH and, a relatively low endogenous metabolisms 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, streptomycetes counts decrease markedly, 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 growth of streptomycetes inoculated into sterile soil occur at moisture tensions between pH 1.5 and 2.5. Some streptomycetes isolated from acid soil are able to grow on media at high osmotic potentials (Wong and Griffin, 1974)

Soil, fodder and composts are the primary reservoirs for streptomycetes. Specific growth rate and doubling time for streptomycetes in laboratory culture are approximately intermediate between those of bacteria and fungi (Flowers and Williams, 1977). PH is clearly an important factor determining the distribution and activity of streptomycetes. Acidophilic which is neutrotolerant streptomycetes grows between pH 3.5 and 7.5, but optimally around pH 5.5 are common in acid soils (Khan and Williams, 1975).

## 2.1 Criteria used for classification and identification of *Streptomyces* species

The 15 criteria used for classification and identification of *Streptomyces* species are summarized in Table 2.2 (Holt, 1989).

**Table 2.2 Criteria for classification and identification *Streptomyces* species**

| Characters | Character states |
|---|---|
| 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, blue, green, red-orange or violet. 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. |

Table 2.2 Criteria for classification and identification *Streptomyces* species

| Characters | Character states |
| --- | --- |
| 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. |
| 13. Growth in the presence of inhibitory compounds (% w/v) | Crystal violet (0.0001), phenol (0.1), phenylethanol (0.1, 0.3), potassium 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. |

## 3. Antibiotics from *Streptomyces* species

Antibiotic is a chemical substance, produced by microorganisms, has a capacity to inhibit growth or destroy bacteria and other microorganisms (Waksman, 1953). Antibacterial and antifungal produced by *Streptomyces* recently reported were shown in Table 2.3

Table 2.3 Antimicrobial agents produced by *Streptomyces*

| Antimicrobial agents | 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 grampositive bacteria and some fungi | Bertasso *et al.*, 2002 |
| Benzanthraquinone YM-181741 | *Streptomyces* 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 spectrum antimicrobial activity against gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) | Igarashi *et al.*, 1998 |

Table 2.3 Antimicrobial agents produced by *Streptomyces*

| Antimicrobial agents | Strains | Activity | References |
|---|---|---|---|
| Cyclomarins A, B and C | *Streptomyces* sp. CNB-982 | Potent anti-inflammatory 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'-demethyinovobiocins | *Streptomyces* sp. TP-A0556 | Antibacterial activity against Gram-positive and negative bacteria | Sasaki *et al.*, 2001 |
| Dihydrophencomycing | *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, *et al.*, 1996 |
| Feigrisolide B | *Streptomyces* sp. *griseus* | Strong antibacterial activity, as well as medium cyctotoxic and antiviral activity | Tang *et al.*, 2000 |
| Hygromycin B | *Streptomyces hygroscopicus* | Against bacteria, fungi and higher eukaryotic cells by inhibiting protein synthesis | Sugden, B. *et al.*, 1985 |

Table 2.3 Antimicrobial agents produced by *Streptomyces*

| Antimicrobial agents | Strains | Activity | References |
|---|---|---|---|
| Lactonamycin | *Streptomyces* sp. *rshiriensis* MJ 773-88K4 | Antimicrobial activity against Gram-positive bacteria including MRSA and vancomycin-resistant *Enterococcus* (VRE) | Matsumoto *et al.*, 1999 |
| Mannopeptimycins ε | *Streptomyces hygroscopicus* LL-AC98 | Antimicrobial activity against Staphylococccus and enterococcus | Singh *et. al.*, 2003 |
| Mathemycin B | *Streptomyces* sp. | Inhibition of *Fusarium culmorum* 100 | Mukhopadhya y *et al.*, 1999 |
| 2-methyl-heptyl isonicotinate | *Streptomyces* sp. 201 | Antimicrobial activity against *B. subtilis*, *Shigella* sp., *Klebsiella* sp., *E. coli*, *Proteus mirabilis* and pathogenic fungi | Bordoloi *et al.*, 2002 |
| Methylsulfomycin 1 | *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 *et al.*, 1999 |

Table 2.3 Antimicrobial agents produced by *Streptomyces*

| Antimicrobial agents | Strains | Activity | References |
|---|---|---|---|
| Niddamycin and celesticetin | *Streptomyces* sp. caelestis | Antimicrobial activity against gram-negative and gram-positive bacteria | Mellouli, *et al.*, 2003 |
| Pentaene macrolide TPU-0043 | *Streptomyces* sp. TP-A0625 | Antifungal activity | Yasuhiro, *et al.*, 2005 |
| Radamycin | *Streptomyces* sp. RSP9 | Potent antibiotic activity against several Gram-positive bacteria | Gonzalez-Holgado *et al.*, 2002 |
| Rubiginones $D_2$, H and 1 | *Streptomyces* sp. Go N1/5 | Growth inhibition of Gram-positive bacteria and cytostatically active against different tumor cell lines | Puder, *et al.*, 1999 |
| Spirofungin | *Streptomyces* sp. *violaceusniger* Tu 4113 | Antimicrobial activity against various fungi and particularly yeasts | Holtzel *et al.*, 1998 |
| Streptocidins A-D | *Streptomyces* sp. Tu 6071 | Antibiotic against Gram-positive bacteria | Gebhardt, *et al.*, 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 |

Table 2.3 Antimicrobial agents produced by *Streptomyces*

| Antimicrobial agents | Strains | Activity | References |
|---|---|---|---|
| Watasemycins A and B | *Streptomyces* sp. TP-A0597 | Antibiotic activity against Gram-positive and negative bacteria, and yeasts | Sasaki *et al.*, 2002 b |
| Yatakemycin | *Streptomyces* sp. TP-A0356 | Antifungal activity against *Aspergillus fumigatus*, *Candida albicans* also cytotoxicity against cancer cell line | Yasuhiro *et. al.*, 2002 |
| Zelkovamycin | *Streptomyces* sp. K 96-0670 | Antimicrobial activity against *Xanthomonas oryzae*, *Acholeplasma laidlawii* and *Staphylococcus aureus* | Zhang *et al.*, 1999 |

## 4. Screening of antimicrobial producing actinomycetes

### 4.1 Primary screening

Waskman and Starkey (1987) reported that during a study of actinomycetes plate counting, some of actinomycetes colonies exhibited inhibition zone, an area where was free from bacterial and fungal growth. By far testing of these antagonistic properties are the most successful method for screening of antibiotic producing actinomycetes. Actinomycetes which demonstrate an antagonistic property by producing a diffusible substance effective upon the test microorganisms must also demonstrate that their diffusible substances are produced in liquid media (Waskman, 1967). Since antibiotics must be obtained in liquid media for large-scale production.

Agar diffusion method is a classical primary screen method for detection and isolation of antibiotic producing microorganisms. The method has also been used to monitor an antibiotic production in fermentation process (Shiring and Gottlieb, 1966). Primary screening can be both qualitative and quantitative approaches. The qualitative approach reveals a spectrum or range of microorganisms, which is sensitive to the antibiotic. The quantitative approach reveals the yield of the antibiotic produced.

### 4.2 Secondary screening

Secondary screening emphasizes on factors affected on growth and antibiotic production, for example, pH, aeration, nutrient requirement. These formations will be used for decision making on their commercial potential.

## 5. Antibiotic production of *Streptomyces*

Optimal conditions for antibiotic, secondary metabolite, production are different from optimal conditions for growth, and primary metabolite production. Antibiotic production depends on several factors. One of them is medium composition (Luria, 1960).

## 5.1 Effect of nutrient on antibiotic production by *Streptomyces*

Medium composition used for antibiotic production by various strains of *Streptomyces* were shown in Table 2.4

### 5.1.1 Carbon source

Nearly all species of *Streptomyces* are chemoheterotrophs. The organisms are usually found naturally in soil and have an ability to utilize a variety of organic carbon sources. The most common carbon substrates used for antibiotic production are starch, oil, and various types of simple sugars. Since, the carbon substrate is usually the major medium component and frequently, is the major cost of medium. Therefore, suitable carbon substrate should be evaluated from both antibiotic yield and the carbon substrate cost.

### 5.1.2 Nitrogen source

The streptomycetes can assimilate various form of nitrogen. The nitrogen sources usually employ in industrial antibiotic fermentations are protein, ammonia and ammonium salt, urea, and nitrate salt. The streptomycetes generally utilize the nitrogen sources in the order presented above (Waksman. 1967). When carbon source is depleted, the streptomycetes will utilize protein and release ammonia to the medium. This causes a rise in the pH of the culture broth. Excessive ammonia level in culture broth inhibits antibiotic production (Young, 1981; Masuma *et al.*, 1983).

### 5.1.3 Phosphorus source

Phosphorus is supplied to the fermentation medium in the form of inorganic phosphate (Waksman, 1967) and/or complex organic phosphate. The complex phosphate is generally present in the natural products used to supply protein to the medium. Excess phosphate is a strong inhibitor or repressor of antibiotic production.

#### 5.1.4 Sulfur source

Sulfur is usually supplied to medium as sulfate, but some sulfur is also available from cysteine and methionine of protein. Most streptomycetes media contain excess of sulfur, as sulfur does inhibit neither growth nor antibiotic production.

#### 5.1.5 Major cations

The major cations; including sodium, potassium, calcium and magnesium; are supplied to the medium as counter ions of sulfate, phosphate, chloride, etc., which naturally occur as minerals in water, and as minerals in other complex substrates. The effect of the major cations on the antibiotic production is complicate. In some cases, high level of a particular cation can either stimulate or inhibit product formation. Most of microorganisms require a proper balance of sodium and potassium. Calcium and magnesium can form insoluble salts and resulted in removal of nutrients from medium.

#### 5.1.6 Trace minerals

Numerous enzymes require certain metal ions as cofactors. The ions those are often required including manganese, iron, cobalt, copper, zinc, and molybdenum. They are generally required in very low concentration but are essential. Excessive level can inhibit product formation. Many of these ions are readily available in water and complex nutrients.

Table 2.4 Medium composition and conditions for antibiotic production of *Streptomyces* strains

| Strains | Antibiotics | Seed | | Production | | Reference |
|---|---|---|---|---|---|---|
| | | medium | conditions | medium | conditions | |
| *Streptomyces* sp. Tu-6071 | Streptocidins A-D | Mannitol, soybean meal | pH 7.5, 27°C, 120 rpm, 2 days | Mannitol, soybean meal | pH 7.5, 27°C, 120 rpm, 6 days | Gebhardt *et al.*, 2001 |
| *Streptomyces* sp. WK-6326 | 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 al.*, 2002 |
| *Streptomyces* sp. Q27107 | 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 | Kobayashi *et al.*, 2001 |

Table 2.4 Medium composition and conditions for antibiotic production of *Streptomyces* strains

| Strains | Antibiotics | Seed | | Production | | Reference |
|---|---|---|---|---|---|---|
| | | medium | conditions | medium | conditions | |
| *Streptomyces* sp. TP-A0584 | Goadsporin | Soluble starch, glucose, NZ-case, yeast extract, Bacto Tryptone, $K_2HPO_4$ $MgSO_4.7H_2O$, $CaCO_3$ | pH 7.0, 30°C, 2 days | Soluble starch, glucose, glycerol, pharmamedia, yeast extract, Diaion HP-20 | pH 7.0, 30°C, 4 days | Onaka *et al.*, 2001 |
| *Streptomyces* sp. RSP9 | Radamycin | Yeast extract, sucrose, xylose, $MgCl_2$ | pH 7.2, 28°C, 200 rpm, 1 days | Yeast extract, sucrose, xylose, $MgCl_2$ | pH 7.2, 28°C, 200 rpm, 4-7 days | Gonzalez-Holgado *et al.*, 2002 |
| *Streptomyces* sp. TA-0403 | 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 | Matsuura *et al.*, 2002 |

## 6. Characteristics of the genus *Micromonospora*

*Micromonospora* formed well-developed, branched, septate substrate mycelium and single conidia are produced. This microorganisms, normally lacking aerial mycelium, forming light yellow-orange to orange-red colonies (occasionally brown, maroon or blue green) composed of tightly woven, fine hyphae (0.2-0.6 $\mu$m in diameter). The dark brown to black spores are formed within and at the surface of the colonies which darken as a result of sporulation and usually turn black and may become mucoid. The single spores are borne in dense clusters on repeatedly branched sporophores (cluster type) or are well dispersed throughout the mycelium. Sporophore branching may be monopodial or sympodial (Sykes and Skinner, 1973). The most *Micromonospora* strains are sensitive to pH below 6.0. Growth occurs normally between 20$^{\circ}$C and 40$^{\circ}$C but not above 50$^{\circ}$C (Holt, 1989). This organism could grow in 1.5%-5% NaCl concentration, normally could grow in 3% NaCl. The temperature range for growth is 15-45$^{\circ}$C, and the optimal temperature is 25-30$^{\circ}$C. All strains of *micromonospora* showed positive results for gelatin liquefaction.

Chemotaxonomically, the genus *Micromonospora* is characterized by a cell wall type II (Lechevalier and Lechevalier, 1970), and a phospholipids type II (Lechevalier, DeBievre, and Lechevalier, 1977). The cell walls of *Micromonospora* have been found to contain glycine, glutamic acid, *meso*-diaminopimelic acid (*meso*-DAP), and D-alanine in a molar ratio of 1:1:1:0.6-0.8. The phospholipids contained in the cells are diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, and phosphatidylethanolamine, but phosphatidylcholine is not detected. The predominant cellular fatty acids are *iso*- and *anteiso*- branched fatty acid. The predominant menaquinone components can be divided into three groups, MK-9, MK-10 and MK-12 groups. The range of G+C contents of the DNA was 71-73 mol%. *Micromonospora* is the large group of rare actinomycetes that can produce a large number of antibiotics. Approximately 6% of actinomycetes antibiotics came from *Micromonospora*. Bioactive compounds produced from *Micromonospora* strains are summarized in Table 2.5

Table 2.5 Antimicrobial agents produced by *Micromonospora*

| Antimicrobial agents | Strains | Activity | References |
|---|---|---|---|
| Arisostatins A and B | *Micromonospora* sp. TP-A0316 | Antibiotic activity against Gram-positive bacteria and antitumor activity | Furumai *et. al*., 2000 |
| Calicheamicins | *Micromonospora echinospora* subsp. calichensis | Antitumor activity against P388 leukemia and B16 melanoma in vivo. | Maiese *et. al*., 1989 |
| Echinosporamicin | *Micromonospora echinospora* | Antibacterial activity | He *et. al*., 2004 |
| Galtamycin B, retymicin, and ribofuranosyllu-michrome | *Micromonospora* sp. strain Tu 6368 | Cytostatic effects to various human tumor cell lines | Stroch *et. al*., 2005 |
| Kosinostatin | *Micromonospora* sp. TP-A0468 | Antitumor activity | Furumai *et. al*., 2002 |
| Micromonosporin A | *Micromonospora* sp. strain TT1-11 | Antibacterial activity, antimalarial activity, and antimycobacterial activity | Thawai *et. al*., 2004 |
| Saquayamycin Z | *Micromonospora* sp. strain Tu 6368 | Cytostatic effects to various human tumor cell lines and active against Gram-positive bacteria | Stroch *et. al*., 2005 |
| Thiocoraline | *Micromonospora* sp. strain L-13-ACM2-092 | Antimicrobial activities | Romero *et. al*., 1997 |