CHAPTER II

LITERATURE REVIEW

1. Actinomycetes

Actinomycetes are prokaryotes that look like the fungal because they grow as filamentous mycelia and form spores. There are two essential features that distinguish actinomycetes from fungi, first they are prokaryotic that have no cell nucleus, second hypha are from 0.5 to 1.0 μm in diameter, which are much smaller than fungal hypha (which are 3-8 μm in diameter). Most actinomycetes are saprophytes, growing by decomposing organic matters. They compose 10% to 50% of the total microbial population in soil. Actinomycetes are found in soil (most commonly), composts and sediment (Coyne, 1999). They are divided into 9 families including Actinomycetaceae, Mycobacteriaceae, Frankiaceae, Actinoplanaceae, Dermatophilaceae, Nocardiaceae, Pseudonocardiaceae, Micromonosporaceae and Streptomycetaceae (Goodfellow and Board, 1980; Lechevalier *et al.*, 1986; Embley *et al.*, 1988; Warwick *et al.*, 1994; Kim *et al.*, 2003).

1.1 *Streptomyces*

The genus *Streptomyces* are gram-positive bacteria in the family Streptomycetaceae, order Actinomycetales. Nowadays *Streptomyces* contain about 527 species. They are aerobic bacteria processing vegetative hypha (0.5-1.0 μm in diameter), which produce an extensive branched mycelia that rarely fragment. They are long chain spores. Spores are non-motile (Goodfellow, 1988).

1.1.1 Characteristics of *Streptomyces*

Morphological and culturals characteristics:

Cultural characteristics of the genus *Streptomyces* on various culture media are such characters as the colors of soluble pigments, the colors of the vegetative growth, the aerial mycelium and the micromorphology of the sporulation structures have been used as criteria for descriptions the species of *Streptomyces*. Spore chain morphological is spiral, retinaculiaperti,

rectiflexibiles. Spore surface have been characterized by terms rugose, hairy, smooth, warty or spiny. Spore color is blue, gray, green, red, violet, white or yellow. Pigmentation of substrate mycelium is yellow-brown, blue, green, red-orange or violet and colonies on agar media are granular, powdery, velvety or floccose (Goodfellow, 1988; Cross *et al.*, 1994; Collin *et al.*, 1977, Holt, 1989).

Physiological and biochemical characteristics :

Physiological and biochemical properties such as hydrolysis reaction (starch, gelatin, or milk), nitrate reduction, melanin formation and utilization of carbon sources have been used extensively to characterize *Streptomyces* strains and species. The optimum temperature for growing is 25-37 ^{o}C and the optimum pH range for growth is 6.5-8.0 (Brock *et al.*, 1993).

Chemotaxonomic characteristics:

The cell walls of *Streptomyces* strains have been found to contain N- acetylglucosamine, N- acetyl muramic acid, D- alanine, D- glutamic acid and glycine with LL- diaminopimelic acid. No characteristic sugar pattern .Phospholipids pattern is phospholipids type II (Lechevalier *et al.*, 1977). The predominant cellular fatty acid profile are saturated iso- and anteiso-branched fatty acid, but lack of mycolic acid (Kroppenstedt, 1985). Major menaquinones are MK-9 (H_6) and MK-9 (H_8). DNA G+C content is in the range 69-78 mol% (Goodfellow, 1988; Cross *et al.*, 1994; Collin *et al.*, 1977).

Streptomyces are ubiquitous in nature. Their ability to colonise the soil in greatly facilitated to growth as a vegetative hyphal mass which can differentiate into spores that assist in spread and persistence. The spores are a semi-dormant stage in the life cycle that can survive in soil for long periods. Moreover they are found in aquatic habitats such as lake, mud, river sediment, marine sediment and forest. They also occur in estuarine environment such as sandy sediment, intertidal sediment and sediment samples collected from the eutuarine environment at the mouth of stream (Goodfellow and William, 1983; Jensen *et al.*, 1991; Takahashi and Omura, 2003). Soil fodder and composts appear to be the primary reserviors for *Streptomyces* (Flower and Williams, 1977).

1.1.2 Antimicrobial compounds from *Streptomyces*

Antibiotics are secondary metabolites. Most of them are produced from microorgamisms in the group of actinomycetes. They have been extensively studied and have been used in agriculture, food, nutrition, and especially medical science (Goodfellow, 1988).

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).

The genus *Streptomyces* was found to produce many groups of antibiotics such as aminoglycosides, macrolides or ansamycins, β-lactam antibiotics, peptides, glycopeptides, anthracyclines, tetracyclines, nucleosides, polyenes, and quinines (Okami and Hotta, 1988).

The strains of *Streptomyces* are valuable because they produce most of commercial antibiotics. Examples include amphotericin B produced by *Streptomyces nodosus*, tetracycline produced by *Streptomyces aureofaciens*, erythromycin produced by *Streptomyces erythraeus*, neomycin produced by *Streptomyces fradiae*, nystatin produced by *Streptomyces noursei* and streptomycin produced by *Streptomyces griseus* (Tortota *et al.*, 1995). Examples of antimicrobial compounds produced by *Streptomyces* strains were shown in Table 2.1.

Table 2.1 Antimicrobial compounds from *Streptomyces* strains

Compounds	Strains	Activity	References
Actinomycin Z	*S. fradiae*	Growth inhibition of *B. subtilis* ATCC 6051	Lackner *et al.*, 2000
Antimycins	*Streptomyces* sp. SPA 10191 and SPA 8893	Antifungal activity	Hosotani *et al.*, 2005
Arylomycin A and B	*Streptomyces* sp. TU 6075	Antimicrobial activity against gram-positive bacteria	Schimana *et al.*, 2002
Bafilomycin B1 and C 1	*S. halstedii* K 122	Antifungal activity	Frandberge *et al.*, 2000
Bagremycin A and B	*Streptomyces* sp. TU 4128	Antimicrobial activity against gram- positive bacteria and fungi	Bertasso *et al.*, 2001

Table 2.1 Antimicrobial compounds from *Streptomyces* strains (continued)

Compounds	Strains	Activity	References
Cedamycin A and B	*Streptomyces* sp. TP- A0456	Antimicrobial activity against gram-positive and gram-negative bacteria and fungi	Sasaki *et al.*, 2001
Chalocomycin B	*Streptomyces* sp. B 7064	Antimicrobial activity against gram-positive bacteria	Asolkar *et al.*, 2002
Demethyl mutactimycins	*Streptomyces* sp. GW 60/1571	Antimicrobial activity against gram- positive bacteria	Speitling *et al.*, 1998
5' – and 7'-demethylnovobiocins	*Streptomyces* sp. TP- A0556	Antimicrobial activity against gram-positive and gram-negative bacteria	Sasaki *et al.*, 2001
(*E*)-4-Oxonon-2-enoic acid	*S. olivaceus*	Growth inhibition of gram-positive and gram-negative bacteria	Ballini and Bosica, 1998
Enterocin	*Streptomyces* sp. BD-26T	Antimicrobial activity against gram-positive and gram-negative bacteria	Sitachitta *et al.*, 1996
Feigrisolide B	*S. griseus*	Strong antimicrobial activity and antiviral activity	Tang *et al.*, 2000
Geldanamycin and 17-0-demethylgeldanamycin	*S. hygroscopicus*	Antibacteria, Antitumor, and herbicide	Glasby, 1993
Istamycins	*S. tenjimariensis* SS-37	Antimicrobial activity against gram-positive and gram-negative bacteria including aminoglycoside resistant strains	Hotta *et al.*, 1980
Kanchanamycins	*S. olivaceus* TU 4081	Antibacterial and antifungal especially effective against *Pseudomonas fluorescens*	Fiedler *et al.*, 1996

Table 2.1 Antimicrobial compounds from *Streptomyces* strains (continued)

Compounds	Strains	Activity	References
Lactonamycin	*S. rishiriensis* MJ 773-88K4	Antimicrobial activity against gram-positive bacteria including MRSA and vancomycin-resistant *Enterococcus* (VRE)	Matsumoto *et al.*, 1999
Lydicamycin	*S. platensis* TP-A0598	Antimicrobial activity against gram-positive bacteria including MRSA	Furumai *et al.*, 2002
Macrolide TPU-0043	*Streptomyces* sp. TP-AO625	Antifungal activity	Igarashi *et al.*, 2005
Meroparamycin	*Streptomyces* sp. MAR01	Antimicrobial activity against gram-positive and gram-negative bacteria and *Candida albicans*	Naggar *et al.*, 2006
2- methylheptyl isonicotinate	*Streptomyces* sp. 201	Antimicrobial activity against gram-positive and gram-negative bacteria and fungi	Bordoloi *et al.*, 2001
Methylsulfomycin I	*Streptomyces* sp. HLI Y-9420704	Antimicrobial activity against gram-positive bacteria including MRSA and vancomycin and teicoplanin resistant strain	Vijaya, 1999
Midecamycin	*S. mycarofaciens*	Antimicrobial activity against gram-positive bacteria and against *Legionella* and *Campylobacter* sp.	Harold, 1983
4-phenyl-3-butenoic acid	*S. koyangensis* VK-A60	Antifungal activity	Lee *et al.*, 2005

Table 2.1 Antimicrobial compounds from *Streptomyces* strains (continued)

Compounds	Strains	Activity	References
Resistoflavine	*S. chibaensis* AUBN1/7	Antimicrobial activity against gram-positive and gram-negative bacteria and potent cytotoxic against cell lines (Gastric adenocarcinoma and Hepatic carcinoma)	Gorajana *et al.*, 2006
Ripromycin	*Streptomyces* sp. TU 6239	Antimicrobial activity against gram-positive bacteria and cytostatic effects on various tumor cell lines	Bertasso *et al.*, 2003
RS-22A, B and C (macrolide antibiotics)	*S. violaceusniger*	Antimicrobial activity against gram-positive bacteria and fungi	Ubukata *et al.*, 1995
SRB-22 polyketide antibiotic	*S. psammoticus*	Antimicrobial activity against methicillin resistant *Staphylococcus aureus*	Sujatha *et al.*, 2005
Spectinomycin, Actinospectin, Desertomycin, Spectomycin, Streptovaricin, Spectinabilin	*S. spectabilis*	Antimicrobial activity against *Neisseria gonorrhoea* and especially in treatment of penicillin-resistant *gonorrhea*, and against gram-positive and gram-negative bacteria	Yu and Fan, 1994; Ivanova, 1997; Staley and Rinehart, 1994; Spasova *et al.*, 1997; Kakinuma *et al.*, 1997
Spinamycin	*S. albospinus*	Antitumor	Wang *et al.*, 1996
Streptocidins A-D	*Streptomyces* sp. TU 6071	Antimicrobial activity against gram-positive bacteria	Gebhardt *et al.*, 1999

Table 2.1 Antimicrobial compounds from *Streptomyces* strains (continued)

Compounds	Strains	Activity	References
Swalpamycin	*Streptomyces* sp. Y-84, 30967	Antimicrobial activity against gram-positive bacteria including erythromycin resistant strains	Franco *et al.*, 1987
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	Antimicrobial activity against gram-positive and gram-negative bacteria and yeast	Sasaki *et al.*, 2002
Yatakemycin	*Streptomyces* sp. TP-A0356	Antifungal activity	Igarashi *et al.*, 2003
Zelkovamycin	*Streptomyces* sp. K 96-0670	Antimicrobial activity against *Xanthomonas oryzae*, *Acholeplasma laidlawii* and *Staphylococcus aureus*	Zhang *et al.*, 1999

1.2 *Amycolatopsis*

The genus *Amycolatopsis* was established by Lechevalier *et al.*, (1986) and was assigned to order Actinomycetales, family Pseudonocardiaceae (Embley *et al.*, 1988; Warwick *et al.*, 1994), which also contains the genera *Actinibispora, Actinopolyspora, Kibdelosporangium, Prauserella, Pseudonocardia, Saccharomonospora, Saccharopolyspora, Thermocrispum* (Kim and Goodfellow, 1999; Labeda and Kroppenstedt, 2000). Recently, increasing interest has been shown in *Amycolatopsis*, as it contains that produce commercially significantly bioactive compounds. Nowadays *Amycolatopsis* contain about 33 species namely *Amycolatopsis alba*

(Mertz and Yao, 1993), *A. albidoflavus* (Lee and Hah, 2001), *A. australiensis* (Tan *et al.*, 2006), *A. azurea* (Henssen *et al.*,1987), *A. balhimycina* (Wink *et al.*, 2003), *A. benzoatilytica* (Majumdar *et al.*, 2006), *A. coloradensis* (Labeda,1995), *A. decaplanina* (Wink *et al.*, 2004), *A. eurytherma* (Kim *et al.*, 2002), *A. fastidiosa* (Henssen *et al.*, 1987), *A. halotolerans* (Lee, 2006), *A. japonica* (Goodfellow *et al.*, 1997), *A. jejuensis* (Lee, 2006), *A. kentuckyensis* (Labeda *et al.*, 2003), *A. keratiniphila* (Almusallam *et al.*, 2003), *A. lexingtonensis* (Labeda *et al.*, 2003), *A. lurida* (Lechevalier *et al.*, 1986), *A. mediterranei* (Lechevalier *et al.*, 1986), *A. methanolica* (Boer *et al.*, 1990), *A. minnesotensis* (Lee *et al.*, 2006), *A. orientalis* (Pittenger and Brigham, 1956), *A. palatopharyngis* (Huang *et al.*, 2004), *A. plumensis* (Saintpierre *et al.*, 2005), *A. pretoriensis* (Labeda *et al.*, 2003), *A. rifamycinica* (Bala *et al.*, 2004), *A. rubida* (Huang *et al.*, 2001), *A. rugosa* (Marco and Spalla 1957), *A. sacchari* (Goodfellow *et al.*,2001), *A. sulphurea* (Lechevalier *et al.*, 1986), *A. taiwanensis* (Tseng *et al.*, 2006), *A. thermoflava* (Chun *et al.*, 1999), *A. tolypomycina* (Wink *et al.*, 2003), and *A. vancoresmycina* (Wink *et al.*, 2003).

1.2.1 Characteristics of *Amycolatopsis*

Morphological, cultural, physiological and biochemical characteristics:

Members of the genus *Amycolatopsis* produce branched, fragmenting aerial and substrate mycelium. *Amycolatopsis* strains able to form aerial mycelium and spores that produced on differentiated hyphae, spores borne in chains and resembling *Streptomycetes* (Goodfellow *et al.*, 1988). Colonies are colorless or yellow to orange. Soluble pigment rarely formed. The growth temperature range is 10-55 $^{\circ}$C and the pH range is 6.0-9.0 (Cross, 1994).

Chemotaxonomic characteristics:

The cell walls of *Amycolatopsis* strains have been found to contain N- acetylglucosamine, N- acetyl muramic acid , arabinose, galactose and *meso*-diaminopimelic acid. Phospholipids pattern is phospholipids type II (Lechevalier *et al.*, 1977). The absence of mycolic acids. The predominant cellular fatty acid profile are saturated iso- and anteiso-branched fatty acid. Major menaquinones are MK-9 (H_4) with a minor amount of MK-9 (H_2). DNA G+C content is in the range 66-73 mol% (Lechevalier *et al.*, 1986). The differential characteristics of A*mycolatopsis* species were shown in Table 2.2.

Table 2.2 Differential characteristics of A*mycolatopsis* species

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Soluble pigment	-	-	-	+	+	+	-	-	-	-	+	-	-	+	+	+	+	+	-	+	-
Acid production																					
Arabinose	+	+	+	+	-	w	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	ND	ND	ND
D-Galactose	+	+	+	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	ND	ND	+
meso- Inositol	+	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+
Lactose	w	+	+	+	-	-	+	+	-	+	-	+	-	+	-	+	+	+	ND	ND	+
Maltose	-	+	+	+	+	w	+	+	-	+	-	+	+	-	-	+	+	+	ND	ND	ND
D-(-)-Manitol	+	+	w	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	ND
Raffinose	-	+	-	+	w	+	+	-	+	-	-	-	+	+	-	-	w	-	-	+	+
L-(+)-Rhamnose	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+
D-(-)-Sorbitol	+	-	-	-	-	-	-	w	+	-	-	-	-	+	-	+	-	w	ND	ND	ND
D-(+)-Xylose	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	ND
Decomposition																					
Casein	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	ND	ND	ND
Gelatin	+	+	+	+	+	-	+	+	+	+	w	+	+	-	+	+	+	+	ND	ND	ND
Growth at																					
10 ° C	-	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	-	ND	ND	+
45 ° C	+	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	ND	ND	-
Growth in 5% NaCl	+	-	+	+	+	-	w	w	+	w	+	+	-	+	w	ND	+	+	ND	ND	+

Taxa:

1 *A. eurytherma* DSM 444348 ^{T}

2 *A. alba* DSM 44262 ^{T}

3 *A. albidoflavus* KCTC 9471 ^{T}

4 *A. azurea* NRRL 11412 ^{T}

5 *A. coloradensis* NRRL 3218 ^{T}

6 *A. fastidiosa* NRRL B - 16697 ^{T}

7 *A. japonica* DSM 44213 ^{T}

8 *A. mediterranel* ATCC 13685 ^{T}

9 *A. methanolica* IFO 15065 ^{T}

10 *A. orientalis* NRRL 2450 ^{T}

11 *A. ruubida* JCM 10871 ^{T}

12 *A. sacchari* DSM 44468 ^{T}

13 *A. sulphurea* DSM 46092 ^{T}

14 *A. thermoflava* IFO 14333 ^{T}

15 *A. rifamycinica* DSM 46095 ^{T}

16 *A. kentuchyensis* NRRL B - 14129 ^{T}

17 *A. lexingtonensis* NRRL B - 24131 ^{T}

18 *A. pretoriensis* NRRL B - 24133 ^{T}

19 *A. tolyponycina* DSM 44544 ^{T}

20 *A. vancoresmycina* DSM 44592 ^{T}

21 *A. keratiniphila* KCTC 19104 ^{T}

+,Positive W, Weak reaction

-, Negative ND, No data

1.2.2 Antimicrobial compounds from *Amycolatopsis*

Amycolatopsis is one of the important genera in the antibiotics industry. It produces some of the most widely used anti-infectious agents, such as rifamycin, vancomycin. Among them, rifamycim is one of the major drugs for clinical treatment of HIV-relate tuberculosis, and vancomycin is currently considered as the last line of those defense against some microorganisms that are resistant to β- lactam antibiotics (Yao *et al.*, 2002). Considerable interest is being shown in the genus *Amycolatopsis*, as it contains many strains that are the source of many bioactive compounds as shown inTable 2.3.

Table 2.3 Antimicrobial compounds from *Amycolatopsis* strains

Compounds	Strains	Activity	References
Amythiamicins	*Amycolatopsis* strain MI481-42F4	Antimicrobial activity against gram-positive bacteria including multi-drug resistant strains	Shimanaka *et al.*, 1994
Azicemicins A and B	*Amycolatopsis* strain MJ126-NF4	Antimicrobial activity against gram-positive bacteria and *mycobacterium*	Tsuchida *et al.*, 1995
Balhimycin	*A. balhimycina*	Antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA)	Wink *et al.*, 2003
Decaplanin	*A. decaplanina*	Antimicrobial activity against gram-positive bacteria including *enterococci* and against methicillin resistant *Staphylococcus aureus* (MRSA)	Wink *et al.*, 2004
MJ347-81F4 A and B	*Amycolatopsis* strain MJ347-81F4	Antimicrobial activity against gram-positive bacteria and against methicillin resistant *Staphylococcus aureus* (MRSA)	Sasaki *et al.*, 1998
Nogabecin	*A. keratiniphila*	Antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA)	Wink *et al.*, 2003
Rifamycin	*A. mediterranei*	Antimicrobial activity against *M. tuberculosis* and *M. leprae*	Mejia *et al.*, 1997

Table 2.3 Antimicrobial compounds from *Amycolatopsis* strains (Continued)

Compounds	Strains	Activity	References
Tolypomycin	*A. tolypomycina*	Antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA)	Wink *et al.*, 2003
Vancomycin	*A. orientalis*	Antimicrobial activity against gram-positive bacteria and some microorganisms that are resistant to β-lactam antibiotics	Pittenger and Brigham, 1956
Vancoresmycin	*A. vancoremycina*	Antimicrobial activity against gram-positive bacteria and against vancomycin resistant strains like *Enterococcus* sp.	Hopmann *et al.*, 2002

1.3 *Kitasatospora*

The genus *Kitasatospora* was proposed by Omura *et al.*, in 1982 for actinomycete strains (Omura *et al.*, 1982), re- established by Zhang and coworkers in 1997. The *Kitasatospora* are gram- positive bacteria in the family Streptomycetaceae, order Actinomycetales (Zang *et al.*, 1997). Nowadays *Kitasatospora* contain about 20 species namely *Kitasatospora arboriphila* (Groth *et al.*, 2004), *K. azatica* (Zhang *et al.* 1997), *K. cheerisanensis* (Chung *et al.*, 1999), *K. cineracea* (Tajima *et al.*, 2001), *K. cochleata* (Zhang *et al.* 1997), *K. cystarginea* (Kusakabe and Isono, 1992), *K. gansuensis* (Groth *et al.*, 2004), *K griseola* (Takahashi *et al.*, 1985), *K. kifunensis* (Groth *et al.*, 2003), *K. mediocidica* (Labeda, 1988), *K. niigatensis* (Tajima *et al.*, 2001), *K. nipponensis* (Groth *et al.*, 2004), *K. paracochleata* (Zhang *et al.*, 1997), *K. paranensis* (Groth *et al.*, 2004), *K. phosalacinea* (Takahashi *et al.*, 1985), *K. putterlickiae* (Groth *et al.*, 2003), *K. sampliensis* (Mayilraj *et al.*, 2006), *K. setae* (Mayilraj *et al.*, 2006), *K. terrestris* (Groth *et al.*, 2004), *K. viridis* (Liu *et al.*, 2005).

1.3.1 Characteristics of *Kitasatospora*

Morphological, cultural, physiological and biochemical characteristics:

The *Kitasatospora* strains were phenotypically similar to the *Streptomyces* strains.The substrate mycelium, and the aerial mycelium bears long spore chains containing more than 20 spores. No fragmentation of substrate mycelium occurs. No sporangium are formed. The growth temperature range is 15-42 ^{o}C and the pH range is 6.5-8.0 (Zang *et al.*, 1997).

Chemotaxonomic characteristics:

The cell wall of *Kitasatospora* strains have been found to contain N- acetylglucosamine, N- acetyl muramic acid , glycine, galactose and *meso* and LL- diaminopimelic acid, depending on the type of cells analyzed. When cell are grown on agar media, the aerial spores contain LL-DAP, whereas the substrate mycelium contain *meso*-DAP. When cells are grow in liquid media, submerged spores are formed, and the spores contain LL-DAP and the filamentous mycelia contain *meso*-DAP. Phospholipids pattern is phospholipids type II (Zang *et al.*, 1997). The predominant cellular fatty acid profile are saturated iso- and anteiso-branched fatty acid, but lack of mycolic acid (Kroppenstedt, 1985). Major menaquinones are MK-9 (H_6) and MK-9 (H_8). DNA G+C content is in the range 66-76 mol% (Zang *et al.*, 1997)

The genus *Kitasatospora* can be distinguished from the genus *Streptomyces* by the ratio of *meso*-DAP to LL-DAP in whole cell hydrolysates. The *meso*-DAP content is 49 to 89 % in *Kitasatospora* strains and 1 to 16% in *Streptomyces* strains. Galactose is present in the whole-cell hydrolysates of *Kitasatospora* strains but not in the whole- cell hydrolysates of *Streptomyces* strains. The genus *Kitasatospora* can be readily distinguished from the genus *Streptomyces* by specific nucleotide signatures in the sequences of 16S rDNA. (Omura *et al.*, 1982; Zang *et al.*, 1997; Wellington *et al.*, 1992). The differential characteristics of *Kitasatospora* species were shown in Table 2.4.

Table 2.4 Differential characteristics of *Kitasatospora* species

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aerial mycelia	R	R	S	S	R	R	R	S	R	R	R	R	R	R	R
Melanin formation	-	-	+	-	-	-	-	+	-	+	+	-	-	-	+
Nitrate reduction	+	+	-	-	-	-	+	+	+	+	-	-	+	-	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Gelatin liquefaction	+	-	-	-	-	-	-	-	-	+	-	ND	ND	+	+
Utilization of carbohydrate															
L-Arabinose	+	+	+	-	+	+	+	-	-	-	+	+	+	-	+
D-Fructose	-	+	-	-	-	+	-	+	+	+	-	-	-	+	+
D-Manitol	-	-	-	-	-	ND	-	-	-	-	+	-	-	-	-
D-Sucrose	-	-	-	-	-	+	-	-	-	+	+	-	-	+	+
D-Xylose	+	+	-	-	+	+	+	-	+	-	+	+	+	-	+
G+C content (mol%)	75.8	70.5	72.4	70.6	66.0	74.0	73.2	73.1	73.3	ND	ND	73.7	73.5	ND	ND

Taxa: 1 *K. cheerisanensis* KCTC 2395 ^{T}

2 *K. azatica* IFO 13803 ^{T}

3 *K. cochieata* IFO 14768 ^{T}

4 *K. cystarginea* IFO 14836 ^{T}

5 *K. griseola* IFO 14371 ^{T}

6 *K. mediocidica* IFO 14789 ^{T}

7 *K. setae* KM - 6054 ^{T}

-, Negative; +,Positive; ND, No data

R, Rectiflexibiles; S, Spiral

8 *K. paracochleata* IFO 14769 ^{T}

9 *K. phosalacinea* IFO 14372 ^{T}

10 *K. putterlickiae* DSM 44665 ^{T}

11 *K. kifunesis* DSM 41654 ^{T}

12 *K. cineracea* IFO 16452 ^{T}

13 *K. niigatensis* IFO 16453 ^{T}

14 *K. nipponensis* DSM 44787 ^{T}

15 *K. terrestris* DSM 44789 ^{T}

1.3.2 Antimicrobial compounds from *Kitasatospora*

The data about antibiotics produced from *Kitasatospora* seem to be a little amount when compare with the antibiotics produced from *Streptomyces*, it may be that the study in this genus is still limited. Examples of antimicrobial compounds produced by *Kitasatospora* strains were shown in Table 2.5.

Table 2.5 Antimicrobial compounds from *Kitasatospora* strains

Compounds	Strains	Activity	References
Bafilomycin	*K. cheerisanensis*	Antifungal activity	Chung *et al.*, 1999
Cystargin	*K. cystarginea* RK-419	Antifungal activity	Kusakabe and Isono, 1988
Kimorexins	*K. kimorexae* 90-GT-302	Antifungal activity	Yeo *et al.*, 1994
Phosalacine	*K. phosalacinea* KA-338	Herbicidal activity	Takahashi *et al.*, 1984
Sch 725424 and Sch 725428 antibiotics	*Kitasatospora* sp. SPRI-0408	Antimicrobial activity against *Staphylococcus aureus* and weak antifungal activity against *Saccharomyces cerevisiae*	Yang *et al.*, 2005
Setamycin	*K. griseola* AM-9660	Antifungal activity	Takahashi *et al.*, 1984

1.4 Fermentation

In the production of antibiotic actinomycete strains are usually cultivated in various natural liquid media in shaking condition at 180-250 rpm at 27-28^{o}C. Most media consist of carbon (e.g. glycerol, glucose, starch) and nitrogen (e.g. ammonium sulphate, meat extract, peptone, soybean meal) sources in combination with inorganic phosphate, cations such as Ca, Mg and Na, or trace elements like Co, Cu, Fe, Mn and Zn. Since regulatory mechanisms for antibiotic

production are so varied, no single medium can be chosen for the production of all types of antibiotics. It is, therefore, partical to cultivate actinomycetes strains in media with varied comparisions in order to screen for diverse antibiotics (Goodfellow, 1988). Example of composition of media and condition for antibiotics production of *Streptomyces, Amycolatopsis*, and *Kitasatospora* strains were shown in Table 2.6.

Table 2.6 Composition of media and condition for antibiotics production of *Streptomyces, Amycolatopsis*, and *Kitasatospora* strains

Strains	Antibiotics	Medium		References
		Seed medium	Production medium	
Streptomyces sp. Tu-6071	Streptocidins A-D	Mannitol, soybean meal Condition : pH 7.5, 27°C, 120 rpm, 2 days	Mannitol, soybean meal Condition: pH 7.5, 27°C, 120 rpm, 6 days.	Gebhardt *et al.*, 2001
Streptomyces sp. RSP9	Radamycin	Yeast extract, sucrose, xylose, $MgCl_2$ Condition : pH 7.2, 28°C, 200 rpm, 1 day	Yeast extract, sucrose, xylose, $MgCl_2$ Condition : pH 7.2, 28°C, 200 rpm, 4-7days	Gonzalez *et al.*, 2002
S. avermectinius	Avermectins	Glycerol, cerelose, starch, beef extract, aradamine, $MgSO_4.7H_2O$, KH_2PO_4, Na_2HPO_4, $CaCO_3$ Condition : pH 7.0, 28°C, 220 rpm, 2 days	Cerelose, peptonized milk nutrient, ardamine, polyglycol P Condition : pH 7.0, 28°C, 220 rpm, 5 days	Burg *et al.*, 1979
S. chibaensis AUBN1-7	Resistoflavine	Soybean meal, corn steep solids, glucose, $CaCO_3$ Condition : pH 7.0, 28°C, 220 rpm,2 days	Soybean meal, corn steep solids, soluble starch, glucose, $CaCO_3$ Condition : pH 7.0, 28°C, 220 rpm, 3 days	Gorajana *et al.*, 2006

Table 2.6 Composition of media and condition for antibiotics production of *Streptomyces*, *Amycolatopsis*, and *Kitasatospora* strains (Continued)

Strains	Antibiotics	Medium		References
		Seed medium	Production medium	
Streptomyces sp. TP-AO625	Macrolide TPU-0043	Starch, glucose, NZ-case, yeast extract, tryptone, K_2HPO_4, $MgSO_4.7H_2O$, $CaCO_3$ Condition : pH 7.0, 30°C, 200 rpm, 4 days	Glucose, glycerol, soluble starch, pharmamedia (protein), yeast extract, KH_2PO_4, Na_2HPO_4, HP-20 resin Condition : pH 7.0, 30°C, 200 rpm, 7 days	Igarashi *et al.*, 2005
A. mediteeranei	Rifamycin B	Soybean meal, $CaCO_3$, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_20$ Condition : pH 7.2, 25°C, 250 rpm, 3 days	Glucose, Soybean meal, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, K_2HPO_4, $CaCO_3$, $CuSO_4.5H_20$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.7H_20$, $CoCl_2.6H_20$, MoO. Condition : pH 7.2, 25°C, 250 rpm, 6 days	Mejia *et al.*, 1997
Amycolatopsis strain MJ347-81F4	MJ347-81F4 A and B	Glycerol, galactose, dextrin, bacto soytone, corn steep liquor, $(NH_4)_2SO_4$, $CaCO_3$ Condition : pH 7.0, 27°C, 200 rpm, 4days	Glycerol, galactose, dextrin, bacto soytone, corn steep liquor, $(NH_4)_2SO_4$, $CaCO_3$ Condition : pH 7.0, 27°C,200 rpm, 11 days	Sasaki *et al.*, 1998
Kitasatospora sp. SPRI-0408	Sch 725424 and Sch 725428 antibiotics	Glucose, trehalose, tryptone, soyflour, yeast extract Condition : pH 7.2, 28°C, 250 rpm, 4 days	PD-650 dextrin, proflo flour, cerelose, $CaCO_3$, $CoCl_2$ Condition : pH 7.2, 28°C, 250 rpm, 4 days	Yang *et al.*, 2005