CHAPTER II

HISTORICAL

Man has been living his life on nature since pre-historical age, thousand years ago. Natural products have been used in basic needs of human-being such as house hold, food, cloth and the last but not the least important, medicine. Great deals of natural origins were used in traditional medication since early of age, both plant and animal materials including microorganisms. Nowadays, researchers pay large attention to seek for novel bioactive metabolites from microorganism. However the increase of antibiotic resistant pathogenic bacteria has become a serious problem in infectious disease therapy nowadays. Therefore, extensive screening lead to the discovery of new species and bioactive metabolites are needed, and microorganisms are good sources for this study (Goodfellow, 1988).

1. ACTINOMYCETES

The term *Actinomyces* appeared in the literature as early as 1887, this name means "ray-fungus" in Greek, and suggests that these microorganisms were observed, cultured, and studied as fungi in the early days. In 1937, Chatton classified microorganisms according to their general cellular organization, a method which is supported by abundance of biochemical, physiological, and microscopic evidence that has accumulated since then.

Actinomycetes are prokaryotes that look like fungi because they grow as filamentous mycelia and form spores. There are two essential features that distinguish actinomycetes from fungi: they are prokaryotic that have no cell nucleus and form hyphae that are from 0.5 to 1.0 μm in diameter, which are much smaller than fungal hyphae (which are 3 to 8 μm in diameter). Most actinomycetes are saprophytes, growing by decomposing organic matters. Some actinomycetes are human pathogens, like most other microorganisms. However, actinomycetes are usually harmless soil organisms. Some actinomycetes are particularly beneficial. Actinomycetes compose 10% to 50% of the total microbial population in soil. They are found in soil (most commonly), composts, and sediment. These microorganisms, with some exception, are aerobic. Actinomycetes are not tolerant of desiccation, but the spores they produced can tolerant desiccation (Coyne, 1999). The classification of actinomycetes is based on 16S rRNA, morphology, physiological characteristics, the composition of cell constituents such as cell walls, and the presence of characteristic lipids, sugars, quinones and, GC content. The GC content of

DNA of actinomycetes is generally in the range of 65-75%, although certain thermophilic actinomycetes have a low GC content (44-45%). The GC content of *Streptomyces*, *Kitasatospora*, and *Micromonospora* are high (70-75%) (Stackebrandt, Rainey, and Ward-Rainey, 1997; Holt, 1989).

1.1 *STREPTOMYCES*

The genus *Streptomyces* is a member of the family *Streptomycetaceae* in the order *Actinomycetales*. This family contained fourteen genera, including *Streptomyces*, *Actinopycnidium, Actinosporangium,* and related genera in the family *Streptomycetaceae*. *Streptomyces* species have been the subject *Chainia, Elytrosporangium, Intrasporangium, Kitasatoa, Kitasatosporia, Nocardioides, Sporichthya, Streptospora, Streptoverticillium, Kineosporia, Microellobosporia* (Goodfellow, 1990).

In 1974, Pridham and Tresner described the characterization of the genus *Streptomyces* of intensive investigation as sources of antimicrobial agents since the early 1940s. *Streptomyces* and related genera can be readily distinguished by chemotaxonomic and morphologic properties. Nowadays *Streptomyces* contained more than 450 species (Holt, 1989).

1.1.1 CHARACTERISTICS OF *STREPTOMYCES*

Streptomyces are gram-positive aerobic bacteria possessing vegetative hyphae (0.5-2.0 μm in diameter), which produce an extensive branched mycelia that rarely fragment. The mature aerial mycelium forms three to many spore chains. Spores are non-motile. Initially, colonies are relatively smooth surface, but later they develop a weft aerial mycelium that may appear floccose, granular, powder or velvety. *Streptomyces* produce a wide variety of pigments responsible for the vegetative and aerial mycelia. Colored diffusible pigment may also be formed (Goodfellow, 1990). 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*. The optimum temperature for growing is 25-37 ^{o}C; some species grow at psychrophilic and thermophilic range of temperature; the optimum pH range for growth is 6.5-8.0 (Brock, Madigan, Martinko *et al.*, 1993).

Chemotaxonomically, the genus *Streptomyces* is characterized by a cell wall type I (Lechevalier and Lechevalier, 1970) (Table 1), no characteristic sugars pattern (Lechevalier and

Lechevalier, 1970) (Table 2), and a phospholipids type II (Lechevalier, DeBievre, and Lechevalier, 1977) (Tables 3 and 4). The cell walls of *Streptomyces* have been found to contain N-acetylglucosamine, N-acetylmuramic acid, D-alanine, D-glutamic acid, and glycine with *L*-diaminopimelic acid (*L*-DAP) as the diamino acid. The phospholipids pattern contained in the cells are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmanosides, and but phosphatidylcholine is not detected. This pattern corresponds to phospholipid type II as reported by Lechevalier *et al.*, (1977). The predominant cellular fatty acids profile are saturated iso- and anteiso-branched fatty acids, but lack of mycolic acid (Kroppenstedt, 1985). Major menaquinones possess isoprene units MK-9 (H_6), MK-9 (H_8) (Collin, Pirouz, Goodfellow *et al.*, 1977).

Table 1. Cell wall chemotypes of the actinomycetes

Major constituent	**Chemotype**							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
L-Diaminopimelic acid	+							
meso-Diaminopimelic acid		+	+	+				
Diaminobutyric acid							+	
Aspartic acid						v		
Glycine	+	+					+	
Lysine					+			
Ornithine					+			+
Arabinose				+				
Galactose				+		v		

+, present; v, variable amounts

Table 2. Whole-organism sugar patterns of the actinomycetes containing *meso*-diaminopimelic acid (chemotype II-IV)

Pattern	**Sugar**				
	Arabinose	**Fucose**	**Galactose**	**Madurose**[a]	**Xylose**
A	+		+		
B				+	
C			No diagnostic sugars		
D	+				+
E		+			

+, present; [a], 3-O-methyl-D-galactose.

Table 3. Phospholipid types in actinomycetes

Phospholipid type	**PIMs**	**PI**	**PC**	**PG**	**PE**	**PME**	**GluNU**	**APG**	**DPG**
I	+	+	-	v	-	-	-	v	v
II	+	+	-	v	+	-	-	v	+
III	v	+	+	v	v	+	-	v	v
IV	ND	+	-	-	v	v	+	-	+
V	ND	+	-	+	v	-	+	v	+

+, present; v, variable; -,absent

type I, No nitrogenous phospholipids;

type II, Phosphatidyl ethanolamine;

type III, Phosphatidyl choline;

type IV, GluNu (unknown glucosamine-containing phospholipids);

typeV, Glu and phosphatidyl glycerol

1.2 *KITASATOSPORA*

The genus *Kitasatospora* (formely *Kitasatosporia*) was proposed by Omura (Omura, Takahashi, Iwai *et al.*, 1982), transferred to the genus *Streptomyces* by Wellington *et al* (1992), and re-established by Zhang and coworkers in 1997). The taxon is now widely recognized and encompasses eighteen speices, namely, *Kitasatospora arboriphila, K. azatica, K. cheersanensis, K. cineracea, K. kifunensis, K. cystarginea, K. gansuensis, K. griseola, K. kifunensis, K. mediociica, K. niigatensis, K. nipponensis, K. paracochelata, K. paranensis, K. phosalacinea, K. putterlickiae, K. setae*, and *K. terrestris* (Chung, Sung, Mo *et al.*, 1999; Zang *et al.*, 1997).

1.2.1 CHARACTERISTICS OF *KITASATOSPORA*

The genus *Kitasatospora* comprises a group of filamentous and aerobic Gram-positive bacteria that form an extensively branched substrate mycelium, the aerial mycelium bearing long spore chains of more than 20 spores and is phenotypically similar to *Streptomyces* strains (Omura *et al.*, 1982). The cell walls have been found to contain of N-acetylglucosamine, N-acetylmuramic acid. The phospholipids contained in the cells are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylmanosides, and but phosphatidylcholine is not detected (phospholipid type II Lechevalier *et al.*, 1977). The predominant cellular fatty acids profile are saturated iso- and anteiso-branched fatty acid, but lack of mycolic acid (Kroppenstedt, 1985). Predominant menaquinones possess with nine isoprene units MK-9 (H_6), MK-9 (H_8) (Collins *et al*, 1977).

Differential characteristics of the genus *Kitasatospora* which can be used to distinguish it from the genus *Streptomyces* are the higher ratio of *meso-* DAP to LL-DAP and the presence of galactose in the whole cell hydrolysates (Omura *et al.*, 1982; Zang *et al.*, 1997; Wellington *et al.*, 1992) proposed to unify two taxa on the basis of rRNA-targeted oligonucleotide probes and phenotypic properties. However, proposed to revise the genus *Kitasatospora* as they form a significant monophyletic clade and contain differential chemotaxonomic makers. *Kitasatospora* species have been known to produce several antifungal and antibacterial agents such as cystargin (Nakamura *et al.*, 1989).

1.3 *MICROMONOSPORA*

The genus *Micromonospora* is a member of family *Micromonosporaceae*. In 1997 Stackebrandt *et al.*, described that this family contained seven genera, including *Micromonospora, Actinoplnes. Dactylosporangium, Catellatospora, Catenuloplanes,*

Couchioplanes, and *Pilimelia*. Sub-sequently, the genera *Spirilliplanes* (Tamura *et al.*, 1997) and *Verrucosispora* (Rheims *et al.*, 1998) were found. Lee, Goodfellow and Hah, 1999 transferd *Catellatospora ferruginea* and *Catellatospora ishikariense* to *Asanoa* as *Asanoa ferruginea* and *Asanoa ishikariense*. Recently, two new genera namely, *Virgisporangium* (Tamura *et al.*, 2001) and *Longispora* (Matsumoto *et al.*, 2003) and have been described.

The genus *Micromonospora* was first described by Ørskov (1923) for actinomycete strains producing spores singly borne on sporophores branched from substrate hyphae. This genus is considered to accommodate 15 species at present, i.e. *M. aurantiaca*, *M. carbonacea*, *M. chalcea*, *M. chersina*, *M. coerulea*, *M. echinospora*, *M. gallica*, *M. halophytica*, *M. inositola*, *M. matsumotoense*, *M. nigra*, *M. olivasterospora*, *M. pallida*, *M. purpureochromogenes*, and *M. rosaria* (Kawamoto *et al.*, 1983; Szabo and Fernandez, 1984; Horan and Brodsky, 1986; Tomita *et al.*, 1992; Lee *et al.*, 1999; Kroppenstedt, 1985; Kawamoto, 1989; Koch *et al.*, 1996a 1996b; Lee *et al.*, 1999; Kasai, Tamura, and Harayama 2000). Recently new species were described as *M. eburnea*, *M. aurantinigra* and *M. endolithica* (Thawai *et al.*, 2004a; Thawai *et al.*, 2004b; and Hirsch *et al.*, 2004).

1.3.1 CHARACTERISTICS OF *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, fine hyphae (0.2-0.6 μm 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 viscid or mucoid. The mycelial pigments appear to differ little diagnostic value in strain or species recognition.

Sporulation appear almost as readily in submerged broth culture as on agar media. The formation of single spores on substrate mycelium is one of the well-defined criteria in the genus *Micromonospora*. They have been characterized by terms "smooth", "rough", "warty" or "blunt spiny". Mature spores are unaffected by ultrasonication but mycelia are quickly killed. Spore survival of most strains is >50% after 20 min at 60 ^{o}C in phosphate buffer and <0.5% after 20 min at 80 ^{o}C. *Micromonospora* spores are more resistant to ketones than to alcohols and dioxin (Kawamoto, 1989). The optimum temperature for growth is 25-30 ^{o}C; the optimum pH for range growth is pH 6.0 and 8.0. All strains of *Micromonospora* showed positive results for gelatin liquefaction (Vobis, 1989).

The cell walls of *Micromonospora* comprised of N-acetylglucosamine, N-acetylmuramic acid, D-alanine, glutamic acid, glycine, *meso*-diaminipimelic acid (*meso*-DAP) (including its 3-hydroxy-derivative). They are classified as cell wall type II (Lechevalier and Lechevlier, 1970), a whole cell sugar pattern D (Lechevalier and Lechevalier, 1970), and a phospholipid type II (Lechevalier *et al.*, 1977). The pentoses, xylose, and arabinose are always constituents of the cell wall, although the amounts vary to some extents. Hexoses, glucose, and galactose are detected more frequently than mannose and rhamnose. The predominant cellular fatty acids are iso- and anteiso-branched fatty acid. Unsaturated or 10-methyl fatty acids may be found in certain strain, that were iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$. anteiso-$C_{15:0}$, $C_{17:0}$, and anteiso-$C_{17:0}$. This pattern corresponds to fatty acid type 3b of Kroppenstedt (1985), but mycolic acid and cyclic fatty acids are not presented.

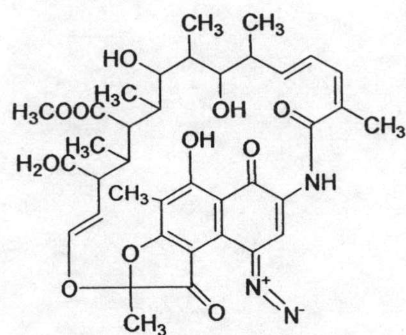
The genus *Micromonospora* currently consists of 18 validly described species, and they (*except for M. gallica*) can be divided into three groups based on the predominant menaquinone components, i.e. MK-9, MK-10, and MK-12 groups. Species containing hydrogenated MK-9 as major menaquinones (MK-9 group) are *M. carbonacea, M. halophytica, M. nigra, M. chersina,* and *M. eburnea,* while the MK-12 group is found in only one species, *M. pallida.* The species, *M. chalcea, M. inositola, M. coerulea, M. purpureochromogenes, M. olivasterospora, M. echinospora, M. matsumotoense, M. rosaria, M. aurantiaca, M. aurantinigra* and *M. endolithica* contained MK-10 as a major menaquinone. The major menaquinones were MK-9, MK-10, and MK-12. The range of G+C contents of the DNA was 71-73 mol%

2. Antibiotics from *Streptomyces*

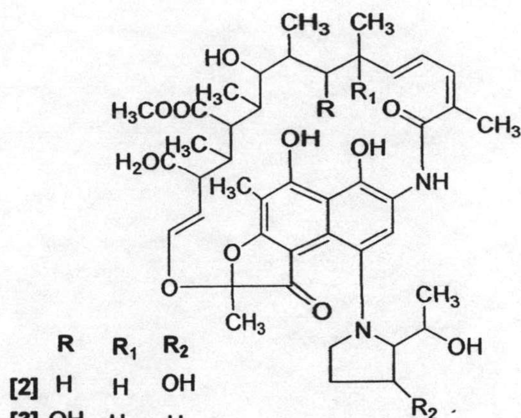
Actinomycin, the first antibiotic from *Streptomyces*, was discovered by Waksman and Woodruff in 1940, followed by antituberculosis antibiotic streptomycin. In addition, the ansamycins are a class of macrocyclic compounds. These structures consist an aromatic nucleus in which two non-adjacent positions are linked by a long aliphatic chain of up to twenty-four atoms (Thomson, 1987; Dewick 1997). The aromatic portion may be benzenoid, napthlenic, or quinonoid. Ansamycins displayed many biological activity such as antibacterial activity, antitumor activity, and herbicidal activity. Most of ansamycins are obtained from *Streptomyces* (Glasby, 1993) (Table 4).

Table 4. Sources and biological activity of ansamycins

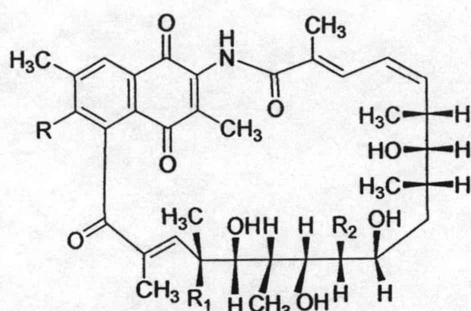
No.	compounds	Strains	Activities	References
1	Rifamycin X	*S. mediterranei*	Antibacterial	Glasby, 1993
2 3 4	Halomicin A Halomicin B Halomicin C	*S. halophytica*	Antibacterial	Glasby, 1993
5 6 7 8 9	Protostreptovaricin I Protostreptovaricin II Protostreptovaricin III Protostreptovaricin IV Protostreptovaricin V	*S. spectabilis*	Inhibit Rauscher Leukemia virus RNA-dependent DNA polymerase	Deshmukh *et al.*, 1976 Glasby, 1993
10 11 12 13 14 15	Streptovarcin A Streptovarcin B Streptovarcin C Streptovarcin D Streptovarcin E Streptovarcin F	*S. spectabilis*	Inhibit the incorporation of mucleosides into Hela cells	Rinehart Jr. *et al.*, 1971.
16 17	Ansatrienin A_2 Ansatrienin A_3	*S. collinus*	Antifungal	Damberg *et al.*, 1982
18 19	Mycotrienol I Mycotrienol II	*S. rishiriensis*	Antifungal , Antileukeia	Glasby, 1993
20 21	Geldanamycin 17-O-demthylgeldanamycin	*S. hygroscopicus*	Antibacterial, Antitumor, Antifungal and Herbicide	De Boer *et al.*, 1970 Heisey and Patunam, 1986
22 23 24 25 26 27	Herbimycin A Herbimycin B Herbimycin C Dihydroherbimycin A Dihydroherbimycin B Dihydroherbimycin C	*S. hygroscopicus*	Antibacterial, Herbicide, and Cytotoxic	Omura *et al*, 1979



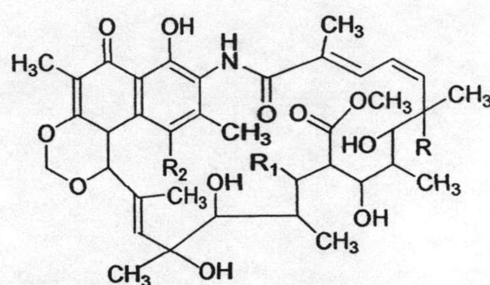
[1]



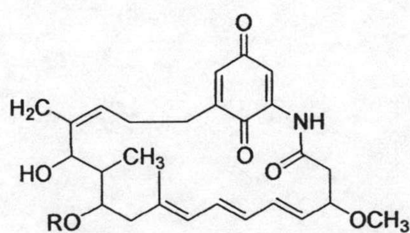
	R	R_1	R_2
[2]	H	H	OH
[3]	OH	H	H
[4]	H	OH	H



	R	R_1	R_2
[5]	OH	H	CH_3
[6]	OCH_3	H	CH_3
[7]	OH	OH	CH_3
[8]	OCH_3	OH	CH_3
[9]	OH	H	H

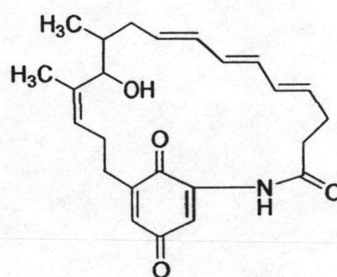


	R	R_1	R_2
[10]	OH	$OCOCH_3$	$OCOCH_3$
[11]	H	$OCOCH_3$	$OCOCH_3$
[12]	H	OH	$OCOCH_3$
[13]	H	H	$OCOCH_3$
[14]	OH	H	$OCOCH_3$
[15]	OH	OH	$OCOCH_3$



[16] R = $COCHCH_3NHCOCHCH_3CH_2CH_3$

[17] R = $COCHCH_3NHCOCHCH_3CH_2CH_3$



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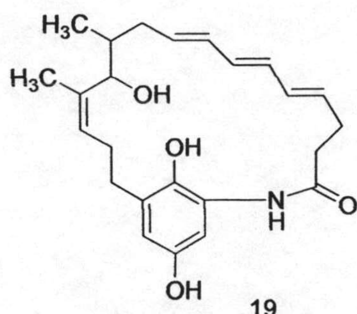
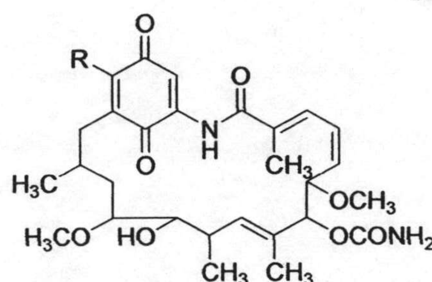
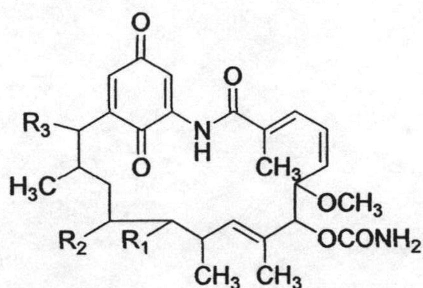


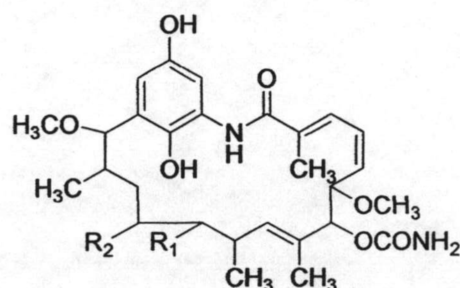


[20] R = OCH_3

[21] R = H



	R_1	R_2	R_3
[22]	OCH_3	OCH_3	OCH_3
[23]	OH	OCH_3	H
[24]	OH	OCH_3	OCH_3



	R_1	R_2
[25]	OCH_3	OCH_3
[26]	OH	OH
[27]	OH	OCH_3

3. *MONASCUS*

The genus *Monascus* belonging to the class *Ascomycetes* under the family *Monascaceae* was established in 1884 by Van Tieghem, who divided this genus into two species, *M. mucorioides* and *M. ruber*. Thereafter F. Went isolated the third species, *M. purpureus* from the Chinese Anka (Ang-quac, ang-kak) in 1985.

For several years, scientists have been studying the production of pigments and enzymes, as well as the classification of this genus. *Monascus* species comprises: *M. albidus*, *M. albus*, *M. anka*, *M. araneosus*. *M. barkeri*, *M. bisporous*, *M. floridanus*, *M. fuliginosus*, *M. kaoliang*, *M. major*, *M. mucoroides*, *M. olei*, *M. paxii*, *M. pilosus*, *M. pubigerus*, *M. purpureus*. *M. ruber*, *M. rubiginosus*, *M. rubropunetatus*, *M. serorubercens*, *M. vini*, and *M. vitreus* (Iizuka and Lin, 1981; Hawksworth and Pitt, 1983; NishiKawa and Iizuka, 1993)

3.1 CHARACTERISTICS OF *MONASCUS*

Monascus formed well-developed, irregularly branched, and reddish mycelium. Mycelium initially white, becoming strongly pigment as colonies mature, in orange-red shades.

Colonies (12-18 μm in diameter) plane and sparse, surface texture usually floccose. Conidia usually terminal but sometimes intercalary are produced. Terminal conidia (4-9 x 3.5-9 μm) are formed by swelling of the apex of the fertile hypha. This portion then becomes thicker-walled and a septum is formed below the swollen portion delimit the conidium (Hawksworth and Pitt, 1983).

3.2 Strain improvement

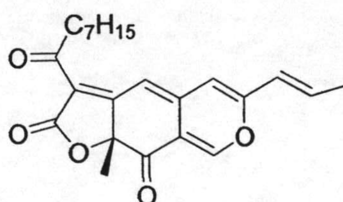
Irradiation of wild *Monascus* strains by UV light, neutron or X-rays, mutation using or combinations of these methods can result in mutants with advantageous properties (rapid growth, superior pigment production, elimination of ascospore formation) or albino mutants (Wong and Koehler, 1981). Yongsmith *et al.*, (1994) obtained a mutant of a *Monascus* species which produced a high concentration of yellow pigments instead of the red pigments formed by its parent strains.

3.3 SECONDARY METABOLITES FROM *MONASCUS*

The genus *Monasucs* produced a mixture of six major pigments of polyketide origin Secondary metabolites from *Monascus* species are shown below.

3.3.1. Monascorubrin

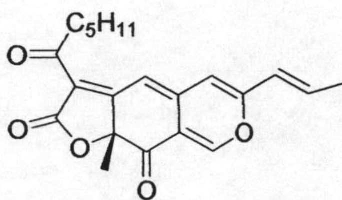
In 1932, Nishikawa isolated monascorubrin **[28]**, an orange pigment from *M. purpuseus* Wenti (Fielding *et al.*, 1960). A note describing substantial inhibitory effects of rubropunctatin and monascorubrin on *Bacillus subtilis* and *Escherichia coli* appeared in a paper by Nozaki *et al.* (1991)



[28]

2.3.2. Rubropunctatin

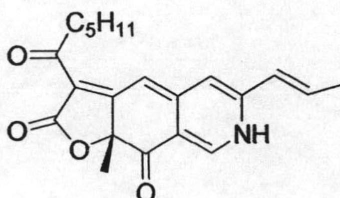
In 1959, Haws and co-workers isolated a new orange pigment **[29]** from *M. rubropunctatus* Sato (Haws *et al.*, 1959). Rubropunctatin had teratogenic effect and embryo toxicity on chicken.



[29]

3.3.3. Rubropunctamine

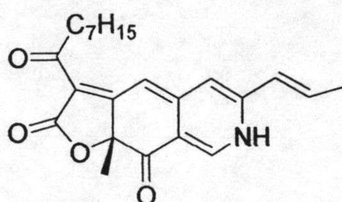
Rubropunctamine **[30]**, the red compound was rapidly formed by adding dilute ammonium hydroxide to rubropunctatin at room temperature (Haws *et al.*, 1959).



[30]

3.3.4. Monascorubramine

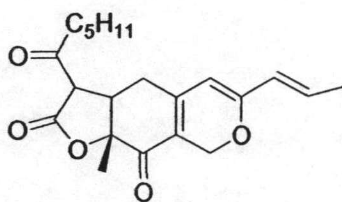
Monascorubramine **[31]**, was formed by treatment with dilute ammonium hydroxide on monascorubrin. Monascorubramine and rubropunctamine **[31,30]** were the water-soluble red pigments (Blanc *et. al*, 1994).



[31]

33.5. Monascin (Monascoflavin)

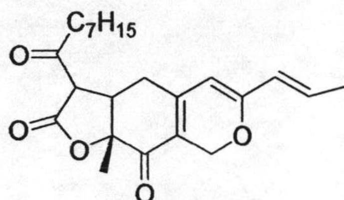
Monascin **[32]**, the yellow pigment is produced by *M. purpureus*. Martinkova *et al.* (1999) reported that monascin and its derivatives are not toxic.



[32]

3.3.6. Ankaflavin

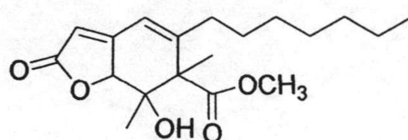
In 1973, Manchand and Whalley isolated yellow pigment ankaflavin **[33]**, from *M. anka*. Ankaflavin had toxic towards chicken embryos and immunosuppressive activity on mouse T-splenocyte. Carels and Shepherd (1977) supposed that ankaflavin and monascin originated from chemical oxidation of monascorubrin and rubropunctatin.



[33]

3.3.7. Ankalactone

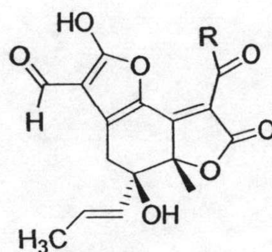
Nozaki *et al* (1991) isolated a novel α,β-unsaturated γ-lactone derivative **[34]** named ankalactone, from a culture filtrate of *M. anka* that grown in a glucose-peptone medium for 7 days.



[34]

3.3.8. Xanthomycins [35]

In 1992, Sato *et al.*, isolated yellow pigments xanthomycin A and xanthomycin B produced by *M. anka* U-1. Xanthomycins A and B showed antimutagenic properties against Trp-P-2(NHOH). In the Ames salmonella assay, these pigments showed individually no mutagenic activity, and inhibited the mutagenicity of 3-hydroxyamino-1-methyl-5H-pyrido [4,3-**b**] indole [Trp-P-2(NHOH], the activated form of Trp-P-2(3-amino-1-methyl-5H-pyrido [4,3-**b**] indole (Izawa *et al.*, 1997).



[35]

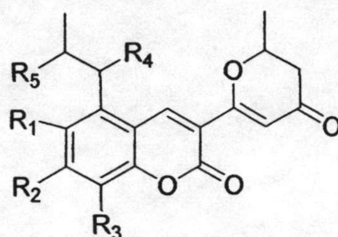
Xanthomycin A R = $-C_5H_{11}$

Xanthomycin B R = $-C_7H_{15}$

3.3.9. Monankarins [36]

Monankarins A-F, a series of non pigment having conjugated pyrano-coumarin skeleton had been isolated from *M. anka.*

Monankarins A-D exhibited monoamine oxidase (MAO) inhibitory activity, while the activity was not observed in monankarins E and F (Hossain, Okuyama and Yamazaki, 1996).



[36]

Monankarin

Monankarins A and B: R1= CH_3, R2 = OH, R3 = H, R4 = CH_3, R5 = OH

The spectral data of monankarin B were similar to monankarin A except for the circular dichroism. (monankarin B was a diastereomer of monankarin A)

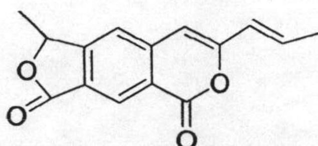
Monankarins C and D: R1= CH_3, R2 = OH, R3 = CH_3, R4 = CH_3, R5 = OH (monankarin D was diastereomer of monankarin C)

Monankarins E: R1= H, R2 = OH, R3 = CH_3, R4 = H, R5 = OH

Monankarins F: R1= CH_3, R2 = OH, R3 = CH_3, R4 = H, R5 = OH

3.3.10. Monascodilone [37]

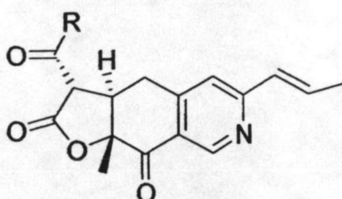
Wild, Toth and Humpf (2002) isolated nonpigment component of red yeast rice from *M. purpureus*.



[37]

3.3.11. Monascopyridine A and B [38]

Wild, Toth, Humpf (2003) isolated monascopyridine A and monascopyridine B from *M. purpureus*.



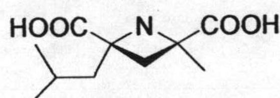
[38]

Monascopyridine A R = $-C_5H_{11}$

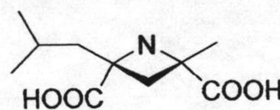
Monascopyridine B R = $-C_7H_{15}$

3.3. 12. (±) Monascumic acid

In 2004, Akinisa *et al.*, isolated (+) monascumic acid **[39]** and (-) monascumic acid **[40]** [(±) (-) syn-2-Isobutyl-4-methylazetidine-2,4 dicarboxylic acid] from the n-butanol-soluble fraction of the 70% ethanol extract of red fermented with *M. pilosus* (Akinisa *et al.*,2004).



[39]



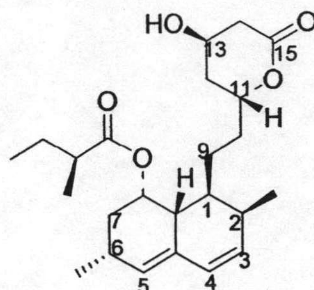
[40]

3.3.13. Monacolins

- Monacolin K (Mevinolin)

In 1979, Endo A. isolated monacolin K **[41]** from *Monascus* species. Mevinolin is produced as a mixture of a lactone and a free hydroxyl acid [Monacolin K was potent inhibitors of cholesterol biosynthesis in humans and possess potential in treatment of artherosclerosis and coronary heart disease (Changling, Zhu, Wang *et al.*, 1998). It blocked isoprenoid formation

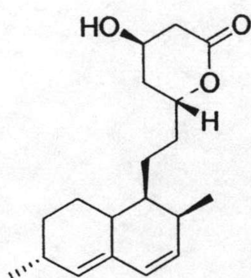
because of competitive inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) (Endo, 1981). Mevinolin related compounds vary in composition of the C_4 side chain (Endo, Komagata, and Shimada, 1986).



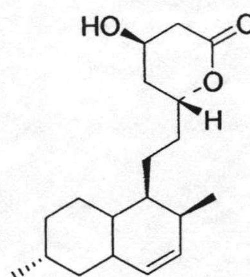
[41]

- Monacolin L

In 1985, Endo A. isolated monacolin L **[42]** and dihydromonacolin **[43]** (lack of the side chain of the C_4) from *M. ruber*. The results of Komagata *et al* (1989) indicated that monacolin L **[42]** is the precursor of monacolin J **[44]**, which in turn, can be converted to monacolin K **[41]** (Kimura *et. al*, 1990).

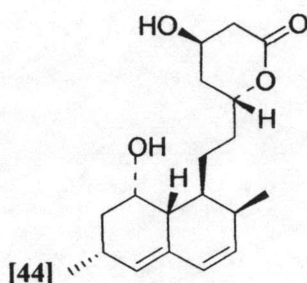


[42]



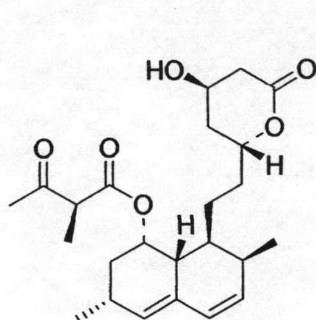
[43]

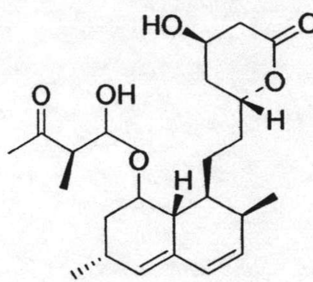
- Monacolin J



- Monacolin X

In 1986, Nakamura *et al.*, isolated monacolin X **[45]** and monacolin M **[46]** from *M. ruber* (Endo, Komagata, and Shimada, 1986). Growth experiments with *M. ruber* using ^{14}C-labled monacolin J or L suggested that both compounds are precursors of monacolin K (Endo *et al.*, 1985).





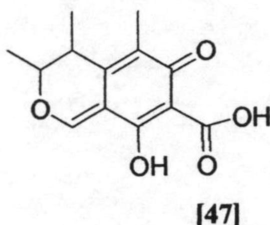
[45] **[46]**

Monacolin K (mevinolin) and its related compounds, monacolins J, L, X, dihydromonacolin L and monacolin M are metabolites of *M. ruber.* These compounds specifically inhibit 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the rate limiting enzyme in chlolesterol synthetic pathway.

3.3.14. Citrinin (monascidin A) [47]

Citrinin [(3R,4S)-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid has been identified previously as a secondary fungal metabolite produced by a variety of *Aspergillus* and *Penicillium* species (Sabater-Vilar *et al.*, 1999). Citrinin induced nephrotoxicity has been demonstrated in various cellular and animal models (Speijers and Speijers, 2004). However, the first scientific report on the antibacterial effects of this fungus

appeared in 1977 when Wong and Bau found monascidin A from *M. purpureus* on *Bacillus*, *Streptococcus* and *Pseudomonas*. In addition, it induced cytotoxic effect on human embryonic kidney cell (HEK 293) and a positive dose depending mutagenic response in the *Salmonella*-hepatocyte assay applying strain TA-98.



[47]

3.4 Biological activity and application

Monascus was used in the fermentation industry for production of red rice wine, red Shao-Hsing wine and native food such as red soybean cheese. Recently, as the synthetic food colors have been prohibited for their toxicity, the development of comparatively safe natural food colors are now bringing about the great interest of researchers. The monascus-pigment has been considered at the hopeful pigments (Iizuka and Lin, 1981). *Monascus* pigments, has been well-known as a food dye in Japan and China for centuries (Juzlova, Martinkova, and kren, 1996).

Some biological effects of the secondary metabolites of *Monascus* species remain to be investigated. In this respect, additional studies on the antimicrobial and immunosuppressive effects of the pigments merits further research. Because *Monascus* species are used in the production of food additives, it is necessary to take into account the toxicity of some of their metabolites. According to Blanc *et al.* (1994) there is some risk of contamination of the colorants with citrinin which could be avoided

Mevinolin and their derivatives obtained by chemical modifications (pravastatin, simvastatin) have provided a new mode of therapy for patients with hypercholesterolemia (disease characterized by an elevated plasma concentration of the low density lipoprotein (LDL)/cholesterol complex). Mevinolin inhibitits sterol synthesis not only in hepatocytes and other types of mammalian cells but also in fungi (Jounoud, 2004).