CHAPTER II

REVIEW OF LITERATURE

1. Antibiotics from *Micromonospora* species

The word "antibiotics" was used to describe a type of association in which one living creature was destroying another in order to sustain its own life. Waksman. (1947) 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 microorganisms." 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.

Table 1 showed antibacterials, antifungals , antitumors, antibiotics, and enzyme inhibitors produced by *Micromonospora*.

Additional examples are *Micromonospora* sp. SA246 producing 1-hydroxycrisamicin A (Lee *et al.*, 1998), and *Micromonospora echinospora* subsp. *echinospora* producing YM-47515 (Sugavara *et al.*, 1997). A strain of *Micromonospora* strain is known to produce thiocoraline (Romero *et al.*, 1997). *Micromonospora* sp. C39217-R109-7 produces prolosporin A (Schroeder *et al.*, 1996), and *Micromonospora* sp C39500 produces korkormicins an antitumor antibiotics (Lam *et al.*, 1995).

Table 1 Antibiotics from genus *Micromonospora*

Compounds	Strains	Activity	References
Micromonosporin	*Micromonospora* sp.	antibacterial	Glasby, 1993
Neomycin B [1]	*Micromonospora* sp.69-683	antibacterial	Glasby, 1993
Microcin A	*Micromonospora* sp.	antibacterial antifungal	Glasby, 1993
Gentamicin A_1 [2]	*M. echinospora*, *M. purpurea*	Antibacterial	Glasby, 1993
Gentamicin A_2 [3]	*M. echinospora*, *M. purpurea*	Antibacterial	Glasby, 1993
Gentamicin A_3 [4]	*M. echinospora*, *M. purpurea*	Antibacterial	Glasby, 1993
Gentamicin A_4 [5]	*M. echinospora*, *M. purpurea*	Antibacterial	Glasby, 1993
Gentamicins B [6], B_1 [7]	*M. echinospora*, *M. purpurea*	antibacterial antiprotozoal	Glasby, 1993
Gentamicin C_1 [8], C_1 [9], C_2 [10]	*M. purpurea* (NRRL 2953)	antibacterial antiprotozoal	Glasby, 1993
Gentamicin X [11]	*M. echinospora*, *M. purpurea*	antibacterial, antiprotozoa	Glasby, 1993
Megalomicin A [12], B [13], C [14], $C_{2\alpha}$ [15] and $C_{2\beta}$ [16]	*M. inositola* MK-41	antibacterial, antifungal	Glasby, 1993
Antibiotic 67-694	*M. rosaria*	antibacterial	Glasby, 1993
Antibiotic G-52 [17]	*M. zionensis*	antibacterial	Glasby, 1993
Antibiotic Sch14342 [18]	*Micromonospora* spp.	antibacterial	Glasby, 1993

Table 1 (continued)

Compounds	Strains	Activity	References
Mutamicin 1 [19], 1a [20], 1b [21] and 2 [22]	*M. inyouensis*	antibacterial	Glasby, 1993
Mutamicin 2a [23]	*M. inyoensis*	antibacterial	Glasby, 1993
Antibiotic 66-40 [24]	*M. inyouensis*	antibacterial	Glasby, 1993
Antibiotic 66-40D [25]	*M. inyouensis*	antibacterial	Glasby, 1993
Fortimicin A [26]	*Micromonospora* sp.	antibacterial	Glasby, 1993
Fortimicin B [27]	*Micromonospora* sp.	antibacterial	Glasby, 1993
Sagamicin [28]	*M. sagamiensis*	antibacterial	Glasby, 1993
Verdamicin I [29]	*M. grisea*	antibacterial	Glasby, 1993
Antibiotic LL-E33288ε [30]	*M. echinospora* var. *calichensis*	antibacterial antitumor	Glasby, 1993
Antibiotic LL-E33288 [31]	*M. echinospora* var. *calichensis*	antibacterial antitumor	Glasby, 1993
Dapiramicin [32]	*Micromonospora* sp.	antibacterial	Glasby, 1993
Antibiotic XK-62-6 [33]	*M. sagamensis* var. nonreductans	antibacterial	Glasby, 1993
Antibiotic XK-62-8 [34]	*M. sagamensis* var. nonreductans	antibacterial	Glasby, 1993
Clostomicin [35]	*M. echinospora* subsp.*ameniaca* subsp. nov	antibacterial	Omura *et al.*, 1986
Antibiotic K-259-2 [36]	*M. oligovasterospora*	inhibitor Ca^{2+}	Yazuru *et al.*, 1987
Antibiotic K-13 [37]	*M. halophytica* subsp. exillisia	angiotensin I converting enzyme	Kase *et al.*, 1987

Table 1 (continued)

Compounds	Strains	Activity	References
Antibiotic BU-3420T [38]	*M. chersina*	antibacterial antifungal anti tumour	Glasby, 1993
Antibiotic LL-E19085[39]	*M. citrea*	antibacterial	Glasby, 1993
Antibiotic G-418 [40]	*M. rhodoranges*	antibacterial	Glasby, 1993
Mycinamicin VIII [41]	*M. griseorubida*	antibacterial	Glasby, 1993
Antibiotic JI-20A [42]	*M. purpurea*	antibacterial	Glasby, 1993
Antibiotic JI-20B [43]	*M. purpurea*	antibacterial	Glasby, 1993
MS-444 [44]	*Micromonospora* sp. KY7123	inhibitor of myosin light chain kinase	Nakamishi *et al.*, 1995
Dynemicin [45]	*M. chersina* sp. nov M9561	antibacterial antitumor	Konishi *et al.*, 1990
Macquarimicin [46]	*M. chalcea*	antibacterial antitumor	Jackson *et al.*, 1995
Antibiotic I-SKA$_1$ [47]	*Micromonospora* sp.	antibacterial	Glasby, 1993
Antibiotic I-SKB$_1$ [48]	*Micromonospora* sp.	antibacterial	Glasby, 1993
Antibiotic I-SKB$_2$ [49]	*Micromonospora* sp.	antibacterial	Glasby, 1993
Antibiotic M-4365 A$_1$ [50]	*M. capillata*	antibacterial	Glasby, 1993
Antibiotic M-4365 A$_2$ [51]	*M. capillata*	antibacterial	Glasby, 1993
Antibiotic M-4365 A$_3$ [52]	*M. capillata*	antibacterial	Glasby, 1993
Antibiotic M-4365 G$_1$ [53]	*M. capillata*	antibacterial	Glasby, 1993
Antibiotic M-4365 G$_2$ [54]	*M. capillata*	antibacterial	Glasby, 1993
Halomicin A [55], B [56] and C [57]	*M. halophytica*	antibacterial, antifungal	Glasby, 1993
Quinolidomicins A$_1$ [58], B$_1$ [59] and A$_2$ [60]	*Micromonospora* sp. JY16	antitumor	Hayakawa *et al.*, 1993

Table 1 (continued)

Compounds	Strains	Activity	References
Cororubicin [61]	*Micromonospora* sp. JY16	antitumor	Ishigami *et al.*, 1994
Everninomicin B [62], C[63] and D [64]	*Micromonospora* sp.	antibacterial	Glasby, 1993
Antibiotic X-14847 [65]	*M. echinospora*	antibacterial	Glasby, 1993
Sibanomicin [66]	*Micromonospora* sp. SF-2364	antibacterial antitumour	Glasby, 1993
Citreamicin α [67], β [68], γ [69], δ [70] and γ [71]	*M. citrea*	antibacterial	Carter *et al.*, 1990
Antibiotic 42752	*M. saitamica*	antibacterial	Glasby, 1993
Antibiotic 43038	*M. saitamica*	antibacterial	Glasby, 1993
Antibiotic 43139	*M. saitamica*	antibacterial	Glasby, 1993
Antibiotic SF-1854	*Micromonospora* strain SF-1854	antibacterial	Glasby, 1993
Antibiotic K-26	*Micromonospora* strain K-26	antibacterial	Glasby, 1993
Antibiotic SF-2312	*Micromonospora* sp. SF-2312	antibacterial	Glasby, 1993
Neihumicin	*M. neihuensis*	against KBcell	Wu *et al.*, 1988
Antibiotic LL-D42067	*M. purpureochromogenes* subsp. *wuxiensis*	antibacterial antitumour	Glasby, 1993
Antibiotic SF 2448A, B and C	*Micromonospora* strain SF 2448	antibacterial	Glasby, 1993

2. Characteristics of genus *Micromonospora*

This genus is in the group of actinomycetes which produce a large number of antibiotics. Although the actinomycetess posses cytologic and physiologic structures and filamentous properties which support a morphological basis for classification, colony characterizatión has been used extensively for describing *Micromonospora* species in terms of texture and color of mycelium and spores.

Micromonospora are characterized by the absence of aerial mycelium, and this trait is quite reliable, particularly with the return of *Micromonospora vulgaris* to the genus Thermoactinomyces . Occasionally on old *Micromonospora* colonies, a limited white aerial bloom may occur that seems to have little diagnostic significance . An exception is in colonies of *Micromonospora echinospora*, where a bloom of very short aerial hyphae of a lavender gray many sporadically occur and is helpful, although a non dependable, recognition unit. The generally smooth surface of a *Micromonospora* colony is composed of tightly woven hyphae within which sporulation occurs. The color of the nonsporulating colony surface is generally of light yellow , orange, or orange red . The colony surface mycelium may darken often characteristically, just prior to massive sporulation . Sporulation most often causes the production of dark brown to black spores, and as these spores develop, the colonies take on a progressively darker hue . A careful study of the color change occuring as a colony matures may have recognition on unit value. Another colonial characteristic that appears frequently in the micromonospora is the conversion of the dry or waxy plicate colony into a viscid (mucoid) dark-colored mass. This feature appears in cultures in which sporulation and mycelial fragmentation appear to consume the entire colony mass.(Holt, 1989).

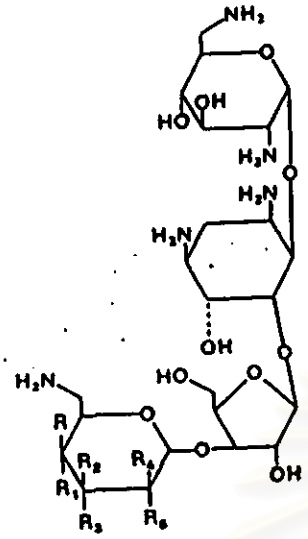
Table 2 Antimicrobial activity and enzyme inhibitors activity of antibiotics from *Micromonospora* species

compounds	Activity	References
Gentamicin	*Enterobacter, E. coli, Krebsiella, Samonella, Seratia , P. aeroginosa , S. aureus , Bacillus , Clostridium, Corynebacterium*	Glasby, 1993.
Antibiotic G-418	*S. aureus* 209P	Glasby, 1993.
Antibiotic G-52	*Escherichia, Pseudomonas,* and *Staphylococcus*	Glasby, 1993.
Antibiotic M-4365A_1	*Klebsiella pneumoniae, Mycoplasma* species	Glasby, 1993.
Crisamicin A	*S. aureus* 6538P, *Micrococcus luteus, B. subtilis* ATCC 7972, B16 murine melanoma cells	Ling *et al.*, 1986.
Clostomicin	*Clostridium perfringens*	Takasashi *et al.*, 1987.
Antibiotic K-259-2	Inhibitor of Ca^{2+} and calmodulin dependent cyclic nucleotide phosphodiesterase	Yuzuru *et al.*, 1987.
Antibiotic K-13	Angiotensin I converting enzyme inhibitor	Kase *et al.*, 1987.
Antibiotic Sch 37137	*Candida* sp., dermatophytes	Cooper *et al.*, 1988.
Sibaminomicin	P-388 lukemia cells	Glasby, 1993.
Antibiotic BU-3420T	Inhibit or of mamalian tumors, P-388 lukemia cells	Glasby, 1993.
Mycinamicin VIII	*Streptococcus* species, *Micrococcus luteus* ,*Corynebacterium diphthelia*	Glasby, 1993.

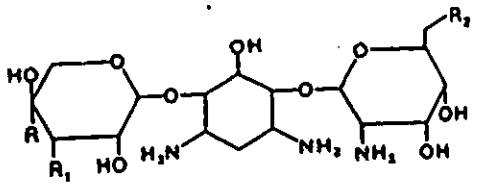
Table 2 (continued)

compounds	Activity	References
Dynemicin	B. subtilis PCI 219, *S. aureus* Smith , *S. epidermidis* 11-1168, Mouse melanoma , P-388 lukemia cell	Konishi *et al.*, 1991.
Cororubicin	KB cells	Ishigami *et al.*, 1994.
MS-444	Inhibitor of myosin light chain kinase	Nakamishi *et al.*, 1995.
Maquarimicin	*Bacteroides* sp., leukemia P-388 cells	Jackson *et al.*, 1995.
Quinolidomicin	Human lukemia (K562), P-388 llukemia cells , HT-29 (Human colon cancer)	Hayakawa *et al.*, 1993.

Mycelial pigments and pigments that are produced in the substrate mycelium and diffuse out into the medium have been used as criteria for descriptions of *Streptomycetes*. Pigments produced by the *Micromonospora* are numerous and often unpredictable but , in spite of their shortcomings , are helpful when present as ancially recognition units . Pigments are biochemical products and often are associated with certain species groups. The problem is to differentiate between strain-specific pigments and group-shared pigments. We may devide these pigments into the readily diffusible pigments and mycelium bound pigments; however, mycelial bound pigments in order cultures may diffuse, possibly due to mycelial autolysis. The yellow-orange and orange-red mycelial pigments appear to dffer little diagnostic value in strain or species recognition. Maroon mycelial pigments are often recognition units for the *Micromonospora echinospor* and *M. purpurea* species group. These pigments are soluble in water and acid alcohol and act as acid-base indicators, being red in the acid range and blue-green and precipitable in the basic range.



[1] Neomycin B

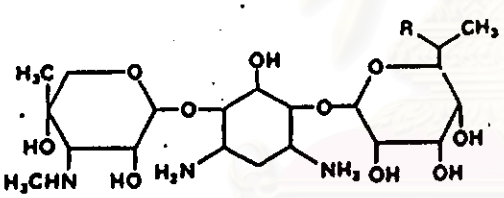


[2] Gentamicin A_1; R=H, R_1=$NHCH_3$, R_2=OH

[3] Gentamicin A_2; R=H,R_1= R_2=OH

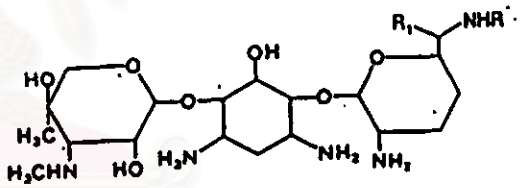
[4] Gentamicin A_3; R=H, R_1=$NHCH_3$, R_2=NH_2

[5] Gentamicin A_4; R=CHO, R_1=$NHCH_3$, R_2=OH



[6] Gentamicin B; R=H

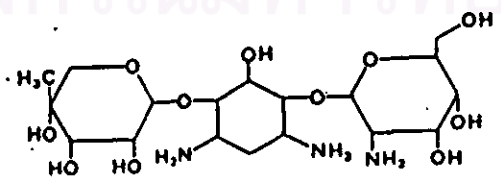
[7] Gentamicin B_1; R=CH_3



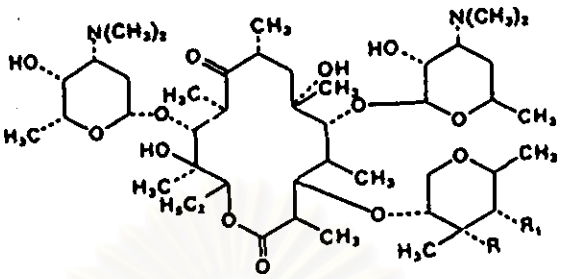
[8] Gentamicin C_1; R= R_1=CH_3

[9] Gentamicin C_{1a}; R= R_1=H

[10] Gentamicin C_2; R=H, R_1=CH_3



[11] Gentamicin X



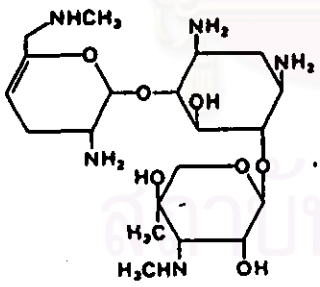
[12] Megalomicin A;R= R_1=OH

[13] Megalomicin B;R=OH, R_1=$OCOCH_3$

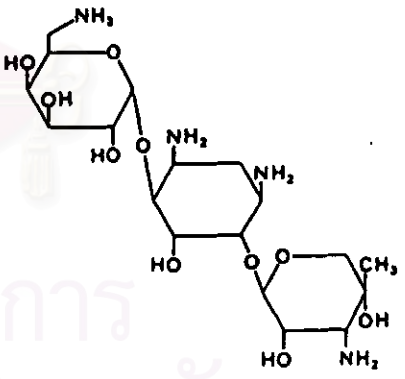
[14] Megalomicin C;R=R_1=$OCOCH_3$

[15] Megalomicin $C_{2\alpha}$;R=$OCOCH_3$, R_1=$OCOCH_2CH_3$

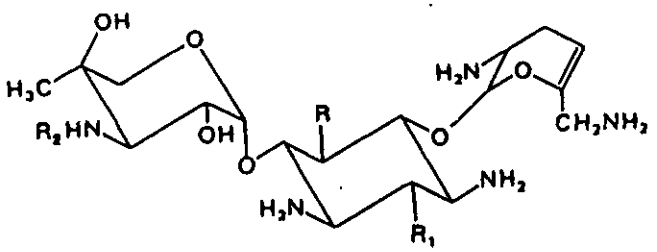
[16] Megalomicin $C_{2\beta}$;R=$OCOCH_3$, R_1=$OCOCH_2CH_3$



[17] Antibiotic G-52



[18] Antibiotic Sch 14342

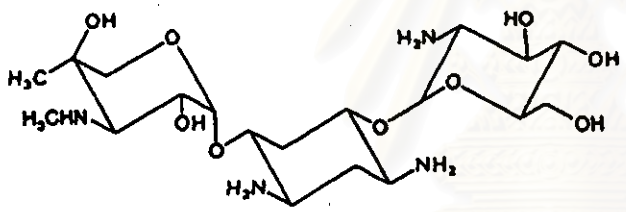


[19] Mutamicin 1; R= R_1=OH, R_2=CH_3

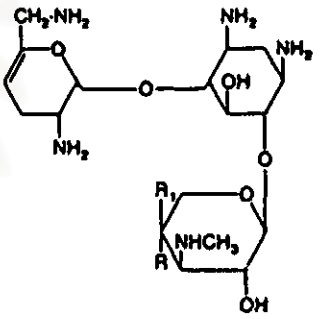
[20] Mutamicin 1a; R=R_1=OH, R_2=$COCH_3$

[21] Mutamicin 1b; R=R_1=OH, R_2=H

[22] Mutamicin 2; R=R_1=H, R_2=CH_3

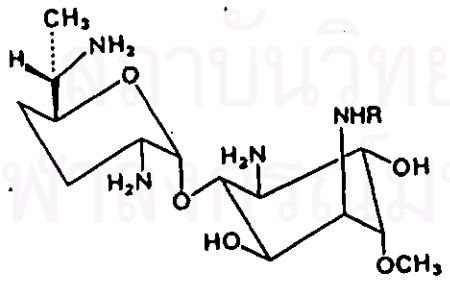


[23] Mutamicin 2a; R=R_1=OH, R_2=CH_3



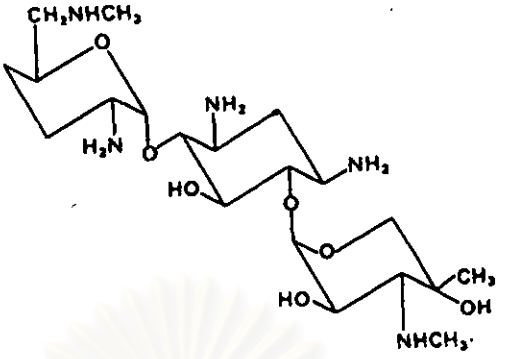
[24] Antibiotic 66-40; R=OH, R_1=H

[25] Antibiotic 66-40D; R=H, R_1=OH

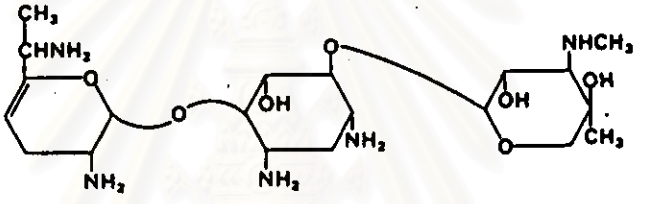


[26] Fortimicin A; R=$COCH_2NH_2$

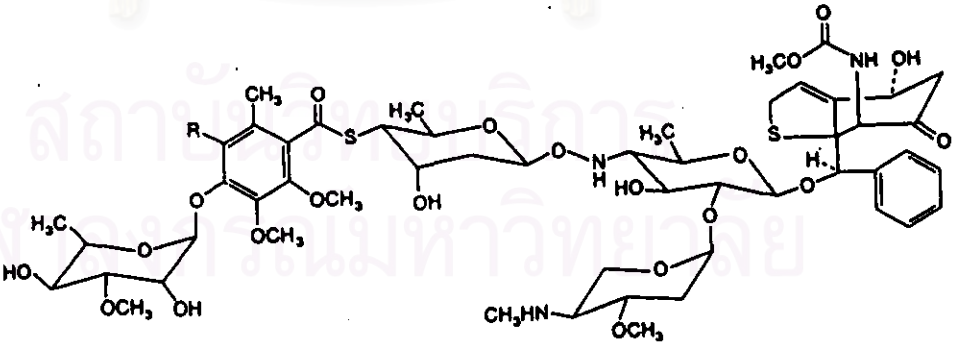
[27] Fortimicin B; R=H



[28] Sagamicin

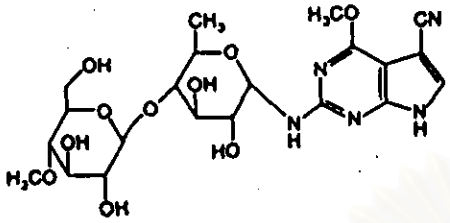


[29] Verdamicin I

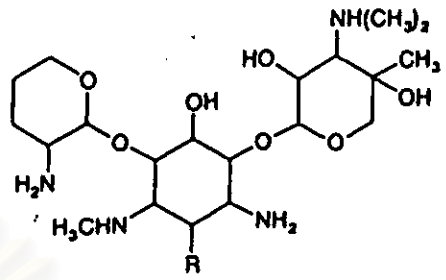


[30] Antibiotic LL-E33288$_8$; R=Br

[31] Antibiotic LL-E33288$_8$; R=I

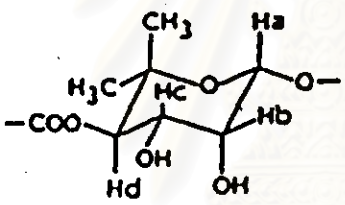


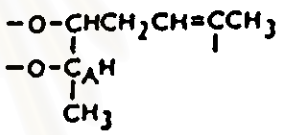
[32] Dapiramicin A



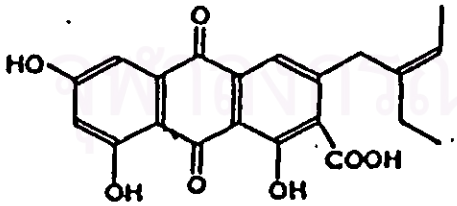
[33] Antibiotic XK-62-6; R=CH_2NHCH_3

[34] Antibiotic XK-62-8; R=$CH(CH_3)NHCH_3$

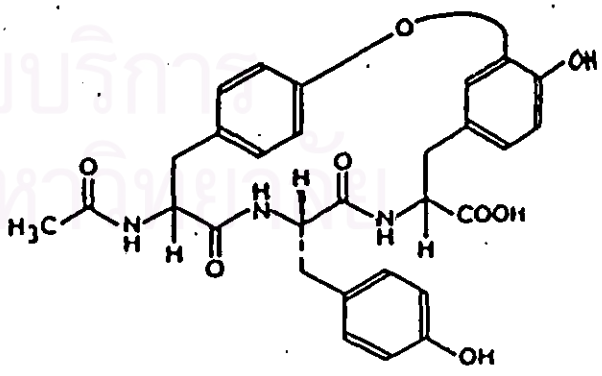




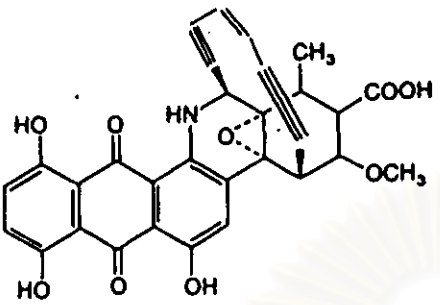
[35] Clostomicin B



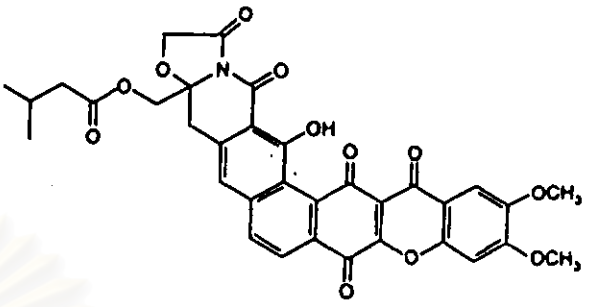
[36] Antibiotic K-259-2



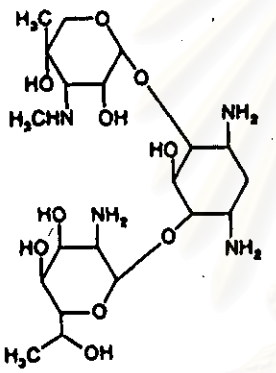
[37] Antibiotic K-13



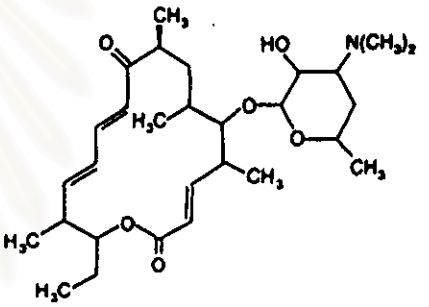
[38] Antibiotic BU-3420T



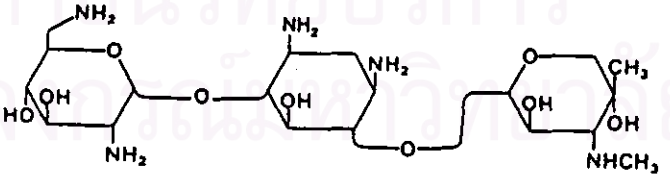
[39] Antibiotic LL-E19085



[40] Antibiotic G-418

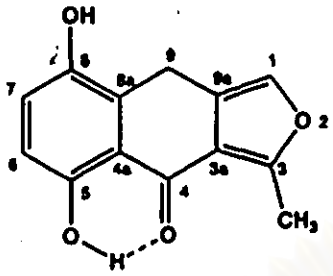


[41] Mycinamicin VIII

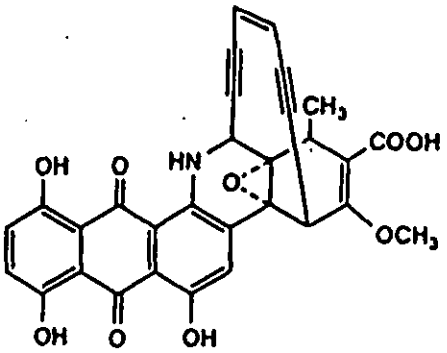


[42] Antibiotic JI-20A; R=H

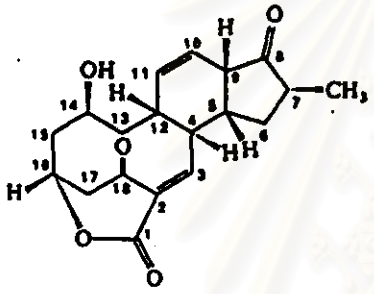
[43] Antibiotic JI-20B; R=CH_3



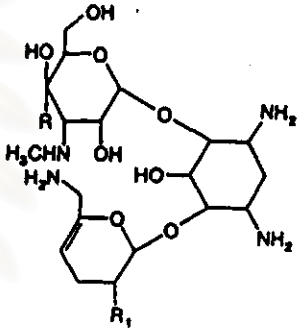
[44] MS-444



[45] Dynemicins



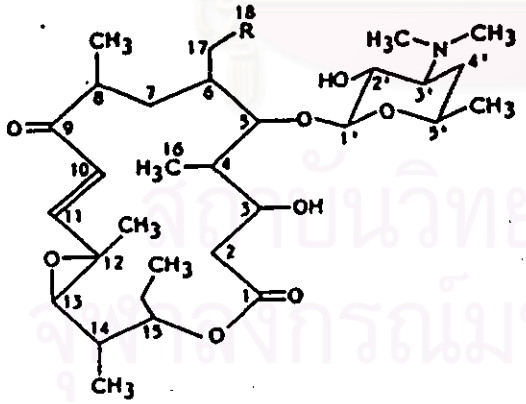
[45] Macquarimicin



[47] Antibiotic I=SKA$_1$;R=CH_3, R_1=OH

[48] Antibiotic I=SKB$_1$;R=CH_3, R_1=NH_2

[49] Antibiotic I=SKB$_2$; R=H, R_1=NH_2



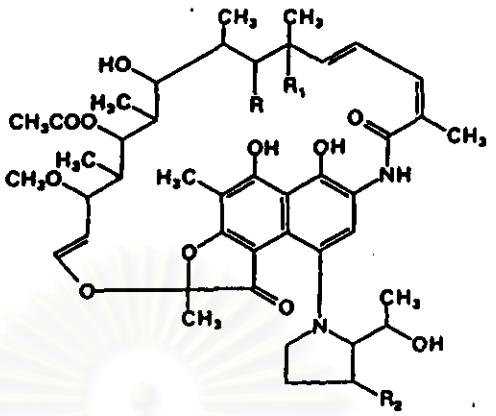
[50] Antibiotic M-4365A$_1$; R=CH_3

[51] Antibiotic M-4365A$_2$; =Rosaramicin

[52] Antibiotic M-4365A$_3$; =Juvenimicin

[53] Antibiotic M-4365G$_1$; R=CHO

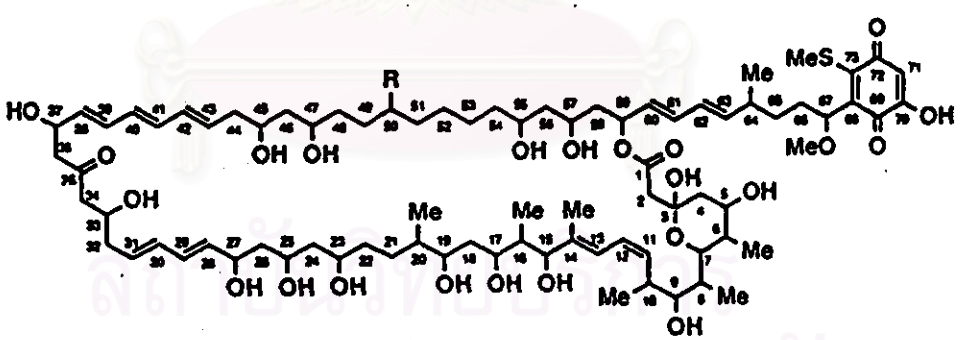
[54] Antibiotic M-4365G$_2$; R=CH_2OH



[55] Halomicin B; R=R_1=H; R_2=OH

[56] Halomicin C; R=R_2=H, R_1=OH

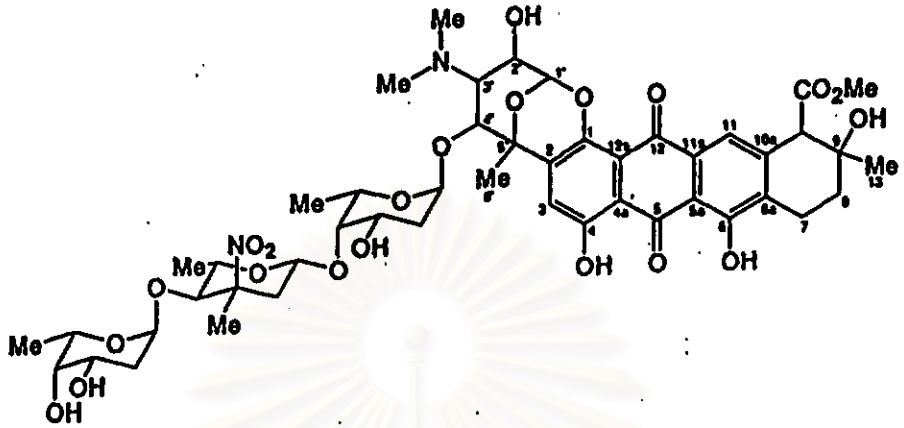
[57] Halomicin D; R=R_2=H; R_1=OH



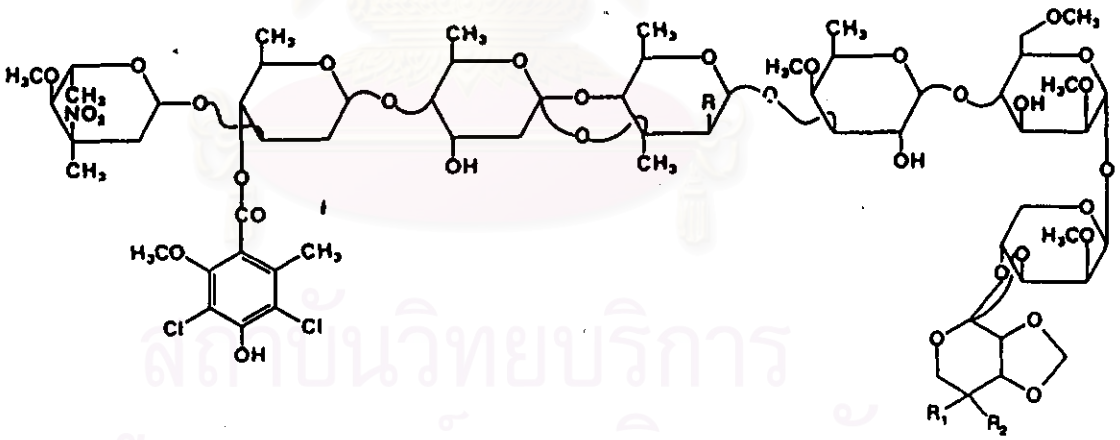
[58] Quinolidomicin A_1; R=CH_3

[59] Quinolidomicin B_1; R=H

[60] Quinolidomicin A_2; R=CH_3



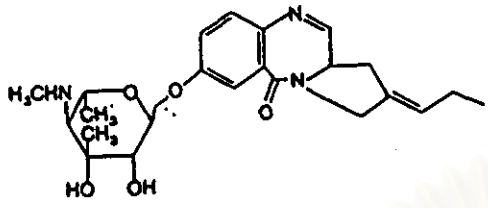
[61] Cororubicin



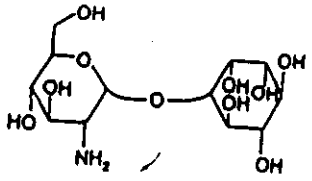
[62] Everninomicin B; R=R_1=OH, R_2=$CH(OCH_3)CH_3$

[63] Everninomicin C; R=R_2=H, R_1=OH

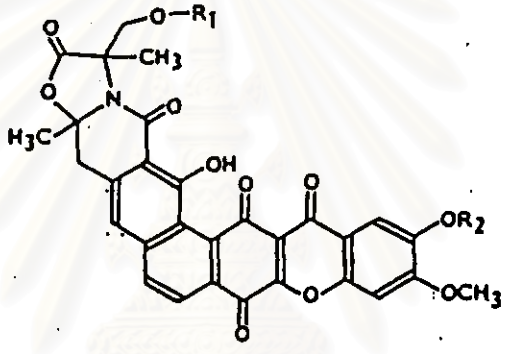
[64] Everninomicin D; R=H, R_1=OH, R_2=$CH(OCH_3)CH_3$



[65] Antibiotic X-14847



[66] Sibanomicin



[67] Citreamicin α; R_1=$COCH_2CH(CH_3)_2$, R_2=CH_3

[68] Citreamicin β; R_1=$COCH(CH_3)_2$, R_2=CH_3

[69] Citreamicin γ; R_1=$COCH_3$

[70] Citreamicin δ; R_1=$COCH_2CH(CH_3)_2$, R_2=H

[71] Citreamicin η; R_1=H, R_2=CH_3

Some *Micromonospora* appear quite sensitive to acid conditions. Potato plug after autoclaving have pH of 5.8 to 6.2. This can easily be determined with various pH indicators such as by adding a small amout of $CaCO_3$ directly to the potato plug or by testing an isolate's ability to grow on both the acid and neutralized potato plug.

Ability to grow on various organic and inoganic nitrogen sources has yielded little differential information. Czapek's agar with 1 % soluble starch is an exception. Most strains of *Micromonospora echinospora* and *M. purpurea* grow well on this substrate. Hydrolysis reaction, such as starch, gelatin, and milk , appear positive for most *Micromonospora* tested Carbohydrate utilization has been used extensively to characterize *Streptomyces* strains and species . It is also useful for characterizing strains and species of *Micromonospora*. The basal inorganic salts medium of pridham and Gottlieb has been modified to accomodate the *Micromonospora*.

Organisms of the genus *Micromonospora* occur infrequently in soils but in relatively high numbers in aqutic habitats such as lake mud and river sediments. Their occurrence in soil was first reported by Jensen (1932) for Astralian soils and later by Kriss (1939) for Russian soils. Early work on lakes in Wisconsin showed that *Micromonospora* comprised 10-50 % of the microbial population in the water mass, were the only actinomycetes in mud samples, but were rarely isolated from adjacent soils (Umbreit and McCoy, 1940) *Micromonospora* have also been isolated from marine environments , such as beach sand (Watson and Williams , 1974) , deep marine sediments (Weyland ,1969) and sediment from White Sea and Black Sea.

Differential characteristics of the genus *Micromonospora* are shown in Table 3 (Holt, 1989).

3. Antibiotic screening

3.1 Primary screening

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

The classical agar diffusion method have 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. (Colegate and Molyneux, 1993).

Waskman and Starkey, (1987), by 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 (Waksman, S.A.,1962). This is of prime importance, since antibiotics must be obtained in liquid media for large scale production.

Table 3 Differential characteristics of the genus *Micromonospora*

Characteristics	*M. carbonacea* subsp. *carbonacea*	*M. carbonacea* subsp. *aurantiacea*	*M. halophytica* subsp. *halophytica*	*M. halophytica* subsp. *nigra*	*M. chalcea*	*M. inositola*
colony colour	orange	orange-red	brown	orange-black	orange-black	-
spore colour	brown (0.7-1.0 μm)	brown (0.7-1.0 μm)	orange-black (1.2 μm)	-	dark brown (0.7-1.0 μm)	blight orange (0.8-1.0 μm)
diffusible pigment	-	pale yellow	reddish brown	-	pale yellow	-
nitrate reduction	+	-	+	+	Weak	-
NaCl tolerance (%)	3	3	4	4	5	1.5
melanin pigment	-	-	-	-	-	-
gelatin liquefaction	+	+	+	+	+	+
milk peptonization	+	+	+	+	-	-
growth temperature	27-37°C	27-37°C	18-40°C	18-40°C	27-37°C	25-40°C
main menaquinone	9	9	9	9	10	10
carbon utilization	raffinose, salicin	raffinose, melibiose	melibiose, raffinose	arabinose, galactose, β-lactose, galactose, fructose	D-galactose, fructose	D-galactose, fructose, lactone

Table 3 (continued)

Characteristics	*M. coerulea*	*M. purpureochromogenes*	*M. olivasterospora*	*M. echinospora* subsp. *echinospora*	*M. echinospora* subsp. *feruginea*	*M. echinospora* subsp. *pallida*
colony colour	orange-black	dark brown	light-brown to dark yellow	orange-brown	orange	ivory
spore colour	blue green (0.8-1.5 μm)	brown (0.8-1.2 μm)	dark green (1.0 μm)	purplish black (1.0-1.5 μm)	blue	blue
diffusible pigment	-	dark brown	olive green	maroon to purple	maroon-purple	-
nitrate reduction	-	-	-	weak	-	+
NaCl tolerance (%)	1.5	1.5	3	3	3	3
melanin pigment	-	-	-	-	-	-
gelatin liquefaction	+-	-	+	+	+	+
milk peptonization	+-	+-	+	+	+	+
growth temperature	24-37°C	27-37°C	28-38°C	27-37°C	27-37°C	27-37°C
main menaquinone	10	10	10	10	10	12
carbon utilization	D-galactose, fluctose, lactose	galactose, fructose	galactose, fructose	L-arabinose	fluctose -	L-rhamnose

3.2 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 determines which microorganism are able to produce a compound without providing much idea of the production or yield potential for the organisms.

Secondary 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 are pH, aeration or 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.

4. Fermentation conditions

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. (Table 4).

Table 4 Nutrents and condition for antibiotic production of the genus Micromonospora

Strains	antibiotics	seed		production		reference
		medium	condition	medium	condition	
M. megalomicia	Megalomicin	beef extract, tryptone, dextrose, potato starch , yeast extract $CaCO_3$		yeast extract, dextrose, starch, casein, $CaCo_3$		Weinstein *et al.*, 1969: 253-258
M. purpureochromogenes subsp. *halotolerans*	Crisamicin	glucose, soluble stach, yeast extract, $CaCO_3$	28°C, 250 rpm	glucose, potato dextrin, $CaCO_3$		Nelson *et al., 1985*
M. echinospora subsp. *armeniaca*	Clostomicin	glucose, starch, peptone, meat extract, yeast extract, $CaCo_3$	27°C	soluble starch, dry yeast, $CaCO_3$	27°C	Omura *et al* ., 1985: 1407-1417
M. halophytica subsp. *exillisia*	K-13	glucose, soluble starch, beef extract, yeast extract, tryptone, $CaCO_3$	ph 7.2, 28°C, 220 rpm	dextrin, soybean meal, corn steep liquor, K_2HPO_4, $MgSO_4.7H_2O$	pH 7.8, 30°C, 350 rpm	Kase *et al.*, 1986: 450-458
M. oligovasterospora	K-259-2	glucose, soluble starch, beef extract, yeast extract, tryptone, $CaCO_3$	28°C, 300 rpm	glucose, soluble starch, soybean meal, corn steep liquor, yeast extract, pharmamedia, $CaCO_3$	28°C, 200 rpm	Matsuda *et al., 1987*: 1092-1100

Table 4 (continued)

Strains	antibiotics	seed		production		reference
		medium	condition	medium	condition	
M. neihuensis	neihumicin	glucose, tryptone, yeast extract, K_2HPO_4	pH 7.0, 28°C, 200 rpm	glucose, molass, peptone, $CaCO_3$	pH 7.0, 28°C, 200 rpm	Wu *et al.*, 1987: 481-486
Micromonospora sp.	Sch 37137	beef extract, tryptone, yeast extract, potato starch, $CaCO_3$	30°C, 300 rpm	NZ-amine, yeast extract, $COCl_2$	30°C, 300 rpm	Cooper *et al.*, 1987: 13-24
M. fastidiosa nov.	rosaramicin	glycerol, maltose syrup, yeast extract, pharmamedia, wheat germ meal, meat extract, $(NH_4)_2SO_4$, KH_2PO_4, NaCl, $MgSO_4.2H_2O$, $CaCO_3$		glucose, yeast extract, soluble starch, skim milk, K_2HPO_4, NaCl, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$		Funaishi *et al.*, 1989: 938-947
M. citrea	Citreamicin	glucose, dextrin, yeast extract, $CaCO_3$	32°C	dextrin, glucose, nutrisoy, corn steep liquor, $CaCO_3$	28°C, 110 rpm	Carter *et al.*, *1989: 504-512*

Table 4 (continued)

Strains	antibiotics	seed		production		reference
		medium	condition	medium	condition	
M. chersina sp. nov. M956-1	Dynemicins	lactose, soluble starch , fish meal, $CaCO_3$., $CaSO_4$. $2H_2O$	pH 7.0, 32°C, 200 rpm	soluble starch, glucose, beet molasses, fish meal,$CaCo_3$	pH 7.0, 28°C, 250 rpm	Konishi *et al.*, 1991
Micromonospora sp.	M-444	glucose, soluble stach, yeast extract, beef extract, tryptone, KH_2PO_4, $MgSO_4$.$7H_2O$	28°C	glucose, corn steep liquor, soluble vegetative protein, cotton seed oil, $CoCl_2$.$6H_2O$	28°C	Ishigami *et al.*, 1994: 1219-1225
M. chalcea	macquarimicins	glucose, soluble starch, yeast extract, tryptone, beef extract, $CaCO_3$	pH 7.0, 30°C, 250 rpm	sucrose, cotton seed flour, yeast extract, K_2HPO_4, $CoCl_2$.$6H_2O$, $MgSO_4$.$7H_2O$	pH 7.3, 30°C, 200 rpm	Jackson *et al.*, 1995: 462-470

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 stand point 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. (Yuwapin, 1997).

5. Isolation, purification and elucidation of structures of antibiotics

5.1 Study of antibiotic by thin layer chromatography

Over the years many investigators have devised numerous procedures for classification and identification of antibiotics by use of 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. Sephadex has been used in thin layer chromatography for the identification of antibiotics by Zuidweg et al.,1969. The solvent selected by the thin layer chromatography can be applied to column chromatography for preparative separation.

5.2 Bioautographic detection of antibiotics in preparation chromatogram

Bioautography 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 coontact 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 inocubation perioud , 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 h. The presence of antibacterial compounds is indicated by a clear spot against a colored background. The assay, using *Bacillus subtilis* and *E. coli*, proved insensitive to a number of cytotoxic compounds belonging to the camptothecin, quassinoid and lignan series . Dispersal of bacteria can be reduced by containing the assay system in a glove bag. While these techniques are useful as a front-line screen, selective search strategies have been devised and have identified a variety of new antibiotics (Colegate, 1993).