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# Applications of Actinobacterial Fungicides in Agriculture and Medicine

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## 1. Introduction

Actinobacteria are found in virtually every natural substrate, such as soils and compost, freshwater basins, foodstuffs and the atmosphere. Deep seas, however, do not offer a favorable habitat. These organisms live and multiply most abundantly in various depths of soil and compost, in cold and in tropical regions. Alkaline and neutral soils are more favorable habitats than acid soils and neutral peats are more favorable than acid peats.

The application of fungicides and chemicals can control crop diseases to a certain extent, however, it is expensive and public concern for the environment has led to alternative methods of disease control to be sought, including the use of microorganisms as biological control agents. Microorganisms are abundant in the soil adjacent to plant roots (rhizosphere) and within healthy plant tissue (endophytic) and a proportion possess plant growth promotion and disease resistance properties. Actinobacteria are gram-positive, filamentous bacteria capable of secondary metabolite production such as antibiotics and antifungal compounds. A number of the biologically active antifungal compounds are obtained from the actinobacteria. A number of these isolates were capable of suppressing the fungal pathogens *Rhizoctonia solani*, *Pythium* sp. and *Gaeumannomyces graminis* var. *tritici*, both *in vitro* and in plants indicating the potential of the actinobacteria to be used as biocontrol agents.

The principal reason behind the actinobacteria having such important roles in the soil and in plant relationships comes from the ability of the actinobacteria to produce a large number of secondary metabolites, many of which possess antibacterial activity. Actinobacteria produce approximately two-thirds of the known antibiotics produced by all microorganisms. The genus *Streptomyces* produces nearly 80% of the actinobacterial antibiotics, with the genus *Micromonospora* producing one-tenth as many as the *Streptomyces*. In addition to the production of antibiotics the actinobacteria produce many secondary metabolites with a wide range of activities. Activities of the secondary metabolites include antifungal agents

that degrade cell walls and inhibit the synthesis of mannan and  $\beta$ -glucan enzymes, antiparasitic agents and insecticidal agents.

Actinobacteria produce a number of plant growth regulatory compounds, some of which have been used commercially as herbicides. Not all secondary metabolites are antimicrobial. Others are enzyme inhibitors, immunomodulators and antihypertensives. The actinobacteria produce over 60% of secondary metabolites produced by microorganisms, with *Streptomyces* accounting for over 80%.

In some cases actinobacteria form a pathogenic relationship with plants. *Streptomyces scabies* is a soil-borne actinobacterium that is the principal causal agent of scab diseases, which affect a variety of underground tuberous vegetables such as potato. *S. scabies* produces thaxtomin, a family of phytotoxins, that induce the development of necrotic lesions in potato. There is a 100% correlation between pathogenicity and the ability to produce thaxtomin. Scab suppressive soils have been identified and it has been found that the lenticels on these tubers are colonised by *Streptomyces* (Schottel *et al.*, 2001). Suppressive strains of *Streptomyces* isolated from a naturally scab suppressive soil produced antibiotics that inhibited *S. scabies in vitro* (Neeno-Eckwall and Schottel, 1999).

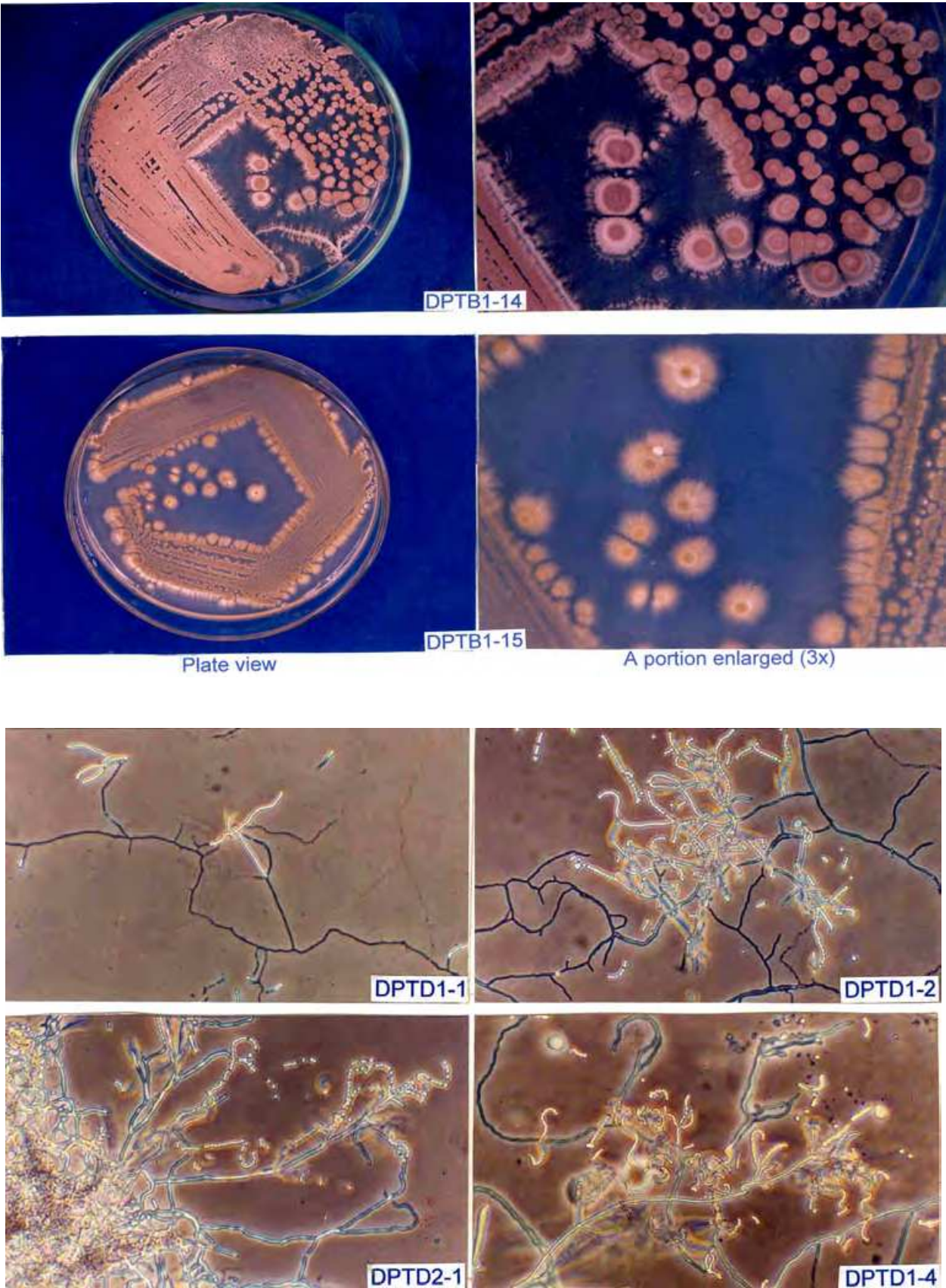
*Streptomyces* species have also been implicated in the biological control of a number of other pathogens. *S. ambifaciens* inhibited *Pythium* damping-off in tomato plants and *Fusarium* wilt in cotton plants. *S. hygroscopicus* var. *geldanus* was able to control *Rhizoctonia* root rot in pea plants and the inhibition was due to the production of the antibiotic geldanamycin. *Streptomyces lydicus* WYEC108 inhibited *Pythium ultimum* and *R. solani in vitro* by the production of antifungal metabolites (Yuan and Crawford, 1995). A number of other actinobacteria that are used in inhibiting the human and animal pathogens such as *Aspergillus niger*, *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. *Candida albicans*, *Cryptococcus neoformans*. This chapter describes the potential applications of fungicidal substances from actinobacterial origin, screening methods, mode of action of fungicides against plant and animal fungal pathogens.

### 1.1 Antagonistic actinobacteria

The actinobacteria first recognized as potential destroyers of fungi and bacteria by Gasperini (1890). Tims (1932) studied an actinobacteria antagonistic to *Pythium* of sugarcane. Waksman (1937) made a detailed survey of actinobacteria possessing antagonistic effect upon the activity of other microorganisms in their studies on decomposition.

Dhanasekaran *et al.*, (2009a) screened 78 *Streptomyces* isolates for their antimicrobial activity against pathogenic fungi by agar overlay assay method. Among the 78 isolates, 18 isolates showed antifungal activity. The maximum percentage of the isolates of *Streptomyces*, which showed antifungal antagonistic activity, was found in sea shore soil (13/27 isolates, 48.14 %) followed by salt pan soil (4/9 isolates, 44.44 %), estuarine soil (3/12 isolates, 25 %) and agricultural field soil (5/30 isolates, 16.6 %). Among the 18 isolates tested, all the isolates showed extracellular antifungal activity including 8 isolates having both extra and intracellular antifungal activity (Fig.1; Plate 1). They also studied the antifungal actinobacteria in marine soil of Tamilnadu against *Candida albicans*, *Aspergillus niger* using agar overlay, diffusion assay method (Dhanasekaran *et al.*, (2005b) and estuarine *Streptomyces* against the *Candida albicans* (Dhanasekaran *et al.*, (2009b)







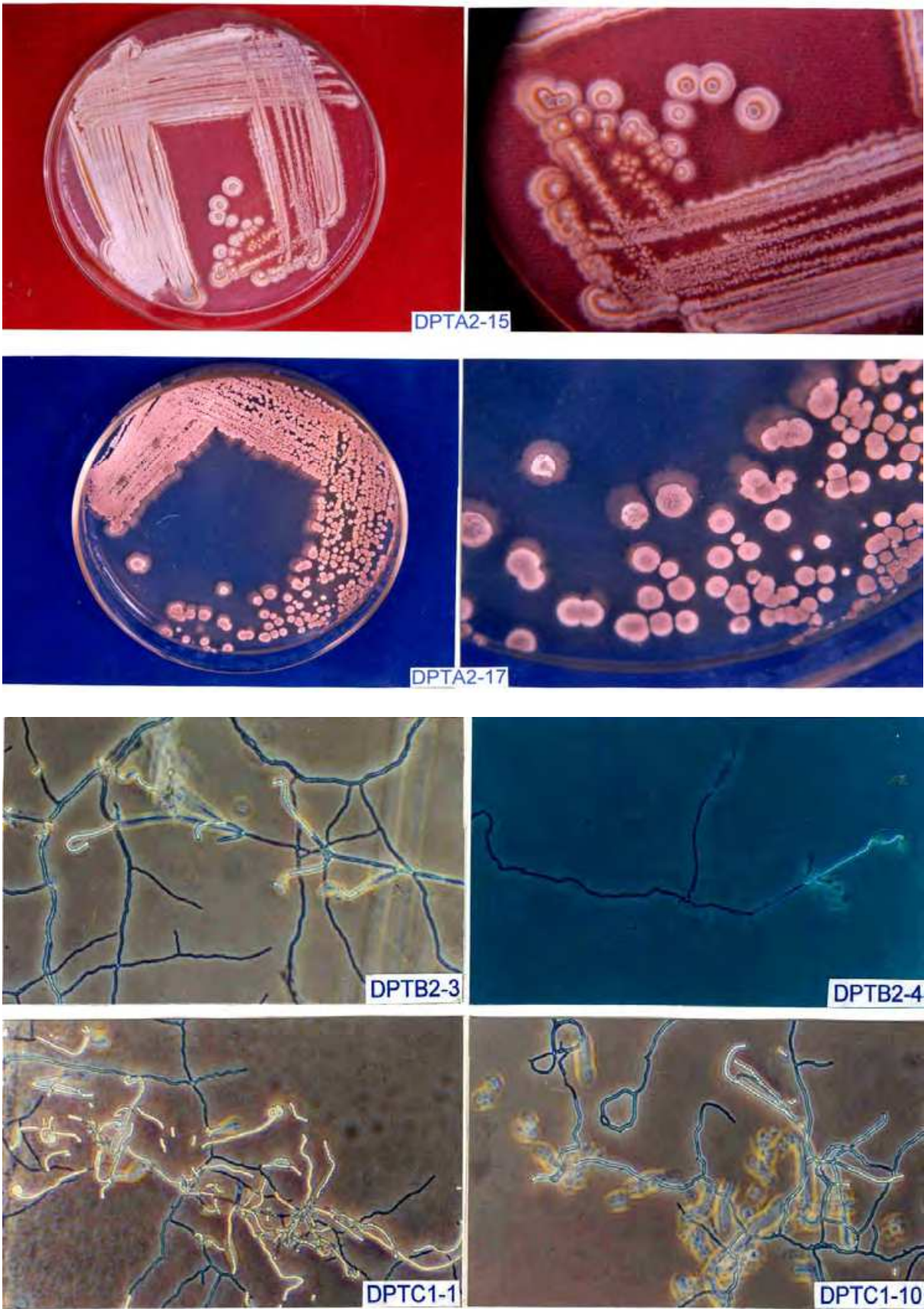


Plate 1. Cultural and microscopic view of *Streptomyces* isolates aerial mycelium with spores

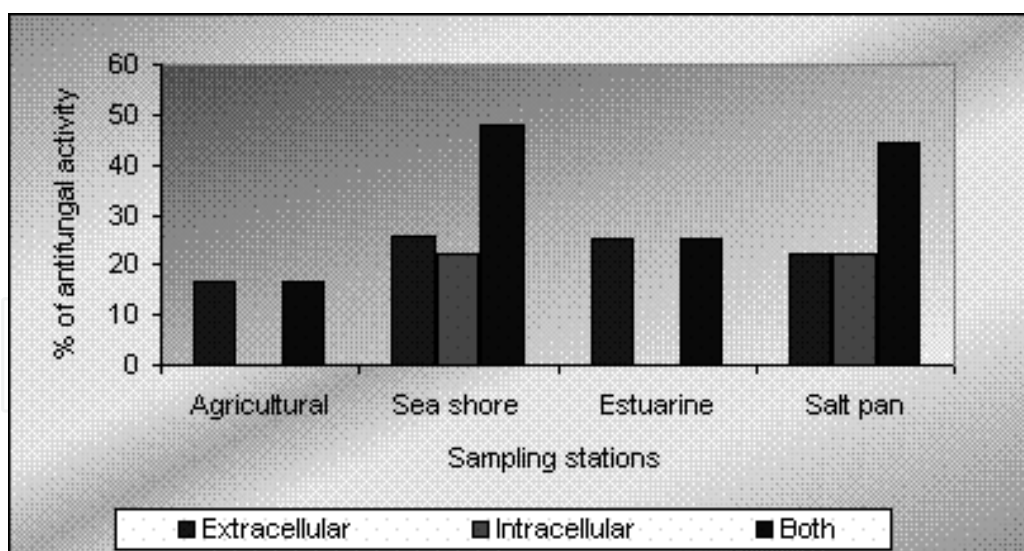


Fig. 1. Antifungal activity of Extra and Intracellular compounds of *Streptomyces* isolates

## 2. *In vitro* screening methods of fungicidal substances produced by actinobacteria

### 2.1 Crowded plate technique

A series of test tubes containing 9 ml of sterile water was taken. From the stock culture, 1 ml suspension was transferred aseptically to the 1<sup>st</sup> tube ( $10^{-1}$ ) and mixed well. Further serial dilutions were made to produce  $10^{-5}$  suspensions. Suspension (0.1 ml) from each test tube was spread on sterile soyabean-casein digest medium (SBCD), actinobacteria isolation agar (AIA) medium and starch-casein agar medium plates aseptically in a laminar air flow cabinet. The plates were incubated at  $27 \pm 2^\circ\text{C}$  for 84 h. The plates were observed intermittently during incubation. After 72 h, whitish pin-point colonies, characteristic of actinobacteria and with clear zone of inhibition around them were observed. The pinpoint colonies with inhibitory or clear zone of inhibition were selected and purified and used as a potent isolate for fungicidal compound production.

### 2.2 Agar streak method

The Fungicidal activity of the soil actinobacterial isolates were analyzed by agar streak method. Each of the isolate was streaked as a straight line on Starch casein agar (SCA) medium and incubated at  $27^\circ\text{C}$  for 6 days. After the 6<sup>th</sup> day, different fungal pathogens were streaked at right angle, but not touching each other, and then incubated at  $28^\circ\text{C}$  for 48 h. If the organism is susceptible to the antibiotic produced by actinobacteria, then it will not grow near the actinobacteria. The zone of inhibition against each test fungal pathogen was noted.

### 2.3 Agar disk method

The *Streptomyces* isolate was smeared on SCA medium as a single streak and incubated at  $28^\circ\text{C}$  for 4-6 days, from well grown streaks 6 mm agar disks of *Streptomyces* colony mass was prepared by using sterile cork borers. Disks were then aseptically transferred to PDA plates having fresh lawn cultures of *Aspergillus* isolate. Controls included using plain disks from

SCA medium. Plates were incubated at 24°C for 4-6 days and fungicidal activity was evaluated by measuring the diameter of inhibition zones (mm).

## 2.4 Dual culture method

Antifungal activity of actinobacterial isolates were tested by dual culture technique using PDA medium. A mycelial disc of the fungal pathogen (5mm dia.) was placed at one end of the Petri plate. The actinobacterial antagonists were streaked 1 cm away from the periphery of the Petri plate just opposite to the mycelial disc of the pathogen. Visual observation on the inhibition of pathogenic fungal growth was recorded after 96 hours of incubation in comparison with the PDA plate simultaneously inoculated with fungal pathogen only as control.

Percent of test pathogen inhibition by the actinobacterial isolate was evaluated by dual culture technique. The radial growth of mycelium in mm was measured and percent inhibition (PI) was calculated.

$$PI = \frac{C-T}{C} \times 100$$

Where, C is the growth of test pathogen (mm) in the absence of the antagonistic isolate; T is the growth of test pathogen (mm) in the presence of the antagonistic isolate.

## 2.5 Agar overlay method

To evaluate the fungicidal activity of the actinobacteria, phytopathogenic filamentous fungi were used as test microorganisms. The actinobacteria were spot inoculated onto SCA medium and incubated at 28°C for 14 days. After this period, the antagonism between actinobacteria and the test fungal pathogen was evaluated using the agar over lay method. For this procedure, 10 ml of Sabouraud soft agar medium was added and inoculated with 10<sup>6</sup> spores/ml of filamentous fungi. All plates were incubated at 28°C and incubation time of 7-10 days for fungi.

## 2.6 Well diffusion method

The sterilized Sabouraud's dextrose agar medium (Dextrose 4.0 g, Mycological peptone 1.0 g, Agar 2.0 g, pH 5.0, Distilled Water 100 ml,) was poured to a petridish in a uniform thickness and kept aside for solidification. Using sterilized swabs, even distribution of lawn culture was prepared using desired fungi such as *A. niger*, *P. notatum*, *C. albicans* in SDA plates. Using sterile well cutter two wells were made in plates at required distance. 20µl of different solvents treated test fungicidal compound was added in to one well and another well was loaded with corresponding control (solvent without compound). The plates were incubated at room temperature for 48 hours. After incubation, the zone of inhibition was analyzed and recorded.

## 2.7 Paper disc assay

### 2.7.1 Preparation of disc

The filter paper disc were impregnated with 5µg/µl antifungal compound + 25µl distilled water, similar procedures were used to prepare the other concentrations of the disc such as 10µg/µl + 20µl distilled water, 15µg/µl + 15µl distilled water, 20µg/µl + 10µl distilled water, 30µg/µl + 1µl distilled water.



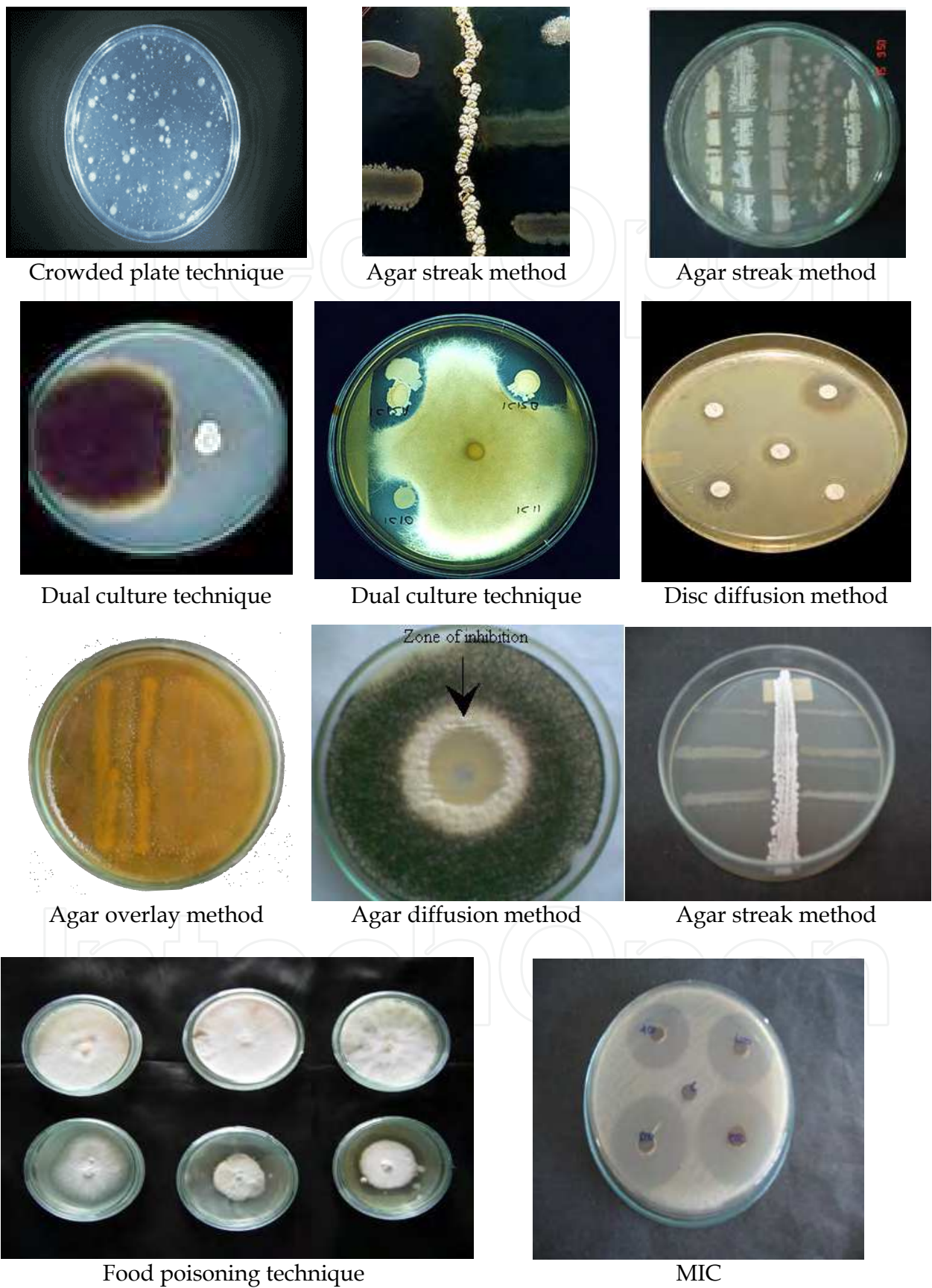


Plate 2. *In vitro* screening methods for Fungicidal substances produced by actinobacteria



### 2.7.2 Inoculum preparation

All the clinical pathogens were prepared in 0.85% saline corresponding to No. 0.5 McFarland turbidity standard. All cultures were incubated on a shaker at 37°C for 18 h and then diluted to 1/10 the concentration to yield a culture density of approximately 10<sup>8</sup>CFU/ml. The pathogens used in the study such as *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus flavus* for sensitivity assay.

### 2.7.3 Antifungal assay

Mueller Hinton agar (Beef extract 0.2 g, Peptone 1.75g, Starch 0.15g, Agar 2.0g, Distilled water 100 ml, pH 7.5) prepared with lawn culture using desired test organisms. The inoculated plates were kept aside for few minutes. The discs with fungicidal compound were placed over the medium. After diffusion, the plates were incubated at 28°C for 48hours for antifungal analysis. After incubation, the zone of inhibition was analyzed and recorded.

### 2.8 Determination of Minimum Inhibitory Concentrations (MIC)

To measure the MIC value, two-fold serial dilutions of 50, 25, 12.5, 6.25, 3.125, 1.562 and 0.781 mg ml<sup>-1</sup> of the fungicidal compound was prepared in solvent and assayed by well diffusion method. The MIC was defined as the lowest concentration able to inhibit any visible fungal growth.

## 3. Fungicidal potential of actinobacteria against phytopathogen in crop plants

Fungal phytopathogens pose serious problems worldwide and cause a number of plants and animal diseases such as ringworm, athlete's foot, and several more serious diseases. Plant diseases caused by fungi include rusts, smuts, rots, and may cause severe damage to crops. Fungi are some of the world's largest and possibly oldest individuals.

Agrochemical treatment may result in environmental impact and pose a threat to humans and animals. As a result, there has been an increase in research on potential Biocontrol agents, aimed at finding a definitive solution or at least reducing pesticide use in the treatment of phytopathogenic diseases.

Actinobacteria have been considered as potential Biocontrol agents of plant diseases. Several investigators have described the *in vitro* and *in vivo* activities of the actinobacteria. Their modes of action includes parasitism of hyphae (El-Tarabily and Sivasithamparam, 2006), oospores or fungal sclerotia (Crawford *et al.*, 1993) competition with pathogens (Kunoh, 2002), antibiotic production (Igarashi, 2004), siderophores (Khamna *et al.*, 2009), as herbicides (Hasegawa *et al.*, 2006), and via enzymes such as cellulases, hemicellulases, chitinases, amylases, and glucanases (Yuan and Crawford, 1995).

In addition, actinobacteria may affect plant growth (Igarashi, 2004). According to Kunoh (2002), endophytic *Streptomyces* may play an important role in the development and health of plants, because it affects plant growth due to its assimilation of nutrients and production of secondary metabolites. The tomato (*Lycopersicon esculentum*) is highly susceptible to phytopathogen attack, and tomato crops are most intensively treated with agrochemicals.

Among the many fungal pathogens that attack tomatoes are *Phytophthora infestans*, *Alternaria solani*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium oxysporum*, Most of these pathogens not only spread disease in tomato plants, but also affect other crops. According to Cao *et al.* (2004a), *R. solani* can develop in both farmed and unfarmed soils, spreading disease in many crops, including rice. *F. oxysporum* attacks banana plants, causing a disease known as fusarium wilt (Cao *et al.*, 2004b) and also infects wheat (Taechowisan *et al.*, 2003). *R. solanacearum* is an important soil pathogen causing bacterial wilt in more than 200 plant species, including the potato, tomato, pea, tobacco, banana and others (Tan *et al.*, 2006).

Marten *et al.* (2001) reported that RhizovitR from *Streptomyces rimosus* is used in the control of a wide range of fungi such as *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*, *Alternaria brassicola*, and *Botrytis* sp. Liu *et al.* (2004a) also reported that *S. rimosus* showed a high antagonism activity against *Fusarium solani*, *F. oxysporium* f sp. *cucumarinum*, *Verticillium dahliae*, *R. solani*, *Fulvia fulva*, *Botrytis cinerea*, *A. alternata*, *Sclerotinia sclerotiorum* and *Bipolaris maydis*. The antifungal antibiotic, which is produced by *S. rimosus*, was purified by silica gel column chromatography. Its ultraviolet (UV) spectrum was consistent with that of polyene macrolide, which had the same absorption peaks at 291, 305, and 318 nm. Antifungal activity can be kept for 20 months at room temperature (12–30°C, pH 5.4) (Liu *et al.*, 2004b). So *S. rimosus* will be employed as a target to search for new biocontrol agents or drugs to satisfy public demands, and much interests will be generated (Table1).

Name of the actinobacteria	Fungicidal activity against	Disease control	Investigator(s) and year
Actinomycetes	<i>Fusarium oxysporum</i> <i>F. cubense</i> <i>Penicillium graminicolum</i>	Vascular wilt in Tomato	Meredith, 1946
<i>Streptomyces antibioticus</i>	<i>Helminthosporium sativum</i>	Seedling blight and root rot of cereals	Stevenson, 1956
<i>Streptomyces aureus</i>	<i>H. oryzae</i> <i>C. lunata</i>	Brown leaf spot of Rice Stem blight disease of Cassava	Chakrabarthy and Chandra, 1979
<i>S. violaceoniger</i>	<i>Macrophomia phaseolina</i>	Charcoal root rot of cotton, soybean, peanut, and corn.	Hussain and El-Gammal, 1980 Ivanova <i>et al.</i> , 1998
<i>S. globus</i>	<i>Alternaria solani</i> <i>A. niger</i> <i>C. pallescens</i> <i>Phytophthora</i> sp.	Early blight in potato and Tomato Pod, seed rot of peanut Black mould of onion Wilt and root rot	Paul and Banerjee, 1983 Nair <i>et al.</i> , 1994 Hwang <i>et al.</i> , 1996.
<i>S. arabicus</i>	<i>A. brassicae</i>	Blight, black leaf spot in cauliflower, cabbage, radish	Sharma <i>et al.</i> , 1985; El-Shahed, 1994
<i>Streptomyces</i> sp.	<i>Botrytis</i> sp. <i>Helminthosporium</i> sp. <i>Fusarium</i> sp. <i>Pyricularia</i> sp. <i>Sclerotinia sclerotiorum</i>	Blights and fruit rots in alliums, beans, carrot, celery, citrus Leaf, crown, and root diseases in bluegrass and rye - Blast in Rice Cottony rot, watery soft rot, stem rot in flowers and vegetables	Tanaka <i>et al.</i> , 1987 De and Gupta, 1991 Matsuyama, 1991 Wang and Shen, 1992
<i>Streptomyces</i> sp.	<i>Phytophthora</i> sp.	Blights of leaves and shoots and root and crown rots in Ericaceae (Rhododendrons, azaleas, etc.)	Omura <i>et al.</i> , 1988; Omura, 1990; Hwang <i>et al.</i> , 1994; Tang <i>et al.</i> , 2000
<i>S.griseochromogenes</i>	<i>S. scabies</i>  <i>Botrytis</i> sp.  <i>Phytophthora</i>	Scab on potatoes, beets, radish, rutabaga, turnip, carrot and parsnips - Late blight or potato blight.	Cheng <i>et al.</i> , 1989 Philips and Mc Closkey, 1990 Eckwall and Schottel, 1997 Xiao <i>et al.</i> , 2002
<i>Streptomyces</i>	<i>Phytophthora capsici</i>	Blight in <i>Capsicum annum</i>	Kook and Kim, 1995
Marine <i>Streptomyces</i> sp. Strain AP77	<i>Pythium</i>	Damping-off and seedling diseases	Woo and Kamei, 2003

Name of the actinobacteria	Fungicidal activity against	Disease control	Investigator(s) and year
<i>Streptomyces</i> sp.	<i>F. moniliforme</i> , , <i>F. oxysporum</i> , <i>Botrytis pumilis</i> <i>C. albicans</i>	Ear-rotting in maize Wilt on banana, tomato - -	Haque <i>et al.</i> , 1992 Abussaud, 1996 Malviya <i>et al.</i> , 1994 Saadoun <i>et al.</i> , 2000 a,b Singh <i>et al.</i> , 2009
<i>Streptomyces</i> sp.	<i>A.fumigatus</i> , <i>A. niger</i> , <i>M. hiemalis</i> , <i>P. roqueforti</i> , <i>P. rariotii</i> , <i>C. albicans</i> , <i>Cryptococcus humicolus</i> <i>P. cinnamoni</i> , <i>Pestalotiopsis sydowiana</i> , <i>B. cinerea</i> , <i>Sclerotinia homoeocarpa</i> , <i>A. alternata</i> , <i>M. circinellodies</i> , <i>P.citirum</i> , <i>P. notinanae</i> , <i>P. aphanidermatum</i> , <i>P. oligandrum</i> , <i>P. porphyrae</i> , <i>P. ultimo</i> , <i>Ustilogo maydei</i>	- - - Roots of disease-Rhododendron  - Corn smut is a disease of maize	Frandsberg <i>et al.</i> , 2000 Shimizu <i>et al.</i> , 2000  Woo and Kamei., 2003 Moncheva <i>et al.</i> , 2002  Augustine <i>et al.</i> , 2004 Augustine and Kapadnis, 2005 Dhanasekaran <i>et al.</i> , 2005 b
Marine actinomycetes	<i>R. solani</i> , <i>Pyricularia oryzae</i> , <i>H. oryzae</i> and <i>Colletotrichum falcatum</i>	- Red rot disease of sugarcane	Kathiresan <i>et al.</i> , 2005 Dhanasekaran <i>et al.</i> , 2005c
<i>Streptomyces</i> sp. GAAS7310	<i>Rhizopus nigricans</i> <i>Geotrichum candidum</i> Link <i>Fusarium oxysporum</i> f. sp.cubense <i>Cladosponum carpophilum</i> Thum. <i>Alternaria solani</i> (E. et M.) Jones et Grout <i>Cladosporium</i> sp. <i>Sclerotinia sclerotiorum</i> (Lib.) de Bary <i>Peronophthora litchi</i> Chen <i>Botrytis</i> sp. <i>Colletotrichum nigrum</i> EL.et Halst <i>Curvularia lunata</i> (Wakker) Boed <i>Phomopsis vexans</i> (Sacc et Syd) Harter	Rot in Peaches, cherries Sour rot in peach, tomato Panama disease in banana Scab in Plum Early blight in tomato Internal blight in papaya fruit White Rot of Sunflower Brown blight in Litchi Leaf rot in Potato Anthracnose in Chilli Leaf Spot in Millet Phomopsis blight in Brinjal	Chen <i>et al.</i> , 2005 Thakur <i>et al.</i> , 2007
<i>Streptomyces</i>	<i>Aspergillus flavus</i> , <i>A. niger</i> , <i>Candida albicans</i> , <i>Fusarium semitectum</i> , <i>Rhizoctonia solani</i> and <i>Botrytis cinera</i>	- - -	Yadav <i>et al.</i> , 2009
<i>Streptomyces</i> , <i>Microbispora</i> , <i>Micromonospora</i> and <i>Nocardia</i>	<i>Bipolaris sorokiniana</i> <i>Verticillium alboatrum</i> <i>Rhizoctonia</i> sp.,	Root rot, Spot blotch of barley Wilt in alfalfa and cotton -	Oliveira, <i>et al.</i> , 2010
<i>Actinoplanes</i> sp. HBDN08	<i>Cladosporium cucumerinum</i> , <i>Corynespora cassiicola</i> , <i>Rhizoctonia solani</i> , <i>Phytophthora capsici</i> , and <i>Phytophthora infestans</i> .	Scab on water melon seedlings Target spot in Tomato - Blight on pumpkin, squash, pepper	Zhang <i>et al.</i> , 2010
<i>Streptomyces</i> sp. 201	<i>Rhizoctonia solani</i>	Damping off-cause of death of seedlings in tomato	Thakur <i>et al.</i> , 2009

Table 1. Fungicidal activity of actinobacterial isolates against plant pathogens

4. Fungicidal potential of actinobacteria against human fungal pathogens

Some species of fungi produce mycotoxins that are very toxic to humans. For example, the fungus *Claviceps purpurea* causes the ergot poisoning. An individual infected with the mycotoxin experiences hallucination, gangrene, and blood flow restrictions in limbs. Humans usually get infected with the fungus after eating cereal grains contaminated with *C. purpurea*.

The incidence of opportunistic mycoses and the number of different fungal pathogens are increasing dramatically. During the 1980s, the frequency of nosocomial candidemia increased as much as 500% over the decade (Mitchell 1998). High mortality and increasing antifungal drug resistance are also major concerns. These trends will continue unless better preventive or treatment measures are developed. The traditional approach is to increase the screening programmes that are still being initiated in various countries for the isolation of antibiotic producing microorganisms from the environment, especially marine and terrestrial soil, which provide a rich source for these organisms, particularly the actinobacteria (Labeda & Shearer 1990). It has been estimated that approximately two-thirds of naturally occurring antibiotics have been isolated from actinobacteria (Takizawa *et al.* 1993). Of these antibiotics, the majority were isolated from the genus *Streptomyces* (Goodfellow & O'Donnell 1989).



Saadoun *et al.* (2000b) identified several *Streptomyces* isolates from soils in northern Jordan which were bioassayed for their antifungal activity against several food-associated fungi and moulds isolated from olive-mill residue. Dhanasekaran *et al.* (2008) reported the antifungal compound 4' phenyl-1-naphthyl-phenyl acetamide from *Streptomyces* sp. DPTB16. It showed significant antifungal activity against *Candida albicans* followed by *Aspergillus niger*, *A. fumigatus*, *A. flavus* and minimum inhibitory activity was observed with *Mucor* sp. and *Penicillium* sp. Kumar and Kannabiran (2010b) reported the antifungal activity of *Streptomyces* VITSVK5 spp. against drug resistant *Aspergillus* clinical isolates from pulmonary tuberculosis patients.

Dermatophyte infections are one of the earliest known fungal infections of mankind and are very common throughout the world. There are three genera of dermatophytes, such as *Trichophyton*, *Microsporum* and *Epidermophyton*. Dermatophytoses are world wide in distribution with high prevalence in tropical and sub-tropical countries due to the hot and humid climate which favours their growth. As the dermatophytes have developed resistance to antimycotic drugs and due to a lack of safe and effective antifungal antibiotics, there is an urgent need for nontoxic, safe and cost effective antifungal antibiotics. Deepika *et al.* (2009) reported the actinobacteria exhibiting antidermatophytic activity against *Trichophyton rubrum* were identified among 100 isolates by cross streak method. Among them only two actinobacterial isolates DKD 6 and DKD 7 exhibiting potential antidermatophytic activity and further characterized and identified as *Streptomyces* sp (Table 2; Plate 3).

Organism	Activity against	Disease control	Investigator(s) and year
<i>Streptomyces</i> spp.	<i>Trichophyton</i> sp., <i>Fusarium</i> sp., <i>Penicillium</i> sp.	Dermatophytoses -Trichophytosis in humans and animals	Leben <i>et al.</i> , 1952
<i>Streptomyces aureus</i>	<i>T. mentagrophytes</i>	Tinea in man and animals	Chakrabarthy and Chandra, 1979
<i>S. hygroscopicus</i>	<i>T. mentagrophytes</i> and <i>C. albicans</i>	Tinea in man and animals Oral thrush in man and animals	Gurusiddaiah <i>et al.</i> , 1979
<i>S. globus</i>	<i>T. rubrum</i> , <i>T. mentagrophytes</i> <i>C. albicans</i>	Tinea capitis in Man and animals -	Hwang <i>et al.</i> , 1996.
<i>S. aerocolonigens</i>	<i>C. albicans</i>	Oropharyngeal candidiasis (thrush) and vulvovaginal candidiasis (vaginal Candidiasis)	Nishio <i>et al.</i> , 1989
<i>S. roseiscleroticus</i> (Sultriecin) and <i>S. hygroscopicus</i> (Yatakemycin)	<i>Cryptococcus neoformans</i> <i>Blastomyces dermatitides</i> <i>A. fumigatus</i> <i>F. moniliforme</i> , <i>Petriellidium boydii</i> <i>C. albicans</i> <i>T. mentagrophytes</i>	Cryptococcal meningitis Dermatitidis in skin bronchopulmonary aspergillosis - Visceral infections- endocarditis - Ringworm infections of man, domestic and captive animals such as horses, chinchillas, dogs, cats, calves, and monkeys, as well as many wild animals such as foxes, muskrats, squirrels	Ohkuma <i>et al.</i> , 1992 Imamura <i>et al.</i> ,1993 Atalan, 1997; Zheng <i>et al.</i> , 2000 Datta <i>et al.</i> , 2001
<i>S. violaceusniger</i> (new-macrolide)	<i>C. neoformans</i> , <i>C. albicans</i> , <i>C. tropicalis</i> , <i>C. parapsiolis</i> , <i>C. glabrata</i> , <i>A. fumigatus</i> , <i>C. gloeosporides</i> <i>A. flavus</i> , <i>T. mentagrophytes</i> , <i>T. rubrum</i> , <i>M. canis</i> , <i>M. gypseum</i> ,, <i>M. grisea</i>	- - - Tinea barbae, unguim, pedis Ectothrix Hair infection, Tinea barbae	Ubukata <i>et al.</i> , 1995 a Fulgueira <i>et al.</i> , 2004
<i>Streptomyces</i> sp.	<i>Candida albicans</i> , <i>Cryptococcus terreus</i> <i>Aspergillus terreus</i> <i>Aspergillus niger</i> <i>Penicillium funiculosum</i> <i>Trichophyton rubrum</i> <i>Alternaria alternata</i> <i>Alternaria brassicicola</i> <i>Fusarium moniliforme</i> <i>Aspergillus parasiticus</i> <i>Aspergillus ochraceus</i>	- Lungs pneumonia - - - - Upper respiratory tract infections and asthma Mycotic keratitis Aflatoxicosis Mycotoxycosis	Banga <i>et al.</i> , 2008 Deepika <i>et al.</i> , 2009 Valan Arasu <i>et al.</i> , 2009 Arumugam <i>et al.</i> , 2010 Kumar and Kannabiran, 2010a Duraipandiyani <i>et al.</i> ,2010
<i>Streptomyces</i> sp. DPTB16	<i>Aspergillus flavus</i> , <i>A. niger</i> , <i>A. fumigatus</i> <i>Mucor</i> sp. <i>Penicillium</i> sp. <i>Candida albicans</i>	Bronchopulmonary aspergillosis Pulmonary Zygomycosis Pulmonary grmuloma, Keratitis	Dhanasekaran <i>et al.</i> , 2008; 2009a

Table 2. Fungicidal activity of actinobacterial isolates against human pathogens

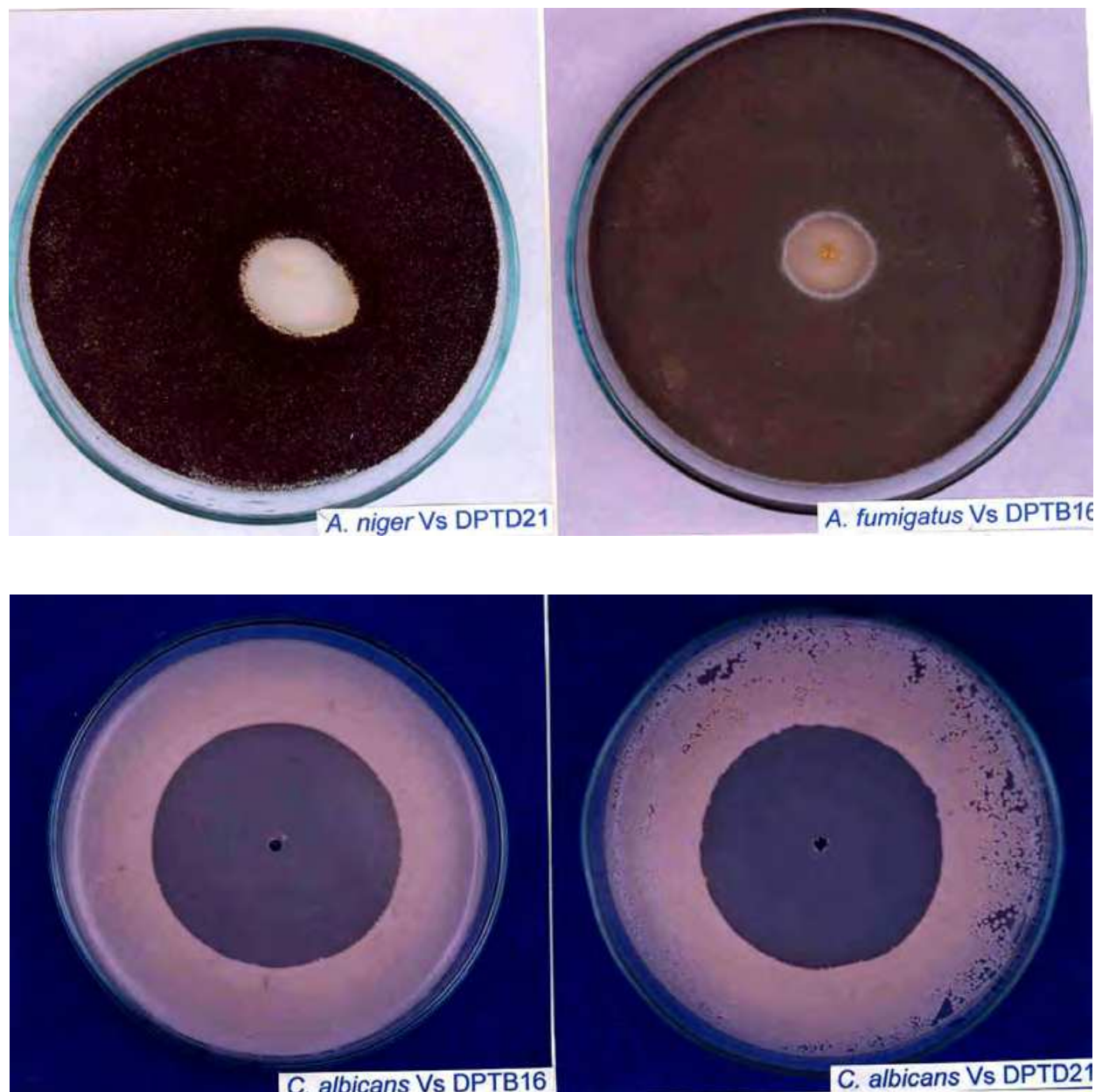


Plate 3. Antifungal activity 4' phenyl-1-naphthyl-phenyl acetamide and methyl substituted  $\beta$ -lactum compounds of *Streptomyces* isolates DPTB16 and DPTD21

## 5. Characterization of actinobacterial fungicidal compounds

A novel antifungal antibiotic, FR-900848 was isolated from *Streptoverticillium ferverns* and its physico-chemical and biological properties have been reported (Yoshida and Horikoshi, 1988). Tomita *et al.* (1989) reported that Pradimicins A, B and C as new antifungal antibiotics from *Streptomyces* sp. Fushimi *et al.* (1989b) reported new phosphate ester antifungal antibiotics phoslactomycins and elucidated the structure of phoslactomycins A to F.

Yamaguchi *et al.* (1989) studied the mode of antifungal action of (S)2-amino-4-oxo-5-hydroxypentanoic acid, RI-331 derived from *Streptomyces* sp. Schwartz *et al.* (1988) studied L-671, 329, a new antifungal agent from *Streptomyces* strain.

Oki *et al.* (1989) studied cispentacin, a new antifungal antibiotic and its *in vitro* and *in vivo* antifungal activities. Novel antifungal antibiotics, Maniwamycins A and B I and II and their structure were studied and reported (Nakayama *et al.*, 1989; Takahashi *et al.*, 1989).

Konishi *et al.* (1989) studied the production, isolation, physico-chemical properties and its structure of cispentacin, a new antifungal antibiotic. A novel hepatoprotective  $\gamma$ -lactone, MH-031 was discovered and their physico-chemical properties and structure have been reported (Itoh *et al.*, 1991). Nishio *et al.* (1989) reported Karnamicin, a complex of new antifungal antibiotic from *Streptomyces* sp. and its taxonomy, fermentation, physico-chemical and biological properties.

Sawada *et al.* (1990) reported new antifungal antibiotics, pradimicins D and E glycine analogs of pradimicins A and C. Water-soluble pradimicin derivatives synthesis and antifungal evaluation of N, N-dimethyl pradimicins derived from *Actinomadura hibisca* was studied and reported (Oki *et al.*, 1990a,b).

Kakushima *et al.* (1990) studied the effect of stereochemistry at the C-17 position on the antifungal pradimicin A. Stephan *et al.* (1996) was observed that Kanchanamycins, new polyol macrolide antibiotics produced by *S. olivaceus*. The structures of the Kanchanamycins were determined by electrospray MS and modern 2D NMR techniques. A Manumycin type antibiotic (SW-B) was isolated from a solid agar culture of *S. flavus* strain A-11. The structure was determined by MS and by 1 and 2D NMR spectroscopy (Kook *et al.*, 1996).

Harindran *et al.* (1999) isolated a new antifungal antibiotic, HA-1-92 from the biomass of *Streptomyces* CDRIL-312. The antibiotic is presumed to be an oxehexaene macrolide and showed promising antifungal activity against yeasts and filamentous fungi. The structure elucidation and antifungal activity of plants an anthracycline antibiotic, daunomycin, isolated from *Actinomadura roseola* against *Phytophthora* blight in pepper have been reported (Kim *et al.*, 2000). A new tetraene polyene macrolide antibiotic was isolated from the culture broth of *S. arenae* var. *ukrainiana* and its structure was determined on the basis of spectral data such as UV, IR,  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and Mass spectroscopy (Gupte *et al.*, 2000). The isolation and structure elucidation of a new antifungal and antibacterial antibiotic produced by *Streptomyces* sp. have been reported (Bordoloi *et al.*, 2001).

Hwang *et al.*, (2001) isolated the antifungal substances SH-1 and SH-2 from *Streptomyces humidus* strains SE 55 cultures by various purification procedures and identified as phenyl acetic acid and sodium phenyl acetate respectively based on the nuclear magnetic resonance, electron ionization mass spectral analysis and inductively coupled plasma mass spectral data SH-1 and SH-2. The two compounds were as effective as the fungicide metalaxyl in inhibiting spore germination and hyphal growth. Raytapadar and Paul (2001) found a broad-spectrum antifungal *Streptomyces* isolate IDA-28 from Indian soil, which was characterized and identified as *Streptomyces aburaviensis* var. *ablastmyceticus* (MTCC 2469).

Frandsberg *et al.* (2000) observed antifungal compounds on solid substrate that inhibit radial growth of fungi among Ascomycetes, Basidiomycetes, Deuteromycetes, Oomycetes and Zygomycetes and strongly affected hyphal branching and morphology of the fungus such as *Aspergillus niger*, *Mucor hiemalis*, *Penicillium roqueforti* and *Paecilomyces variotii*.

Ellis (2002) reported that Amphotericin B is a polyene macrolide antibiotic derived from the actinomycete, *Streptomyces nodosus*. Amphotericin B has a relatively broad spectrum of



action and is useful in treating cases of Candidiasis, extracutaneous sporotrichosis, Mucormycosis and some cases of hyalohyphomycosis and Phaeohyphomycosis.

Igarashi *et al.* (2003) screened for novel antifungal compound, Yatakemycin from the *Streptomyces* species TP - AO 356. Yatakemycin were obtained by solvent extraction of the fermentation broth and preparative HPLC. NMR elucidated the structure of Yatakemycin and CID - MS/MS experiments as a novel antibiotic belonging to a family of CC - 1065 and duocarmycins known to be DNA alkylating agents. Yatakemycin inhibited the growth of pathogenic fungi such as *Aspergillus fumigatus* and *Candida albicans* with the MIC values of 0.01 - 0.03 µg/ml more potent than amphotericin B (MIC 0.1 - 0.5 µg/ml). It also showed potent cytotoxicity against cancer cell lines with the IC<sub>50</sub> of 0.01 - 0.3 µg/ml.

Datta *et al.* (2001) studied the Ju-2 a novel phosphorous-containing antifungal antibiotic from *Streptomyces kanamyceticus* M8. Ellaiah *et al.* (2005) studied the characteristics of oligosaccharide antibiotic by <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra and elucidated the structural formula as C<sub>14</sub>H<sub>86</sub>O<sub>17</sub>. Separation, purification and structural elucidation of Irumamycin and 17-hydroxy-venturicidin were established by IR, ESI-MS, <sup>1</sup>H and <sup>13</sup>C NMR data (Fourati *et al.*, 2005). The numerous fungicidal compounds from actinobacterial genera are summarized in the Table. 3

## 6. *In silico* molecular mechanism of action of fungicidal compounds

Computational biology has now become an indispensable part in research to understand the biological process in a better way at a short period. *In silico* molecular docking is an application area in bioinformatics, which studies the interaction between the molecules by fitting the molecules in a 3D space. The interactions between protein-protein, protein - DNA, protein - small molecule (drug) and even within carbohydrates and lipid molecules can be studied using docking tools.

Docking tools are automated, available both in online and offline, most of the offline tools are commercial tools. Each tool has its own advantage. Some of the online tools are patch dock ([bioinfo3d.cs.tau.ac.il/PatchDock/](http://bioinfo3d.cs.tau.ac.il/PatchDock/)), Zdock server ([zdock.bu.edu/](http://zdock.bu.edu/)), Dock blaster ([blaster.docking.org/](http://blaster.docking.org/)). Few non commercial docking tools are Hex, Autodock, Dock, MS-dock and few commercial tools are Flexidock, GOLD, HADDOCK.

Molecular docking studies can also be performed for fungicides. As fungicides are the chemical compounds used to kill or inhibit fungi or fungal spores. The fungicides act primarily by inhibiting any of the process: electron transport chain, nucleic acid synthesis, mitosis and cell division, protein synthesis, lipid and membrane synthesis, sterol biosynthesis. The enzyme involved in any of the above mentioned process can be considered as a target receptor and the fungicide as ligand.

In case of a fungicide that targets sterol biosynthesis (DM inhibitors), CYP 51 enzyme (14 -α demethylase) involved in the ergosterol biosynthesis can be chosen as a target (Yang, *et al.*, 2009). The docking studies require the 3D structure of the target enzyme. If the 3D structure of the target enzyme is not already available, then homology modeling can be performed.

The docking tools calculate the binding energy between the fungicide and the enzyme target. The binding energy is denoted as E value. The E value for the docked complex should be more negative. The more negative the E value, more stable the docking complex formed.

S.No.	Compound	Source	References
1.	Rapamycin	<i>Streptomyces hygrosopicus</i>	Lomovskaya <i>et al.</i> , (1997)
2.	FK520 Ascomycin	<i>Streptomyces hygrosopicus</i> var. <i>ascomyceticus</i>	Wu <i>et al.</i> ,(2000)
3.	Jinggangmycin	<i>Streptomyces hygrosopicus</i>	Jian <i>et al.</i> ,(2006)
4.	Amphotericin B	<i>Streptomyces nodosus</i>	Caffrey <i>et al.</i> ,(2001)
5.	CE-108	<i>Streptomyces diastaticus</i>	Perez-Zuniqua <i>et al.</i> , (2004)
6.	Rimocidin	<i>Streptomyces diastaticus</i> var. 108	Seco <i>et al.</i> , (2004)
7.	Shurimycins A and B	<i>Streptomyces hygrosopicus</i>	Kamazawa <i>et al.</i> , (1994)
8.	Nikkomycins	<i>Streptomyces ansochromogenus</i>	Luo <i>et al.</i> ,(1998)
9.	Aminoglycoside antibiotics, Istamycins	<i>Streptomyces tenjimariensis</i>	Hotta <i>et al.</i> , (1980)
10.	Scopafungin	<i>Streptomyces hygrosopicus</i> var. <i>enhygrus</i> var. <i>nova</i> UC-2397	Samain <i>et al.</i> ,(1982)
11.	Polyether antibiotic-Ferensimycins	<i>Streptomyces myxogenes</i>	Kusakabe <i>et al.</i> ,(1982)
12.	Auramycins A and B	<i>Streptomyces galilaeus</i> OBB-111	Hoshino <i>et al.</i> , (1982)
13.	Aminoglycoside antibiotic-Boholmycin	<i>Streptomyces hygrosopicus</i> H617-25	Saitoh <i>et al.</i> , (1988)
14.	Maniwamycins	<i>Streptomyces prasinopilosus</i>	Nakayama <i>et al.</i> , (1989)
15.	Phoslactomycins	<i>Streptomyces nigrescens</i>	Fushimi <i>et al.</i> , (1989a)
16.	Griseochelin	<i>Streptomyces griseus</i>	Grafe <i>et al.</i> , (1984)
17.	Resormycin	<i>Streptomyces platensis</i>	Iquarashi <i>et al.</i> , (1997)
18.	Ileumycin	<i>Streptomyces lavendulae</i>	Kawakami <i>et al.</i> , (1978)
19.	Kalafungin	<i>Streptomyces tanashiensis</i> strain Kala UC5063	Johnson and Dietz (1968)
20.	Lomofungin	<i>Streptomyces lomodensis</i>	Johnson and Dietz (1969)
21.	Axenomycins	<i>Streptomyces lisandri</i> nov. sp.	Bruna <i>et al.</i> ,(1973)
22.	Candiplanecin	<i>Ampullariella reguralis</i> subsp. <i>mannitophila</i> subsp. nov.	Itoh <i>et al.</i> ,(1981)
23.	Milbemycins	<i>Streptomyces hygrosopicus</i> sub sp. <i>aureola rimosus</i>	Takahashi <i>et al.</i> ,(1993)
24.	Kanchanamycins	<i>Streptomyces olivaceus</i>	Stephan <i>et al.</i> ,(1996)
25.	Pimaricin	<i>Streptomyces natalensis</i>	Recio <i>et al.</i> ,(2004)
26.	Blasticidin S	<i>Streptomyces griseochromogenes</i>	Zhanq <i>et al.</i> ,(1998)
27.	Leptomycin	<i>Streptomyces lividans</i>	Hu <i>et al.</i> ,(2005)
28.	RS-22 A, B and C	<i>Streptomyces violaceusniger</i>	Ubukata <i>et al.</i> , 1995a,b
29.	Methyl substituted $\beta$ -lactum compound	<i>Streptomyces</i> DPTD21	Dhanasekaran, 2005a
30.	4' phenyl-1-naphthyl-phenyl acetamide	<i>Streptomyces</i> DPTB16	Dhanasekaran <i>et al.</i> , 2008

Table 3. Fungicidal secondary metabolites produced by actinobacteria

Computational advancement also provides way to modify the functional group of the fungicide, leading to the creation of analogues of the fungicide. These docking approaches provide insight into the structure based drug designing. Structure based drug designing assist in the creation of novel fungicides that can be used to treat already existing drug resistant fungal pathogens.

The interaction between the fungicide and the enzyme target will always by hydrogen bond formation between electropositive and electronegative atom. For an enzyme target to get disrupted, the H bond formation of the fungicide should be within the active site of the target.

The knowledge about the active site of the target enzyme can be obtained using online active site predicting tools. Few such tools include: CASTp – Computed Atlas of Surface Topography of proteins (<http://sts.bioengr.uic.edu/castp/calculation.php>), Q-site finder (<http://www.modelling.leeds.ac.uk/qsitfinder/>), and Pocket finder (<http://www.modelling.leeds.ac.uk/pocketfinder/>).

The antifungal compound isolated from the marine *Streptomyces* sp. DPTB16 was characterized as 4-Phenyl-1-Naphthyl Phenyl Acetamide and its 1D structure was also elucidated via spectral analysis (Fig.2). The structure of the compound was submitted to Pubchem compound database with accession number CID: 49786168 (Fig.3,4,5).

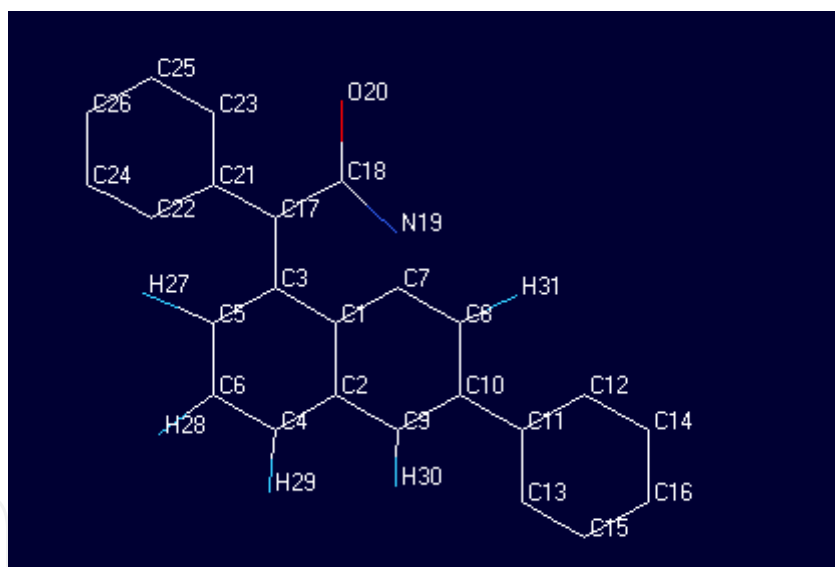


Fig. 2. The elemental representation of 4 - Phenyl 1-Naphthyl Phenyl Acetamide

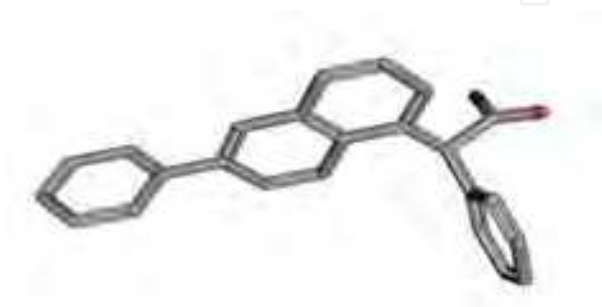


Fig. 3. The 3D structure of 4P1NPA in Pubchem compound database (CID: 49786168)



*In silico* tools are fruitful to gain insight into the mode of action of fungicides and help the research to move a step ahead leading to the rational drug discovery.

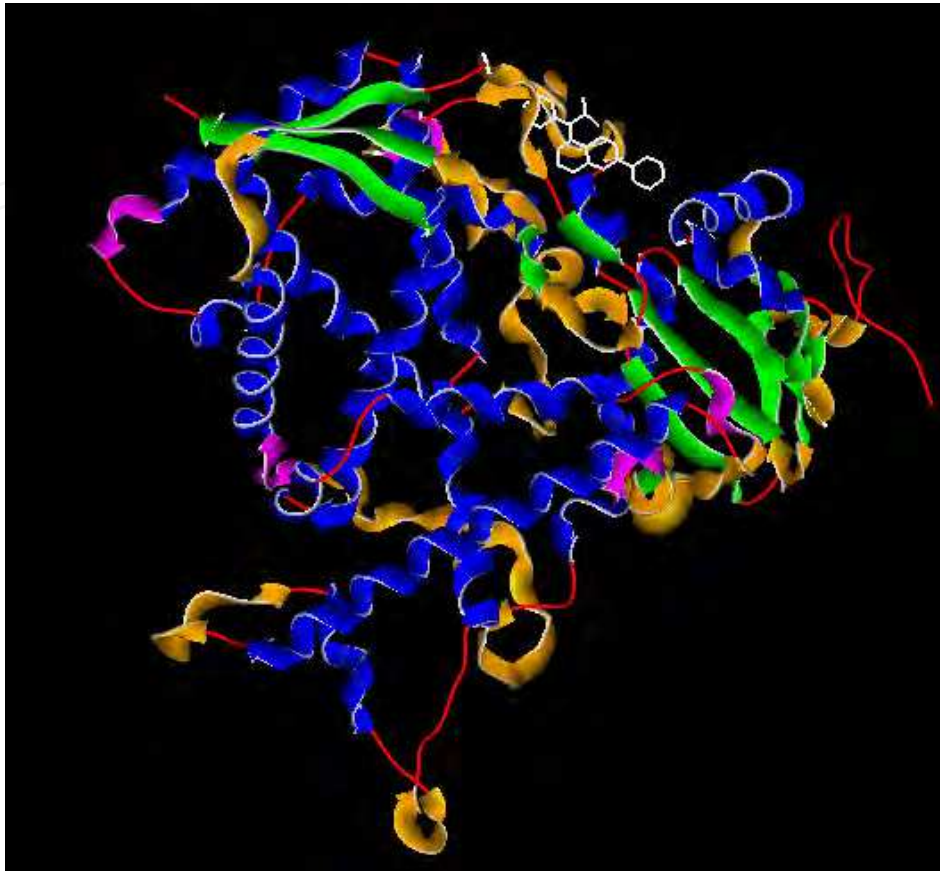


Fig. 4. Cytochrome 51 docking complex with a fungicidal compound(4P1NPA)

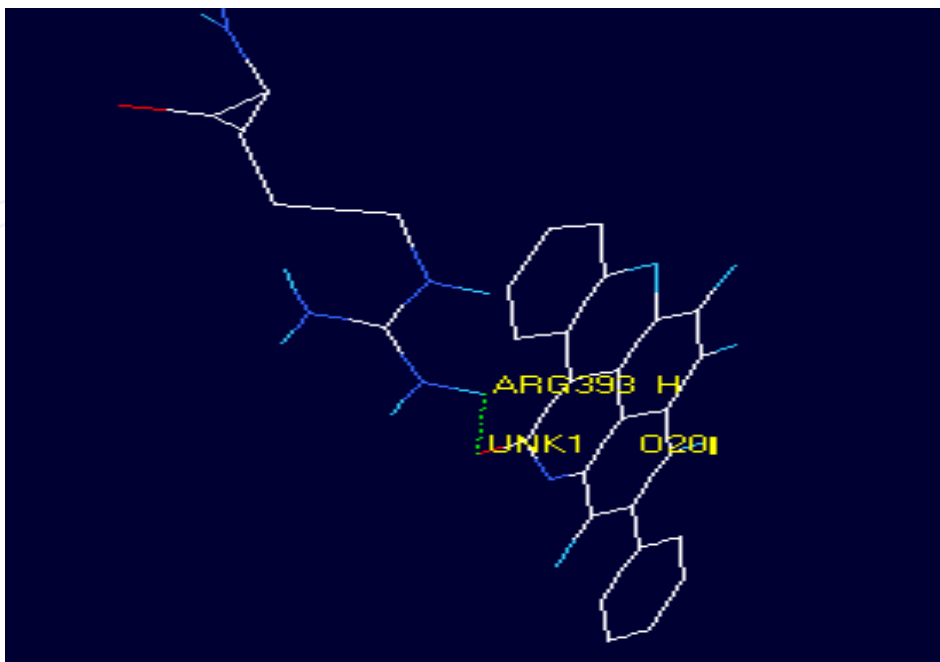


Fig. 5. H bond formation between cytochrome 51 and fungicidal compound(4P1NPA)

## 7. Conclusion

Actinobacteria isolated from marine habitat have potential for novel fungicidal compounds. The significance of finding actinobacteria in marine soil sample lies in the intrinsic economic importance in biotechnological perspectives. The chapters reinforce the view that the unexplored actinobacteria for bioprospecting novel fungicidal compound in the development of Biocontrol agents and formulations of drugs. Further investigation should address the relationship between structure relationship activity of fungicidal compounds, rapid methods for large scale production, purification and application in managing fungal infection in agriculture crops, human and animals.

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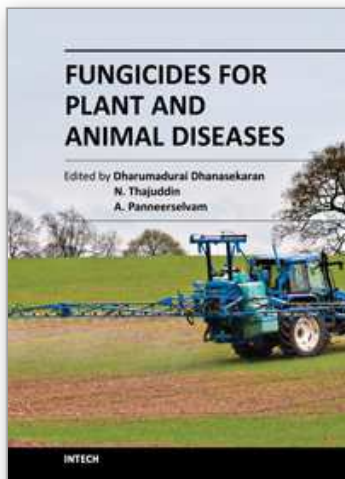
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## **Fungicides for Plant and Animal Diseases**

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A fungicide is a chemical pesticide compound that kills or inhibits the growth of fungi. In agriculture, fungicide is used to control fungi that threaten to destroy or compromise crops. Fungicides for Plant and Animal Diseases is a book that has been written to present the most significant advances in disciplines related to fungicides. This book comprises of 14 chapters considering the application of fungicides in the control and management of fungal diseases, which will be very helpful to the undergraduate and postgraduate students, researchers, teachers of microbiology, biotechnology, agriculture and horticulture.

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