CHAPTER TWO

LITERATURE REVIEW

2.1 Actinomycetes diversity and distribution in nature

Actinomycetes are a group of branching unicellular organisms, which reproduce either by fission or by means of special spores or conidia. They are closely related to the true bacteria; frequently, they are considered as higher, filamentous bacteria. Classified in *Actinomycetales* order, they are also belonged to Gram-positive bacteria with a high Guanine-plus-cytosine (G+C) content in their DNA which is above 55% (Osada, 2001).

2.1.1 Terrestrial habitat

According to Hayakawa (2008), actinomycetes are distributed widely in various habitats but soil remains the most important with the largest population found in the surface layer. In natural soil habitat, streptomycetes exist as a major component of actinomycetes population. There are many studies on isolation of actinomycetes from soil have been reported (Iwami *et al.*, 1986; Jain & Jain, 2007; Kavita & Vijayalakshmi, 2007). *Streptomyces* was encountered to be the most abundant genus isolated in each of the studies, followed by *Micromonospora*. To date, there is interest in assessing antimicrobial potentials among soil actinomycetes (Wijittra *et al.*, 2006). Oskay *et al.* (2004) discovered farming soil-actinomycetes which were antagonistic against few bacteria and had capability of being a producer of novel antibiotics.

Recently, actinomycetes had been isolated from mangrove soil. Ismet *et al.* (2004) and Hong *et al.* (2009) reported isolation of *Streptomyces* and *Micromonospora* from mangrove soils and plants which have potential in producing biologically active secondary metabolites. Though the actinomycetes population in anoxic mangrove

rhizosphere was 1000 to 10000 times smaller than arable lands because of tidal influence, it was diverse and mainly represented by *Streptomyces, Micromonospora*, actinobacteria and nocardioform actinomycetes (Tan, 2007). Recently, studies of mangrove actinomycetes were focusing on their antimicrobial activity against pathogenic bacteria and fungi (Vikineswary *et al*, 1997; Vikineswary *et al*., 2003). Besides, Schneider *et al*. (2007) reported a strain of actinomycete, belonged to genus *Nocardia* isolated from mangrove soil produced new cytotoxic metabolites that strongly inhibited human cell lines such as gastric adenocarcinoma.

Actinomycetes occur universally on the surface of plants and sometimes even in various parts of the plants themselves, especially *Streptomyces* spp. from the root which is in contact with soil. Tian *et al.* (2004) isolated 274 strains of actinomycetes from rice's sterilized root and leaves. Most of them belonged to *Streptomyces* spp., only a few fell into *Streptoverticillium*. In other study, Petrolini *et al*. (1991) reported the isolation of 1755 actinomycetes strains from surface-sterilized roots of 205 plants from 156 species. They identified eighty percent of the strains as *Streptomyces* spp. and the remaining as nocardioforms, *Micromonospora, Actinoplanes, Streptosporangium, Streptoverticillium* and *Saccharomonospora*. Isolation of endophytic actinomycetes from roots of healthy wheat plants was done by Coombs & Franco (2003). They found that the isolates were belonged to *Streptomyces, Microbispora, Micromonospora* and *Nocardiodes*. Vercesi *et al*. (1992) isolated *Streptomyces* spp. from other parts of grapevine berries, which exhibit widespread antagonistic activity against yeasts and fungi inhabiting the same environment.

Besides *Streptomyces* spp., Matsumoto *et al.* (1998) isolated rare actinomycetes like *Microbispora* spp. from surface-sterilized fallen leaves using different methods. Taechowisan *et al.* (2003) used healthy plant tissues from leaves, stems and roots to

isolate endophytic actinomycetes which have antifungal potential, particularly *Streptomyces* spp.

2.1.2 Aquatic habitat

2.1.2.1 Freshwater habitat

Waksman (1959) explained that actinomycetes are abundant in fresh water lakes. An old report stated that thermophilic actinomycetes are found in river water. They were also found in sewage and grew well at 60˚C. This statement was then proved when Cross (1981) reported isolation of members of genera *Actinoplanes, Micromonospora, Rhodococcus, Streptomyces* and the endospore-forming *Thermoactinomycetes* from freshwater habitats. Goodfellow & Haynes (1984) however explained that majority of these actinomycetes most probably are wash-in from land and accumulated in freshwater habitats. According to Makkar & Cross (1982), sporangia of *Actinoplanes* could withstand prolonged dessication and release motile spores when rehydrated. That is why they are common in soils, rivers and lakes. *Actinoplanes* is mainly discovered on allochthonous leaf litter washed to lake shore and twigs submerged in streams.

Waksman (1959) believes that members of the genus *Micromonospora* represents a truly indigenous group of microbial inhabitants of waters and bottom deposites of inland lakes. Therefore, a great number of *Micromonospora* also can be isolated from lake sediments. Complex organic compounds such as chitin, cellulose and lignin accumulated in lake mud were able to decompose by this genus of actinomycetes. Rowbotham & Cross (1977) elucidated that *Micromonospora* spores can survive as dormant propagules as they washed into streams, rivers and lakes. Comparable to *Thermoactinomycetes* endospores found in lake sediments, it was also reported remain dormant in cold waters as they were washed in from surrounding soils.

On the other hand, the presence of *Rhodococcus coprophilus* (also has been isolated from water and sediments of rivers and lakes), a coprophilic species in lakes was believed due to wash in of contaminated herbivore dung.

A study of freshwater actinomycetes isolation from sediments of lakes was carried out by Jiang & Xu (1996) was resulted the discovery of yielded predominantly *Micromonospora* followed by *Streptomyces*. The presence of *Streptomyces* in freshwater habitat was because of their spores being continuously washed into rivers and lakes. This enable the *Streptomyces* spores could be found in foam of rapids at the water-air interface. Isolation of actinomycetes from lake water and sediments by Terkina *et al*. (2002) leads to an interesting conclusion that *Streptomyces* were dominant in water samples while great numbers of *Micromonospora* were found in sediments.

2.1.2.2 Marine habitat

Marine environment is a unique habitat that has exclusive characteristics which terrestrial areas do not have, like high hydrostatic pressure, high salts concentration and low concentration of organic matter. Thus, microorganisms surviving both in the marine and terrestrial environments are expected to be totally different. This makes a point of view that marine habitat would be an excellent sampling point as microbes in seawater and marine sediments are diverse.

Waksman (1959) enlightened that there were very few reports available concerning the occurrence of actinomycetes in sea and sea bottom until year 1959. Early study reported that the existence of actinomycetes in this environment was believed because of soil contamination, or to their presence on algal material floating on the surface of the sea, or to the fact that the samples of water were obtained near the docks.

Isolation of actinomycetes from inshore marine sediments was done by Grein & Meyers (1958), based on absence of apparent morphological and biochemical difference between both marine and terrestrial isolates, they elucidated that the actinomycetes might be originated from terrestrial but adapted to salinity level of sea water. Okami & Okazaki (1974) studied the transportation of actinomycetes spores into shallow sea mud. The study explained that actinomycetes spores could be transferred from land to the sea by rain or river and survived. Spores were precipitated by the NaCl acceleration after reaching the sea into sediments. According to Okazaki & Okami (1975) later, on media formulated with sea water, terrestrial strains could grow well besides showed a wide range of halo-tolerance, whereas halo-tolerance of NaCl-sensitive strains could be induced by stepwise exposure to increasing NaCl concentrations.

However, the existence of indigineous marine actinomycetes had been reported by Weyland (1969) based on abundant actinomycetes isolated from deep sea sediments. Besides, Jensen *et al.* (1991) had also reported that maximum numbers of actinomycetes isolation from near-shore sediments in both shallow and deep sampling sites showed a bimodal distribution in relation to depth. This was characterized by an obvious decrease of streptomycetes and actinoplanetes increment with increasing depth. Furthermore, 98% of the streptomycetes were obtained from depth $\leq$ 3m and the percentage decreased radically with increasing depth. However, the actinoplanetes number was increased with up to 33m of depth increment. These discoveries indicated that the theory that marine-derived actinomycetes are originated from terrestrial could be argued. In addition, they proved that near-shore marine sediments actinomycetes are well-adapted and functional members of the marine microbial community.

Jensen *et al.* (1991) reported that actinomycetes isolated from marine environment are metabolically active and have adapted to life in the sea. Hakvag *et al.* (2008) isolated a total of 217 marine actinomycetes from sea surface microlayer. All

these strains resembled the genus *Streptomyces* spp. Furthermore, studies on isolation of actinomycetes from marine sediments suggested that they were able to survive under marine conditions due to their salt tolerance. In fact they were able to endure for considerable periods of time under marine environment. According to Ghanem *et al*. (2000), marine actinomycetes isolated from sediments counts far exceeded those found in sea water. Thus, sediments are still the best supplier of this microorganism. A total of 192 marine actinomycetes were isolated from sediments, these isolates were identified and resulted in the discovery of novel marine-derived actinomycetes within the family *Micromonosporaceae* (Magarvey *et al*., 2004). Miller *et al*. (2006) also isolated marine *Streptomyces* spp. from the same source. All these studies proved that marine sediments are really a valuable source for isolating marine actinomycetes.

2.1.3 Extreme environments

Actinomycetes were also isolated from uncommon habitat. Alkalophilic actinomycetes (*Streptomyces* and *Nocardiopsis* were the dominant genera) had been isolated from alkaline soils (pH 10-12) surrounding mineral springs (Jiang *et al*., 1993). A new genus and species of alkalophilic actinomycetes from a soda lake soil (pH10) was also been described as *Bogoriella caseilytica* (Groth *et al.*, 1997). In other study, Al-Zarban *et al.* (2002) isolated *Saccharomonospora halophila*, a halophilic actinomycete with optimum growth at 10% NaCl from marsh soil. Mevs *et al.* (2000) reported a study of *Modestobacter multiseptatus*, a psychrophilic strains with optimum growth at temperature 11-13˚C was isolated from transantarctic mountain soils. An obligate psychrophilic actinomycetes, *Cryobacterium psychrophilum*, with optimum growth temperature 9-12˚C and did not grow at temperature higher than 18˚C was isolated from Antartica soil by Suzuki *et al.* (1997). Other than that, Zakalyukina *et al.* (2002) isolated acidophilic actinomycetes from acidic forest and peat soils, mainly *Streptomyces* and

Micromonospora. Dey & Chaphalkar (1998) isolated thermophilic *Streptomyces* spp. from silt and water samples of meteoritic crater. This isolate grew on agar at 55˚C, with only 18 hours sporulation time. It produced protease at 55˚C and the enzyme was stable up to 85˚C at 7.5-12 pH range. Few rare thermotolerant actinomycetes isolated from desert soils of Mojave Desert, California belonged to genera *Microbispora, Nocardia, Microtetraspora, Amycolaptosis, Actinomadura* and *Saccharothrix* were reported by Takahashi *et al.* (1996). These actinomycetes grew at temperature up to 50˚C.

2.2 Role of actinomycetes in ecosystem

Actinomycetes are found in quite a significant number as a major component in most soils. In most ecological systems, they are saprophytic with a major role in soil organic matter decomposition (Arai, 1997). Referring to Goodfellow & Williams (1983), actinomycetes playing an important ecological role in biodegradation of plant litter, particularly in the recalcitrant lignocelluloses component. As they remain as dormant spores, sporangia or resting cocci, actinomycetes will automatically germinated in the occasional presence of exogenous nutrients (Mayfield *et al.*, 1972). Apart from nutrient availability, there are other environmental factors that could affect the growth of actinomycetes such as soil temperature, pH and moisture tension (Goodfellow & Williams, 1983).

In rhizosphere soil, actinomycetes are capable of producing antibiotics and other useful metabolites. Therefore, they have possibility in influencing the rhizospheric pathogens. Getha & Vikineswary (2002) reported the potential of *Streptomyces* spp. as biocontrol agent against fungal pathogens especially in commercial crops. *Streptomyces* spp. potential in biocontrol aspect also proved in other study by Vercesi *et al.* (1992) which reported that *Streptomyces* spp. isolated from grapes have antifungal activity against pathogenic yeast and fungi from the same habitat. On the contrary, there are

certain species of *Streptomyces* considered as pathogen such as *S. turgidiscabies* which causes erumpent scab lesions on potatoes in Hokkaido, Japan. This species was isolated from soil (Miyajima *et al.*, 1998). Besides, Park *et al.* (2003) reported that the potato scab disease was caused by other species of *Streptomyces* (*S. luridiscabiei, S. puniciscabiei* and *S. niveiscabiei.*

2.3 Identification and characterisation of actinomycetes

In actinomycetes classification and characterisation, phenotypic and phylogenetic methods are involved as a polyphasic taxonomic approach (Tan, 2007). These include morphological, physiological and biochemical characterisation in phenotypic approach; while molecular methods are in phylogenetic characterisation part. Some characterisation procedures for identification are outlined in this chapter.

2.3.1 Cultural and morphological observation of actinomycetes

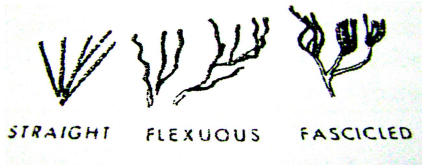
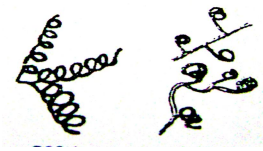
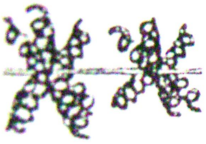
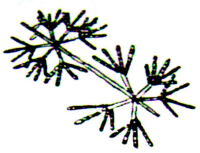
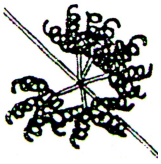
Based on Shirling & Gottlieb (1966) methods for *Streptomyces* spp. characterisation, the basic observation should involved cultivation of cultures on various medium; yeast extract – malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts – starch agar (ISP4) and glycerol – asparagine agar (ISP5). Growth and morphology of *Streptomyces* spp. are observed when cultures turned matured with heavy spore mass in order to determine aerial spore mass colour, substrate mycelium and diffusible pigments colour (Williams *et al*., 1993). Preliminary differentiation could be done by colour grouping when dealing with large number of different isolates. However, this study only involved *Streptomyces* spp.; which proven by Williams *et al.* (1993) that this group of actinomycetes fell into the same group with similar morphological and physiological properties based on their colour grouping.

Morphological observation in this study is referring to sporophores morphology as it is considered stable and clearly defined feature for actinomycetes classification. However, this is only valid without the occurrence of strain degeneration due to subculture or improper maintenance (Pridham *et al.,* 1958). Based on different spore chain morphology, the authors divided *Streptomyces* spp. into seven groups of "morphological sections" (Table 2.1). Whereas every section was further categorized into another six "series" according to the spore mass colour: white, olive-buff (buff to tan to olive-buff), yellow, blue (blue to blue-green to green), red (pink to red to lavender to lavender-grey), grey (light grey to mouse-grey to brown-grey to grey-brown). The spores then could be subdivided into distinct ornamentation: smooth, rugose, spiny, hairy, warty, knobby, tuberculate or verrucose (Vobis, 1997).

2.3.2 Physiological characterisation of actinomycetes

Basically, any actinomycetes physiology consideration involves a study of their growth and nutrition, their metabolic processes and their reaction to environmental conditions (Waksman, 1959). According to Shirling & Gottlieb (1966), only melanoid production and carbon sources utilization were being characterized for *Streptomyces* spp. physiology. However, there are some other physiological characteristics that had been considered to categorize actinomycetes, especially *Streptomyces* spp. depending on number of isolates and objective of study. For instance; optimum temperature range, nitrate reduction test, NaCl tolerance, production of hydrogen sulfide (H_2S), starch hydrolysis, liquefaction of gelatin, nitrogen source utilization, pH sensitivity and sensitivity to some antibiotic are some of the characteristics that are being tested in characterizing any actinomycetes isolates physiologically (Gottlieb, 1960; Williams *et al.,* 1989; Sakai *et al.,* 2004).

Table 2.1: Seven morphological sections of *Streptomyces* spp. (Pridham *et al.*, 1958).

No.	Section	Figures	Description
1	Rectus-flexibilis (RF)		Straight, flexuous or fascicled spore chains; no spirals
2	Retinaculum-apertum (RA)		Hooked, open-looped or greatly extended spiral spore chains
3	Spira (S)		Closed or open spirals
4	Monoverticillus (MV)		Primary verticils or whorls attached to long, straight branches; no spirals
5	Monoverticillus – Spira (MV-S)		Spiraled primary verticils or whorls attached to long, straight branches
6	Biverticillus (BIV)		Compound verticils or whorls attached to long, straight branches; no spirals
7	Biverticillus – Spira (BIV-S)		Spiraled compound verticils or whorls attached to long, straight branches

2.3.3 16S rRNA gene sequence analysis of *Streptomyces* spp.

Stackebrandt *et al.* (1992) explained that 16S rRNA gene sequences used in phylogenetic relationship inference among microorganisms besides to characterize unknown isolates has been commonly accepted. This is due to the fact that relationships

measurement among bacteria can be done because of the nature of universality of this gene in bacteria (Clarridge, 2004). About 1,550 base pairs (bp) long, the 16S rRNA gene sequence is basically composed of both variable and conserved regions. Chen *et al.* (1989) elaborated that *"The gene is large enough, with sufficient interspecific polymorphisms of 16S rRNA gene, to provide sufficient sequence information that permits statistically significant comparisons. Universal primers are usually chosen as complementary to the conserved regions at the beginning of the gene and at either the 540-bp region or at the end of the whole sequence (about the 1,550-bp region), and the sequence of the variable region in between is used for the comparative taxonomy. Although 500 and 1,500 bp are common lengths to sequence and compare, sequences in databases can be of diverse lengths."*

Clarridge (2004) stressed that the 16S rRNA gene sequences comparisons allows organisms differentiation at the genus level. This including strains classification at species and subspecies level. A comparison of partial nucleotide sequences (120 bp) by Kataoka *et al.* (1997) spanning the variable α region of 16S rRNA in 89 *Streptomyces* strains demonstrated that these short nucleotide sequences are useful for *Streptomyces* species rapid identification. At the intra-species level, partial 16S rRNA gene sequence is enough to deduce the *Streptomyces* strains phylogenetic relationships. Similar approach was used by Thamchaipenet *et al.* (2001) to differentiate antifungal-producing streptomycete isolates into different species by partial 16S rRNA sequencing. They found that phylogenetic analyses of the partial sequences indicated possible new species with those of known antifungal-producing streptomycetes.

However, the 16S rRNA phylogenetic marker usage is often criticized because of its heterogeneity of the same genome (Acinas *et al.*, 2004) or according to Pontes *et al.* (2007), due to its lack of resolution at the species level. Fortunately, it is still used as a unique and valuable standard for bacterial identification. Therefore, the

implementation of next-generation sequencing technology has impressively increased the size of 16S rRNA sequence databases (Armougom & Raoult, 2009).

2.3.4 Chemical profiling and compounds isolation

2.3.4.1 Thin layer chromatography (TLC)

Thin layer chromatography is the most commonly used techniques in natural product research (Houghton & Raman, 1998). TLC is generally regarded as a simple, rapid, and inexpensive method for the separation, tentative identification, and visual semi quantitative assessment of a wide variety of substances. In recent years, TLC has come to rival HPLC and GC in its ability to resolve complex mixtures and to provide quantitative results. The evolution of the technique has included improvements in the quality of the TLC plates and detection reagent application techniques, the introduction of new stationary phases and approaches in plate development, and the design of sample application and densitometric scanning (Striegel & Hill, 1996).

The main advantages of TLC are its low cost and the relative speed of analysis. The materials needed to perform TLC are minimal. They include a development chamber, chromatographic plates, solvents, detection reagents, and reference materials. Also, TLC can be applied to the detection and identification of a wide range of materials, like those found in binding media. Disadvantages of TLC analysis include the need for a larger sample size and its lower sensitivity in comparison with other methods, such as HPLC or GC.

An active (antibiotic activity against *Klebsiella* sp. and *Escherichia coli*) yellow crude pigment of *Streptomyces hygroscopicus* subsp. *ossamyceticus* (strain D10) isolated from Thar Desert soil, Rajasthan was purified using TLC (Selvameenal *et al.*, 2009). The Rf value was calculated as 0.768. The purified pigment exhibited greater antibiotic activity against the tested pathogens in disc diffusion method. The pigment

was screened chemically and was identified as group of sugar containing molecules. Gurung *et al.* (2009) detected two spots on TLC plate from three potential antimicrobial actinomycetes strains metabolites. The strains were isolated from soil samples of Kalapatthar, Mount Everest region. The spots were completely different from the spot produced by vancomycin. The active isolates were heterogeneous in their overall macroscopic, biochemical, and physiological characteristics through unweighted pair group method using average (UPGMA) cluster analysis. TLC in this report assisted in determining that the potential strains of actinomycetes were belonged to distinct taxonomic group.

2.3.4.2 Column chromatography

Chromatographic procedures are the most diverse and widely used techniques in the fractionation of extracts, which column chromatography is the oldest form of chromatographic technique (Houghton & Raman, 1998). Conventionally, silica gel was used as the stationary phases in column chromatography which were adsorbents of high polarity. However, many other forms of stationary phase, e.g. reverse-phase silica, ion-exchange resins, exclusion chromatography stationary were introduced in these recent years.

Recently, three novel antitrypanosomal alkaloids were isolated from a new endophytic actinomycete species, *Streptosporangium oxazolinicum* K07-0460(T) culture broth (Inahashi *et al.,* 2011). The isolation of the alkaloids named spoxazomicins A-C was done by silica gel column chromatography and HPLC. Analyses by both NMR and X-ray crystal elucidated the structures of the spoxazomicins which shown to be a novel pyochelin family antibiotic. Spoxazomicin A illustrated strong and selective antitrypanosomal activity in vitro with an IC value of 0.11 g ml without cytotoxicity against MRC-5 cells (IC=27.8 g ml).

2.3.4.3 High performance liquid chromatography (HPLC)

HPLC and TLC are similar in term of their identical of the mobile phase, the stationary phase, and the separation mechanism. While HPLC and TLC are considered complementary techniques, HPLC is considered more efficient than TLC in separating components. Also, an HPLC system is a closed system that allows for greater control of the mobile phase velocity (Striegel & Hill, 1996).

A possibility of strain JF-1 containing a novel compound was detected when five potential actinomycetes crude extracts was screened in preliminary analysis using HPLC (Intra *et al.*, 2011). Isolated from rhizospheric soils plant, strain JF-1 was positive in inhibiting *Colletrotrichum* spp. growth, the causative agent of anthracnose disease. Phylogenetic analysis revealed that strain JF-1 is 99.8% similar to *Streptomyces cavourensis* NRRL 2740.

2.3.4.4 Preparative thin layer chromatography (PTLC)

PTLC is one of the simplest and cheapest methods available for the isolation of a component or components from a mixture, although it is labour intensive and only small amounts can be obtained from each fractionation procedure (Houghton & Raman, 1998).

In a report of Maskey *et al.* (2003), PTLC was done to purify the compound of a new phenazine derivative, phenazostatin D which was isolated from an extract of marine actinomycete *Pseudonocardia* sp. B6273 *via* a TLC-guided chemical screening. The structure of the compound was assigned by spectroscopic methods and found to be the *meso*-form of phenazostatin B. The strain also produced the known phenazine antibiotic methyl saphenate.

2.4 Potential of actinomycetes in biotechnology and industry

Possessing structurally unique secondary metabolites, marine actinomycetes have become a major source of various potent biological activities. Among the actinomycetes, genus *Streptomyces* established a major number of microbially derived antibiotics (Boonlarppradab *et al*., 2008).

2.4.1 Antimicrobials and antitumor potentials of actinomycetes

In a review article, Berdy (2005) discussed about microbial metabolites production since year 2002, which filamentous actinomycetes produced over 10,000 bioactive compounds (45% of all microbial metabolites). 75% or 7600 of the valuable production were of *Streptomyces* spp. origin and 2500 or 25% were from rare actinomycetes (*Micromonospora*, *Actinomadura* and *Streptoverticillium*). The approximately proportion of all actinomycetes products demonstrated antimicrobial activity to anti-tumor activity was 70:30 respectively. Production of a great number of important drugs by actinomycetes is well known. Okami & Hotta (1988) reported some significant drugs provided by actinomycetes such as aminoglycosides, anthracyclines, chloramphenicol, β-lactams, macrolides and tetracyclines. Among the actinomycetes, *Streptomyces* contributed the greatest chemical diversity (Sanglier *et al*., 1993a). A study on obtaining antifungal compounds from marine *Streptomyces* spp. was carried out by Cho *et al.* (1999). These compounds displayed strong antifungal activity against *C. albicans, E. coli* and *P. aerogenosa*. More recently, Ogunmwonyi *et al*. (2010) reported a wide range antimicrobial activity of ten most potent marine *Streptomyces* spp. isolated from the Nahoon beach, a coastal shore of Indian Ocean in the Eastern Cape Province of South Africa. The ethyl acetate extracts of the isolates exhibited activities against at least 6 and up to 26 of the 32 test bacteria screened. IR spectra analysis was done to

characterize the crude extracts and the possibility of terpenoid, long chain fatty acids and secondary amine derivatives compounds in the extracts presence was revealed.

Basically, antitumor compounds are produced naturally mainly by microorganisms. In fact, actinomycete is the major producer of various natural products with different properties including antitumor activity (Olano *et al.,* 2009). Boonlarppradab *et al*. (2008) isolated *Streptomyces* from marine sediments producing two novel spiroaminals, marineosins A and B which possess significant inhibition of human colon carcinoma (HCT-116) in an in vitro assay and selective activities in diverse cancer cell types.

Streptomyces spp. also plays an important role as biocontrol agent of fungal pathogens Getha & Vikineswary (2002). A report by Yuan & Crawford (1995) explained an experiment of coating pea seeds with spores/mycelia of *Streptomyces lydicus* WYEC108 to inhibit *Phytium ultimum,* a kind of fungus in an oospore-enriched soil. They discovered that less than 40% of the coated seeds were infected but all uncoated seeds were infected by the fungus 48 hours after planting. More recently, an Iranian *Streptomyces plicatus* strain 101 was reported having chitinolytic activity and antifungal inhibitory effects on mycelial growth of *Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium graminearum, F. solani, Rosellinia necatrix* and *Pythium aphanidermatum* mainly by extracellular chitinase production (Baharlouei *et al.,* 2010).

2.4.2 Actinomycetes enzymes

Actinomycetes enzymes are the most significant products after antibiotics. Microbial enzymes are widely used in food processing, detergent manufacturing, the textile and pharmaceutical industries, medical therapy, bioorganic chemistry and molecular biology (Peczynska-Czoch & Modarski, 1988). Actinomycetes were best known as antibiotics

sources for many years. These recent years, they also have been detected to be a potential source of a wide range of important enzymes.

Rifaat *et al.* (2005) investigated twenty producing cellulase free-xylanase *Streptomycetes* strains isolated from Egyptian soils. They reported two most active strains and have been identified as *Streptomyces albus* and *Streptomyces chromofuscus*. The increment of the enzyme activity was found when both isolates were grown on yeast extract. Optimum production of xylanase was recorded after five days of fermentation. Xylanase produced with *Streptomyces chromofuscus* showed higher bleaching activity than that from *Streptomyces albus*. The enzyme enhanced the liberation of reducing sugars, which improved pulp bleachability.

Kim *et al.* (2003) reported the isolation and purification of chitinase from culture filtrate of *Streptomyces* sp. M-20. The enzyme was active optimally at pH of 5.0 and at 30˚C, and stable from pH 4 to 8, and up to 40˚C. The purified chitinase showed antifungal activity against *Botrytis cinerea*, and lysozyme activity against the cell wall of *Botrytis cinerea*. Besides, the chitinase activity was completely inhibited by Hg+, Hg2+ and p-chloromercuribenzoic acid. Narayana & Vijayalakshmi (2009) reported that under submerged fermentation, the production of chitinase by a terrestrial *Streptomyces* sp. ANU 6277 was promoted by utilization of starch and yeast extract as carbon and nitrogen sources.

In a study of mannanase screening in actinomycetes, Montiel *et al*. (1999) reported high levels of the enzyme produced in *Streptomyces scabies* CECT3340 and *S. ipomoea* CECT3341 in liquid culture. The mannanase potential in bleachability improvement of pine kraft pulp was tested. Obviously, it released the colour and chromophoric material from the pine kraft pulp besides increased the pulp brightness. The optimization of mannanase production in *Streptomyces* sp. PG-08-3 from Rajasthan dessert, India was investigated by Bhoria *et al.* (2009). Increment of guar gum

concentration in the growth media demonstrated to enhance the production of mannanase.

Adriana *et al.* (2005) published a first report of two Antartic actinomycetes which produced keratinolytic enzymes to enable their growth on keratin-containing wastes. *Streptomyces flavis* 2BG (mesophilic) and *Microbispora aerata* IMBAS-11A (thermophilic) demonstrated as very promising strains for effective processing of native keratinous wastes. Pettett & Kurtboke (2004) explained that keratin-degrading and antibiotic producing actinomycetes such as *Streptomyces, Saccharomonospora, Nocardioides, Nocardiopsis* and *Nonomuraea* have potentials in turning poultry farm feather waste by composting into odourless and complete biological degradation in pathogen-free biofertilizer.

2.4.3 Actinomycetes as agents of biodegradation/bioremediation

Zhou & Zimmermen (1993) reported the role of actinomycetes as agent of decolourization in industrial effluents containing water-soluble synthetic reactive dyes. The report exhibited the capability of actinomycetes in decolorizing between 17% up to 73% of the effluent, by decolourization or adsorption of the dyes to the cells. Azo dyes, azo-copper complex, anthraquinone, formazan-copper complex and phthalocyanine dye were among the included dye in this report. *Streptomyces* spp. have also reported to decolourize paper mill effluent obtained after semichemical alkaline pulping of wheat straw (Hernandez *et al.,* 1994). The highest decolourization level reported were 60 – 65%.

Actinomycetes are also responsible in pesticides degradation with various different chemical structures, including organochlorines, s-triazines, triazinones, carbamates, organophosphates, organophosphonates, acetanilides, and sulfonylureas (De Schrijver & De Mot, 1999; Nawaz *et al.,* 2011). Indigenous soil actinomycetes had

been reported to degrade the herbicide Diuron in soil (Esposito *et al.*, 1998). Diuron, a kind of phenylurea is widely used as weed biocontrol on non-crop areas and certainly on crops like cotton, pineapple, citrus and sugar-cane at low concentration. *In vitro,* by applying Diuron, the selected actinomycetes exhibited up to 37% level of herbicide degradation in seven days.

Rubber-degrading actinomycetes are widespread in nature due to natural rubber degradation as sole carbon source is actinomyctes privilege (Jendrossek *et al.*, 1997). This was concluded after isolation of variety of rubber-degrading actinomycetes (up to 10^5 cfu/g) from soil samples of *Hevea brasiliensis* plantation and from waste water ponds of a rubber-producing company in Malaysia. Among the actinomycetes, *Streptomyces* were found to be dominant followed by *Micromonospora, Actinoplanes, Nocardia, Dactylosporangium* and *Actinomadura.* Novel rubber-degrading *Gordonia* species from fouling water inside a deteriorated automobile tyre: *G. polyisoprenivorans* (Linos *et al.*, 1999) and *G. westfalica* (Linos *et al.*, 2002) have been published.

2.5 Antifungal assays: *Candida albicans* and *Schizosaccharomyces pombe*

Candida albicans is commonly a diploid fungus (a form of yeast) and a causal agent of most opportunistic oral and genital infections in humans (dEnfert & Hube, 2007; Krempl-Lamprecht, 1991). Cooper & Silva-Hutner (1985) explained that yeast infections are among the most fungal infections affecting humans. This is due to the fact that its biofilms are formed on implantable medical devices surface. This lead to a serious health concern of hospital-acquired infections in patients not previously considered at risk. *C. albicans* is commensal and is among the gut flora that survive in mouth and gastrointestinal tract of human being. Although candidiasis caused by overgrowth of *C. albicans*, this fungi is not harmful under normal circumstances in human population. Regularly, Candidiasis is detected

in immunocompromised individuals such as HIV-positive patients. Candidiasis also may occur in the blood and in the genital tract. Known as "thrush", it can be easily treated but not in people who are immunocompromised. Reaction to environmental cues by the unicellular yeast-like form of *C. albicans* caused the host tissue infected when it then switches into an invasive, multicellular filamentous form.

C. albicans plasma membrane retains its structure and properties by virtue of a complex cell wall composed of glucan, mannan, chitin, proteins and lipids (Bossche, 1991). The changes of its size and shape continually occur depending on the needs of reproduction, and require localization and controlled action of both biosynthetic and hydrolytic enzymes. Thus, any imbalance between the synthesis of new wall subunits and the controlled and localized hydrolysis to create the addition sites, will affect the growth, bud formation and/or septation. Therefore, this organelle, in principle, suggested as an ideal target for antimycotic action.

Compared to antibacterial antibiotics, the search for new and safer broad-spectrum antifungal antibiotics has been progressing slowly. This may due to the reason that fungi are eukaryotes; therefore agents that inhibit protein, RNA or DNA biosynthesis in fungi have greater potential for toxicity to the host as well (Georgopapadakou & Walsh, 1994).

Taxonomically, *Schizosaccharomyces pombe* (fission "yeast") has been classified in 'archiascomycetes' group which is to belong to an ancestral assembly of the ascomycetes. This yeast is easy to culture and manipulate, besides is well characterized genetically. Since *S. pombe* genome has been sequenced, it has become an alternative fungal model system comparable to *Saccharomyces cerevisiae* (*Schizosaccharomyces pombe*, 2011).

According to Wood *et al.* (2002), since *S. pombe* is a single-celled fungus, its genome sequencing is significant. They identified fifty genes in *S. pombe* like cystic

fibrosis, hereditary deafness, and diabetes which related to human diseases. This study reported that genes involved in cancer are among the human disease-related genes. They reported about 23 such genes which are responsible in maintaining genomic stability.

Schizosaccharomyces pombe may also act as a powerful tool to screen human genes relevant to cancer. Chung *et al*. (2007) reported that evaluation of relevance to cancer of 437 human full-length cDNAs was done using *S. pombe*. The cDNAs were isolated from liver and/or gastric cancer tissues by microarray analysis. 161 human cDNAs in *S. pombe* were considered cancer-related phenotypes since their over-expression have caused growth inhibition and/or morphological changes. Among the genes, sixteen were chosen for their oncogenic properties validation. The test comes out with a result that these genes were found to be highly expressed especially in liver and/or gastric cancer cell lines. In fact, the proliferation rates of the mouse embryonic fibroblast cell were increased by 32% to 120% when transfected with these genes.

Since *Schizosaccharomyces pombe* has simple actin cytoskeletons which is genetically tractable, it also serves as a model system in exploring the role of actin cytoskeleton. Takaine & Mabuchi (2007) reported that due to the fact that previous fission yeast actin had not been isolated from its native form and not yet characterized, its biochemical assays was performed in their study. Rabbit skeletal muscle actin was used in the biochemical assays due to its possibility of interaction with the fission yeast actin-binding proteins in different manner from previous fission yeast actin. This group of research finally comes out with a novel method to isolate the actin which is functionally active from the fission yeast cells. Once this new method established the relevant interactions between both actin and fission yeast actin-binding proteins will be able to reconstruct physiologically. Besides, it will also lead to classification of the function of actin cytoskeleton in cellular activities.