Chapter 2
LITERATURE REVIEW

There have been many definitions of actinomycetes, though in generalization all are same yet many researchers have put forward respective definitions and interpretations for better understanding. Actinomycetes are in most cases reported as gram positive bacteria that grow in form of mycelia. They are mainly aerobic; inhabit the soil in most of the cases, with very few exceptions. The name for the bacterium is derived from the anaerobic species, *Actinomyces bovis*. (Schlegel 1992).

As put forth by Oskay *et al., (*2004*)* actinomycetes are soil inhabiting microorganisms which are globally distributed. Many actinomycetes have been isolated and screened from the soil system, in recent decades, indicating upto 70-80% of relevant secondary metabolites available commercially (Khanna *et al.,* 2011). Historically, most actinomycetes isolated from the soil have been those belonging to genus *Streptomyces* and *Micromonospora* (Basilio *et al.,* 2003). Out of 22500 biologically active compounds derived in some form or other from microorganisms, 45% are from actinomycetes, 38% from fungi and those from bacteria account 17% (Berdy 2005). 70% of the total antibiotic production is related to species of *Streptomyces*, also those from *Micromonospora* was less than one-tenth as many as *Streptomyces* (Lam 2006)

Abundance of Actinomycetes localization and distribution

Among the abundant isolates that have been isolated by researchers are members of *Actinoplanes*, *Streptomyces*, *Nocardia*, *Actinomadura*, *Micromonospora*, *Nonomuria*, and *Streptosporangium* (Wang *et al*., 1999). Evidently, actinomycetes denote a high share of soil microbial biomass, and seem to be of importance among the microbial flora of the rhizosphere. The interaction found in plant and the Actinomycete, could be both deleterious and beneficial for the host (Couillerot *et al*.,2013). A large number of actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. The population of actinomycetes has been time and again marked as one of the major group of soil population which may vary with the soil types. Among actinomycetes group, streptomycetes is suggested as the most dominant. The non streptomycetes are also called rare actinomycetes.The genus

streptomyces is one of the largest genus of Actinobacteria and also the type genus of the family Streptomycetaceae. Over 500 species of *Streptomyces* bacteria have been described so far. The genus *Streptomyces* belong to the Domain Bacteria, Phylum Actinobacteria, Order Actinomycetales and the Family Streptomycetaceae.

Isolations within India

Screening of actinomycetes have been undertaken for many attributes like four different strains from laterite soil in Guntur region of Andhra in India (Kavitha *et al.,* 2010).15 strains of actinomycetes were isolated from Lucknow in Uttar Pradesh (Pandey *et al.,* 2011) Five actinomycetes strains of *Isoptericola variabilis was* isolated from 25 samples of Cauvery river basin. (Muthu *et al.,* 2013). six strains (in total) of actinobacteria were isolated from the soil samples collected from various arid and semi regions around Jaipur, Jhunjhunu, Sikar of Rajasthan (Masand *et al.,* 2015).10 isolates with distinct respective morphology were isolated and purified on starch casein agar from forest soils of Mahabubnagar district, Andhra Pradesh by Balakrishna *et al*., (2012)

Throughout the world

Similarly, other investigations carried through-out the world shows evidence of the ability of actinomycetes to inhabit many parts of the world. Heng *et al*., (2015) have isolated 110 *Streptomyces* isolates from samples of peat soil of Malaysia.A thermophilic actinomycetes *Thermasporomyces composti* gen. nov., sp. nov. was isolated from compost (Yabe *et al*., 2011). 31 strains of potential antibiotic producing actinomycetes from sediments as well as water of Tana Lake, Ethiopia were isolated by Gebreyohannes *et al*., (2013). 60 actinomycetes isolates were isolated from soil samples that were collected from different selected locations of Saudi Arabia. (Ababutain *et al.,* 2012). A total of foryt four strains of actinomycetes were isolated from Caspian Sea sediments at a depth of 5-10 m (Mohseni *et.al*. 2013).

Understanding Actinomyctes

Evidence indicates that actinomycetes are quantitatively vital within the rhizosphere. (Barakate *et al* 2002, Crawford *et al*.1993, Doumbou *et al*.2001,). Couillerot *et al*.,(2013) during their work on biocontrol and biofertilizer activities evaluation of *Streptomyces anulatus* S37 have stated that they provide protection to various plants, from soil-borne fungal pathogens and the antagonistic property

against pathogenic fungi, which have allowed these bacteria to be used as a bio control agent, with attributes such as fungus-anatagonistic root colonizer. Like the other Actinobacteria, streptomycetes are gram-positive, and have genomes with high GC-content, grow in soil and decaying vegetation, with permanent substrate and aerial mycelia which are mostly branching. Aerial mycelia have characteristic long chains of arthrospores during mature stage in their life cycle, also called as sporophore, which serve to enhance the spread of the organism, through budding of conidia. The morphological structure of the sporophore, colonial morphology, colour, size and odour are diagnostic features, used to differentiate many species and strains. .Streptomycetes can be easily distinguished by their distinct "earthy" odour which is due to production of a volatile metabolite, geosmin which was isolated from *Streptomyces griseus* (Schlegel 1992).

Others too have noted that they are responsible for the earthy smell of freshly upturned healthy soil (Sprusansky *et al.,* 2005). And as outlined by Chaudhary *et al*.,(2013) actinomycetes decomposes complex mixtures of dead plant, animal fungal materials which are conductive for crop production as the by-products are extra cellular enzymes.Actinomycetes also produce auxin and gibberlin like compounds which is realted to plant growth. (Persello-Cartieaux *et al*.2003, Bloemberg *et al.* 2001). Studies of Cummins and Harris (1956) established that actinomycetes have a cytomembrane composition comparable to that of gram-positive bacterium, and conjointly indicated that the chemical composition of the cytomembrane may furnish sensible strategies of differentiating numerous varieties of actinomycetes. Fuentesa *et al.* (2010) reported that growth of 12 out of 18 actinomycetes isolates was closely realted to the presence of other microorganism and the pesticide namely (chlordane, lindane or methoxychlor) and highest growth and pesticide removal were observed with chlordane. Sharma (2014) has said that spores of most actinomycetes endure desiccation and show slightly higher resistance to dry or wet heat than vegetative cells hence are appropriate for soil applications.

Growth after germination during the life cycle of the gram positive , *Streptomyces coelicolor* a soil dwelling bacterium starts when spores come in contact with a suitable source of nutrient . The filamentous vegetative cells known as “substrate hyphae” grow following apical tip extension along with branching, ultimately resulting in a tangled filamentous network. The second filamentous cell types emerge with the

gradual aging of the vegetative colonies, taking to the air as aerial hyphae which is at a distance from the substrate hyphae, by undergoing septation and compartalization into 40 to 60 units of equal size. Known as “prespore” these structures are the precursors for metamphosis into spores. There are many steps to the maturation, ultimately cumulating in the deposition of a grey polyketide pigment, upon the surface of the respective spores and evtentually turning the colour of aerial mycelium to grey from white. (Davis and Chater, 1990). The switch from substrate hyphal growth to aerial growth is understood to coincide with the sensing of environmental stress and nutrient deficiencies. Nitrate depletion, for instance, is known to coincide with initiation of formation of aerial hyphae (Karandikar *et al*. 1997), and while the presence of glucose is understood to inhibit development of the aerial hyphae (Redshaw *et al*., 1976). The emergence of aerial hyphae in turn results in production of various secondary metabolites, and those have subsequent and significant application in varous fields like medical science as antibiotics, antifungal drugs and also varous important chemotherapeutic agents. Further there has been suggestions that physiological parameters through tests can be used as indispensable tools for classification and identification of actinomycetes. (Kampfer *et al*.,1991)

Actinomycetes as source of antibiotic.

As is known antibiotic producing ability is the best known ability of actinomycetes. As reported by other workers as well approximately 70% of all antibiotics known to mankind has been isolated from actinomycetes, (Ayari *et al*. 2012) It is also stated that in comparison to other microbes, novel therapeutic antibiotics are being discovered from this group at a frequent rate in various chemotypic and biologically active forms namely daptomycin, thiednamycin and echinocandins. (Newman and Cragg, 2007). It has been put on record by Marinelli (2009) that of the total marketed microbial drugs two-thirds are produced by streptomycetes.

Table 1. List of antibiotic compound produced by actinomycetes

Type	Active component	Microorganism
Antifungal compound	Nystatin	*Streptomyces noursei*
	Amphotericin B	*Streptomyces nodosus*
	Natamycin	*Streptomyces natalensis*
Antibacterial compound	Erythromycin	*Saccharopolyspora erythrea*
	Neomycin	*Streptomyces fradiae*
	Streptomycin	*Streptomyces griseus*
	Vancomycin	*Streptomyces orientalis*
	Daptomycin	*Streptomyces roseosporus*
	Rifamycin	*Streptomyces mediterranei*
	Chloramphenocol	*Streptomyces venezuelae*
	Puromycin	*Streptomyces alboniger*
	Lincomycin	*Streptomyces lincolnensis*
Other bioactive compounds	Brasilinolide A	*Nocardia brasiliensis* IFM0406
	Tetrodotoxin	Marine actinomycetes
	Niromycin A	*Streptomyces endus* N40
	Salinosporamide A	*Salinispora tropica*

Relevance of Actinomycetes and economic importance

Bignell *et al*. (2010) have reported a new biosynthetic gene cluster found in *Streptomyces scabies* that produce coronafacic acid, which is part of the plant toxin, coronatine which mimics the plant hormone jasmonate, thus playing a major role in contributing to virulence.

Means of Bio-remediation

Due to fact that actinomycetes have been isolated from mostly any type of habitat or agroclimatic zone , it can be inferred that there must be some ability in these organism to be able to auto remedy various type of soil or biological structure. Lin *et al* (2011) demonstrated that a strain of *Streptomyces parvulus* , new in its occurence, isolated from waste-water sludge could even degrade a pyrethroid based insecticide named cypermethrin. Polti *et al*., (2007) put up, that if bioremediation of heavy metals and other organic compounds is to be considered then actinomycetes are relatively well suited owing to the relevance that as they constitute a prevalent microbial component in many soil biota, attributed due to their metabolic diversity and growth characteristics, mycelial form and relatively rapid colonization of selective substrates.

Isolation technique for Actinomycetes

There are reports of many types of techniques, which have been followed as such or with certain modification for isolation of actinomycetes by reserachers from respective area of location and climatic zone. Sahin and co-workers followed simple soil dilution method with starch casein agar as the media for isolation of thermophilic *Streptomyces*. In brief about 1g of soil samples was aspetically transferred to 9ml of sterile Ringer's solution (oxiod) ¼ strength, which was manually shaken for a span of half an hour, with intent to disperse the bacteria.Preheated in water bath at 55^0C for a duration of 6 min was opitimised to heat the suspension at tenfold dilution. Aliquots (0.2 ml) of 10^{-2} to 10^{-5}dilutions were evenly spreed on the dried starch casein plate surfaces at (pH 7.2; 20) with supplements of cycloheximide (only 50mg ml^{-1}) and filter sterilized rifampicrin (0.5 mg ml^{-1}).7 days incubation of inoculated plates in replicas of four was done at 55^0C.Colony forming units (c.f.u) per gm per dry wgt of sample was used in counting the expression of isolates in each plate (Sahin *et al*.2002). Similar method was applied by Lo *et al*. (2002) but by using HV agar medium. Same was done earlier in past too by El-Nakeeb and Lechevakier (1963) followed dilution technique for aerobic actinomycetes but used many types of media like Gaueze's agar medium, benedicts medium modified, chitin medium, soyabean meal glucose medium, Czapek's agar medium and gave a conclusive report that AGS medium or Arginine-glycerol-salt medium supports isolation of many types of actinomycetes. Hsu and Lockwood (1975) on the other hand pointed out that chitin agar was superior for isolation and at the same time enumeration. Moncheva further said that starch-casein-nitrate agar can be used for isolation, cultivation as well as maintenance of isolated soil actinomycetes (Moncheva *et al*., 2002). Other related works include isolation of a moderate halophilic actinomycets, strain called as HA-9 or *Nocardiopsis kunsanensis* from salt urns in Kunsan in Republic of Korea, by simply amending seawater in the Bennet medium. Through their work, another research group Hayakawa *et al*.,(2000) formulated an experimental, termed rehydration and centrifugation (RC) method, wherein supplementation of media by yet another simple enrichment technique allowed direct isolation from soil and litter of selective and rapid isolation of many types of zoosporic actinomycetes.

Similarly,Takahashi and Omura (2003) described selective isolation of novobiocin or Actinoplanes strains of *Kitasatospora* using chemotactic (KCl)

substituted by gellan gum as a solidifying agent and appraised nine novel compounds and proposed two new genera, five new species and one new subspecies. Couillerot *et al.,* (2013) in their work have used supplements in Olsons media and nutrient agar, namely actidione to inhibit fungus and nalidixic acid to inhibit bacteria capable of swarming. Soil dilution method was used by Srividya *et al*., (2012) to isolate Streptomycetes strain from solanaceae rhizospheric soils of brinjal, capsicum and chilli grown in Bangalore and Assam. Serial dilution method diluted upto 10^6 dilutions with plating on starch casein agar following spread plate technique was used by Gopalakrishnan *et al*., (2011) to isolate 137 cultures from herbal compost. Similarly Heng *et al*., (2015) have isolated 110 actinomycetes isolates from peat soil samples of areas in Malaysia, by agitating soil sample with orbital shaker and plating on starch casein agar at 28^0C. Modified standard dilution technique was followed by Ara *et al*.,*(*2012a), for collection of 105 actinomycetes strains from the soils of Riyadh in Saudi Arabia.

Study of morphology of actinomyctes

Studies have been made to understand each aspect of actinomycetes growth and survival under various condition. In the past, Shirling and Gottlieb (1966) examined isolates for pigmentation, colour of aerial mycelium and related morphological features. Abbas using the same method, did grow cultures for 4 weeks and observation were made at weekly intervals for morphological properties of colony, cells and spores (Abbas 2006).

Even variation of the media for growth was done for morphological study, like Sahin *et al.,*(2002) chose oatmeal agar as the medium of growth and visual examination on the basis of aerial spore mass colour, substrate mycelia pigmentation and colouration of media by diffusible pigments was done, and at the same time peptone-yeast extract in iron agar plates were used to observe production of any dark colored melanin pigments. Also there is confinement of study and investigation of fine structure of germinating spores to *Streptomyces* genera as put forth by Kalakoutswl and Agre (1973). Hence, there has been suggestions for need of modification of colour grouping method, and objective color determination method (Pridham 1965).

Production of melanine

Actinomycetes have another feature of being able to synthesize as well as excrete, melanin and melanoid, which are uaully dark colored and forms the basic criteria of taxonomical study, tests have been conducted by growing actinomyctes in peptone-yeast extract agar and synthetic tyrosine agar, to study the melanine production. This was done by dispensing 10 µl of selected media in liquid form in test tubes and then inoculating loop full of *Streptomyce*s spore, sunjecting the same to a stationary stage, facilitating better observance at 27^0 c for seven days (Dastager 2006)

Enzyme activity

Enzyme activity of actinomycetes, mainly fungal cell wall degrading enzymes such as cellusase, chitinase, β 1-3-glucanase have been extensively studied by different scientists. Production of chitinase from endophytic *Streptomyces aureofaciens* CMUAC130 and likewise effect on phytppathogenic fungi has been reported Taechowisan *et al*., (2003). Srividya *et al* (2012) reported chitinase, Glucanase, cellulose, protease production by *Streptomyces* sp. 9p.

IdentificationTechniques

Various techniques are adapted by the researchers for identification of different groups of actinomycetes. Identification can be done by the conventional classical method where the morphological attributes are noted for the identification and classification. The identification key as described in Bergey's Manual of Determinative Bacteriology(Buchanan and Gibbon 1974) is very much useful for identification of Streptomycetes group.Colour of the aerial spore mass, production of melanoid pigment, spore chain morphology, spore structure,sporulation types all these characters are routinely followed(Li *et al*., 2016) for the morphological identification procedure. Chemotaxonomy is another method of identification of organisms where the chemical variation in the organisms are considered for classification. Being Gram positive bacteria the presence of Diaminopimelic Acid (DAP) isomers in the cell wall is the most important chemical characteristic of Actinomycetes as described by Schon and Groth (2006). Molecular identification of the actinomycetes is done by the nucleic acid sequencing method. The sequence of 16S ribosomal DNA is being used by various researchers for accurate identification of the actinomycetes up to genus level. With help of the 16S rDNA sequences phylogenetic relationship of different actinomycetes can be

confirmed. Yu *et al*., (2015) isolated actinomycetes from wetland and successfuly performed their molecular profiling following the method. Other workers like Jami *et al*.,(2015), Labeda *et al.,* (2014), Muthu *et al.*,(2013) also reported molecular identification of the actinomycetes based on the 16S rDNA technique.

Plant protection by biocontrol agents.

Plant diseases and pathogenic microorganisms are a major and chronic threat to crop production as well as crop loss affecting food production. Evidently, owing to dependancy on agrochemicals and proven protection against pathogens agriclutral production has increased manyfold in the decades gone by. However, the flip side of the same has been deterioting health related conditions to humans upon comsumption or contact and resulting environmental pollution. Also increase of resistance against pathogens to wide range of fungicides is there. (Prapagdee *et al*., 2008). Furthermore, the growing effective cost of pesticides, predominantly in developing countries of the world, and increase in awareness of people against harmful effect of these chemicals has led to a search for substitutes for chemical properties.On similar lines, various microrganism to microorganism interaction for growth enhancement , antagomisn and overall soil system uogradation has been underaken as experimental set ups against pathogens and pest using either bacteria, fungi, actinomycetes etc. For instance Xiao *et al*. (2002) observed that application of isolated strains of actinomycetes rather significantly reduced severity of root rot in host alfalfa and soybean caused by *Phytophthora medicaginis* and *Phytophthora sojae.* in sterilized vermiculate as well as naturally infested field soil.

Similarly, *Streptoverticillium albireticuli* upon isolation and cross with fungal pathogens *Rhizoctonia solani*, *Phytophthora cinnamomi* and *Fusarium oxysporum* showed remarkabile ability towards antifungal activity(Park *et al*., 2002).Phytophthora blight pathogen of red pepper, *Phytophthora capsici* growth was inhibited by thermostable low molecular weight substance of *Streptomyces halstedii* (Joo, 2005)

Mechanism of biocontrol

Biological control for direct protection of plants from pathogens involve the use of antagonistic microorganisms.The machanism by which these microorganisms carry out their function are their abillity to parasitize the pathogen directly, production of antibiotic against the pathogen, their ability to compete for space and nutrients and to

survive in the presence of other microorganisms. These mocroorganisms produce enzymes which attack the cell component of pathogen, induce defence response in plants they surround. Although thousands of microorganisms have been shown to interfare with the growth of plant pathogens in the laboratory , only a few are effective in the field condition. The most important of them are *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* (Agrios 2005).The genus *Streptomyces* is gaining reputation as a suitable alternative for much needed organic method of desease control in plants, as there is an unprecendented awareness in the global market aimed at not only crop protection but a general need for use or consumption of natural products, having genuinely low or nil toxic trail, upon application for the same (Behal 2000). Actinomycetes isolates as biocontrol agents exert a direct inhibitory effect on hyphal growth and structure of fungal pathogens like *Botrytis cinerea* to reduce disease incidence, even though the exact mechanisms by which actinomycetes isolates operate is not elucidated. (Couillerot *et al*., 2013)

Table 2. Actinomycetes as bio control agent

Actinomycetes	**Host Plant**	**Disease**	**Pathagen**	**Reference**
Streptomyces Sp. *S. thermotolerans* and *Streptomyces sp.* N0035	Yam	Spot diseases in Yam	*Curvularia eragrostides* (Henn.) *Meyer Colletotrichum gloeosporioides* (Penz.)	Soares *et al* 2006
Streptomyces GS 93-23	Alfa alfa	Phytophthora root rot on alfalfa	*P. medicaginis*	Xiao 2002
Streptomyces griseus	Tomato	Fusarium disease of tomato	*Fusarium oxysporium f. sp.* Lycopersici	Anitha and Rabeeth. 2009
Strepromyces griseus, S. *hygroscopicus* var. *geldanus* and *S. noursei* *S.cellulosae* *S. herbaricolor* *S. coeruleofuscus*	Pea	Rhizoctonia root rot on pea	*Rhizoctonia solani* *Phytophthora megasperma* var.*sojae*	Rothrock and Gottlieb., 1981
Strepromyces	Cucumber	Damping off	*Pythium aphanidermatum*	Costa *et al.* 2013
Streptomyces	Sunflower	Sunflower Head and	*Sclerotium sclerotiorum*	Baniasadi *et al.*, 2009

		Stem rot disease		
Actinomycetes isolate 19	Radish	Rhizoctonia root rots	*Rhizoctonia solani*	Sahaya *et al*.,2012
Streptomyces sp.	Sugar beet	Root rot	*Sclerotium rolfsii*	Errakhi *et al.* 2009
Nocardia sp. AzL025 Streptosporangium sp. AzR 021 *and* 048	Lettuce	Root rot	*Pythium and phytophthora sp.*	Verma *et al.*, 2009
Actinomycetes isolate A5005 *and* A 5314	Rice	Rice Blast	*Magnaporthe grisea*	Hong-Sik and Yong-Hwan , 2000
Streptomyces sp.	Chilli pepper	Chilli anthracnose	*Colletotrichum gloeosporioide*	Suwam *et al.*, 2012
Streptomyces Sp.	Tomato	Bacterial wilt in tomato	*Ralstonia solanacearum*	Sreeja and Surendra., 2013
Streptomyces hydroscopicua	Chilli	Stem rot disease of Chilli	*Sclerotium rolfsii*	Pattanapipitpaisa Kamlandharn., 2012
*Streptomyces sindeneusis*isolate *263*	Rice	Rice Blast	*Magnaporthe oryzae*	Zarandi *et al.*, 2009
Actinomycetes	Soybean	Damping off	*Sclerotium rolfsii*	Sastrahidayat *et al.*, 2011
Strepromyces sp.	Sweet pea	Powdery mildew disease	*Oidium sp.*	Sangmanee *et al.*, 2009
Streptomyces nigellus NRC 10	Tomato	Dumping off	*Pythium ultimum*	Helmy *et al*. 2010
Streptomyces lydicus WYEC108	Pea	Pythium seed rot and root rot.	*P. ultimum*	Yuan and Crawford., 1995
Actinomycetes	Lettuce	Damping - off	*Pythium lutimum*	Crawford *et al.* 1993
Streptomyces Viridodiasticus and *Micromonospora carbonacea*	Lettuce	Basal drop disease of lettuce	*Sclerotinia minor*	El-Tarabily *et al.*, 2000

Antimicrobial activity.

Sahin and co-workers (2003) studied *Streptomyces* isolates under, *in vitro* condition for microbial activity against gram positive and gram-negative bacteria and

yeast. The result of the study inferred that 5 isolates, 3 identified as *Streptomyces antibioticus* (MU106, MU107), *S. rimosus* (MU114) showed prominent level of activity against chosen microbe of coagulase-negative *Staphylococcus* (CoNS) as well as yeast. Similar reports have been on effective antimicrobial activity of *Streptomyces* isolates have been put forth by reserachers during various time Ilic (2005), Laidi *et.al*., (2006) and Charoensopharat *et al*.,(2008).

Lytic enzyme production

The ability of any organism to act as antagonist against other microbes or pathogen is due to various ability, and production of lytic enzyme capable of destroying fungal cell wall resulting in hyperparasitic activity is one such character, supported by results and accounts from many sources, mostly or coveniently found among actinomycetes.Spore germination and at the same time germ tube elongation in *Botrytis cinerea* was inhibited by chitinase produced by *Serratia plymuthia* (Frankowski *et al*., 2001).The ability of *Serratia marcescens* to produce extracellular chitinase is what helps it to act as antagonist against *Sclerotium rolfsii* (Ordentlich *et al*.,1988). *Pseudomonas stutzeri* synthesize extracellular chitinase as well as laminarinase that digest and lyse *Fusarium solani* mycelia (Lim *et al.*, 1991). Evidence has been reported that *Paenibacillus* sp. strain 300 and *Streptomyces* sp. strain 385 synthesize β-1,3-glucanase, which undertakes cell wall lyses of *Fusarium oxysporum* f. sp. *cucumerinum* (Singh *et al*., 1999). Similarly cell walls of *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium ultimum* are destroyed by the same β-1,3-glucanase but synthesized by *B. cepacia* (Fridlender *et al*., 1993). Endophytic actinomycetes also produce lytic enzymes which inhibit fungal growth. For example, Castillo *et al.* (2002) demonstrated that an endophytic bacterium *Streptomyces* sp. strain NRRL 30562 isolated from *Kennedia nigriscans* produced munumbicins, an antibiotic which can inhibit *in vitro* growth of phytopathogenic fungi, *P. ultimum*, and *F. oxysporum* (Compant *et al.*, 2005).*Streptomyces griseus* upon interactive setups showed evidence for production of a metabolite which inhibited soil-borne plant pathogens (*Alternaria alternate*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Fusarium solani*) and two isolates of *Aspergillus flavus. In vitro* lytic activity presdisposition provides setup of appropriate condition and the effect of biocontrol organism in field level treatment. (Anitha and Rabeeth, 2010)

In-built resistance in plants.

Systemically activated resistance (SAR) is a phenomenan which comes, after primary infection takes place upon invasion by a necrotizing pathogen resulting in increased level of salicylic acid and pathogen related protiens and subsequent hypersentitive reaction (Agrios 2005). Whereas Induced sustemic resistance (ISR) is triggered by non-pathogenic strains of root colonizers. ISR does not cause visible sympotoms on the host plants and first PGPB-elicited ISR was observed on *Dianthus caryophillus* with reduced susceptibility to wilt desease caused by *Fusarium sp* (Van Peer 1991). Similarly, in *Cucumis sativa* with reduced susceptibility to wilt caused by *Fusarium* sp. (Van Peer1991) and on cucumber (*Cucumis sativus*) with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare* (Wei *et al*.,1991). Traditionally, reports supported that only rhizobacterial strains of PGPB, brought about ISR through physiological mediation, but evidence of same by *Pseudomonas fluorescens* an endophytic bacteria against red rot caused by *Colletotrichum falcatum* on sugarcane has also been reported (Viswanathan and Samiyappan.,1999), as has *Burkholderia phytofirmans* PsJN against *Botrytis cinerea* on grapevine (Barka *et al*.,2000).

Defence mechanisms of ISR- mediated by PGPB

The chronology or sequential networking of various reactions within the plant tissue system when a pathogen attackes is dynamic both interanlly and externally too. For optimum defence PGPB triggered ISR leads to enhancement of plant defence enzyme systhesis which in turn strengthens plant cell wall and host response to metabolic responses and physiology. Duffy *et al.,* (2003). Indicated in responses to pathogen colonization of epidermal and hypodermal cells, endophytic *P. fluorescens* WCS417r induce outer peripheral and outer radial end of the first cortical cell wall thickening. Similarly, phenolic compound accumulation along with exodermal or cortical cell wall stregthening was reported in *Burkholderia phytofirmans* PsJN-grapevine interaction upon colonization. (Compant *et al*.,2005).

***Streptomyces* as bio controlling agent**

Evidently *Streptomyces* sysnthesize a variety of fungal cell wall-degrading enzymes, such as chitinase, cellulases, hemicellulases, amylases, glucanases, etc and other antifungal compunds. Fungal inhibition can be related to chitinase production

(Gupte *et al.,* 2002; Dahiya *et al.,* 2006), *Streptomyces viridodiasticus* syntesized chitinase extract has been found to suppress basal drop disease causing fungal pathogen *Sclerotinia minor* in lettuce (El-Tarabily *et al.* 2000).It is further supported that fungal growth inhibition is related to chitinase production in various plants (Gupte *et al*., 2002; Dahiya *et al.,* 2006). Evidence is also there that *Aspergillus* sp. and *Fusarium subglutinans* growth is inhibited by *Streptomyces* sp of maize rhizosphere (Bressan 2003). Control of foliage diseases by culture filtrates of streptomycetes has also be worked out with satisfactory results wherein nine out of ten samples controlled or suppressed one or other disease under green house condition (Pridham *et al.,* 1956). Even under artificial condition *Streptomyces ambofaciens* controlled *Fusarium* wilt in cotton and *Pythium* damping-off in tomato plants (Reddi and Rao.,1971).

***Streptomyces* as growth enhancer**

There is a wide array of compound that assist and contribute towards the vitality and vigour of plants. Indole-3-acetic acid (IAA) is one such hormone, it is the principal form of auxin in regulating cellular processes like cell division, elongation, differentiation. At the same time plays a key role in shortening of root length and root hair formation. Hence IAA assists in increasing the nutrient absorption ability of the palnt. Other role in developmental activity includes embryo and fruit development, vascular tissue differentiation, tropism of plant, apex formation and apical dominance (Shrivastava *et al.,*2008). There has been observation regarding induction of IAA systhesis by *Streptomyces* species, six in number when tryptophan is present, viz *S. violaceus* and *S. exfolitus* through catabolization of IAM, ILA, IET, IAAld into IAA, with other possible pathways into IAA biosynthesis (Manulis *et al.,* 1994). Igarashi *et al*.(2002) reported secretion of indole-3-acetic acid (IAA) by *S.violaceus, S.scabies, S.griseus, S. exfoliates, S. coelicolor* and *S. lividans* when L-trytophan was induced. So are reports that strain of *Streptomyces* sp MBR52 augmented elongation and emergence of plant adventitious roots (Meguro *et al*., 2006)

***Streptomyces* as Plant growth inhibitors or herbicidal agents**

There have been many reviews and works on the many beneficial aspects of plant actinomycetes interaction. There is support to the fact that *Streptomyce*s also inhibit the growth of certain plants and in doing so can be exploited as potential herbicide. Certain metabolite systhetesized by *Streptomyces* sp strain SANK 63997

produced herbicidal antibiotics called Herbicidin H, the strain was isolated from leaves of *Setaria virdis* var *pachystachys* (Hasegawa *et al.*, 2006). It is not only *Streptomyces* but other actinomycetes which happen to have herbicidal properties, there has been reports of strain SANK 61299 of *Dactylosporangium sp.* from *Cucubalus* sp. producing two growth inhibitors along with streptol acting on adverse germination of *Brassica rapa* (Okazaki 2003).

Endophytic actinomycetes as biocontrol agents

Actinomycetes are found at various types of habitat and in different roles in the ecosystem, most of the time in soil rhizosphere, in lakes or pond, in sediments or in riverine soil as free living, sprophytes etc. Moreover there are groups which are in direct interaction with the living tissue of plants, these are known as endophytic actinomycetes, and may be beneficial like *Frankia* which has suggestive role in nitrogen fixation and *Rhizobium* which is believed to do the same in legume plants. Endophytic actinomycetes have been demonstrated to improve and promote growth of host plants, as well as to reduce disease symptoms. Management of beneficial potentials of endophytic actinomycetes to favour plant growth could be realized by a better understanding of the physiological and molecular interactions between these microbes and plants. (Simizu ,2011).Thirty-eight strains belonging to *Streptomyces, Microbispora, Micromonospora* and *Nocardia* were isolated from surface sterilized healthy wheat tissues (Coombs and Franco, 2003)Similarly, 59 endophytic isolates isolated from root tissues of *Zingiber Officinale* and *Alpinia galangal* showed maximum antifungal activity against *Candida albicans*, mostly *Streptomyces aureofaciens* (Taechowisan *et al*, 2005)

Actinomycetes metabolites that affect plant's life

Endophytic colonizers do get shielded from external factors and get nutrition from the host, and in turn different kinds and forms of bioactive metabolites are synthesized by them which aids in the plant vitality and vigour. The control point of such attributes may be the ability to fix nitrogen, produce phytohormones, inhibit phytopathogen growth or incidence through related phenomemen like antibiotic secretion, siderophore production, competition for nutrient and most importantly directly or indirectly brining about systemic disease resistance.

Use of *Streptomyces* as biocontrol agent for plant disease management

Okazaki *et al.,* (1995) and Matsumoto *et al.* (1998) reported that a variety of actinomycetes inhabit a wide range of plants as symbionts, parasites or saprophytes, and most of them belonging to the genera, *Streptomyces* and *Microbispora.*

Actinomycetes and their role in various plant pathogen interaction
***Pythium* seed and root rot of pea.**

Extracellular metabolites produced by *Streptomyces lydicus* WYEC108 was instrumental in combating fungal pathogens of pea, which was evident by inhibition of *Pythium ultimum* and *Rhizoctonia solani* together with *Streptomyces lydicus* WYEC108 grown in liquid medium. Even spore coating of pea seeds by the respective actinomyetes showed maximum inhibition of infection or invasion by test pathogen *P ultimum,* even under conditions of high oospore count in the soil of growth. Even seed and root was indicatively mimimized or suppresed under controlled condition. Also formulations of the strain as spore and peat moss-sand media in sterile and non sterile soil under pathogen infestation, was able to positively affect pea plant growth and vigour. With proven ability to lyse fungal cell wall and distort fumgal hyphae the strain *S. Lydicus* WYEC108 can be said to be a potential biocontrol agent when controlling Pythium seed and root rot (Yuan and Crawrford., 1995).

Lettuce damping-off caused by *Pythium ultimum*

Seed germination of lettuce under open and glass house condition in pathogen infested soil was optimal in works carried out in England inferreing that damping off by *Pythium ultimum* was controlled when formulations of actinomycetes was applied, isolated from both rhizospheric and non rhizospheric soil. Same isolates inhibited growth of other root pathogens as well (Crawford *et al*., 1993).

Basal drop disease of lettuce.

Isolation and screening of microbes from lettuce growing fields of Al-Ain, United Arab Emirates resulted in availalibity of countless number of bacterial, *Streptomyces* and non-Streptomyces isolates, which in turn showed ability to sysnthesize higher level of Chitinase. Further *in vitro* assay of these isolates against a known pathogen *Sclerotina minor* casual organism of basal drop disease resulted in marking three isolates namely *Serratia marcescens, Streptomyces viridodiasticus* and

Microsperma carbonacea as most effective in disease supression. The trio, upon further assay for β-1,3-glucanase showed prominent results, of not only for enzyme synthesis but also *in vitro* pathogen incidence and infection reduction under glass house condition (EL-Tarabily *et al*., 2000)

Leaf spot diseases of yam

Reports indicate that *Streptomyces* strain AC26 was effective in halting the growth of both spore and mycelium of *Curvularia eragrostides* (Henn.) causing leaf spot disease of yam. At the same time *Streptomyces thermotolerance* and *Streptomyces* sp N0035 did the same towards *Colletotrichum gloeosporioides*. The result was in tune with the chitinolytic activity of the strains and sysnthesis of secondary metabolites (Soares *et al*., 2006)

***Phytophthora* root rot on alfalfa**

Xiao et al., (2002) put forward results stating post emergence damping-off caused by *Phytophthora* on alfalfa was inhibited by application of formulations of *Streptomyces* isolates with the added advantage of increase in plant vigor and forage yield. Similarly, supportive was findings that a *Streptomyces* strain GS 93-23 acted as prominent bio control agent against *Phytophora medicaginis* in alfalfa plants even in infested conditions. The same strain under *in vitro* conditions inhibited growth of alfalfa and soybean pathogens *Pythium ultimum, Phoma medicaginis Aphanomyces euteiches.* Further it also showed ability against diverse soil borne pathogens for integrated control.

Fusarium disease of tomato

Experiments with *Streptomyces griseus* in testing inhibition of *Fusarium oxysporum f.sp. lycopersici* under *in vitro* condition showed prominent sign of pathogen growth inhibition supported by indiciation of presence of inhibitory substance, antibiotic, and enzymes such as protease, Glucanase etc. Even cell wall lysis was observed (Anitha and Rabeeth 2009).

Rhizoctonia root rot on pea

Experimental set ups with known antibiotic producing *Stretomyces* namely *Streptomyces griseus, Streptomyces hygroscopicus.var.geldanus,Streptomyces noursei* showed prominent zones of inhibition against pathogens *Rhizoctonia solani* and

Phytophthora megasperma var sojae. *Streptomyces hygroscopicus* provided complete control over disease development upon pre application of strain seven days before pathogen infestation of planting soil and *Streptomyces herbaricolor* and *Streptomyces coeruleofuscus* was found to provide consistent control over different conditions against the pathogens (Rothrock and Gttleieb, 1981)

Disease of interest as per present investigation.

There are several records of fungi belonging to ascomycetes, deuteromycetes, basidiomycetes as being causal agent of root rot and wilt disease, prominant among them are *Sclerotium rolfsii, Rhizoctonia solani, Thielaviopsis, Acermonium, Fusarium solani* as well as its other species which even cause stem rot in various crop plants.

Fusarium root rot

Fusarium solani and *Fusarium oxysporium* are prominent pathogens of root rot in non grain crops. *Fusarium solani f.sp.phaseoli* causes *Fusarium* root rot and it is a prominent disease effecting common bean (*Phaseolus vulgaris* L.) and also other crops like soybean, peanut, asparagus etc. The symptomology is evidently initial reddish colour of young tap roots, which eventually attain dark tinge over some time and the affect is enhanced with the gradual cracks along main root and ultimate killing of secondary roots.

Further disease development marks yellowing of leaves, growth retardation, leaf fall or simple death of the plant even without visible wilting.

Generally production of asexual spores is there in *Fusarium solani* , which are either micro conidia, macro conidia, chlamydospores which are thick walled. Chlamydospores are profusely found in killed plant tissues or colonized organic stock in soil. Chlamydospore helps the fungi pass on unfavourable condition of low temperatures and drought as inactive stage and attains active state with proximity to seedling root system. This happens during an early phase of the growing season when warm conditions prevail.

Sclerotial rot

Sclerotium rolfsii is very harmful soil – borne fungus which is pathogenic to an array of host, it over winters in its mycelial form within the infected host tissue or the plant debri. A round structure called sclerotia, either in free condition or association

is the main over wintering structure and is the primary inoculum bringing about disease by persistingly dwelling near the soil surface. (Aycock 1966; Punja 1985). Sclerotial dissemination is either by the cultural practices, infested transplanted seedling, wind, or water itself, and like other sclerotium producing fungi has capability of overwintering as sclerotia or sterile mycelium.(Akram *et al.,* 2008). The form genus *Sclerotium* is characterized by dark brownish, black to tanned sclerotia mostly spherical in shape and on internal examination has a rind, cortex and medulla.(Punja and Rahe 1992). It also has a teleomorphic state (Punja 1988) and is prevalent in occurrence where weather is warm. Infection initiates as a lesion in the soil line area of the stem and gradually moves upward in a cottony at times fluppy appearance of mycelium, at times parallel leaf wilting, yellowing and die back is also there. Of the diseases that it causes collar rot is the most frequent as well as prominent in terms of occurrence and loss (Singh and Pavgi 1965).

Table 3: List of different Diseases of *Vigna radiata* and bio control agent

Bio-control agent	Disease	Pathogens	References
Trichoderma spp.	Root rot of *Vigna mungo*	*Macrophomina phaseolina*	Leo *et al.* 2010
Pseudomonas spp.	Seedlings damping off and stem rot of cowpea (*Vigna unguiculata* L. Walp)	*Pythium aphanidermaturm*	Dieudonne *et al* 2007
Trichoderma viride	Root rot diseases of *Vigna radiata*	*Rhizoctonia solani Sclerotium rolfsii Macrophomina Phaseolina, Alternaria alternate, Furarium solani and Colletrichum capsicii*	Mishra *et al* 2011
T. harzianum	Root rot diseases of *Vigna radiata*	*Macrophomina phaseolina*	Kumari *et al.* 2012
Burkholderia sp. Strain TNAU-1	Root rot diseases of *Vigna radiata*	*Macrophomina phaseolina*	Satya *et al*, 2011
Fluorescent *Pseudomonas* (MRFP)	Root rot diseases of *Vigna radiata*	*Fusarium solani Rhizoctonia solani Macrophomina phaseolina*	Ara *et al*, 2012a

Table 4: List of different Diseases of *Phaseolus vulgaris* and bio control agent

Bio control agent	Disease	Pathogen	References
Trichoderma harzianum	Root rot of *Phaseolus vulgaris*	*Fusarium solani,Rhizoctonia solani,Fusarium oxysporium,Sclerotium rolfsii* and *Pythium* spp.	El-Mohamedy *et al.* 2013
*Glomus*spp	Root rot of *Phaseolus vulgaris*	*Rhizoctonia solani*	Hathout *et al.* 2010
Trichoderma harzianium, Glomus intraradices, Azotobacter chroococcum	Root rot of *Phaseolus vulgaris*	*Rhizoctonia solani, Fusarium solani* f.sp. *phaseoli*	Matloob *et al*. 2013 Kilicoglu and Ozkoc, 2013 Bilgi *et al.* 2008

Disease reporting

Fusarium* and *Sclerotium* root rot disease in *Phaseolus vulgaris

Commonly known as either bean, dry bean, French bean, *Phaseolus vulgaris* is from the family Fabaceae. The main desirable part of the crop is its pod which has value in terms of nutrient consumption as well as commercial importance. Root rot is the most prominent and loss-making soil borne disease of *Phaseolus vulgaris* L ,with a broad spectrum of causal agents or pathogens , which includes *Fusarium* sp. *Pythium* sp. *Rhizoctonia solani*.

The occurrence is global in disposition and distributed over all bean growing locations. Abawi and Pastor-Corales (1990), Mukankusi (2011), Abeysinghe (2007) reported Fusarium root rot of *Phaseolus vulgaris* from different parts of the world characterized by reddish to brown lesions evidently at the lower hypocotyls and along tap root of the plant. Infected portions enlarge with gradual aging of the host and its pathogen, attaining a brown colour, at the same time longitudinal cracks form within the older lesions , with discolouration and decay of the ortical cells. The outlined fungus as pathogen is very persistant into disposition for survival and distribution in the soil, with remarkable tools to dwell in the soil for a long duration of time, hence has been deemed difficult to control and contain (Abeysinghe 2007). In similar line of work Abawi and Pastor-Corrales (1990) reported Sclerotium root rot in *Phaseolus.*

Sclerotium* root rot and collar rot disease of *Vigna.

Mungbean or *Vigna radiata* is an annual legume crop belonging to the Fabaceae family, which is an important food supplement in many parts of the world and has high economic value. *Sclerotium rolfsii* root rot and collar rot disease of *Vigna* reported by Yaqub and Shahazad (2005) and Sharma *et al*.,(2002) is the prominent and important disease as per crop loss and disease development.

Formulation

Studies in Kyrgyzstan reported a laboraty made biofertilizer (Patent # 1703, registered by10/12/2012 in the State Register of Kyrgyz Republic) on the basis of *Streptomyces fumanus gn*-2 for the treatment of wheat and bean seeds, before planting them in soil with low fertility in order to determine the effect of this biological agent on germination rate, growth of seedlings and shoots, maturation phase of plants. And resistance of these plants to pathogens confirmed that the introduction of *Streptomyces fumanus* as a biological agent in soil together with the seeds stimulated the growth and reproduction of useful and important microorganisms in the soil environment.(Doolotkeldieva *et al* ,2015).

Yuan and Crawford designed and undertook a process of mass production of *Streptomyces lydicus* WYEC108 as a peat moss-sand formulation. Spores were collected from SPA plates where heavy sporulation was there, aided with a sterile spatula for transfer to sterile sand , which in turn was mixed into sterile sand-peat moss carrier and was maintained at room temperature for future use and storage.(Yuan and Crawford 2005)

Another mode of formulation preparation involves autoclaving 500 ml conical flasks with 50g moist wheat bran for 20 minutes at 121^0C, on three simultaneous occasions (Roiger and Jeffers 1991). Yet another of formulations involved inoculation of substrate with 25ml of spore suspension in 10% glycerol, under aseptic condition with incubation at 28^0C under dark condition for a period of three weeks and routine shaking for uniform growth and colonization. (EL-Tarabily *et al.,* 2000). Simple yet seemingly relevant formulations involve direct seed coating with *Streptomyces* spore, tried on sterilized tomato seeds by soaking in *Streptomyces* spore suspension for half an hour (Dhanasekharan *et al*., 2005)

Scenario at national level

India being a country with a huge population which is increasing exponentially over the years needs tons of food grain each year to feed its people. As the cultivable land is decreasing day by day and the cost of manure and other materials for agricultural use are increasing at the same time the use of biocontrol agents is the only way out. By using the indigenous micro organisms present in soil this aim can be fulfilled. Indian scientists who are working in this field are Dhanasekharan *et.al* (2005) working on biological control *Rhizoctonia* dumping off of tomato by *Streptomyces*. Shrivastava and co workers working on Production of indole-3-acetic acid by immobilized actinomycete (Shrivastava *et al.,* 2008). Anitha and Rabeeth (2010) working on *Streptomyces griseus*.Patel *et al* (2014) working at Gujrat with the Actinomycetes having antibacterial and anti fungal properties. Masand and Menghani (2015) are working at Rajasthan with the same aim. Srividya *et al*.(2012) working with *Streptomyces* sp. 9p having biocontrol ability against chilli fungal phytopathogens. Goplakrishnan *et al*.(2011)evaluated Actinomycetes isolates for biological control of *Fusarium* wilt of chickpea. Janaki *et al* (2016) isolated actinomycetes from mangrove plant rhizosphere and tested their antifungal properties. Shrivastava *et al*., (2017) isolated *Streptomyces aureofaciens* K20 and found its biocontrol ability against *Macrophomina phaseolina.*