CHAPTER 2 LISTERATURE REVIEWS

2.1 The Actinomyces, Genus *Streptomyces*

2.1.1 Definition of *Streptomyces*

Taxonomy: Kingdom Bacteria; Phylum: Actinobacteria; Class: Actinomycetes; Order: Actinomycetales; Family: Streptomycetaceae; Genus: *Streptomyces*, they are gram positive filamentous bacteria within the classis Actinobacteria (Waksman and Henrici, 1943; Williams, 1983). They are the major population of soil microorganisms which playing an important role in the soil community as saprophytes. Nowadays, they are including nearly six hundred validly published *Streptomyces* species and containing about 38 subspecies (Euzeby, 2008). Almost the *Streptomyces* also have a complex growth, characteristic and secondary metabolite production similar to filamentous fungi, in contrast the structural and chemical of vegetative cells are similar to gram-positive bacteria (Flardh, 2003a; Flardh, 2003b). Normally, they are formed branching substrate and aerial mycelium and then developed cross-walls in aerial hyphae as directly as spores (Figure 2.1).

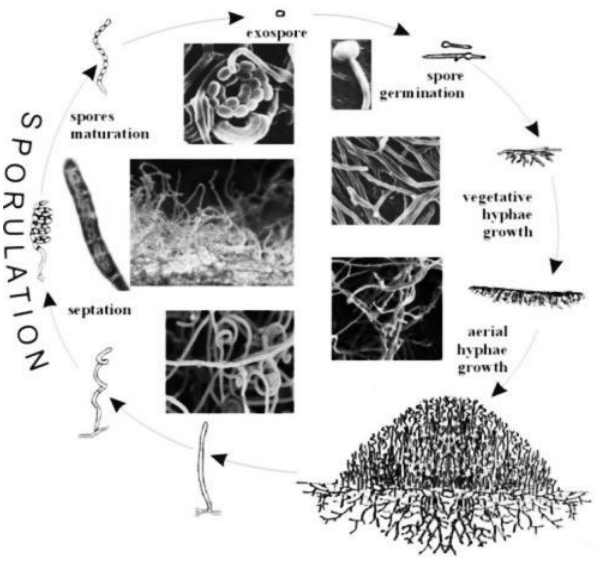

Figure 2.1 The life cycle of *Streptomyces* species
Source: (Jakimowicz, 2007)

A germ tube was emerged from the swelled exospore and developed to hypha by tip extending and branching becomes a vegetative mycelium that grows cover and deep down into the substrate as a vegetative colony. The aerial hyphae was formed from the substrate mycelium by the response of protein signals for breaking the surface tension,

escaping the aqueous environment, and grow into the air. The mature spore was developed at the end of aerial hyphae tips by producing thick wall and specific characteristics (Claessen et al., 2006). Within the developmental life-cycle, they can produce a wide range of secondary metabolites including the hydrolytic enzyme and bioactive compounds especially in sporulation phase.

Table 2.1 The observed properties for bacterial classification and identification

The definition	Property
1. Nutritional type	(i) Autotrophy (ii) Heterotrophy
2. Energy release	(i) Lithotrophy (ii) Organotrophy
3. Cell wall: Gram reaction	(i) Gram negative (ii) Gram positive
4. Cell morphology	(i) Cell shapes (ii) Cell aggregation (iii) Flagellation – motility (iv) Spore formation and location (v) Special staining, e.g., Ziehl–Nielsen
5. Physiological properties	(i) Utilization of various sugars (ii) Utilization of various polysaccharides (iii) Utilization of various nitrogenous substrates (iv) Oxygen requirement (v) Temperature requirements (vi) pH requirement (vii) Production of special enzymes
6. Antigenic properties	
7. Molecular methods	(i) G + C composition (ii) DNA:DNA hybridization (iii) Ribotyping (iv) Fluorescent in situ hybridization (FISH)
8. Chemical analysis (Chemotaxonomy)	(i) Lipid analysis (ii) Protein analysis

Source: (Okafor, 2011)

2.1.2 Identification of *Streptomyces*

In the same genus, the *Streptomyces* species are quieted similar the definition. The various approaches are used to identify and classify the species of *Streptomyces.* For commonly method, the classical approaches is followed by the identification key

(Nonomura, 1974) and Bergey's Manual of Determinative Bacteriology (Buchanan, 1974) based on the character of morphological, physiological, and biochemical (Table 2.1). The observed properties comparing or the parallel analyzing of all factors was clearly considered to report as same or new species.

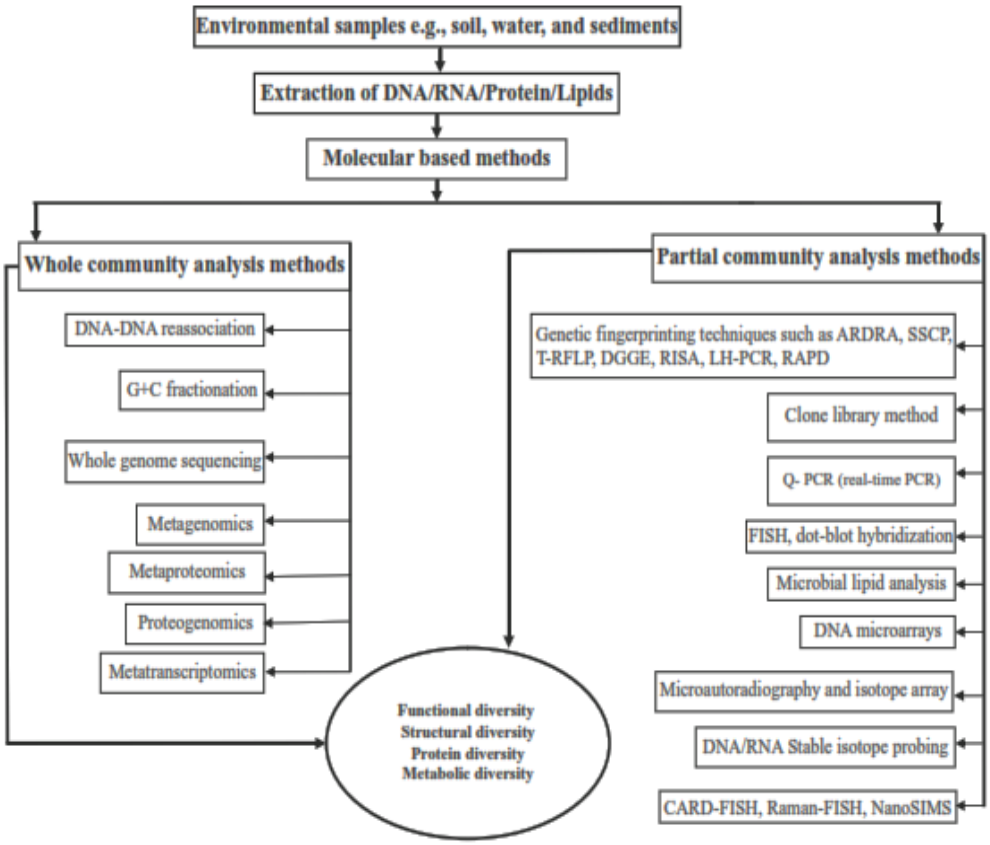

Figure 2.2 The molecular toolbox to characterize the structural and functional diversity of microorganisms in the environment.

Source: (Rastogi and Sani, 2011)

1. Molecular Approach

The molecular approach is the most powerful results to confirm as the basic method for identification. The whole genomes or a selected gene such as 16S rDNA was used for amplifying by the polymerase chain reaction (PCR). The DNA sequencer was used to determine the nucleic acid sequences and the phylogenetic tree was analyzed by computer programs (Saitou and Nei, 1987; Kumar et al., 2001). Many researchers suggested that not only phylogenetic studies can use to identify the genus or specie, but

a wide variety of molecular techniques can also use to describe the diversity of microorganisms (Figure 2.2).

2. Chemotaxonomical Approach (Chemical analysis)

Chemotaxonomy or Chemical analyses are valuable methods to identify the genera of actinomycetes as the chemical composition in organisms to help in the classification and identification. The cell wall composition of *Streptomyces* such as lipid, protein and sugar in same specie has been performed the similar components or patterns. They can be used to explain the homologous or differences between the two organisms. Many reports show that the sugar pattern was used to classification and identification of *Streptomyces* (Mordarska, 1977).

3. Classical Approach

The classical method based on a morphological, physiological, and biochemical characters have been used to describe in the identification of *Streptomyces*. The general methods as the identification key or Bergey's Manual of Determinative Bacteriology (Buchanan, 1974) have been used for long time in the species level identification (Nonomura, 1974). They are as follows.

- Aerial Mass Colour
- Melanoid Pigments
- Reverse Side Pigments
- Soluble Pigments
- Spore Chain Morphology
- Spore Surface
- Assimilation of Carbon Source

The spore chain features of genus *Streptomyces* can be subdivided into seven sections, namely rectiflexibiles (RF), retinaculiaperti (RA), Spirales (S), mono-verticillus (MV), mono-verticillus-spira (MV-S), Biverticillus (BIV), and Biverticillus-spira (BIV-S) (Figure 2.3). In addition, the spore surface can also be separated into five types by scanning electron microscopy (SEM) observing included warty, smooth, hairy, spiny, and rugose as shown in Figure 2.4. Almost data of morphological, physiological and biochemical characters were used to confirm the other approaches for specifying specie of *Streptomyces*.

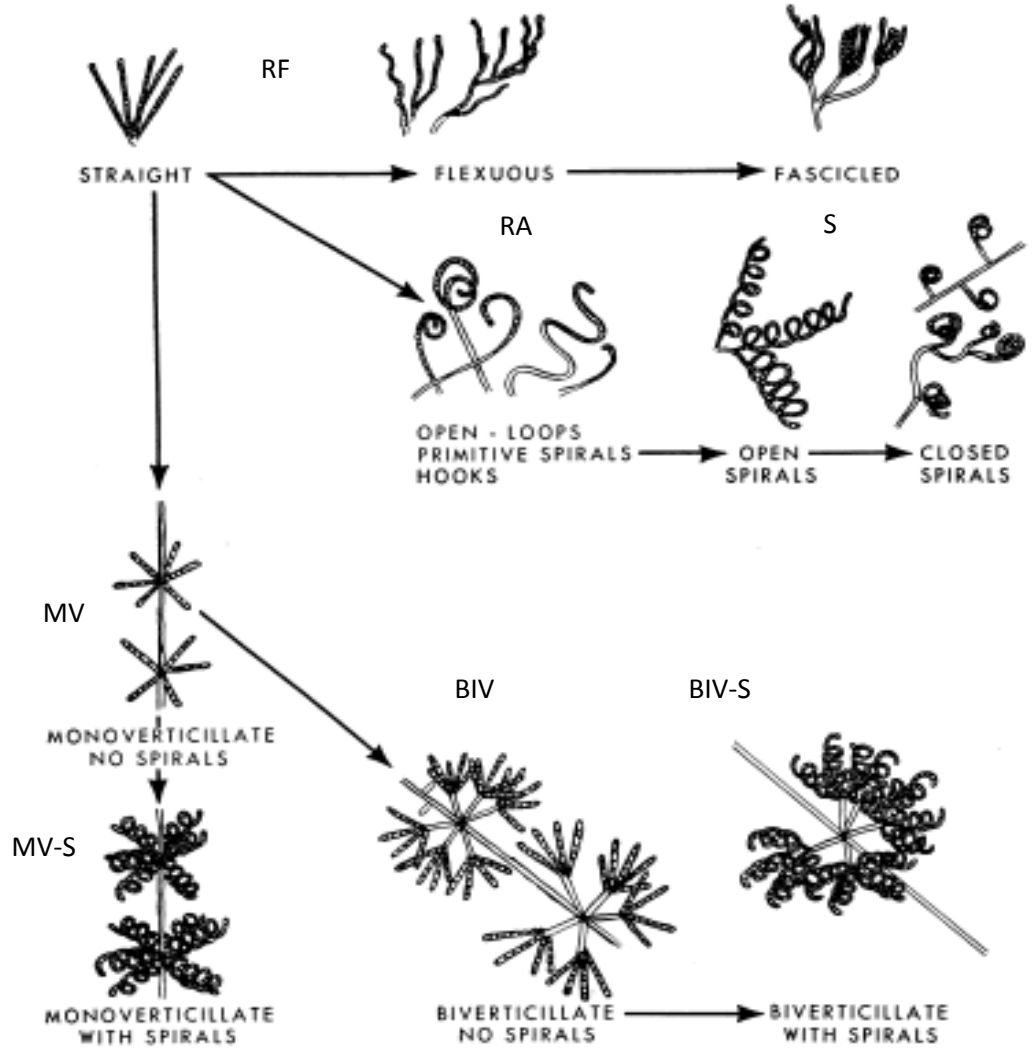

Figure 2.3 Seven sections of spore chain in the genus *Streptomyces*
Source: (Pridham et al., 1958)

4. Numerical Taxonomy Approach

The numerical taxonomy has been converted from classical approach into digits and coded by either Zero (means negative or absent) or one (means positive or present). The large number of *Streptomyces* was observed to arrange a cluster based on the similar characters (Williams, 1983). Several researchers have reported the numerical classification can be used to group the genus *Streptomyces* (Paszkiewicz, 1972; Kurylowicz et al., 1975; Goodfellow et al., 1992). Generally, all methods have been used to confirm each other for classifying the genus or species of organism.

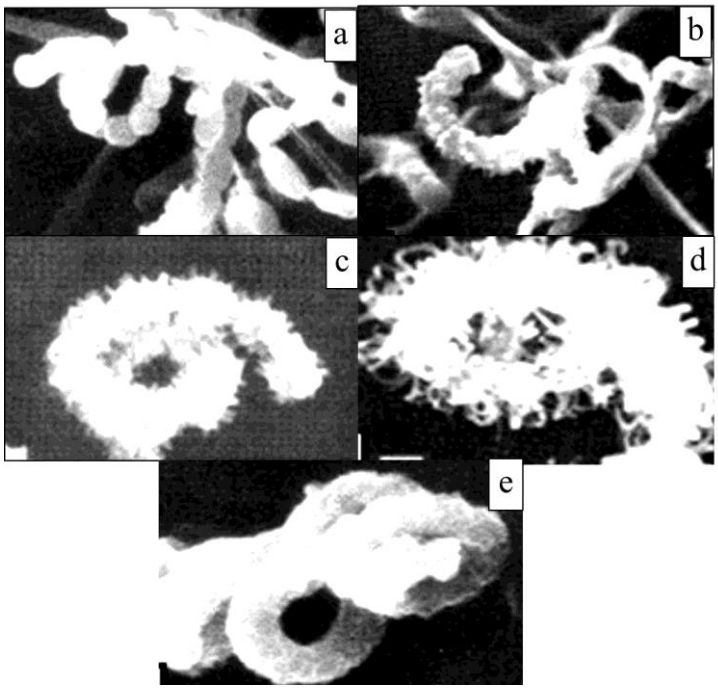

Figure 2.4 The spore surface types; smooth (a), warty, (b), spiny, (c), hairy (d), and rugose (e)
Source: (Dietz and Mathews, 1969)

2.1.3 The Cultivation of *Streptomyces*

The optimum conditions of microbial growth are generally influenced by many factors upon that organism. The *Streptomyces* growths are also depended on environmental conditions and the physiological state of *Streptomyces*.

1. Temperature

Temperature (ºC) can be affected to the mechanism in cell metabolism and cell growth. *Streptomyces* are found at the different temperature range such as mesosphilic (15-40°C), thermophilic (45-60°C) and hyperthermophilic. The optimums temperature for metabolite production is quite different with the optimum temperature for growth. *Streptomyces venezuelae* ISP 5230 has been reported as mesophilic *Streptomyces* which can grow between 15 and 40°C. The previous results reported that optimum temperature for growth of *S. venezuelae* was 30ºC ± 2 ºC (Wang, Y., 1994; Wang, L. F. and Hong, 2003). However, the optimum temperature for stimulate sporulation and antibiotic production of *S. venezuelae* was observed at 42ºC (Meinke and Jones, 1971). In addition, *S. thermogriseus* isolated from hot spring can be grown at 65-68ºC (Xu et al., 1998).

2. Hydronium Ion Concentration (pH)

The pH ranges of *Streptomyces* spp. growth are generally preferred between pH 6.5 to 8.0 as neutrophilic. In addition, the acidophilic and alkaliphilic strains can be found in pH range between 4.0-5.5 and 10.0-11.5. Many researchers have reported that some species of *Streptomyces* can be grown at low pH values (pH 4.5) such as *S. guanduensis* and *S. yanglinensis* (Xu et al., 2006) which according to *S. yeochonensis* (Kim et al., 2004). However, the previous results shown that many alkaliphilic and alkalitolerant *Streptomyces* can isolate from alkaline environments such as alkaline soils, soda lakes and saline-alkaline lakes such as *S. sodiiphilus* (pH 9.0–10.0) (Li et al., 2005), *S. deccanensis* (pH 7.0–12.0) (Dastager et al., 2008) and *S. griseus* (pH 9.0) (Liu et al., 2005). The pH balance in *Streptomyces* cells were adjusted close to neutrality by different membraneous pH-modulating mechanisms (Padan et al., 1981)

3. Dissolved Oxygen

The dissolved oxygen available to bacterial cells within the media can be a limiting factor to each bacteria species. Genus *Streptomyces* has been known as aerobic bacteria which the growth rate is independent of the media dissolved oxygen concentration. The production of secondary metabolites can also be influenced through dissolved oxygen concentration. During antibiotic production by *S. clavuligerus*, increased oxygen saturation above the critical dissolved oxygen concentration resulted in additional product and biomass yield up to the maximum saturation limit of oxygen in the media (Rosa et al., 2005).

4. Substrate (Carbon and Nitrogen)

Genus *Streptomyces* is generally found as saprophytes in organic rich soils which can be utilized various carbon and nitrogen sources for growth and metabolites production (Basak and Majumdar, 1973; Holembiovska et al., 2010). Many polysaccharide-degrading enzymes are secreted to degrade the complex carbon sources such as rice brain, rice straw, corn cobs, soluble starch, shrimp shell, and fish hydrolyses. Similarly with nitrogen sources, *Streptomyces* can utilize both of amino acids and inorganic nitrogen sources. The previous reports indicated that the carbon and nitrogen sources have been affected to growth and antibiotic compounds production (Abou-Zeid and el-Gammal, 1971; Stoichev et al., 1982). Majumdar reported that the yield of neomycin or

kanamycin production was depended on the utilization of carbon and nitrogen compounds of *Streptomyces* (Majumdar and Majumdar, 1967).

2.1.4 The metabolism of *Streptomyces*

The metabolism is commonly exhibited and used for growth as metabolites which can be classified into primary and secondary metabolites. Metabolites are compounds produced by a cell through the process of cellular metabolism**.**

1. Primary Metabolites

The organism has been produced the primary metabolites for using in growth, development, and reproduction process. Amino acids, acetyl-coenzyme A, mevalonic acid, sugars, and nucleotides are normally found as primary metabolites of energy in growth phase.

2. Secondary Metabolites

The secondary metabolite is modified from primary metabolites as organic compounds which produced at nearly or the end of stationary phase. Many antibiotics, pigments, aroma and flavor compounds have been produced by *Streptomyces* species which important use in various application. Olano (2008) reported that secondary metabolites had been produced at growth-phase as aerial mycelium formation on solid medium and at stationary phase in liquid-grown cultures. The major source of novel secondary metabolite was *Streptomyces* and related actinomycetes. According to many metaboilte compounds are produced from actinomycetes such as doxorubicin, jadomycin, tetracycline, actinorhodin, mithramycin, pradimicin and tetracenomycin in Figure 2.5. Bordoloi (2002) reported that fungal phytopathogens have been inhibited by antifungal compound from *Streptomyces* sp. MML1042. According to Woo (2002) *Streptomyces* sp. strain AP77 can be found to against *Pythium porphyrae* by using antifungal protein.

2.1.5 The Application of *Streptomyces*

Several *Streptomyces* are widely known as a major source of antibiotic compounds which applied in pharmaceutical. In agricultural application, they can be used to control in plant disease or activate plant growth by various bioactive compounds. According to environmental application, many *Streptomyces* can also be used to remove the organic

and inorganic pollutants in soil and water. The *Streptomyces* has been sustainable for applying to bioremediation, biodegradation, and bio-sorption processes of wastewater treatment, agricultural and industrial wastes. Nowadays, *Streptomyces* is found to be an important microbial in the area of genetics, protein engineering, and bioinformatics.

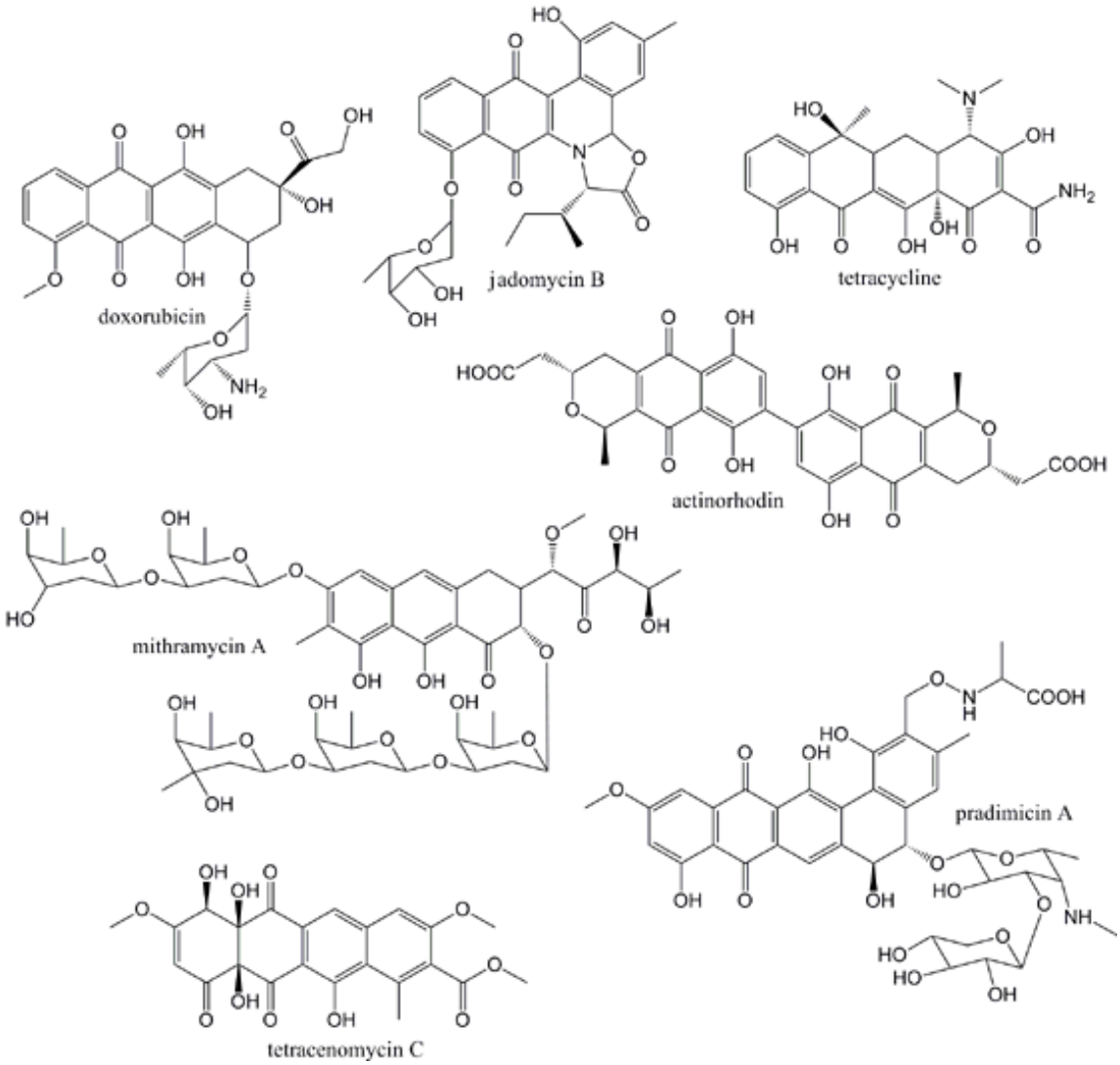

Figure 2.5 The polyketide compounds
Source: (Kallio, 2008)

2.2 Chitin

Chitin is natural poly-amino-saccharides as most as the major component of the shells of crustaceans, the exoskeletons of insects and the cell walls of fungi. Chitin is composed of (1→ 4) linked 2-acetamido-2-deoxy-β-d-glucose units (or *N*-acetyl-d-glucosamine), forming a long chain linear polymer. The chemical structure of chitin is similar to cellulose, having one hydroxyl group on each monomer substituted with an

acetyl amine group (Figure 2.6). The chitin structure can be modified by removing the acetyl groups, which are bond to amine radicals in the C_2 position on the glucan ring, by means of a chemical hydrolysis in concentrated alkaline solution at elevated temperature to produce a de-acetylated form. The difference between chitin and chitosan is the degree of acetylation of D-glucosamine residues.

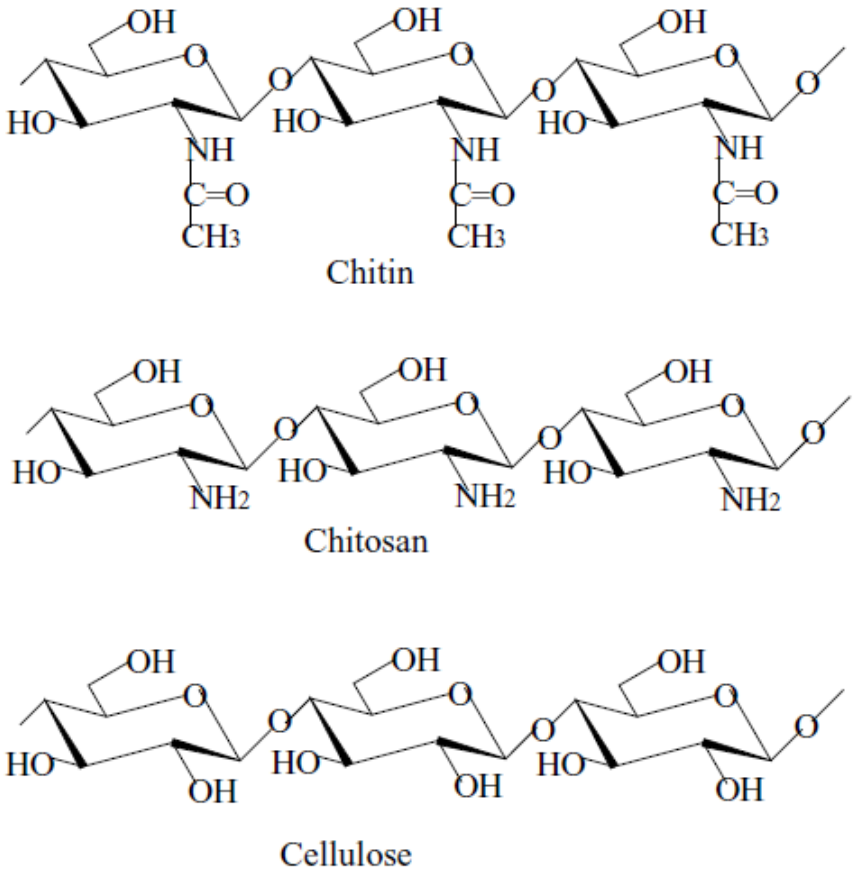

Figure 2.6 Structure of chitin, chitosan and cellulose.
Source: (Krajewska, 2004)

2.2.1 Sources of Chitin

Chitin is widely known that it can be found in the nature, arthropod shells have been the most easily accessible sources of chitin. It is clear that, many shells of marine crustaceans were available as the organic waste from the processing of shrimp products and used for commercial production of chitin. In addition, the other sources of chitin are included as krill, clams, oysters, insects, and fungi (Table 2.2).

Table 2.2 Contents of Chitin

Source	Chitin (%)
Crab cuticle	15-30
Shrimp cuticle	30-40
Krill cuticle	20-30
Squid pen	20-40
Clam/oyster shell	3-6
Insect cuticle	5-25
Fungi cell wall	10-25

Source: (Kurita, 2006)

2.2.2 Biosynthesis Properties of Chitins

The natural biosynthesis of chitin duplicated in vitro has nothing in common with the artificial synthesis of oligomeric chitin by chitinase-catalyzed polymerization of chitobiose oxazoline, or by other techniques involving organic media. The biosynthesis of chitin has been processed in three steps consisted of the first step, it was formed the polymer in the cytoplasmic site. Then, in the second step it was formed the nascent polymer a crossing the membrane and released into the extracellular space. In finally, the third step it was completed to form crystalline microfibrils as single polymers (Merzendorfer, 2006). The arthropoda was become one of the most groups of organisms members like insects, crustaceans and chelicerae. The material science is a hierarchically structured fiber-based composite material based on chitin (Figure 2.7) as the polysaccharide macromolecule of chitin.

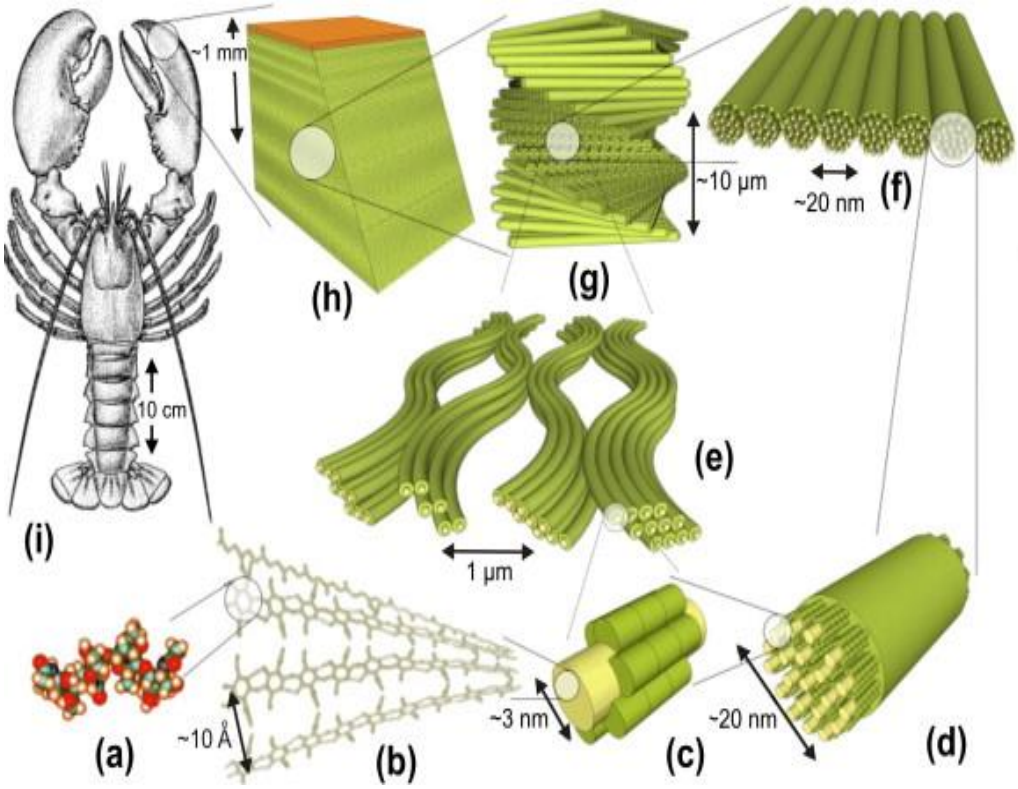

Figure 2.7 The cuticle of the arthropod Homarus americanus displays a variety of different architectures at multiple length scales. (a) N-acetylglucosamine molecules make up (b) chains of alpha-chitin. (c) These chains in turn form nanofibrils wrapped in protein, which bundle together to form (d) larger protein-chitin fibres and (e) mineralized honeycomb-like lamellae. These honeycomb lamellae and (f) lamellae containing more parallel-oriented fibres stack together to form (g) a "twisted plywood" structure making up (h) the multi-layered structure of the bulk cuticle of exoskeleton of the lobster (i).

Source: (Nikolov et al., 2011)

2.2.3 Structural Properties of Chitins

The presence of the amino groups, N-acetylglucosamine are usually linked as the linear polymer and expected to have a high potential as a functional material and structural material. Three polymorphic forms of chitin (α-, β-, and γ-chitins) have been differentiated due to their crystal structure as shown in Figure 2.8. The α-chitin is arranged in an anti-parallel configuration while β-chitin is organized in a parallel configuration. γ-Chitin is a mixture of α- and β-chitin. α-chitin is the most abundant form found in nature. The anti-parallel configuration gives α-chitin a highly ordered

crystalline structure with strong hydrogen bonding between chitin chains. The strong hydrogen bonding leads to the rigid and insoluble properties of α-chitin.

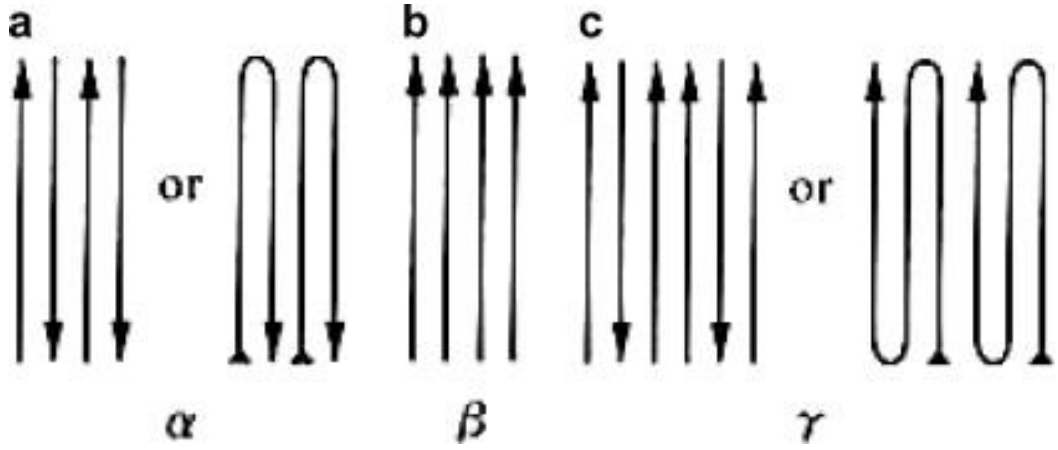

Figure 2.8 The three polymorphic configurations of chitin.
(a) α-chitin, (b) β-chitin, (c) γ-chitin

2.2.4 Applications of Chitin and Its Derivatives

1. Biomedical Applications

Chitin and chitosan have been used as therapeutic agents which less side effects and exhibit high antitumour activity. The chitin can hybrid or conjugate with D-glucose analogue of muramyl dipeptide (GADP) which can against cultured macrophages. In addition the sulphated carboxymethyl chitin was also used as an inert heparinoid for inhibition of blood clotting. According to Song (1992) reported that carboxymethyl chitin has been used as drug carriers and released the drug into the target cell. Nishiya and Chang (1994) reported that the toxic reduction of in blood and lipid mixing inhibition can also be effected from carboxymethyl chitin. The previous reports showed that chitin is possibility used as a wound dressing materials and it derivatives are also used as blood substitutes (Singh et al., 2008; Singh and Singh, 2012)

2. Biotechnology Applications

- Application in bio-separations, carboxymethyl chitin has been used to adsorb the specific compound and separate by calcium blocking.
- Applications in fermentations to enhance production, chitin is usually used for microbial biomass production as a substrate by conversing chitin to N-acetyl – glucosamine. In addition it can effect to ethanol production by reduced the fermentation

time. The chitnase activity was enhanced by addition of chitin in cultivation of chitin deacetylase producing organisms (Young et al., 1985a; b).

- Applications in immobilization of biomolecules, chitin can covalently immobilize to various biomolecules such as glucoamylase, lipase, endo-1, 4-b-xylanase and protease which showed high enzyme activity (Gomes et al., 2004). Chitin as sliced shrimp chitin is used for enzyme supporting then the specificity of the enzyme also increased (Chen and Chang, 1994)

3. Environment and Pollution Control Applications

- Applications in removal of heavy metals, chitin has been studied to adsorb cadmium and lead on its surface from sea water (Zhou et al., 2004). The chitin which presented in fungal cell wall was responded for bio adsorbing of metal ions such as silver, zinc, lead, copper, nickel, cobalt, cadmium, iron and chromium. In addition the compound product of chitin was been used for cadmium, lead and copper ion binding.
- Applications in food industry, chitin had a good emulsifying properties, superior thickening, and gelling agent for stabilizing foods. It is also used as a dietary fiber in baked foods. In addition, chitin can use for solving the problems of flavor, color, and shelf-life, posed by other sources of fiber.
- Applications in Agriculture, chitin has been treated the seed plant to have growth accelerating and growth enhancing effects. Chitins or materials contended chitin was added to the potting mixtures/soil which can help to reduce the infestations and suppression of fungal pathogens.
- Other applications, in paper manufacture and cosmetic production, chitin been used as an additive gradient. Chitin is also added into waste water as a flocculent agent in primary treatment. Solid chitin particles have been used as stabilizers for oil-water emulsions. Commercially chitin is available in a variety of grades and qualities.

2.2.5 Chitin-Degrading Enzymes

Chitin can be cleaved by the enzyme in the glycosyl hydrolases family such as the glycosyl hydrolases family 18, 19 and 20 (Henrissat, 1999; Fukamizo, 2000). Chitinase are various found in many species of microorganism, plant and animal as endo-chitosanase, exochitobiohydrolase (exo-chitosanase), and chitobiase (endo-glucosaminidase) or exoglucosaminidase. The chitinase was hydrolyzed the chitin polymer to multimers and monomers of N-acetyl-glucosamine (GlcNAc). The chitinase

enzymes are divided into the sub-families based on the amino acid sequences of their catalytic domain. Chitinase as member of glycoside hydrolases family 18 can produce in various bacterial, yeast, fungi, and plant. Family 19 chitinase was also consisted of plant chitinases classes I, II, and IV and some *Streptomyces* chitinases (Gherbawy et al., 2012). Family 20 includes the β-N-acetyl-hexosaminidases from bacteria, *Streptomyces* and humans. Several authors have reported bacterial chitinases are consisted of multiple domains, such as chitin-binding domains and fibronectin type III-like domains and catalytic domain (Fujii and Miyashita, 1993; K and Fujii, 1993; Little et al., 1994; Watanabe et al., 1994; Ikegami et al., 2000; Miyashita et al., 2000). The chitinase can be produced in various substrates and condition such as liquid batch fermentation, continuous fermentation, and fed-batch fermentation. In addition, the chitinase production is usually affected by medium components and other physical factors such as aeration, pH, and temperature. Dahiya reported that the maximum chitinase yield (616 U/g) had been observed in solid substrate fermentation using flake chitin as the solid substrate after cultured at 30ºC and 75% moisture level for 168 hours. According to the yield of chitinase production in wheat bran to flake chitin ratio, 1; moisture, 80%; and inoculum, 2.6 ml after 168 h was also observed at 1,475 U/g (Dahiya et al., 2005).

2.3 Damping off Disease in Greenhouse

The damping off disease has been caused the serious problems in greenhouse and plant nurseries. Causative pathogenic fungi of seedling damping off disease in plants were reported to be *Pythium* spp., *Phytophthora* sp. (Joo, 2005), *Rhizoctonia solani* (Asaka and Shoda, 1996), *Sclerotium rolfsii* (Errakhi et al., 2009), and *Fusarium oxysporum* (Getha and Vikineswary, 2002).

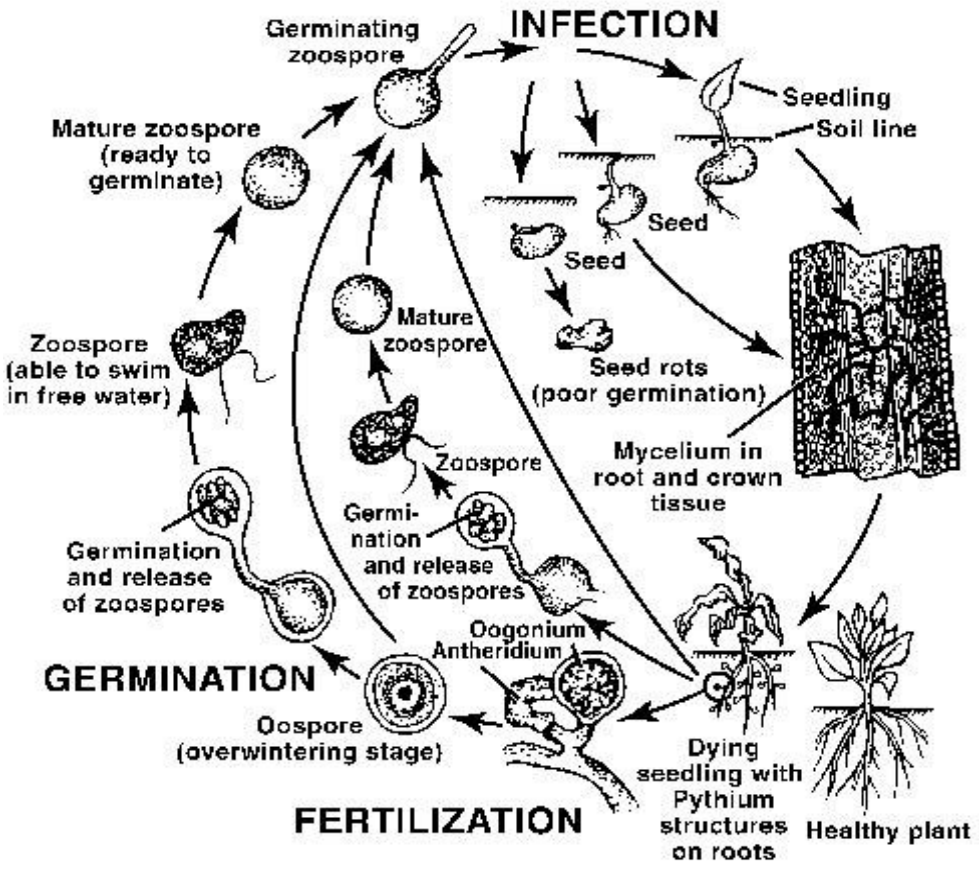

Figure 2.9 Disease Cycle of Damping-off and Seed Decay
http://www.omafra.gov.on.ca/english/crops/pub370/pub370ch8.htm

2.3.1 Symptoms of Common Diseases

The damping off disease was caused by a wide species of fungal pathogens. The infected plant are appeared different symptoms depended on several factors such as hosts, density of pathogens, water contented in soil, temperature, pH, and light intensity. For infection of seedling, the pathogen is usually infected to seed or seedlings in two stage of emerge as pre-emergence damping-off and post-emergence damping-off (Figure2.9).

1. Pre-Emergence Damping-off

In pre-emergence damping-off, the pathogen is infected to seeds and the emerging radicle while developing root from the seed coat below ground. Therefore seedlings have never emerged from soil as low quality of seeds. The symptom can be observed as decayed seed, rotten or soften root, turned dark brown, and died. In addition, the poor

seedling emergence in pre-emergence damping-off can be continued causing to post-emergence damping-off.

2. Post-Emergence Damping-off

In post-emergence damping-off, the pathogen is infected young seedling (the stems, tissues and roots) at both below and aboveground shoots. The infected stem or tissue was appeared as dark brown lesion or dark water-soaked area. Then, the wilting and collapse seedling was observed until the stem breaks off or rots away as top rot or top damping-off and root rot. The causative pathogens are presented and survived in organic matter as spores or other structures for long periods of time. The major group of damping-off pathogens is called oomycetes, including the genus of *Pythium* and *Phytophthora.*

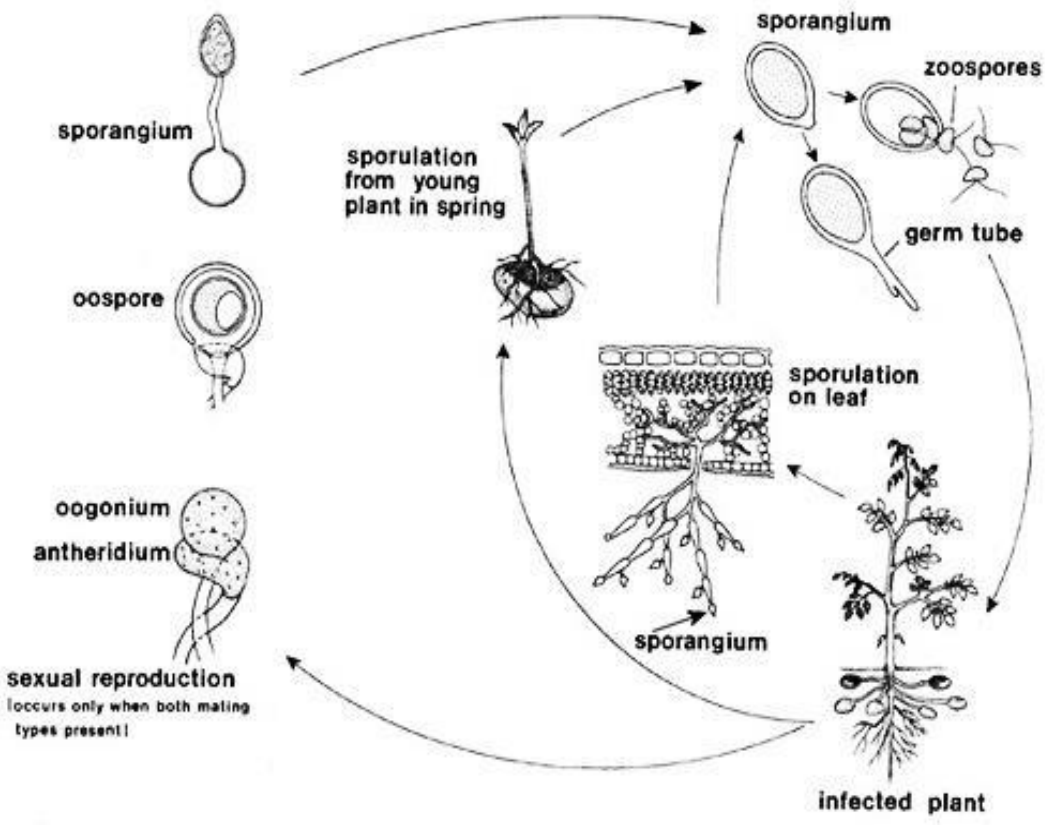

Figure 2.10 This is a simplified disease cycle for late blight of potato and tomato. http://bioweb.uwlax.edu/bio203/s2007/benrud_jaco/index_files/image364.jpg

2.3.2 Seedling Damping Off Pathogens

Pythium and *Phytophthora* species are major soil-borne pathogens that cause seed rot and pre-emergence damping-off and post-emergence damping-off of a broad range of host plants. The damaged seedling from both of pathogens can quickly spread from the early stage of seed germination.

1. Genus *Phytophthora*

Phytophthora spp. was usually associated with root rots of in seedling plants and also involved in damping-off. *Phytophthora* is a water mould which produces the asexual spores and mycelium spread quickly in water and soil. These species enter the root tips and cause a water-soaked brown to black rot similar to *Pythium. Phytophthora* species are regarded as a fungus-like organism and currently classified as an oomycetes belong to order Peronosporales. This disease is especially infected in wet and cold conditions that can significantly reduce both establishment and yield. *P. infestans* has been occurred in cool weather and high moisture content. The host range of *P. infestans* is mainly limited to solanaceous crops, including tomato and potato in Figure 2.10.

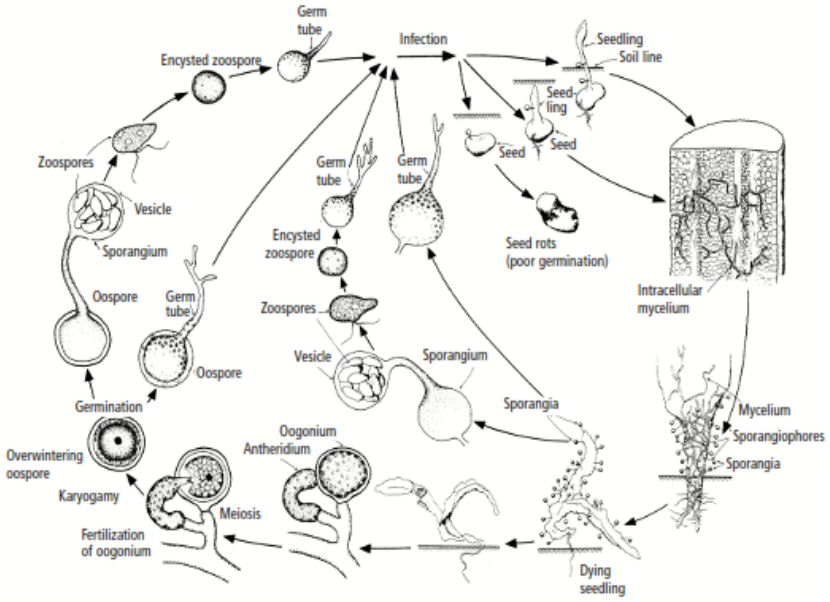

Figure 2.11 *Pythium* Root Rot Life Cycles.

Source: (Agrios, 1997)

2. Genus *Pythium*

Pythium spp. can be caused the damping-off disease similar with genus *Phytophthora* and *Rhizoctonia* in poorly-drained soils, and cool weather. The *Pythium* species can

survive in soil and greenhouse equipment for several years. The symptom of the infected plant has been showed the rot begins as soft to slimy and water-soaked brown root tips and stem (Figure 2.11). In addition, the other symptoms are appeared the wilting and yellowing seedling at above-ground. *Pythium* spores are germinated into root then continued branching mycelium and quickly produced two types of spores. Asexual zoospores and sexual oospores were produced and germinated to start a new infection. Several authors have reported that many *Pythium* species, including *P. aphanidermatum, P. irregular,* and *P. ultimum* are known to cause damping off and crown and root rot in cucumbers, soybeans, chickpeas, peppers, and tomatoes in greenhouses (Georgakopoulos et al., 2002; Sharma et al., 2007; Chen and Nelson, 2008; El-Tarabily et al., 2009; Kamala and Indira, 2011).

2.4. Biological Control of Damping off Disease in Greenhouse

The principal for biological control is inhibition of growth, infection or reproduction of one organism using another organism. The biological control of plant disease has been known as more environmental friendly way than chemical to carry plant disease (Kiss, 2003; Saenz-de-Cabezon et al., 2010). Many microorganisms isolated from soil are reported and used as potential biological control agents (BCAs) such as *Trichoderma* spp, *Gliocladium* spp, *Pseudomonas* spp, and *Streptomyces* spp. The biological control activity can be occurred in various mechanisms including competition, antibiosis, mycoparasitism, and induced resistance.

2.4.1. The Interactions Types of Biological Control

The biological control interactions were associated to the mechanisms of biological control. The types of interaction have been reported as mutualism, protocooperation, commensalism, neutralism, competition, amensalism, parasitism, and predation. From the different interactions for controlling and inhibiting were reported as directed or indirect interaction through the pathogen and they can also be controlled via both of interactions (Table 2.3).

1. The Predation Mechanism

The predation mechanism is controlled by hunting and killing of one organism by another organism such as some antagonistic fungi killed pathogen (nematodes and

arthropods). Biological control can result in varying degrees from all of these types of interactions, depending on the environmental context within which they occur. Significant biological control, as defined above, most generally arises from manipulating mutualisms between microbes and their plant hosts or from manipulating antagonisms between microbes and pathogens.

2. The Parasite Mechanism

The parasite mechanism is related with two organisms coexist over a prolonged period of time. The one organism is usually found smaller physical as the parasite than the other organism as the host. The activities of various hyperparasites have been reported to infect the plant pathogens as biocontrol activity. According to the interaction between *Trichoderma* spp. and *Gliocladium* spp. to pathogenic *Pythium* spp. can consider to mycoparasitism (Sreenivasaprasad and Manibhushanrao, 1990; Paulitz and Belanger, 2001; Inglis and Kawchuk, 2002). The parasite can produce a range of enzymes that are directed against cell walls of fungi to enable the parasite to enter the hyphae of the pathogen such as chitinase, glucanase, cellulase and protenase. Therefore, the activity of mycoparasitism might be important in reducing the secondary spread of the pathogen in greenhouses or fields.

3. The Competition Mechanism

The competition mechanism is resulted by limiting the nutrient in that environment to decreased growth, activity and/or fecundity of the pathogens. The important for limiting disease incidence and severity is shown as the competition of nutrient between pathogens and non-pathogens around the host plant. The nonpathogenic plant associated microbes are rapidly colonized at the rhizosphere to protect the plant. Then the available substrates limited that are not enough for pathogens to grow. In addition essential micronutrients which using as a co-factor in various chemical path way or reaction can limited pathogens growth. Iron limited as increasing efficiency of iron uptake by microorganisms in the rhizosphere can also control the pathogens growth. According to competition of carbon by non-pathogenic fungi were involved as a one mode of competition mechanism.

4. The Antibiosis Mechanism

The antibiosis mechanism proved as the secondary metabolites from one microorganism is toxic to other microorganisms. Many microorganisms such as *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp., and *Trichoderma* spp. can be produced various antimicrobial to suppression the several plant pathogens (Joo, 2005; Bae et al., 2011; Barahona et al., 2011). They include not only antibiotics but also bacteriocines, enzymes and volatile compounds with an antifungal activity (Zhao et al., 2007). The production of secondary metabolites is to a high degree substrate dependent. Thus, it is difficult to predict their actual importance in biocontrol within *in vivo* systems based on experience from *in vitro* experiments. The metabolites in soil and rhizospheres have been developed. Sometimes, it is an obligatory lifelong interaction involving close physical and biochemical contact, such as those between plants and mycorrhizal fungi. In addition, many BCAs have been reported to induce systemic resistance in plants.

2.4.2 Biological Control Product

The organism as the biological control agent (BCA) has been broadly applied to the use of the natural products extracted or fermented from various sources (Saenz-de-Cabezon et al., 2010). The formulations methods are simple mixed together with natural ingredients which specified activities or completed mixtures. The activities of living organisms and non-living inputs should more properly be referred to use as biopesticides or biofertilizers, (Perez-Garcia et al., 2011). The biological control can be resulted in different types of interactions. The development of efficacious fungal BCAs include: speed of action; greater ecological fitness; production; virulence; formulation; application; improved targeting; packaging and storage; bioactive compounds; safety. Formulation can be affected the performance, shelf life, and safety of biocontrol. In biological system, many parameters are greatly affected such as water, food, and environment. In addition, the stage of microbial cell was important such as bacterial endospores, yeasts, and the resting-spore stages of many fungi. However, in stage of spore or conidia was sometime slower to germinate and colonize in plant or soil. Many carrier substrates can be used in the formulation of BCAs such as kaolin, vermiculite, talcum powder, alginate and starch in terms of liquid, semi-solid and powder. Delivery systems that are well deliver the biocontrol agent depends on the biocontrol agent, the pathosystem, and the cropping system. The basics of method to apply an agent as seed coat or spray, dip and drench have been helped to control the disease in the systems.

Table 2.3 Types of interspecies antagonisms leading to biological control of plant pathogens.

Type	Mechanism	Examples
Direct antagonism	Hyperparasitism Predation	Lytic/some nonlytic mycoviruses *Ampelomyces quisqualis* *Lysobacter enzymogenes* *Pasteuria penetrans* *Trichoderma virens*
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol Phenazines Cyclic lipopeptides
	Lytic enzymes	Chitinases Glucanases Proteases
	Unregulated waste products	Ammonia Carbon dioxide Hydrogen cyanide
	Physical/chemical interference	Blockage of soil pores Germination signals consumption Molecular cross-talk confused
	Competition	Exudates/leachates consumption Siderophore scavenging Physical niche occupation
Indirect antagonism	Induction of host resistance	Contact with fungal cell walls Detection of pathogen-associated, molecular patterns Phytohormone-mediated induction