Chapter 2

REVIEW OF LITERATURE

Hevea brasiliensis

Hevea brasiliensis, a forest tree which is indigenous to the tropical rain forest of Central and South America and the only major commercial source of natural rubber, is one of the most recently domesticated crop species in the world. The modern age of natural rubber actually started during the 1870s when the British successfully transported *Hevea* seeds from Brazil for planting in the then British India (Markham, 1876; Petch, 1914). Proudlock (1908) found that the para rubber trees on experimental tapping yielded latex of excellent quality and recommended its cultivation on an extensive scale on the coastal belts of the country lying between the sea and the foot hills of the Western Ghats. This plant is now widely cultivated in Southeast Asia (Backhaus, 1985).

Among the genus *Hevea, Hevea brasiliensis* is the only species grown commercially as the source of natural rubber (George and Panikkar, 2000 and Cornish *et al*., 1993). It was introduced into India in 1873 by the British with the aim of establishing an assured source of this important industrial raw material and is being commercially cultivated since 1902 (George and Jacob, 2000). Owing to the pace of development in the industrial sector, the demand for rubber has been dynamic (Lalithakumari and Jacob, 2000) and ever increasing. Rubber plantations have a green image and are inherently environment friendly (Jones, 1994; Wan and Jones, 1996). Rubber plantations are generally self-sustainable ecosystems and can maintain a fair degree of biodiversity, if properly managed (Sethuraj and Jacob, 1997).

The production of natural rubber is now concentrated in only a few countries. Three major Asian producers - Malaysia, Indonesia, and Thailand - account for 80 percent of the world's total production. Two other Asian producers - Sri Lanka and India - and two African producers - Liberia and Nigeria - account together for another 12 percent of the world total (Grilli and Enzo, 1980).

The economic significance of Para rubber (*Hevea brasiliensis* Muell. Arg.), had long been recognized (Purseglove, 1987) because of its ability to produce latex. Although it can thrive on a wide range of environment, it however does well where the temperature is high and in fertile soil with adequate moisture. Beside these factors, the productivity of the crop is also influenced by the type of clone used (Opeke, 1987). Wood (1986) reported that among the factors affecting rubber productivity, genotype has been the most limiting factor. Similarly, Wycherley (1963) stated that soil constitutes the major aspects of the environment that greatly affect the growth and productivity of rubber trees. Opeke (1987) noted that rubber does well on deep, porous, red-clay top soil with sandy sub-soil.

Extension of *Hevea* cultivation to non traditional areas

The usage of rubber has become increasingly important in daily life. This scenario has triggered rubber planting to be expanded to marginal areas, such as dry areas. FELDA (1989) observed that poor plant growth might be due to inappropriate choice of planting location. Tripura state in Northeast India (22-24° N, 91-92° E) is a non-traditional region for rubber cultivation with sub-optimal conditions (Rao *et al*., 1993; Priyadarshan *et al*., 1998a; Priyadarshan, 2003), where *H. brasiliensis* clones differ in yield when compared to traditional rubber growing areas because of specific adaptation (Priyadarshan *et al*., 1998b).

Abiotic stresses in plants

Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way (Vinebrooke *et al*., 2004).

Abiotic stress factors are naturally occurring, often intangible factors such as intense sunlight or wind that may cause harm to the plants and animals and are essentially unavoidable. Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide (Gao, 2007). Research has also shown that abiotic stressors are at their most harmful when they occur together, in combinations of abiotic stress factors (Mittler and Ron, 2006).

Abiotic stress comes in many forms. The most common stress factors are the easiest for people to identify, but there are many other, less recognizable abiotic stress factors which affect environments constantly. The most basic stress factors include: high winds, extreme temperatures, drought, flood, and other natural disasters, such as tornadoes and wildfires. The lesser-known stressors generally occur on a smaller scale and are less noticeable, but they include: poor edaphic conditions like rock content and pH, high radiation, compaction, contamination and other highly specific conditions like rapid rehydration during seed germination (Palta *et al*., 2006).

Abiotic stress, as a natural part of every ecosystem, will affect organisms in a variety of ways. Although these effects may be either beneficial or detrimental, the location of the area is crucial in determining the extent of the impact that abiotic stress will have. The higher the latitude of the area affected, the greater the impact of abiotic stress on that area (Wolfe, 2007).

Plants are extremely sensitive to climate change of a few degrees, and do not generally adapt quickly (Lane and Jarvis, 2007). Plants also adapt very differently from one another, even from a plant living in the same area. When a group of different plant species was prompted by a variety of different stress signals, such as drought or cold, each plant responded uniquely. Hardly any of the responses were similar, even though the plants had accustomed to the same home environment (Mittler and Ron, 2006).

Environmental stresses, such as drought, salinity, cold and heat cause adverse effects on growth and productivity of crops. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. Among the abiotic factors that have shaped and continue shaping plant evolution, water availability is the most important. Water stress in its broadest sense encompasses both drought and salt stress. Drought and salinity are becoming particularly widespread in many regions, and may cause serious salinization of more than 50% of all arable lands by the year 2050 (Bray *et al*., 2000).

Drought and salt stress, together with low temperature, are the major problems for agriculture because these adverse environmental factors prevent plants from realizing their full genetic potential. Abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang *et al*., 2001). Drought, salinity, extreme temperatures and oxidative stress are often interconnected, and may induce similar cellular damage. They are very complex stimuli that possess many different yet related attributes, each of which may provide the plant cell with quite different information. For example, low temperature may immediately result in mechanical constraints, changes in the activities of macromolecules, and reduced osmotic potential in the cellular level (Xiong *et al*., 2002).

Plants respond to abiotic stresses at molecular and cellular levels as well as physiological level. Physiological manifestations of environmental stress in plants often implicate cell membranes as the primary site of injury (Orvar *et al*., 2000). At low temperatures, a decrease in membrane fluidity leads to a decrease in the activity of membrane-bound enzymes and loss of semi permeable membrane properties (McMurchie and Raison, 1979; Levitt, 1980). This is due to the transition of membrane lipids from a fluid liquid-crystalline phase to a viscous gel crystalline phase (Cropp *et al*., 2000). Expression of a variety of genes has been demonstrated to be induced by these stresses. The products of these genes are thought to function not only in stress tolerance but also in the regulation of gene expression and signal transduction in stress response (Yamaguchi-Shinozaki *et al*., 2002; Shinozaki *et al*., 2003; Thomas *et al*., 2011).

Abiotic stress and *Hevea*

Rubber is a rain fed plantation crop (except in nurseries where it needs to be regularly irrigated). The best rubber growing regions in the world are free from soil moisture deficit, because these regions receive showers frequently. Soil moisture stress can significantly reduce the growth and yield of rubber. But different clones vary in their capacity to tolerate drought stress. The climate change and global warming are affecting both the total amount and distribution of rainfall. Too much rain is also not advisable to rubber. Excessive down pour leading to prolonged stagnation of water in the soil can be particularly damaging to young plants. Both extreme deficiency of soil moisture and excessive rainfall are detrimental for rubber cultivation. Three environmental stresses operate simultaneously in summer. They are soil moisture deficit, high temperature and high sunlight intensity. This combination can be particularly damaging to the young rubber plants both in nursery and in the field. For a tropical plant like natural rubber, the combination of low temperature and high light is very harmful. It is also possible that soil moisture stress puts an additional stress on the trees during summer (Vijayakumar *et al*.,2000).

Diseases of *Hevea brasiliensis*

Heavy rainfall intakes make the tree trunks wet, preventing tapping. When the wet trees are tapped there will be loss of latex because of spillage and wash out. Tapping panels will be prone to fungal diseases when tapped on rainy days. Prolonged rains/cloudy days also favour incidence of several fungal diseases of leaves and buds. Rubber trees are affected by several diseases like leaf diseases, stem diseases and root diseases causing severe damage in most rubber growing countries. The unfavourable climatic conditions adversely affect growth and yield of rubber plants and also act as predisposing factors for various diseases.

Of the many leaf diseases affecting rubber plants, abnormal leaf fall and powdery mildew are the most significant ones noticed in India. The others include shoot rot, *Corynespora* leaf disease, *Gloeosporium* leaf disease, bird’s eye spot, anthracnose and thread blight (Edathil *et al.,* 2000).

The stem, including the tapping panel region, of the rubber tree is susceptible to various diseases. Pink disease, black stripe (black rot), patch canker, dry rot, mouldy rot, bark necrosis, etc. are the major diseases which have attained varying importance in the natural rubber producing countries (Kothandaraman and Idicula, 2000).

Brown root disease, white root disease, red root rot, dry root rot, stinking root rot, *Poria* root rot, black root disease, purple root disease are the major root diseases causing damages in rubber (Rajalakshmy and Jayarathnam, 2000). It is also attacked by *Bacterium albilineans,* and parasitized by *Loranthus* spp. Nematodes isolated from *Hevea brasiliensis* include: *Helicotylenchus cavenessi,H. dihystera, H. erythrinae, Meloidogyne incognita acrita, M. javanica, Pratylenchus coffeae* and *P. brachyurus.* Insect pests include the following species: Scale insects (*Aspidiotus* *cyanophylli* and *Parasaissetia nigra*). White ants cause serious damage to trees at all ages. Snails can be serious pests to young trees. Various animals can damage the trunks (Reed, 1976).

Corynespora leaf fall disease

In India, *C. cassiicola* (causative organism of leaf spot of rubber) was first found in some iron deficient nursery plants in the year 1960 (Ramakrishnan & Pillai, 1961) and later in Malaysia (Newsam, 1963). It has since been occasionally seen in bud wood nurseries of certain clones (Rao, 1975; Ramli *et al*., 2000). Fig. RLI shows *Hevea* tree affected with *Corynespora* leaf fall disease.

Fig. RLI. *Hevea* tree affected with *Corynespora* leaf fall disease

The disease was reported from Nigeria (Awodern, 1969), Indonesia (Soepena, 1983; Sinulingga, *et al.,* 1996), Sri Lanka (Liyanage,*et al*., 1986), Thailand (Kajornchaiakul, 1987) and Malaysia **(**Tan, 1990**).** The disease has now been found in almost all rubber growing regions (Chee, 1988). Severe leaf fall due to infection of *Corynespora* was reported from Sri Lanka (Liyanage *et al*., 1986), Malaysia (Tan, 1990) and Indonesia (Sinulingga *et al*., 1996).

Severity of the disease mainly depends upon factors like climate, existence of virulent pathogens and susceptibility of host genotypes, etc. The conidia of the fungus, produced abundantly on infected leaves, are carried by wind and cause rapid spread of the disease. Conidia remain viable for about a month. Development of new pathogen or race could be suspected where a mild disease turns out to be an epidemic within a short time.

Fig. RL2. A healthy young leaf of *Hevea brasiliensis*

Fig. RL3. Leaves of young plant of *Hevea* infected with *Corynespora cassiicola*

Young leaves (upto 4 weeks) at light green stage are most susceptible to *Corynespora* infection (Fig. RL3). The first symptom appears as brown spots which enlarge into circular or irregular lesions of varying sizes. Several lesions may coalesce to form large blighted area. If the infection is near or on veins it is characterized by the browning or blackening of the veins giving a 'fish bone' or 'railway track' like appearance. The most common symptom observed in India is the presence of circular or irregular lesions measuring 1-10mm in diameter (Ramakrishnan and Pillai, 1961; Rajalakshmi and Kothandaraman, 1996). Even a single leaf spot can cause defoliation. Severe infection on the midrib causes leaf blight. When leaf petioles are infected, greyish black lesions are formed causing defoliation without any symptoms on the lamina. Repeated defoliation and refoliation lead to shoot die-back. The disease also affects mature fields where it causes severe defoliation of newly matured leaves produced during a dry period following an earlier wintering in December. The disease is present in Malaysia, India and West Africa (Rao, 1975). The disease is generally severe in areas with high rainfall without any prolong dry period (Malaysian Country Report, 2000).

Corynespora leaf disease appears to respond to rain and the eco-climatic conditions that influences simultaneous manifestation of refoliation which is a favourable environment for their development (Wahounou, 2000). Harinidi *et al.* (2000) reported that susceptible clones affected by *C. cassiicola* would suffer continuously in a long period and the crown becomes leaf less for the whole year. Foliar infection by these pathogens could cause dieback, stunted growth while on mature trees it could obviously reduce latex production (Awoderu, 1967; Rao, 1975; Sabu *et* al., 2000).

Corynespora cassiicola was a devastating pathogen attributed to heavy leaf fall in susceptible clones of *Hevea brasiliensis*, in the mid-eighties in Sri Lanka. It is known to have a wide host range. The first report of *C. cassiicola* as a leaf pathogen was in croton, *Codiaeum veriegatum,* an ornamental plant. This report emphasizes the possibility of *C. cassiicola* infecting new hosts which could eventually lead to the development of new and virulent strains that are pathogenic to existing tolerant rubber clones (Jayasuriya and Thennakoon, 2007).

Control measures of *Corynespora* leaf disease in *Hevea brasiliensis*

The disease can be controlled by physical, chemical and biological methods. Base budding and crown budding of affected RRIC 103 trees with resistant clones were recommended in Sri Lanka but did not gain popularity as there was difficulty in peeling the bark of disease affected trees for bud grafting (Liyanage *et al.,* 1989). Light overhead shading in the nursery reduces disease intensity. Vigorously growing seedlings are usually less affected and hence balanced nutrition reduces disease incidence. Root diseases (white, red and brown) are controlled by cutting away diseased tissue and applying prophylactic coatings. Panel diseases, classified as black stripe, rot, and panel necrosis, are minimized by spraying or coating specific fungicides. Stem disease, consisting of pink disease, stem canker and dieback is reduced by brushing specific fungicides. Leaf diseases such as abnormal leaf fall, *Gloeosporium* leaf disease, powdery mildew, and bird's eye spot are controlled by a variety of sprays, including copper oxychloride, sulfur dust applied by spray or dusting techniques (Rogers, 1981).

Several fungicides have been recommended for the control of *Corynespora* leaf disease. Spraying of benomyl (Hashim, 1994), mancozeb (Soepena *et al.,* 1996), captan or propineb, benomyl and mancozeb (Jayasinsinghe and Silva, 1996) are some of them. In laboratory tests and limited field trials, benomyl has been found to be most effective in controlling the disease, but only if spraying is extended for several months.

Bio control

Baker and Cook (1974) defined biological control as the reduction of inoculum density or disease producing activity of a pathogen or a parasite in its active or dormant stage by one or more microorganisms accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists. In recent years, interest in bio control of plant diseases has been revived.

Biological control is less disruptive to ecosystem than chemical pesticides. Bio control agents have the potential to supplement (or) replace chemical pesticides. They offer other advantages like improved plant growth which is not possible with the latter. Bio protectants in the rhizosphere can protect the plant from soil-borne plant pathogens throughout the crop growth periods. The need for an alternate disease control measure to supplement or replace chemical fungicides was felt in view of the high cost of the latter, environmental pollution and resistance development in pathogen. Biological control has emerged as an effective and economic method for management of diseases (Jeyarajan *et al.,* 1999).

Recent trends in the area of research on bio control include the increased use of bio rationale in screening processes to identify microorganisms with potential for bio control, increased testing under semi commercial and commercial production conditions, increased emphasis on combining bio control strains with each other and with other control methods and integrating bio control into an overall system (Fravel, 2005). For control of fungal contamination there are two possibilities, heat treatment or chemical treatment, but it is necessary to replace chemical pesticides or fungicides to avoid soil pollution and health problems. Alternatively, antifungal agents produced by microorganisms may be used as bio control agent. Biological control offers an important alternative to synthetic chemicals. The use of bacteria like *Pseudomonas sp.* and *Bacillus sp.*, have been investigated because of their properties to produce antifungal metabolites and protect plants from fungal infection.

Biological control with micro-organisms

The biological approaches that are currently being developed for the control of a variety of phytopathogenic agents include the use of bio control bacteria that can suppress or prevent the phytopathogen damage (O'Sullivan and O'Gara, 1992; Sivan and Chet, 1992; Sutton and Peng, 1993; Cook, 1993; Chet and Inbar, 1994; Dowling and O'Gara, 1994; Pankhurst and Lynch, 1995; McLaughlin *et al*.,1995).

Bacteria used for biological control will infect insects via their digestive tracts, so insects with sucking mouth parts like aphids and scale insects are difficult to control with bacterial biological control. *Bacillus thuringiensis* is the most widely applied species of bacteria used for biological control, with at least four sub-species used to control Lepidopteran (moth, butterfly), Coleopteran (beetle) and Dipteran (true flies) insect pests (Swan. 1964). Species in the genus *Trichoderma* are used to manage some soil borne plant pathogens. *Beauveria bassiana* is used to manage different types of pest such as whiteflies, thrips, aphids and weevils (Hall and Dunn, 1957).

The antagonistic materials based on microorganisms have following properties: high specificity against target plant pathogens; easy degradability; and low mass production cost. *Bacillus sp.* has the characteristics of being widely distributed in soils, high thermal tolerance, rapid growth in liquid culture and the capability to readily form resistant spores.

Rhizospheric bacteria are excellent agents to control soil-borne plant pathogens. Bacterial species like *Bacillus*, *Pseudomonas*, *Serratia* and *Arthrobacter* have been proved in controlling fungal diseases. Non-pathogenic soil *Bacillus* species offer several advantages over other organisms as they form endospores and hence can tolerate extreme pH, temperature and osmotic conditions. *Bacillus* species were found to colonize the root surface, increase the plant growth and cause lysis of fungal mycelia.

The increased reflection on environmental concern over pesticide use has been instrumental in a large upsurge of biological disease control. Biological disease control has further encouraged the exploitation of potential antagonistic micro flora in disease management. Among the various antagonists used for the management of plant diseases, plant growth promoting rhizobacterium (PGPR) play a vital role. Fluorescent pseudomonas has revolutionized the field of biological control of soil borne plant pathogens. Fluorescent pseudomonads have been implicated in the control of several wilt diseases caused by *Fusarium* spp (Chen *et al.*, 1995) and root rot of important crops like wheat, cucumber and tulip. Red rot in sugarcane caused by *Colletotrichum falcatum* was suppressed by certain strain of fluorescent pseudomonas (Viswanathan and Samiyappan, 1999).

Endophytic bacteria in plants

Endophytic bacteria have been defined by different researchers. Kado (1992) defined them as "bacteria that reside within plant tissues without doing substantive harm or gaining benefit other than securing residency". But this definition excludes those bacteria that form symbiotic relationship with their host. Quispel (1992), considers endophytic bacteria as "bacteria that establish an endo-symbiosis with the plant, whereby the plant receives an ecological benefit such as increased stress tolerance or plant growth promotion". This definition as well, excludes the bacteria that have neutral or deleterious effects on plant. From the functional point of view, Hallmann *et al.* (1997) considers bacterium as an endophyte "if it can be isolated from surface disinfected plant tissue or extracted from inside the plant, and if it does not visibly harm the plant". This definition includes internal colonists which are neutral or symbionts in behaviour.

Among beneficial microbes, bacteria form a major portion of total endophytes which play an important role in the sustainability of agro- ecosystems and are used for plant growth promotion and disease control (Manjula *et al.,* 2002). Moosa *et al.* (1998), Moosa and Ahamed (2002), isolated three endophytic bacteria viz *B. subtilis, B. amyloliquefaciens* and *B. megaterium* from the spear leaf tissues of West Coast Tall (WCT) seedlings of coconut, which had been introduced into the coconut seedlings. Bhowmik *et al.* (2002) isolated five *Pseudomonas* (fluorescent and non fluorescent) which are endophytes, from the root and stem of healthy cotton seedlings. They were found to be antagonistic to *Rhizoctonia solani* and *Sclerotium rolfsii* which cause damping off disease and were also found antagonistic to the bacterial blight pathogen.

The presence of non-pathogenic bacteria was first time proposed by Perrotti (1926) in plant tissues. Now, there are numerous reports of endophytic bacteria in seeds and ovules, roots, stems, leaves, fruits and tubers (Sturz *et al.,* 2000) of most plant species without causing visible damage (Jacobs *et al*., 1985). The genera *Acinetobacter, Agrobacterium, Alkaligenes, Bacillus, Clavibacter, Enterobacter, Erwinia, Klebsiella, Serratia, Pseudomonas* and *Phyllobacterium* have been reported as endophyte from several crop plants (McInroy and Kloepper, 1995; Sturz *et al.*, 1999), but *Bacillus* and *Pseudomonas* are predominant. It was observed that the number of endophytic genera decreased with age of the plant (McInroy and Kloepper, 1991). The diversity of bacterial genera was greater in roots than in stems (Fisher *et al.,* 1992; Sturz, 1995), which was also dependent on the plant genotype and soil type (van Vuurde and Elvira-Recuencom, 2000).

Beneficial effects of endophytic bacteria in agro-ecosystems

Endophytic bacteria are reported to improve early plant growth (Frammel *et al.,* 1991; Nejad and Johnson, 2000; Van Peer and Schippers, 1989) and decrease disease incidence caused by *Fusarium* sp. (Chen *et al*., 1995; Nejad and Johnson, 2000). They are also reported to modify mineral nutrition (Bavaresco *et al.,* 2000), fix atmospheric nitrogen (Baldani *et al.,* 1997; Boddey *et al*., 1991), increase drought resistance, and reduce transplanting shock in plants (Sturz *et al.,* 2000). They have also been used for the delivery of insecticidal endotoxins (Tomasino *et al.,* 1995).

Investigations on signal transduction pathways showed induced systemic resistance elicited by several strains of *Bacillus* species (Kloepper *et al.,* 2004). Workers at Montana State University (Bargabus *et al*., 2002), found two strains of *B. pumilis* (Strain203-6 and 203-7) and one *B. mycoides* (Strain Bac J) that reduced the severity of *Cercospora* leaf spot of sugar beat, caused by *Cercospora beticol.* Murphy *et al.* (2003), used two strain combinations of *Bacillus* species incorporated into potting mix, found significant reduction in disease severity. Van Loon *et al.* (1998) showed disease reduction and increased plant growth in many crops as a result of induced systemic resistance. Studies (Sturz *et al.,* 2000) indicate that endophytic bacteria colonizing the internal tissues of plants conferred systemic resistance to plant. They also promoted the growth of plants by means of solubilising insoluble phosphate in the soil by producing gibberellins (GA), indole acetic acid (IAA),*etc.*

Cardamom seeds coated with *B. subtilis* and *P. fluorescens* had resulted in increased seed germination and enhanced growth and vigour as expressed by increase in seedling height, root length and mean leaf area (Thomas and Vijayan, 2003). Nejad and Johnson (2000) found that seed treatment with endophytic bacteria improved plant growth and significantly reduced wilt diseases of tomato.

Endophytic bacteria as bio control agents in plants

Endophytes can protect host plants from insect herbivory (Clay, 1988; Clark *et al.,* 1989) and other fungal pathogens (Carroll, 1988). They can therefore be used as bio regulators to induce resistance against diseases, as biological control agents against certain pathogens (Bissegger and Sieber, 1994) and also in the biological control of undesirable weeds (Dorworth and Callan; 1996). Endophytes can be isolated from surface of disinfected plant tissue or extracted from inside the plant, if it does not visibly harm the plant, e.g. *Bacillus, Agrobacterium, Enterobacter, Pseudomonas* etc. They occur in seeds, ovules, stems, leaves, fruits and tubers. Endophytic bacteria inhibit growth of pathogens by production of antimicrobial compounds like antibiotics (Leyns *et al.,* 1990) and siderophores (Schroth and Hancock, 1981). They are capable of inducing systemic resistance and other defence responses in the host plant, including the production of phytoalexins (Van Peer and Schippers, 1989), accumulation of pathogenesis related proteins (Zdor and Anderson, 1992), deposition of structural barriers in the cell wall (Benhamou *et al.,* 1996) and thus offering protection to the host plant from the attack of a wide range of pathogens. Inoculation of tomato with a combination of *Bacillus pumilis* and chitosan (Benhamau *et al.,* 1998) and of cucumber seedlings with *Serratia plymuthica* (Benhamou *et al.,* 2000) protected host plants from attack of pathogenic fungi and provided disease control.

Antagonism by endophytes

Most of the endophytic bacteria are having antagonistic action against fungal pathogen. Several mechanisms are known to be involved in antagonistic interaction. Antibiosis is the major mechanism involved in antagonism. Additional mechanisms such as induced resistance, production of bio surfactants, interference with pathogen related enzymes and a number of still unknown mechanisms may complete the microbial arsenal (Elad, 1996). Antibiotics encompass a chemically heterogenous group of organic low molecular weight compounds produced by microorganisms. At low concentrations, antibiotics are deleterious to the growth or metabolic activities of other microorganisms (Fravel, 1998; Thomashow *et al.,* 1997; Tuzun and Kloepper, 1995). The bacterial strains significantly reduced pathogen colonization in the stalk tissues and disease progression in sugar cane (Viswanathan and Samiyappan, 1997; 1999). Chen *et al.* (1995) showed *in vitro* antagonisms of endophytic bacteria against *Fusarium oxysporum* f.sp.*cubense. B. subtilis* (bscbe4), *Pseudomonas chloraphis* (PA23), endophytic *Pseudomonas fluorescens* (ENPF1) inhibited the mycelial growth of stem blight pathogen *Corynespora cassicola* under *in vitro* (Mathiyazhakan *et al.,* 2004).

B. subtilis

Bacillus belongs to the Phylum firmicutes, the low G+C gram-positive bacteria. The genus *Bacillus* is the largest in the order Bacillales. They are gram-positive, rod shaped, endospore forming aerobic bacterium. They are non-pathogenic. They were successfully transformed with purified DNA before it was achieved with *E. coli* (Prescott *et al.,* 2003). Prior to the decision to produce spore, the bacteria might become motile through production of flagella and also take up DNA from the environment.

B. subtilis is a ubiquitous bacterium commonly recovered from water, soil, air and decomposing plant residue. *B. subtilis* produces a variety of natural substrates and contribute to nutrient cycling. However, under most unfavourable condition the organism is not biologically active but exists in the spore form (Alexander, 1977). Under adverse environmental conditions**,** *B. subtilis* produces endospores that are resistant to heat and dessication (Claus and Barkeley, 1986), allowing the organism to persist in the environment until condition becomes favourable.

B. subtilis appears to have a low degree of virulence to humans. It does not produce significant quantities of extracellular enzymes or possess other virulence factors that would make it harmful (Edberg, 1991). Cupins, are proteins produced by *B. subtilis* which share identity with the secreted oxalate degrading enzymes of fungi and plants. It produces a proteolytic enzyme subtilisin, which has been shown to be a potential cure for cancer.

Bulliformin, is an antifungal antibiotic produced by *B. subtilis***.** There are a number reports where *B. subtilis* has been isolated from human infections. Earlier literature contains references to infections caused by *B. subtilis* however, the term *B. subtilis* was synonymous for any aerobic spore forming bacilli and quite possibly many of these infections were associated with *B. cereus*.

B. subtilis, a ubiquitously distributed, low GC gram-positive soil organism, is metabolically versatile and displays sophisticated regulatory responses that allow adaptation to changing environmental conditions (Kunst *et al*., 1997). *B. subtilis* is a non-pathogenic Gram-positive bacterium and is generally regarded as safe organism. *B. subtilis* has long been exploited for industrial and biotechnological application (Kiers *et al*., 2000, Schallmey *et al*., 2004).

B. subtilis can divide symmetrically to make two daughter cells (binary fission), or asymmetrically, producing a single endospore that is resistant to environmental factors such as heat, acid and salt which can persist in the environment for long periods of time. The endospore is formed at times of nutritional stress, allowing the organism to persist in the environment until conditions become favorable. Prior to the process to produce the spore, the bacterium might become motile through the production of flagella.

B. subtilis produces a variety of enzymes, which allow it to degrade many natural substrates and thus cycle nutrients in the soil. This increases nutrient availability to plants, thereby stimulating plant growth. Moreover, *B. subtilis* secretes antifungal antibiotics, which can control fungal diseases in plants and crops. *B. subtilis* is capable of increasing the overall performance of crops and other plants (Devine, 2000).

Application of *B. subtilis* in Biotechnology

B. subtilis is widely used in the field of biotechnology due to its ease at being manipulated and its generally low risk. With the completion of sequencing of the *B. subtilis* genome, post-genomic studies were stimulated. Many coding gene were gradually identified and recognition of regulation machinery and element was enhanced in *B. subtilis* (Kunst *et al*., 1997).

Undoubtedly, the vast information available on control elements of expression such as the regulation element/ promoter, played an important role in genetic engineering of *B. subtilis* and accelerated the biotechnological applications of *B. subtilis* (Yang *et al*., 2006). In genetic engineering of *B. subtilis*, plasmid backbone and promoter are two basic elements. Plasmid instability was once a barrier in genetic manipulation (Ehrlich *et al*., 1991), but recently many a convenient vector systems of *B. subtilis* have been developed for genetic manipulation (Yang *et* *al*., 2006, Nguyen *et al*., 2005, Nguyen and Schumann, 2006). This further makes the *B. subtilis* a potential organism in genetic engineering and industrial application.

B. subtilis produces a variety of enzymes useful to industry. The amylases produced by *B. subtilis* are used in the textile and paper industry. The proteases produced by them are used as additives in laundry detergents and products used for processing leather. Furthermore, *B. subtilis* has been used to convert explosives into harmless compounds and to safely discard radionuclide wastes. They can convert nuclear waste and explosives into harmless compounds of nitrogen, CO_2 and water. Proton binding property of its surface plays a role in safe radionuclide waste disposal. *B. subtilis* is capable of infecting and causing mortality of the 2nd instar larvae of mosquito, *Anophelis culicifacies* which is the primary insect vector of Malaria in Central India (Bio control agent**).** *B. subtilis* are being used successfully with poultry as probiotics, which decreases the need for antibiotics in poultry production and pathogen levels on farms (Lonenshein *et al.*, 1993).

B. subtilis as endophyte and bio control agents

An endophytic bacterial strain, Jaas ed1, was isolated from the interior of egg plant's (*Solanum melongena* L.) stem in Jiangsu province, China. According to the morphological and physiological characteristics and phylogenetic analysis of the 16s rDNA sequence, it was identified as *B. subtilis*. Sixty endophytic rhizosphere strains were isolated from coconut, other crops and virgin soils for the management of basal stem rot disease. The strains which showed high growth promotion were subjected to Ganoderma mycelium inhibition study, *in vitro*. The strains EPC5 and EPC8 showed high growth promotion and strong inhibition in Ganoderma pathogen compared to other strains.

Seed bacterization with *B. subtilis* has been found to be effective against soil-borne plant pathogens. Sridar *et al.* (1994) reported the use of *B. subtilis* for the management of sesame root rot caused by *Macrophomina phaseolina*. *B. subtilis* seed treatment reduced the root rot incidence in urd bean from 65% to 15% (Sridar *et al.,* 1991). *B. subtilis* inhibited the growth of *R. solani* by bulb formation on tip, bursting and lysis of hyphae and degradation of sclerotia. Wang *et al.* (1990), reported that *B. subtilis* strain H31 inhibited the growth of *R. solani* due to the production of active substance. *B. subtilis* produced 6 mm inhibition zone against *M. phaseolina*.

The overuse of chemical pesticides had caused soil pollution and harmful effects on human beings. Accordingly, biological control of soil borne diseases has been attracting attention. Several strains related to *B. subtilis* produce insect toxins, peptide antibiotics and antifungal, some of which have been used in bio control and agricultural crop protection. Both the antagonist and its extract were effective in controlling artificially wound- inoculated fruit pathogens, but the use of the extract had better effect than that of the antagonist.

Competence in *B. subtilis*

Competence in *B. subtilis* is developmentally regulated and is dependent on specific nutritional signals. Thus, competence develops and reaches its maximum in later stages of growth. In addition, competence fails to develop in complex media such as LB broth. Glucose has also been found to be required for maximal development of competence (Albano *et al.*, 1987).

Competence in *B. subtilis*, assayed by the ability of cells to be transformed with bacterial DNA or transfected by phage DNA, has been shown to occur in a single semisynthetic medium with peak activity occurring three hours after the cessation of logarithmic growth. The peak of competence was not affected by marked differences in the rate of growth during the logarithmic phase (Bott and Wilson, 1967). In competent cells, the irreversible binding of DNA was found influenced by temperature, hydrogen ion concentration and aeration. Divalent cations, such as barium, strontium, calcium or magnesium are also required (Young and Spizizen, 1963).

Pathogenesis related proteins in plants

PR-proteins and host–coded proteins are induced by different types of pathogens and abiotic stress (Van Loon *et al.*, 1998). Ineffective isolates of *P. fluorescens* did not trigger accumulation of chitinase and β-1, 3 glucanase and did not induce systemic resistance in tobacco against tobacco mosaic virus. In pea seed, treatment with *P. fluorescens* isolates induced the accumulation of hydrolytic enzymes such as chitinase and β-1,3 glucanase at the site of penetration of fungal hyphae of *Fusarium oxysporum* f.sp. *pisi*. These enzymes act upon the fungal cell wall resulting in degradation and loss of inner contents of cells (Benhamou *et al*., 1996).

Chitinase against fungal infections in plants

Earlier reports showed that micro-organisms capable of lysing chitin, which is a major constituent of the fungal cell wall, play an important role in biological control of fungal pathogens. In filamentous fungi and yeasts, chitinase is involved integrally in cell wall morphogenesis. Chitinase is also involved in the early events of host-parasite interactions of necrotrophic mycoparsites, entomopathogenic fungi and vesicular arbuscular mycorrhizal fungi. In plants, induction of chitinase and other hydrolytic enzymes is one of a co-ordinated often complex and multifaceted defense mechanisms triggered in response to phytopathogen attack (Sahai *et al.,* 1993).

Plant chitinases are mostly endochitinases which hydrolyse chitin, the major component of the exoskeleton of insects as well as cell wall of most fungi and nematodes (Neuhans *et al*., 1991). Chitinases have been shown to exert antifungal activity *in vitro* which represents the rationale behind attempts to establish transgenic plants expressing Class1 chitinases (Stintzi *et al*., 1993).

Pathogenesis related proteins including chitinases and β- 1,3-glucanases are produced by plants in response to infection by pathogens. Chitinase hydrolyses the glucan component (Boller, 1985; Boller, 1992). Schlumbaum (1986), reported that purified plant chitinases had antifungal activity *in vitro* and the expression of chitinase with one chitin-binding domain in transgenic plants enhances their resistance against fungal pathogens (Broglie, 1991; Lin , 1995). Also chitinases act synergistically with β-1,3-glucanases in inhibiting fungal growth.

Genetic manipulation of Plant Growth-Promoting Bacteria and endosymbionts to enhance bio control of phytopathogens

Bio control may be improved by genetically engineering plant growth promoting bacteria (PGPB) and endosymbionts to over express one or more of these traits so that strains with several different anti-phytopathogen traits which can act synergistically are created. Marugg *et al.*, 1989 cloned the genes for iron-siderophore receptors from one bio control PGPB and introduced them into other strains. It was reported that amplification of the CHAO gene from *Pseudomonas fluorescens* encoding the housekeeping sigma factor s70, both enhanced antibiotic production and improved protection against *Pythium ultimum*-induced damping-off of cucumber (Maurhofer *et al*., 1995; Schnider *et al*., 1995).

As on now, there is only one commercially available genetically engineered bio control bacterium. A modified strain of *Agrobacterium radiobacter* strain K84 is being marketed in Australia since 1989 to control *Agrobacterium tumefaciens*-caused crown gall disease which affects stone fruit trees and almond. The antibiotic agrocin 84 that is produced by *A. radiobacter is* normally toxic to *Agrobacteria* carrying a nopaline/agrocinopine A type Ti plasmid (Kerr, 1989; McClure *et al*., 1994). However, agrocin 84 resistant strains of the pathogen *A. tumefaciens* can develop if the plasmid carrying the genes for the biosynthesis of agrocin 84 is accidentally transferred from *A. radiobacter.* To avoid this possibility, the region of DNA responsible for plasmid transfer was removed from the agrocin 84 plasmid. Thus, a mutant of the bio control *A. radiobacter* strain was constructed which no longer can transfer the modified agrocin plasmid to pathogenic *Agrobacteria*, thereby retaining the capacity to act as a bio control agent (Jones *et al*. 1988).

In a series of experiments, a chitinase gene was isolated from the bacterium *Serratia marcescens* and then transferred into *Trichoderma harzianum* and *Rhizobium meliloti* cells (Chet and Inbar, 1994). In both cases, the transformed microorganisms expressed the chitinase and subsequently displayed increased anti-fungal activity. When the *S. marcescens* chitinase gene was introduced into a strain of *P. fluorescens* that acts as a bio control PGPB, the transformant stably expressed and secreted active chitinase and was found an effective bio control strain against the pathogen *Rhizoctonia solani* (Koby *et al*.,1994).

In an effort to engineer a more soil persistent bio control bacterium, Colbert *et al*., (1993) transferred the NAH7 plasmid, which carries the genes encoding the enzymes of the naphthalene and salicylate bio degradative pathway, into an established bio control strain. The introduced plasmid was found stably maintained and conferring increased persistence upon the host bacterium when salicylate was present in the soil.

..........ଔଡ଼..........