2.1. Occurrence and distribution

Rice is the staple food for more than three billion people worldwide. Over 600 million people derive more than half of their calories from rice. It is the third largest commercial crop behind wheat and corn. In the year 2005, 700 million metric tons were produced worldwide with a market value of US$ 120 billion. It is estimated that 50 % of the world rice crop is lost due to diseases caused by bacteria, fungi and viruses. In the year 2005, 300 million metric tons of rice was lost due to diseases. This important crop suffers from 40 different microbial diseases, with bacterial leaf blight the most devastating and harmful. One of the most serious bacterial diseases of rice in Africa and Asia is bacterial leaf blight caused by *X. oryzae* pv. *oryzae*. Bacterial leaf blight is one of the oldest recorded rice diseases and has been problematic for over a century. *X. oryzae* pv. *oryzae* spreads rapidly from diseased plant to healthy plant and from field to field in water droplets. Infected leaves develop yellow lesions, and wilt in a matter of days. In severely affected fields, bacterial blight can wipe out half a farmer’s rice crop. Bacterial leaf blight caused by *X. oryzae* pv. *oryzae* was worldwide in distribution particularly destructive in Asian countries during the heavy rains of monsoons. Bacterial leaf blight has become endemic on rice following repeated cultivation. It was first noticed by the farmers in Fukuokka area of Japan in 1849 (Ishiyama, 1922) and the study of the disease commenced in Japan in 1901. In India it was first reported from Koloba district of Maharashtra by Srinivasan *et al.* (1959). The disease was considered to be of minor importance until it broke out in an epidemic form in Shahabad district of Bihar in 1963 (Srivastava and Rao, 1966). Bradbury (1984) has reported disease distribution in several African countries, in India wide spread distribution of the disease has reported from almost all states *viz.*, Andaman and Nicobar Island, Andhrapradesh, Assam, Bihar Delhi, Goa, (Ghosh *et al.*, 1987). Gujarat, Haryana Jammu and Kashmir, Karnataka, Tamilnadu, Maharashtra, Orissa. Uttar Pradesh and West Bengal (Bradbury, 1984; Tikoo *et al.*, 1987; Mondal and Latif 1996). The disease can affect rice plants at any plant growth stages (Jabeen *et al.*, 2012). It is one of the most widespread and destructive diseases of rice in several countries in tropical rice-growing areas of Asia, Australia, United States, Latin America and Africa (Mew, 1987, 1989; Mew *et al.*, 1993; Sere *et al.*, 2005). Bacterial leaf blight was observed to
occur in fields with high incidence of 70 to 80 % in several West African countries (Sere et al., 2005). Under tropical condition bacterial leaf blight is most prevalent in both tropical and temperate areas and endemic to much of Asia and west Africa (Liu et al., 2006). It is a typical vascular disease considered to be one of the destructive diseases in Asia including India, Nepal and Srilanka (Pasueazzi and McCouch, 2007). Bacterial leaf blight has the potential to become a destructive bacterial disease of rice in Pakistan, India and Karnataka (Akhatar et al., 2008; Nayak et al., 2008;Shivalingaiah and Umesha, 2011). Recent studies in West African countries such as Burkina Faso, Niger and Mali revealed the occurrence of bacterial leaf blight causing significant crop damages (Basso et al., 2011). Recent survey reported the occurrence of bacterial leaf blight in most of rice growing ecozones of Togo with high incidence and severity, and the virulence of the pathogen was determined (Dewa et al., 2011). Bacterial blight was observed to occur in fields with high incidence of 70 to 80% in several West African countries (Banito et al., 2012). The X. oryzae pv. oryzae has been the most serious in South East Asia, particularly since the widespread cultivation of dwarf high yielding rice cultivars. It has caused huge yield loss during recent years. In Japan the yield loss reported were ranged between 20-30 %, occasionally increasing up to 80 %.

2.2. Economic Impact

Bacterial leaf blight caused by X. oryzae pv. oryzae is the most damaging disease of rice in south India, Japan, south East Asia and, particularly since introduction of dwarf high yield varieties. In Japan where figures are available up to 4 million ha may be affected annually with a loss of 20-30 % and 50 % still more in tropical regions severe infection results in poor grain development, broken rice and deterioration in chemical and nutritional composition (Ou, 1985a). The disease in its severe form is known to results in yield loss ranges from 74-81 % in susceptible cultivar (Shivalingaiah et al., 2013). In Africa, losses of 3-41 % in grain yield have been found (Awodera et al., 1991). It occurred in an epidemic form during 1998 in Palghat district of Kerala, India and destroyed the rice harvest (Gnananmanickem et al., 1999). Yield losses due to bacterial leaf blight range from 74-81 % in India where it is epidemic. This loss is relatively higher than those reported in other parts of the world which is generally around 20-30 %, occasionally going up to 50 %
Yield losses due to bacterial leaf blight ranging from 50 to 90% have been reported (Ou, 1985b, Sere et al., 2005). Reports from the Philippines, Indonesia and India estimate that losses due to the kresek syndrome of bacterial leaf blight, which affects 60–75%, depending on weather, location and rice cultivar. In addition to reducing yield, bacterial leaf blight may also affect grain quality by interfering with maturation (Liu et al., 2006).

In India millions of hectares were severely infected with the disease causing yield loss up to 40% (Pascuzzi and McCouch, 2007). The disease became prominent in the 1960s, when new high-yielding cultivars were first developed and introduced. Yield loss ranging up to 26% has been reported on susceptible rice cultivars. It is particularly destructive in Asian countries (Adhikari et al., 1995; Ghasemie et al., 2008). Yield losses due to this disease correspond to the plant growth stages, infection at booting stages does not affect yield but results in poor quality and high proportion of broken kernels (Anon, 2009). Recent studies in West African countries such as Burkina Faso, Niger and Mali revealed the occurrence of bacterial leaf blight causing significant crop damages (Basso et al., 2011).

Recent survey reported the occurrence of bacterial leaf blight in most of rice growing eco zones of Togo with high incidence and severity, and the virulence of the pathogen was determined (Dewa et al., 2011). Losses due to bacterial leaf blight in tropical Asia vary from 2 to 74% depending upon certain factors such as location, weather conditions, crop stage and cultivars. Similarly yield losses due to bacterial leaf blight disease range from 20 to 30% though in severely infected fields the losses may reach up to 80% (Chaudhary et al., 2012). Bacterial blight is reported to have reduced Asia's annual rice production by as much as 60%. For example, in Japan, about 300,000 to 400,000 hectares of rice were affected by the disease in recent years. There was 20% to 50% yield loss reported in severely infected fields. In Indonesia, losses were higher than those reported in Japan. In India, millions of hectares were severely infected, causing yield losses from 6% to 60%. To develop rational and economical control measures, the extent of crop losses must be evaluated and related to the potential gain obtained from control practices. Disease severity and crop loss appraisal can be used to determine the economic impact.
2.3. Disease cycle and epidemiology

The development of bacterial leaf blight depends on many environmental factors, presence of rice stubbles and ratoons of infected plants, presence of bacteria in the rice and irrigation channels, warm temperature, high humidity, rain and deep water, over fertilizer handling of seedlings at transplantation (Singh and Paroda 1994). The infected seed, plant debris perpetuate the disease from one season to another season. The bacteria are usually found in the glumes. *X. oryzae* pv. *oryzae* enters the rice leaf through hydathodes at the leaf tip and leaf margin (Ou, 1985b). The spread pattern in a rice field has been analysed by Nayak and Reddy (1985). Potential inoculum source include infected plant material volunteer rice plant, infected chaff, weed host and infected seeds in temperate regions (EEPO, 1997). *X. oryzae* pv. *oryzae* also penetrate the leaf mainly through stomata multiplies in the substomatal cavity and colonizes the intercellular spaces of parenchyma with in few days bacterial cells and exopolysaccharides fill the xylem and oozes out from the hydathodes forming beads or strands of exudates on the leaf surface, a characteristic sign of the disease and a source secondary inoculums. *X. oryzae* pv. *oryzae* may also gain access to the xylem through wounds or openings caused by emerging roots at the base of leaf sheath with in the xylem, *X. oryzae* pv. *oryzae* presumably interact with xylem parenchyma but may also penetrates into the endosperm (EEPO, 1997; Shen et al., 2002). The transmission of the pathogen is favored by the intense wind driven rainfalls that facilitates bacterial entry into plant tissue through wounded leaf edges. Bacteria may also be disseminated in irrigation water as well as by humans, insects and birds (Liu et al., 2006). Out brakes of bacterial leaf blight are more likely to occur during the monsoon seasons of the south East Asia and India (from June to Sep) than at other times of the year (Liu et al., 2006). Cells on the leaf surface may become suspended in guttation fluid as it exudes at night and enters the plant by swimming movement or passively as fluid is withdraw into the leaf in the morning. The bacterium multiplies in the inetracellular spaces of the underlying epithem then enter and spread into the plant through xylem (Noda and Kaku, 1999; Liu et al., 2006). *X. oryzae* pv. *oryzae* can survive in rhizosphere of weeds of genera *Leersia* and *Zizania* as well as in the base of the stem and the roots of rice stubble. *X. oryzae* pv. *oryzae* can also survive in the soil for 1-3 months depending on the soil moisture and
acidity. In the tropics high temperature, humidity and abundance of host plants typically allow *X. oryzae pv. oryzae* to persist throughout the year (Liu *et al.*, 2006).

Severe epidemics often occur following typhoons, the wind blown rain, both disperse bacteria. Bacterial leaf blight is more severe in highly managed system such as irrigated paddy fields or with high nitrogen fertilizer application the disease is aggravated by warm humid and wet conditions (Pascuzzi and McCouch, 2007). Once inside the vascular system, the bacterium multiplies and moves in both directions. Spread takes place in wind and rain, but primarily in flood and irrigation water (Ghasiame *et al.*, 2008).

### 2.4. Morphology and biochemical characterization

*Xanthomonas oryzae pv. oryzae* is a rod shaped, rounded, gram negative bacteria individual cells varies in length from approximately 0.7 - 2.0 µm and 0.4 -0.7 µm in width. Cells are motile by means of single polar flagellum and colonies on solid media contain glucose are round, convex, mucoid and yellow in color due to production of pigment xanthomonadin characteristic of the genus (Bradbury, 1984). *X. oryzae pv. oryzae* cells are producing copious capsular extra cellular polysaccharides, which is important in the formation of droplets or strands of bacterial exudates from infected leaves providing protection from desiccation and aiding in wind and rain-borne dispersal (Swings *et al.*, 1990). To isolate the bacteria, sections of leaf tissues are surface-sterilized and macerated in distilled water, and the resulting suspension is streaked on 1 % dextrose nutrient agar or Wakimoto agar (Reddy and Ou, 1974) and incubated at 25-28°C. Colonies of *X. oryzae pv. oryzae* are slow-growing, mucoid and straw-coloured to yellow in colour, the isolated bacteria stained pink-red and showed thin viscid mucoid strand indicating positive for KOH solubility test and gram negative nature of the bacteria. A clear zone of hydrolysis was formed around the bacterial colonies, when the plates were flooded with Lugol’s iodine. Hence the bacterium indicated positive for starch hydrolysis. The inoculated Tween 80 agar plates showed the presence of white precipitate around the colonies of the bacteria, hence the bacterium indicated positive for lipase activity. Test isolates showed the liquefaction of the gelatin, acid production from glucose. (Vera Curz *et al.*,1984). Necrosis was observed in tobacco plants indicating positive for hypersensitive reaction and also positive for pathogenesis test (Bradbury, 1984).
2.5. Molecular characterization

The battle between pathogens and plants is never-ending due to the coevolution of the parasites and their hosts. Pathogenic bacteria interact with plants by secreting proteins into host cells. It is true even with *X. oryzae* pv. *oryzae*. The isolation and characterization of *X. oryzae* pv. *oryzae* from rice plant and seed by conventional techniques is often difficult usually due to the masking effect of fast growing saprophytes yellow pigmented bacteria. Agar media used in the isolation of *X. oryzae* pv. *oryzae* are not enough to eliminate fast growing contaminants. Biochemical tests, serological assays, fatty acid and metabolic profiling have been used in the identification of pathogen. These assays however, have short comings including lack of sensitivity and specificity. Due to the problems encountered in conventional methods, polymerase chain reaction (PCR) technology has found widespread application in detecting plant pathogenic bacteria (Lang *et al.*, 2010).

Accurate identification and early detection of pathogens is a crucial step in health care, agriculture, environmental monitoring including the fight against bioterrorism (Schaad and Frederick, 2002; Ivnitski *et al.*, 2003; Schaad *et al.*, 2003) and plant disease management. The failure to adequately identify and detect plant pathogens using conventional, culture based, morphological techniques has led to the development of nucleic acid based, molecular approaches (Lopez *et al.*, 2003; Lievens *et al.*, 2005). The new versions of the official European Union protocols for *Clavibacter michiganensis* subsp. *sepedonicus* and *Ralstonia solanacearum* incorporated PCR as screening test in an integrated protocol, including serological techniques, isolation and bioassays, for higher accuracy of the detection of these quarantine pathogens. This approach, not only increases our ability to detect plant pathogens but also can provide new insights into their ecology and epidemiology (Martin *et al.*, 2000; Alvarez, 2004). With the advancement of molecular techniques, the last ten years have seen increasing emphasis on molecular diagnosis using PCR-based assays. PCR-based assays resulting in rapid amplification of the bacterial genome are the mainstay of new analysis methods, including *Vir* typing using restriction fragment length polymorphism (RFLP), fluorescent RFLP, immuno-PCR, and real-time PCR. Although PCR-based methods have clearly facilitated the
detection of phytopathogenic bacteria, these detection techniques require multistep procedures and special laboratory setups and are subject to equivocal results.

Developing sensitive, reliable and accurate detection and diagnostic methods of *X. oryzae* pv. *oryzae* from rice plant material is increasing, but the fact is latent infection is difficult to detect. Although isolation of this quarantine organism is usually required in detection protocols, culture independent molecular techniques can be used as a screening method to circumvent the problems associated with the possible presence of stressed, injured, or viable but nonculturable cells (Swanson *et al.*, 2005). Molecular diagnostics began to develop a real momentum after the introduction of polymerase chain reaction in the mid 1980s and the first PCR based detection of a pathogen in diseased plants was published in the beginning of 1990s (Rasmussen and Wulff, 1991). To date an increasing number of diagnostic laboratories is adapting molecular methods for routine detection of pathogens. With the advances in Molecular Biology and biosystematics, the techniques available have evolved significantly in the last decade and besides conventional PCR, others technologically advanced methodologies such as the second generation PCR known as the real time PCR and microarrays which allows unlimited multiplexing capability have the potential to bring pathogen detection to a new and improved level of efficiency and reliability (Mumford *et al.*, 2006).

The PCR is a powerful technique that has widespread application in molecular biology. This technique is used to amplify a specific nucleic acid fragment that lies between two regions of known nucleotide sequence, and often from an extremely small amount of target nucleic acid in biologically complex samples. Amplified fragments can then be further characterized by size fractionation on agarose gels, restriction enzyme digestion and hybridization with probes or by DNA sequencing. The specificity of the PCR is based on the use of oligonucleotide primers that are complementary to the regions flanking the fragment to be amplified. Because of the sensitivity and specificity of PCR techniques, these procedures will likely to have widespread application to the detection of plant pathogens and can be used to answer precise questions about identification of pathogens, populations and variability (Potter *et al.*, 2007). Nucleic-acid based tests offer greater sensitivity, specificity, reliability and may be quicker than many conventional methods used to detect plant-pathogenic
bacteria in different plant hosts and environments. With the development of polymerase chain reaction and especially real-time PCR, such high sensitivity is achieved, improving the accuracy of pathogen detection and identification (Mullis, 1987; Holland et al., 1991; Vincelli and Tisserat, 2008).

Nucleic acid-based methods are sensitive, specific and allow genetic relationships to be determined. In plant pathology, the most frequently used molecular techniques have been, first, molecular hybridisation and afterwards, the polymerase chain reaction. Compared to traditional methods, PCR offers several advantages, because organisms do not need to be cultured before their detection. It affords high sensitivity at least theoretically, enabling a single target molecule to be detected in a complex mixture and it is also rapid and versatile. In fact, the different variants of PCR, have increased the accuracy of detection and diagnosis and opened new insights into our knowledge of the ecology and population dynamics of many pathogens, providing a valuable tool for basic and applied studies in plant pathology (Lopez et al., 2009).

Globalisation implies that state borders have become more open due to increased in free-trade agreements and this can facilitate the introduction and dissemination of foreign pathogens. This in turn, leads to emerging diseases, which are a growing reality for phytopathologists worldwide. A guiding principle for disease prevention is that when key inoculum sources have been identified, effective measures must be taken to prevent further spread and subsequent disease outbreaks. Consequently, detection of the causal organisms becomes essential, as most bacterial diseases are transmitted through contaminated seeds or propagative plant material. Plant quarantine policies and regulations have been implemented in many countries to avoid pathogens from spreading and to prevent exotic pathogens from being introduced with plant material. To achieve this goal, complex control systems have been designed, which often includes guidelines for rapid, sensitive and specific pathogen detection and diagnosis and among them, PCR is the technique of choice for rapid screening.

Compared to conventional diagnostic methods, PCR offers several advantages, because organisms do not need to be cultured prior to detection; moreover it is highly sensitive, relatively simple and fast to perform. There has been a shift towards DNA-
based protocols developed for diagnostic purposes as well as for etiological or epidemiological studies as reported by reviews published over the past fifteen years (Henson and French, 1993; Louws et al., 1999; Lopez et al., 2003; Schaad et al., 2003; Alvarez, 2004; Lopez et al., 2006; Vincelli and Tisserat, 2008; Lopez et al., 2009). Application of PCR techniques in diagnostic laboratories for routine purposes is also increasing and will continue in the near future, especially for the rapid screening of samples. PCR is now considered a routine technique and recommended in most protocols recently developed by the European Union and The European and Mediterranean Plant Protection Organization (EPPO) (Lopez et al., 2006).

Expansion of global trade in agricultural seed and produce increases the difficulty in controlling the movement of plant pathogens into new agroecosystems. Rapid detection and accurate identification of pathogens are critical steps to guide responses for containment or elimination of pathogens. However, robust and inexpensive diagnostic tools are not available for identification and classification of many plant pathogens. Historically, the primary hurdle in developing highly specific, easily used diagnostic tools for any pathogen has been the difficulty in finding unique features, whether they are cell surface antigens or DNA sequences that have been validated against an extensive collection of representative of a pathogen population. Due to this rate-limiting step, diagnostic tools often target only one feature (Vincelli and Tisserat, 2008; Lang et al., 2010). Nucleic acid based detection methods currently applied in pathogen detection are based on nucleic acid hybridization or PCR. These methods can be designed to detect either DNA or mRNA. However, DNA based detection method is often more straightforward than that of mRNA, the stability of DNA leads to the possibility that DNA based methods yield positive results from non-viable or dead pathogens. One of the main goals of pathogen detection system, besides determining the presence and absence of the pathogen, is the viability since in the event of positive result it is important to know whether the pathogen detected poses threat to crop production, public health or food safety. The lack of discriminating viable from dead cells is a pitfall common to the nucleic acid based detection systems including microarrays and diagnostic PCR (Keer and Birch 2003; Call, 2005). Artz et al. (2006) demonstrated that prolonged detection of non-viable cells led to potential overestimation in the quantitative real time detection of Escherchia coli. The bio-polymerase chain reaction assays to detect bacterial leaf
blight caused by *X. oryzae* pv. *oryzae* in rice is successful in control of disease. Thus, the PCR-based method can be used for the rapid and specific detection of *X. oryzae* pv. *oryzae* and will potentially simplify and facilitate diagnosis and monitoring of this pathogen and guide plant disease management (Ngadze *et al.*, 2012). Mean while, the specificity and sensitivity of detection of pathogens are greatly improved and pathogen detection is becoming simpler and faster, there are still major challenges, technical and economic nature, which need to be addressed to ensure the emergence of reliable detection system for routine applications. The existing and emerging technologies are developing to fulfil these demands and what limitations still exist. In developing a tool for pathogen detection, issues such as detection specificity and sensitivity are very important. In addition multiplexing, quantification and cost effectiveness are increasingly becoming important features of a diagnostic technology. There is also a growing need for a field deployable portable rapid detection system that provides the capability for pathogen testing and identification in the field.

### 2.6 Defense mechanism in plants

Identification of host genes involved in defense responses is one of most critical steps leading to the elucidation of disease resistance mechanisms in plants. Induction of disease resistance in plants involves a complex network of signal perception, amplification and transduction, which includes receptor-mediated recognition of invading pathogens, protein phosphorylation cascades, ion fluxes, and oxidative bursts, generation of secondary signals and activation of various defense genes (Yang *et al.*, 1997). During the past decades, extensive efforts have been made to identify signalling molecules as well as regulatory and structural genes involved in host defense responses. In addition a large number of defense-related genes whose products include receptor protein kinases, mitogen-activated protein (MAP) kinases, ion transporters, NADPH oxidase components, transcription factors, pathogenesis-related proteins and phytoalexin biosynthetic enzymes have been isolated and characterized. Some of these genes may serve as useful targets for genetic engineering of disease resistant crops. To date, the large majority of research in defense signal transduction has been performed on dicotyledonous species such as tobacco and *Arabidopsis*, whereas in economically important cereal crops, our understanding of
the signalling mechanisms leading to disease resistance is largely unknown (Piffanelli et al., 1999). Only few signalling components such as a small-GTP binding protein and MAP kinase (Kawasaki et al., 1999; He et al., 1999; Kim et al., 1999) as well as a dozen defense genes such as PR-1, β-glucanase, Chitinase, phenylalanine ammonia lyase and HMG-CoA reductase (Nelson et al., 1994; Simmons et al., 1992; Zhu et al., 1995; Xu et al., 1996) have been isolated from rice and implicated to be involved in the host defense responses. Most recently, differential display analysis has been used to identify a large number of fungal elicitor-responsive genes from rice cells suspensions (Kim et al., 2000). Most plants have the ability to escape invasion of pathogens by using defence systems even if they do not have a specific disease resistance gene. There is a delicate relationship between plant and pathogen. When environmental conditions such as temperature and humidity are favourable for the pathogen, the pathogen can easily invade the plant. When the defence system of the plant functions effectively on the other hand, the plant can overcome pathogen attack (Iwata, 2001).

Due to the agronomic importance of rice, understanding the molecular mechanisms underlying infection by the pathogen is of utmost importance. In general, the outcome of plant pathogen interactions depends on a molecular interplay between the two organisms, with the pathogen attempting to control the plant cell for the establishment of a compatible interaction (Schulze and Panstruga, 2003). To gain access to nutrients, plant pathogens have evolved different life styles, biotrophs derive their needs from living host cells, whereas necrotrophs destroy host tissue for their own nourishment (Lewis, 1973; Glazebrook, 2005). Magnaporthe oryzae belongs to an intermediate class, the hemibiotrophic pathogens, combining biotrophic and necrotrophic features. Typically hemibiotrophs initially grow biotrophically and then switch to necrotrophic growth, killing the infected tissues (Perfect and Green, 2001; Munch et al., 2008).

Plants are exposed to number of pathogens as a result they have evolved intricate defense mechanisms to recognize and defend themselves against wide range of these diseases causing agents by inducing a set of defense responses that can be defeat the invading pathogen. Plants are resistant to most of the pathogens in their environment, as they are not host plants for particular pathogen or host plants but the
resistant genes, allowing them to recognize specifically distinct pathogen races. Plants possess a range of active defense responses that contributed to resistance against a variety of pathogens. They respond to bacterial pathogens attack by activating various defense responses that are associated with the accumulation of several factors like defense-related enzymes and inhibitors that prevents the pathogen infection. The interaction between the host and pathogen induces some changes in the cell metabolism, primarily in the enzyme activities, including peroxidise (POX) and polyphenol oxidase (PPO). The battle between pathogens and plants is never-ending due to the co-evolution of the parasites and their hosts. Pathogenic bacteria interact with plants by secreting proteins into host cells. The proteins known as effectors, these are injected into host cells by the type III secretion system, which is highly conserved in plant and animal pathogens and these effectors play essential roles in pathogenicity in plants. The type III effectors with known functions have either enzymatic or transcription activator-like (TAL) activities that modify or degrade host proteins or regulate host gene expression (Kay and Bonas, 2009). Some host plants have evolved sophisticated strategies to counter bacterial effectors and avoid diseases. For example, one strategy uses host disease resistance (R) gene promoters to trap the TAL effectors mutation of R gene promoters results in induction of dominant R genes by specific effectors and subsequent host defense responses (Gu et al., 2005; Romer et al., 2007, 2009, 2010). Another strategy is mutation of a host susceptibility gene promoter to become unresponsive to the TAL effectors, this mutation results in a recessive R gene that has lost pathogen-induced expression and subsequent avoidance of disease (Chu et al., 2006; Yang et al., 2006). Although different pathogen effectors have been characterized, it is largely unknown how the host targets of these effectors act to facilitate pathogen infection.

Plants have evolved mechanisms that protect against pathogen effectors-mediated susceptibility of which the resistance (R) genes are an important component (Chisholm et al., 2006; Ellis et al., 2009). Resistance gene products have been proposed to guard important defense signalling complexes that are targeted by virulence effectors by sensing perturbations upon the interaction of the complex with a pathogen virulence effectors or alternatively, by acting as target decoys, intercepting effectors upon their entry into the host (Hogenhout et al., 2009; Antony et al., 2010).
The co-expression patterns of bacterial disease resistance genes and their transcriptional regulators in transgenic rice have indicated that the resistance genes trigger an immune response (Sana et al., 2010; Zhou et al., 2010; Gupta et al., 2012).

Plants have endogenous defense mechanisms that can be induced in response to attack by plant parasitic nematodes. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant’s own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy (Ramamoorthy et al., 2001; Anita and Samiyappan, 2012).

2.6.1. Peroxidase

Peroxidases (EC 1.1.1.7) are found in the host pathogen interactions throughout the plant kingdom. An increase in peroxidase activity has been associated with environmental stresses on plants. A definitive role for peroxidases in plants has eluded plant scientists so far. There have been numerous reports in the literature with respect to their general involvement in lignin synthesis and oxidation of the endogenous IAA. A trend has emerged in the last few years, where lignin synthesis has been associated with the anionic peroxidases and the oxidation of IAA with the cationic peroxidase isoenzymes. Little attention has been paid in the purification of peroxidase to a homogenous protein preparation. Plant peroxidases involved directly in the defense mechanism as catalyst for the polymerization of phenolic compound to form lignin and suberin in the cell wall, which act as barrier for the entry of pathogen (Kavitha and Umesha, 2008). The defense gene products include polyphenol oxidase (PPO), peroxidase (POX) that catalyzes the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis. POX participate in a variety of plant defense mechanism in hydrogen peroxidas is often supplied by an oxidative burst. It is involved in substrate oxidation cell wall lignifications, photosynthesis, and respiration and growth regulation. An increased activity was demonstrated during various pathogenesis (Mohammadi and Kazemi, 2002) showed that increased POX activity occurred in two stages in Lycopersicon species resistant to Oidium neolycopersici. The involvement of POX in induction of defense reactions in resistant and susceptible plants have been reported by many workers where activities of POX as well as inductions of POX isoforms and genes
are considered for disease resistance (Zhang, et al., 2008). The application of salisalyic acid, calcium chloride and oxalic acid reduce the disease incidence in pear fruit caused by *Alternaria alternata* by inducing the activities of β-1, 3-glucanase, PAL, PPO and peroxidase (POX) (Tian et al., 2006; Raju et al., 2008). Peroxidise activity was higher in resistant tomato cultivars than in susceptible cultivars after inoculation with the bacterial spot pathogen *Xanthomonas axonopodis* pv. *vesicatoria* (Kavitha and Umesha, 2008). Peroxidase activity has been shown in several species of plants. Thus, there is a continual search for novel peroxidases for various applications (Padmarajaiah et al., 2009). PAL, PPO, and POX are associated with plant defense against insects. However, little is known about the dynamic changes of these enzymes during plant development and the influence of aphid infestation on their activity in resistant vs. susceptible cultivars, especially in wheat (Han et al., 2009). An increase in peroxidase activity has been reported as an early response to different stresses and may provide cells with resistance against formation of H$_2$O$_2$ which is formed when plants are exposed to stress factor. Also peroxidase is involved in a large number of biochemical and physiological processes and may change quantitatively and qualitatively during growth and development. Indeed accumulation of H$_2$O$_2$ may cause change in plant metabolism (Zhi et al., 2003; Zolfaghari, et al., 2010). Enzymes peroxidise and catalases are high-molecular, which are capable of eliminating the hydrogen peroxide formed during non enzymatic or enzymatic dismutation (Chkhubianishvili et al., 2011).

Peroxidise are widely distributed in nature and are found in plants, micro-organisms and animals, where they catalyze the reduction of hydrogen peroxide (H$_2$O$_2$) to water, rendering it harmless. H$_2$O$_2$ is a common end product of oxidative metabolism and being a strong oxidizing agent, could prove toxic if allowed to accumulate. Thus peroxidises serve to ride plant cells of excess H$_2$O$_2$ under normal and stress conditions. Peroxidases are versatile biocatalyst with an ever increasing number of applications. Roots of horse radish serves at present as the major source of commercially available peroxidise however, the researchers still investigate for new peroxidases of elevated stability and properties suitable for different biotechnological, biomedical and other applications e.g., spring cabbage peroxidase was suggested as a potential tool in biocatalysis and bioelectro catalysis Thus, there is a continual search for novel peroxidases for various applications. Although relevant reviews on
peroxidase activity in plants are available, broad effort for their characterization are very limited (Bania and Mahanta, 2012).

2.6.2. Polyphenol oxidase

Many studies have shown that Polyphenol oxidase (E.C.1.14.18.1; PPO) is induced in response to mechanical wounding, fungal and bacterial infection and by treatment with signalling molecules such as jasmonic acid /methyl jasmonate (MeJA), systemin and salicylic acid (Constabel et al., 2000; Stewart et al., 2001). In addition systemic induction of PPO expression in response to wounding and pathogens might provide an additional line of defense to protect plants against further attack by pathogen and insects (Thipyapong and stiffens, 1997; Stout et al., 1999).

Polyphenol oxidase specific activity significantly increased in wheat heads of resistant and susceptible cultivars following inoculation with Fusarium graminearum during induced resistance (Mohammadi and Kazemi, 2002). Some oxidative enzymes such as POX and PPO can catalyze the formation of lignin and other oxidative phenols and contributes in formation of defense barriers by changing the cell structure defense system get activated against pathogens (Thilagavathi et al., 2007). The defense gene products include polyphenol oxidase (PPO) peroxidase (POX) that catalyzes the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis. (Raju et al., 2008). Resistant cultivars exhibited greater constitutive PAL activity than susceptible ones at the tillering stem elongation and flag leaf stages of chick pea. Aphid infestation enhanced levels of PAL activity in the flag leaf and ear stages in both resistant and susceptible wheat cultivars. Constitutive PPO activity was higher in the resistant cultivars of wheat at all developmental stages (Han et al., 2009). The very earliest work on the mechanisms of induced resistance demonstrated an association with systemic and local accumulation of PR proteins and oxidases such as peroxidase and phenoloxidase (Hammerschmidt, 2009).

Tomato plants treated with native bioagents of Trichoderma virens followed by challenge inoculation of Fusarium oxysporum enhance induction of defence related enzyme such as POX, PPO and PAL than other isolates which could be very effective in the control of Fusarial wilt of tomato (Christopher et al., 2010). The rice
seedling treated with *Adathoda vasca* leaf extract will enhance the production of PPO, POX and PAL activity in rice plants which control the seed borne pathogen *X. oryzae pv. oryzae* (Govindapp *et al.*, 2011). Some members of Bacillus genera are able to produce various lytic enzymes and antibiotics along with induction of systemic resistance of plants, such as increasing the activities of plant defense related enzymes of peroxidase, polyphenol oxidase and phenylalanine ammonialyase (Jayaraj *et al.*, 2004). Extracts of four plants (*Azadirachta indica, Aegel mermelos, Cassia auriculata and Vitex negundo*) against bacterial blight were analyzed. Bacterial blight was more effectively controlled by the water and methanol extracts of *V. negundo* than the other plant extracts. The extracts induced the defense related enzymes such as PPO, PO and β-1,3-glucanase in both pre and post inoculation of *X. oryzae pv. oryzae* (Nisha *et al.*, 2012). The live *Fusarium oxysporum* inoculation to banana plant showed to induced 3-fold PPO activity but the dead pathogen treatement was failed to show the significant activity (Janki *et al.*, 2013).

### 2.7. Management

Chemical control of bacterial leaf blight in rice field began in the 1950s with the preventive application of Bordeaux mixture. In the 1960s, different kinds of agrochemicals were developed from repeated field trials and made available on a large scale, mostly in Japan. They were based on chloramphenicol, nickel-dimethylidithiocarbamate, dithianon and fentiazon. Most were unreliable, however owing to variability in sensitivity among the pathogen population. However, chemical control of bacterial leaf blight in tropical monsoon tropical Asia is impractical and no truly effective bactericide is commercially available for disease control (Mizukami and Wakimoto, 1969; Gnanamanickam *et al.*, 1999; Lee *et al.*, 2004; Liu *et al.*, 2006). Application of chemical derivatives has effectively controlled the plants from bacterial disease but threatens the environment hindering the management of disease in crops and agricultural products (Burhan *et al.*, 2009).

Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while.
However, the blind dependence on synthetics is coming to end and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

The severity of losses incurred due to the disease necessitates the development of strategies that are ecology-conscious and cost effective. Bacterial leaf blight disease management centres around methods that reduce the initial inoculum and subsequent development of the pathogen on host plants and this can be accomplished through the use of chemicals, disease resistant cultivars and biological agents. Effective chemical control measures against the disease are lacking and breeding for disease resistance is the most important approach to its management. Disease-resistant cultivars with one or two major resistance genes are unsustainable in the field because of high pathogenic variability. Development of rice cultivars with durable resistance is ideal but success in this regard is limited. However, it is well known that plants have evolved an array of defence mechanisms to combat invasion by plant pathogens. Besides pre-existing physical and chemical barriers, a variety of defence mechanisms are activated upon pathogen infection (Kessmann et al., 1994; Chen et al., 1999; Hong et al., 1999; Mahmood et al., 2006). This induced resistance is at first localised around the point of pathogen infection. Subsequently, the resistance spreads systemically and develops in distal, uninfected parts of the plant, thereby conferring an elevated level of protection (Sticher et al., 1997). The use of induced resistance in plants is a promising, environment-friendly strategy for controlling plant diseases; including those caused by bacteria (Mohanbabu et al., 2003). Bacterial leaf blight of rice can be effectively managed by using Bordeaux mixture, a copper compound and a copper mercury mixture. An application of copper-oxychloride and streptomycin completely inhibited of bacterial growth. A
streptomycin mixture was tested in India for disinfection of rice seeds and it proved to be effective (Srivastava, 1972). Bacterial leaf blight lesions in rice were reduced by using bleaching powder with 30 % chlorine (2 kg/ha) to disinfect rice seeds (Chand et al., 1979). However, excessive chemical use has a detrimental effect on the environment, farmers’ and consumers’ health, and also causes severe foliar damage to the standing crop and harms beneficial predators and parasitoids. Certain plants have been known for their medicinal and antimicrobial properties since ancient times. Their products can offer advantages because they are relatively safe and easily biodegradable. Biologically active compounds that effectively control various pests and pathogens are known from approximately 2400 plant species. The antibacterial activities of different plant extracts against plants diseases have been previously investigated (Okigbo and Nmeka, 2005). Antibiotics, fungicides, and even organics such as cow dung were attempted for the control of Bacterial leaf blight of rice, but so far, only a partial control of the disease has been possible (Mary et al., 2001). Plant diseases constitute an emerging threat to global food security. Many of the currently available antimicrobial agents for agriculture are highly toxic, non-biodegradable and cause extended environmental pollution (Vyvyan, 2002). The disease is difficult to control but a number of interventions can be employed to manage and control it such as cultural and chemical control methods. However, some of these control measures such as the use of chemicals is costly. Therefore, the long term solution to the problem is to deploy resistant cultivars. Borines et al. (2003) reported that the use of resistant cultivars has been the most effective and economical way of controlling bacterial leaf blight of rice. A study is being conducted with the overall objective of developing resistant rice genotypes against bacterial leaf blight in Uganda. Bacterial leaf blight is one of the important rice diseases in Myanmar and the occurrence of this disease is increasing year by year with no effective control measures. The lack of highly effective and economical pesticides leads to the development and use of resistant cultivars as the most appropriate method of controlling this disease (Ezuka and Sakaguchi, 1978). Numerous resistant cultivars have been rendered ineffective due to occurrence of highly virulent strains or new races. Prevalence of new virulent strains is always a problem to scientists in releasing the resistant cultivars. Therefore, other methods should be developed for the effective control of this disease, such as biological control methods (Shivalingaiah et al., 2013). Seed treatment with bleaching powder (100 grams per liter) and 2 % zinc sulphate may help to reduce the infection.
Other chemicals such as copper compounds and antibiotics have not been proven to significantly control Bacterial leaf blight infection. Farmers in a community may also practice synchronized planting to reduce the occurrence of inoculum over an extended period of time. Proper fertilizer management particularly nitrogen application should be considered as well as proper plant spacing. Bacterial leaf blight disease is difficult to control effectively but rice cultivars with race-specific resistance have been the most important method to control Bacterial leaf blight disease. Unfortunately race specific resistant can promote the build-up of new *X. oryzae* pv. *oryzae* race and result in the failure of resistant rice cultivars. On the other hand, chemical pesticides which applied preventively and creatively can be an effective method to control Bacterial leaf blight, but it is toxic to users, consumers and other non-target organisms. It may persistent in nature thus accumulate in ecosystems. This condition may have a negative impact on the environment in the long term. Application with bactericides such as Kasugamycin, Phenazin and Streptomycin can suppress the intensity of the Bacterial leaf blight in the field but farmers will still have an obstacle because of the cost and not environmentally friendly. Alternative control methods can be developed for effectively controlling Bacterial leaf blight in rice plant.

Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish *et al.*, 1999; Okigbo and Ogbonnaya, 2006; Shariff *et al.*, 2006; Bouamama *et al.*, 2006; Ergene *et al.*, 2006; Kiran and Raveesha, 2006; Mohan and Raveesha, 2006). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental pollution and danger to consumers in contrast to the synthetic pesticides. This leads to screen *in vitro* a large number of plants for antibacterial activity against important seed borne *X. oryzae* pv. *oryzae* with the ultimate aim of developing plant based formulations for plant disease management. Antibacterial active principles isolated from higher plants is appears to be one of the important alternative approaches to contain antibiotic resistance and the management of diseases. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics (Shariff *et al.*, 2006).

Plants are constantly exposed and threatened by a variety of pathogenic microorganisms present in their environments. Diseases caused by pathogens
including bacteria and fungi significantly contribute to the overall loss in crop yields worldwide (Savary et al., 2006). In an effort to combat diseases, plants have devised various mechanisms and compounds to fend off microbial invaders. Despite the existence of plant defense mechanisms, a major difficulty encountered is the lack of effective control agents against some severe plant bacterial diseases. On the other hand, application of chemical derivatives has effectively controlled the plants from bacterial disease but this threatens to contaminate the environment, hindering the management of diseases in crops and agricultural products (Burhan, 2009). Bacterial diseases caused by *Xanthomonas* have devastated various host-plants, resulting in considerable losses in productivity and quality of harvests (Cavalcanti et al., 2006). Pathovars of *Xanthomonas* are reported to have developed resistance to several antibiotics such as kanamycin, ampicillin, penicillin and streptomycin that are generally used in agriculture field (Rodriguez, 1997). In addition, control of the disease is difficult, often requiring expensive and complex integrated pest management, including the use of contamination-free seeds, sanitization practices and the use of chemicals. Moreover, antibiotics and synthetic pesticides are forbidden in many countries as they exert a negative impact such as high and acute toxicity, long degradation periods and accumulation in the food chain. Consequently there is an obvious need to search for alternative natural antimicrobial agents or biopesticides to control bacterial plant diseases for agricultural applications, which are nontoxic and non-polluting (Costa et al., 2000). Compounds are specially sought that have activity against plant pathogenic bacteria that have acquired resistance to commercial compounds. Researches focused on plant-derived natural bactericides and their possible applications in agriculture to control plant bacterial diseases has intensified as this approach has enormous potential to inspire and influence modern agro-chemical research. Naturally occurring and biologically active plant products such as essential oils and organic extracts could be a source of alternative classes of natural biopesticides to serve as templates for new and more effective compounds in controlling plant pathogenic microorganisms (Vivek et al., 2010).

Rice is an important cereal crop affected by various fungal, bacterial and viral diseases, management of these disease using chemicals causes several adverse effects i.e. development of resistance in the pathogen, residual toxicity, pollution in the environment, high cost and etc. Therefore, it has become necessary to adopt eco-
friendly approaches for better crop health and yield. The practical use of natural compounds as control agents is receiving increased attention and this is partly due to their non-toxicity to humans, their systemicity and biodegradability (Singh et al., 1984; Mason et al., 1996). Volatile compounds from plants, especially essential oils have been demonstrated to possess potent antifungal, antibacterial, insecticidal and nematicidal activity (Wilson 1997; Isman, 1999; Oka et al., 2000; Nguefack et al., 2004; 2007). Furthermore, biocides of plant origin are non-phytotoxic, systemic and easily biodegradable (Kagale et al., 2004). Investigations on mechanisms of disease suppression by plant products have suggested that the active principles present in them may either act on the pathogen directly (Ansari, 1995; Amadioha, 2000) or induce systemic resistance in host plants resulting in reduction of disease development (Pal et al., 2011). Rice plays a pivotal role in the economy of Pakistan after cotton as an export item. Rice diseases especially bacterial leaf blight are a limiting factor faced by the farmers in all rice growing tracts of Pakistan. The severity and significance of damages caused by infection necessitate the development of strategies to control and manage the disease for minimizing the crop loss, which is reported to be 50% or even more in severe cases. Losses due to bacterial leaf blight in tropical Asia vary from 2 to 74% depending upon certain factors such as location, weather conditions, crop stage and cultivars (Srivastava and Kapoor, 1982). A lot of work has been done to control the disease through copper based chemicals and antibiotics. Bactericides, particularly antibiotics such as streptomycin satisfactorily control several important bacterial diseases of crops. Copper compounds have served as fungicides and also as bactericides. Copper oxychloride performed the best for bacterial leaf blight control followed by Vitigran Blue with 43.25 and 48.19% disease incidence, respectively against control (71.08%) and these treatments gave the highest paddy yield 3.63 and 3.58 t/ha (Ahmad et al., 2005). Recommended spray of Cupravit or Vitigran Blue (3 g of water) to check further spread of disease. Spraying of copper fungicides alternately with streptomycin (250 ppm) is reported to be effective in controlling the bacterial leaf blight. The earlier studies have identified some chemicals and antibiotics with relative efficacy against the disease. However, effective control of the disease has not been recorded. Bordeaux mixture is the most widely known and generally useful and economical among all sprays used against parasitic fungi as well as for bacteria (Nyvall, 1999). As there is no single effective control measure is available against this disease (Choudary et al., 2012). Plant
diseases caused by bacteria are a major economic liability to agricultural production. Disease control has been a major challenge for many bacterial diseases. Bacterial plant diseases affecting important agricultural crops can result in considerable damage and serious economic loss worldwide. Hence, researches focused on plant-derived natural bactericides and their possible applications in agriculture to control plant bacterial diseases has intensified because of enormous potential to inspire and influence modern agro-chemical research.

2.8. Biological control

Plant diseases are responsible for annual crop losses at a total value of more than 200 billion (Shivalingaiah and Umesh, 2012). Resistant plants and chemicals are often used to control plant diseases. Resistance does not exist against all diseases and the breeding of resistant plants takes many years. The use of microbes to control diseases, which is a form of biological control, is an eco-friendly approach. In contrast, the majority of molecules of agrochemicals do not reach the plant at all. Moreover, the molecules of biological origin are biodegradable compared with many agrochemicals that are designed to resist degradation by microbes. Bacteria that produce antibiotics, which kill pathogens, act via antagonism, if their mutants defective in structural genes in the synthesis of this antibiotic are biocontrol negative. For a bacterium to be suitable for biocontrol, it must not only synthesize and release the antibiotic, but also compete successfully with other organisms for nutrients from the root and for niches on the root to deliver the antibiotic along the whole root system. Also, the bacterium should escape in sufficient numbers from predators feeding on rhizosphere bacteria, so-called protozoan grazers (Jousset et al., 2006). Furthermore, the bacterium should produce the antibiotic in the right micro niche on the root surface (Pliego et al., 2008).

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere describes the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and
activity (McCully, 2005). A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates (Lynch, 1990). Creating a very selective environment where diversity is low (Marilley and Aragno, 1999; García et al., 2001). Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization (Antoun and Kloepper, 2001). Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens whereas other trigger beneficial effects. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980). In the last few years, the number of PGPR that have been identified has seen a great increase, mainly because the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere. Various species of bacteria such as Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus and Serratia have been reported to enhance the plant growth (Kloepper et al., 1989; Glick, 1995; Joseph et al., 2007). Pseudomonas spp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. The most effective strains of pseudomonas have been fluorescent pseudomonads. Considerable research is underway globally to exploit the potential of one group of bacteria that belong to Fluorescent pseudomonads (FLPs). FLPs help in the maintenance of soil health and are metabolically and functionally most diverse (Lugtenberg and Dekkers, 1999; Lata and tilak 2002). The presence of fluorescent pseudomonads inoculant in the combination of microbial fertilizer plays an effective role in stimulating yield and growth traits of chickpea (Rokhzadi et al., 2008). Isolates of FLPs from roots, shoots, and rhizosphere soil of sugarcane provides significant increases in fresh and dry masses (Mehnaz et al., 2009).

PGPR are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of plant pathogens. They can suppress a broad spectrum of bacterial, fungal and nematode diseases. PGPR can also provide protection against viral
diseases. The use of PGPR has become a common practice in many regions of the world. Although significant control of plant pathogens has been demonstrated by PGPR in laboratory and greenhouse studies, results in the field have been inconsistent. Recent progress in our understanding of their diversity, colonizing ability and mechanism of action, formulation and application should facilitate their development as reliable biocontrol agents against plant pathogens. Some of these rhizobacteria may also be used in integrated pest management programmes. Greater application of PGPR is possible in agriculture for biocontrol of plant pathogens and bio fertilizer (Siddiqui, 2006). *Pseudomonas fluorescens* is adapted to survive in soil and colonization of plant roots (Kiely et al., 2006) and this applies also to the particular case of biocontrol agents from this species. They are effective at utilizing seed and root exudates for growth and can colonize the rhizosphere aggressively. Strains with biocontrol ability may represent in the order of 10% of all rhizosphere strains and they have been isolated from a very wide range of soils, climatic regions and host plants (Rezzonico et al., 2007). A major group of rhizobacteria with potential for biological control is the Pseudomonads (Kremer and Kennedy, 1996). Tremendous progress has been made in characterizing the process of root colonization by pseudomonads, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to rhizosphere competence and the mechanisms of pathogen suppression (Weller, 2007). Biocontrol agents from *P. fluorescens* are rather nonspecific in their ability to protect plants from soil phytopathogens. Indeed, each biocontrol strain can typically act in more than one pathosystem i.e., protect more than one plant species from often distinct pathogens, provided the rhizosphere is successfully colonized. They have been mostly studied for protection of crop plants from phytopathogenic organisms. Plant protection may also result from direct interactions with the host plants, especially in the case of induced systemic resistance (ISR) (Bakker et al., 2007; Pal et al., 2011).

Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (Weller, 1988). These include the ability to (i) grow rapidly *in vitro* and to be mass produced; (ii) rapidly utilize seed and root exudates; (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; (iv) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles and growth-promoting substances); (v) compete
aggressively with other microorganisms; and (vi) adapt to environmental stresses. In addition, pseudomonads are responsible for the natural suppressiveness of some soils to soil-borne pathogens. There are two contexts in which biological control mediated by *P. fluorescens* strains and related pseudomonads has important practical implications. The first context corresponds to the use of biocontrol agents as inoculants of soil or plants, which has been successfully implemented in agronomic field trials (Amein *et al*., 2008; Karthikeyan and Gnanamanickam, 2008).

Current disease control methods are primarily based on chemicals, however, exclusive reliance on the use of fungicides often causes problems in disease management. Research reports to date concerning the biological control of the rice diseases, have described the isolation of many effective microorganisms and their efficacy has been tested in greenhouse and small field experiments (Charigkapakorn *et al*., 1991; Kanjanamaneesathian *et al*., 1998). Biological controls have been employed to reduce damage caused by bacterial leaf blight. Chemical control and host plant resistance, two of the most common management practices, have their limitations. Chemical pesticides harm the environment and host-plant resistance is based on a single gene, may not be durable in the field leading to frequent resistance breakdowns. It is imperative to develop environmental-friendly and sustainable control strategies. Biological control is an ecology-conscious, cost-effective, and sustainable alternative method in bacterial leaf blight management. This approach can also be integrated with other management practices to afford greater levels of protection and sustain rice yields. Antagonistic bacteria are considered as ideal biological control agents with obvious advantages. They are easy to handle, grow rapidly and colonize the rhizosphere aggressively (Weller, 1988). Certain strains of Bacillus spp. and Pseudomonas spp. have been used as biocontrol agents to suppress rice bacterial leaf blight (Vasudevan *et al*., 2002). A novel plant growth-promoting strain of *Delftia suruhatensis* HR4 has been shown to be a promising bio control agent against Bacterial leaf blight (Han *et al*., 2005).

In recent years the focus has shifted to the control of insect pests and diseases using biocontrol agents, which are a safe and promising alternative to synthetic pesticides. There is some evidence that endophytes can contribute to the control of plant disease (Kleopper *et al*., 1992; Ramesh *et al*., 2009). In India, limited work has
been done on the isolation of endophytic bacteria *viz.*, *Pseudomonas fluorescens* from stem and roots of chilli seedlings (Muthukumar, 2008) *Bacillus* sp., *P. fluorescens* and *Erwinia herbicola* from chickpea (Rangeshwaran et al., 2008). The internal tissues of plants provide a uniform and safe environment when compared to the rhizosphere and phylloplane where the introduced bacterial population must compete for nutrients and also endure temperature changes and exposure to UV rays. These advantages envisage the use of endophytic bacteria for more successful biological control of plant diseases (Sturz and Christie, 1995; He et al., 1999). Accordingly, *Pseudomonas fluorescens* commercial formulation is recommended as seed treatment, soil application and foliar spray to the transplanted rice crop (Jeyalakshmi et al., 2010).

Plant growth promoting rhizobacteria are a wide range of root colonizing bacteria with the capacity to enhance plant growth by increasing seed emergence, plant growth and crop yield. Soil or seed application of PGPR have been used to enhance growth of the several crops as well as to suppress the growth of the plant pathogens. Germinating seeds and growing plants influence the activities of soil microorganisms in the adjoining volumes of soil known as the spermosphere and the rhizosphere respectively. The microorganisms that colonize the rhizosphere profoundly affect root and plant biology in relation to nutrition, development and health. PGPR, promote plant growth and yield either directly or indirectly (Anandaraj and Bini, 2011).

Studies aimed at replacing chemical pesticides with environmentally safer methods are currently being a greater importance at this juncture. The biological control of soil-borne pathogens with antagonistic bacteria, particularly *Pseudomonas* spp. belonging to plant growth promoting rhizobacteria, has received prominent attention because of the dual role of these bacteria in plant-growth promotion and disease control (Zehnder et al., 2001). The genus *Pseudomonas* has been heterogeneous since Migula first named it in 1895. He designated and described the species associated with the genus in 1895 (Migula, 1895). Pseudomonas is Gram-negative, strictly aerobic, polarly flagellated rods. Soil pseudomonads possess a variety of promising properties, which make them better biocontrol agents (Cook, 1993). They are aggressive colonizers of the rhizosphere of various crop plants, and have broad spectrum antagonistic activity on plant pathogens, such as antibiosis (Cartwright et al., 1995; Rosales et al., 1995), siderophores production and nutrition or site competition (Bull et al., 1991). The importance of bacteria and fungi as sources
of valuable bioactive metabolites is very well established for more than half a century (Basha et al., 2012). As a result, over 120 of the most important medicines (penicillins, cyclosporin, adriamycine, etc.) in use today are obtained from microorganisms (Alanis, 2005). Disease control has been a major challenge for many bacterial diseases. Bacterial plant diseases affecting important agricultural crops can result in considerable damage and serious economic loss worldwide. They are becoming more difficult to control because bactericides in present-day use are not as effective as the past. Antimicrobials for prophylactic treatment of bacterial diseases of plants are limited in availability, use and efficacy and therapeutic use is largely ineffective. (Hanumanthappa et al., 2012). Host Plant resistance is an important component of an integrated management program for this disease. To minimize the risk of attack by bacterial blight, evolving resistant cultivars against the pathogen is the best non chemical method for management of the disease. To develop high yielding varieties with durable resistance to bacterial blight, it is necessary to understand the pathogen population structure.

The use of *P. fluorescens* biocontrol agents is thought to have a limited ecological impact on indigenous saprophytic populations and to take place without negative side-effects on rhizosphere functioning (Loccoz et al., 1998; Mark et al., 2006). Many inoculation products are commercially available (Mark et al., 2006), but Pseudomonas biocontrol strains may loose cell viability during biomass stabilization or subsequent storage of the inoculant product (Haas and De´fago, 2005). However, recent advances show that *Pseudomonas* formulation can be improved for long term storage and efficient antagonistic activity. Investigations on mechanisms of disease suppression by biological control agents including *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* have been well documented (Meera and Balabaskar 2010). However, little is known about disease suppression facilitated by attenuated mutants of *X. oryzae* pv. *oryzae* against subsequent infections with their parental strains. The active principle may act on the pathogen either directly or indirectly through induced systemic resistance (ISR) in host plants resulting in the reduction of disease development. ISR activates multiple defense mechanisms including increased activity of pathogenesis related proteins such as chitinase, β-1, 3-glucanase and peroxidases (POX), as well as the accumulation of phytoalexins (Kagale et al., 2004). Evaluation of biological control agents against various diseases of economic crops on the ISR
mechanism has been attempted under both greenhouse and field conditions (Prathuangwong and Buensanteai, 2006). However, no attempts have been made to understand the mechanisms of disease resistance induced by attenuated mutants of *X. oryzae pv. oryzae* against challenge-inoculation with their parental strains. Seven *Bacillus* plant growth-promoting rhizobacteria spp. were evaluated for growth promotion and induced systemic resistance in rice against *X. oryzae pv. oryzae*. Among the seven strains tested as fresh suspensions, talc and sodium alginate formulations under laboratory and greenhouse conditions, maximum germination of 86% was recorded after seed treatments with fresh suspension of *Bacillus subtilis* followed by 85% germination treated with *Bacillus pumilus* in comparison to only 71% germination in the untreated controls. Seed treatment with talc and sodium alginate formulation challenge inoculation with *X. oryzae pv. oryzae* increased accumulation of phenylalanine ammonialyase, peroxidase and polyphenol oxidase compared to untreated control seedlings. Thus, the results of this study suggest that the PGPR strains used as fresh suspensions and powdered formulations may have commercial potential in plant growth promotion and in management of rice bacterial leaf blight disease (Chithrashree *et al.*, 2011).