Chapter - 2

REVIEW OF LITERATURE

Asia is known as major source of spices from ancient times. Among 70 spice plants of world, 13 important spices are produced in Asia. Spices are important agricultural product which is life of any food. They have influenced the human civilization a lot. They were used by the ancient peoples like the Egyptian, the Arab and the Roman who extensively used them to add flavor to foods and beverages, as medicines, disinfectants, incenses, stimulants and many more. They were treated like gold and precious metals. The trading of spices is done enormously all over the world because only few selected countries can produce spices while on the other hand it is used by all. In India, chilly, turmeric, cumin seed & pepper are major spices produced in terms of their crop size. The contribution of India, Syria, and Turkey is more than 90% of cumin production. India is the world’s leader in production of cumin seeds, followed by Syria & Turkey. (Nazeem, 1995).

Crop production is adversely affected by plant diseases which destroy in agriculture as well as drastically affect modern agriculture system. These diseases are mainly caused by soil borne pathogens like *Pythium, Phytophora, Botrytis, Rhizoctonia, Fusarium* etc. These are responsible for most of the losses as they spread very quickly and affect crops of economic importance very severely. *Fusarium oxysporum* is a soil-borne facultative parasite that causes economically important losses in form of vascular wilt in a wide variety of crops. Fusarium species are the most important plant pathogens in the world (Booth 1971). Members of the *Fusarium oxysporum* species complex are phylogenetically diverse (Donell et al., 2008). It has two types of strains viz. pathogenic and nonpathogenic strains. Wilt disease is caused by plant pathogenic forms and are grouped into formae speciales (f. sp.) based on their host range. (Armstrong and Armstrong, 1981). Some of these are subdivided into pathogenic races. The reason for new outcomes of Fusarium wilt is said to be a recent introduction rather than an independent local origin of the pathotype. Asexual propagation is the dominant influence on population structure in
*F. oxysporum* and the absence of sexual reproduction is not likely to prevent this pathogen from continuing to inflict significant damage on susceptible crop hosts. (Gordon and Martyn, 1997). Fusarium species are filamentous phytopathogenic and mycotoxigenic fungi which seriously affects economically important cereals. Several Fusarium species causes Fusarium head blight (FHB), which reduces both crop yield and quality in cereals by releasing toxins which are dangerous for humans and animals (Mattila). Most of pathogenic fungi survive in soil for several years in the form of resting bodies for example sclerotia of *M. phaseolina* survive for 2-15 years in soil depending on environmental conditions (Cook et al., 1973; Short et al., 1980) *F. oxysporum* can survive in soil by means of chlamydospores for about 3 years (Haware et al., 1996).

In present scenario farming systems which is lifeline for the well-being of communities in many ways has destroyed natural habitats. This has lead to loss of not only species but also their ecosystem (Sachs et al., 2010). Many measures have been taken to protect plants. These may prevent plants from diseases but in the long run causes problems for human health (Horrigan et al., 2002). Greenhouse-gas are emitted by different agricultural practices which is the major cause of global warming (IPCC, 2007). Plant pathogens have tendency to emerge, re-emerge and can be endemic. This is the most important challenge of our ability to prevent plant growth and health worldwide (Miller et al., 2009). Thus we are constantly trying to develop environment friendly and sustainable crop production in this modern scientific era. Microbial inoculants containing microorganisms as beneficial plant-microbe interactions have a great potential in order to reach our target soon (Berg, 2009; Bhattacharjee et al., 2008).

No One knew about the microorganisms like fungi, bacteria until the discovery of microscope by Anton von Leeuwenhoek (1683). The utilization of bacteria to stimulate plant growth has been investigated since ancient times. Theophrastus (372–287 BC) suggested the mixing of different soil samples for remedying defects and adding heart to soil (Tisdale and Nelson, 1975).
The genus Fusarium has around 100 species. The research based upon deoxyribonucleic acid (DNA) sequence comparison is increasing gradually so this number is also being enhanced day by day. Most of the Fusarium species are associated with plants and are generally pathogenic. A lot of secondary metabolites (mycotoxins) are released by many species that cause damage not only to plants but are dangerous to human and animal health. Genetics of biosynthesis of mycotoxins and their mode of action is being reported by many research but still the exact importance and role of these compounds in pathogenic fungus needs to be explained. (Nicholson et al., 2009).

Varied and innovative strategies are used by these fungal plant pathogens to infect their host plants (Schafer 1994, Oliver and Osbourn 1995; Knogge 1998). Many pathogenic and virulent genes have been reported by Idnurm et al., 2001. They have characterized these genes on the basis of their support in the formation of infectious structures, degradation of cell wall, responses to the host environment, biosynthesis of toxins and other novel functions. We should try to have knowledge of these genes because this may help in understanding the complicated process of various diseases and can help in finding a suitable target for disease control.

Chehri et al., 2011 collected diseased cucurbit plants collected from fields in different geographic regions in Kermanshah province, Iran. They isolated 100 Fusarium isolates which were characterized as Fusarium oxysporum, Fusarium proliferatum, Fusarium equiseti, Fusarium semitectum and Fusarium solani based on their morphological characters. Then their pathogenicity on healthy cucumber (Cucumis sativus) and honeydew melon (Cucumis melo) was calculated in the glasshouse. It was found that F. oxysporum caused damping-off in 20–35 days on both cucurbit seedlings tested. Typical stem rot symptoms were observed within 15 days after inoculation with F. solani on both seedlings. This was the first report on identification and pathogenicity of major plant pathogenic Fusarium spp. causing root and stem rot on cucurbits in Iran.
The mechanisms of pathogenicity by this fungus is not understood clearly. (Beckman, 1987). The genes that encode cell wall-degrading enzymes which are the key factors in pathogenicity or virulence, have been cloned from *F. oxysporum* (Arie et al., 1998; Di Pietro and Roncero 1998; Huertas Gonzalez et al., 1999; Garcia et al., 2000). Their exact roles, however, are undefined. Due to difficulty in identification of fungi, studies on the diversity of fungi in natural environments have been hampered.

The distribution of *Fusarium solani f. sp. Phaseolina* was studied by Nash and Synder (1962), Burke *et al.* (1972) and Dryden and Alfen (1984) and they found that the fungus was distributed uniformly throughout the ploughed layer and their population decreased with increase in bulk density of soil with depth. Presence of other microorganism in soil may affect the pathogenicity of *Fusarium oxysporum* so knowledge of its survival and population dynamics in their presence is important. According to Hopkins *et al.*, 1987, when a monoculture of particular watermelon cultivar was grown, the soil became suppressive of fusarium wilt of watermelon. The population dynamics, chlamydospore germination of *Fusarium oxysporum f.sp. niveum* and the colonization of water melon roots by this fungus in relation to other microorganisms in soil was studied by Larkin *et al.*, 1993. He found that fungal population remained stable over six months period and their level was higher when added to conducive soils. At present very little is known about *Fusarium oxysporum f.sp. cumini* in relation to other microorganisms. According to Sunil *et al.*, 2004, the effect of fungi, bacteria, actinomycetes, total microbial population, soil moisture and soil temperature on the population dynamics of *Fusarium oxysporum f. sp. cumini* were studied in soils with or without a cumin (*Cuminum cyminum L.* ) crop at different soil depths. Their population increased progressively with continuous cultivation of cumin for two seasons, but remained almost stagnant in fallow soil without a host.

There are a number of techniques which can be used to observe genetic variation within fungal pathogens. One of these techniques is VCG which is based on the ability of the mycelium of fungi to anastomose to form heterokaryon that determines genetic relatedness. This was employed for the first time by Puhalla
(1985) to distinguish and classify strains of F. oxysporum. VCG diversity can be calculated by dividing the number of total number of VCG by the total number of isolate (Smith White et al., 2001). Vegetative compatibility has been used to study the origins and relatedness among plant pathogenic Fusarium sps. (Katan 1999). Isolates that belong to same VCG generally have pathological and physiological traits as well as geographical origins (Swift et al., 2002). In general, strains within a VCG are said to be more genetically similar than strains in different VCGs (Leslie 1993).

Identification and categorization of fungal pathogens cannot be done only on the basis of phenotypic measurements. Thus molecular identification strategies are employed. These are based on many factors like (i) the changing habitat of medically important fungi (ii) species-specific reports that reveal differences in antifungal susceptibilities of these newly recognized fungi (iii) morphological studies that demonstrates that morphology alone may not be a sufficiently objective method for species determination and (iv) a growing scarcity of bench scientists and microbiologists trained in traditional mycology (Balaji et al., 2011).

Molecular markers including random amplified polymorphic DNA (RAPD) can overcome limitations of other techniques, which are time-consuming. These molecular biology techniques are highly sensitive in differentiating various strains of F. oxysporum (Assigbetse et al., 1994; Kelly et al., 1994; Grajal-Martin et al., 1993). Many studies have been done for molecular characterization of Fusarium sps. in different plants. A study was done by (Arif et al., 2011) in which they identified and analysed genetic diversity among Fusarium isolates collected from malformed mango tissues. They used two texon selective primers, ITS-Fu-f and ITS-Fu-r, for faster identification of Fusarium spp. This study indicated a wide variability among different isolates of Fusarium. However, recent reports indicate that ribosomal DNA sequence analysis is a suitable tool which can be used to infer the phylogenetic relationships of fungi and to analyse the diversity of natural populations (Rodríguez et al., 2004; Ferrol et al., 2004b). Fingerprinting techniques, using gel electrophoresis of PCR-amplified rDNA fragments, can also be applied (Cornejo et
Recent advances in the genetic and genomics of the fungi have been reviewed (Ferrol et al., 2004a; Gianinazzi-Person et al., 2004; Parniske, 2004).

Molecular and pathogenic characterization of isolates of the pathogen from chickpea of diverse geographic origin for the first time by Daniel et al., 2011. They studied that Fusarium redolens caused wilting-like symptoms in chickpea in Lebanon, Morocco, Pakistan, and Spain. Pathogenicity assays were done using three chickpea plants and isolates from different geographic origins which indicated that F. redolens was not so virulent on chickpea. Similarly Kavak et al., 2006 studied resistance level of 26 Sesame breeding lines that were collected from 3 provinces within South eastern Anatolia district of turkey against wilt disease caused by Fusarium oxysporum f.sp. sesami. They performed pathogenicity tests on two local lines under controlled conditions.

With the discovery of chemical compounds it was thought that these soil borne pathogens can be eradicated permanently but it was found that application of these fungicides and insecticides is not safe for environment as they are highly toxic and cause environmental pollution. Not only this, but they adversely effect non-target organisms and cause tremendous harmful effect on human beings also. (Alabouvette and Couteadier, 1992). Certain fertilizers like ammonium sulphate etc. when added to soil, changes its pH due to which there develops a chance for change in community size as soil acidity favors Fusarium solani f. sp. phaseoli and Rhizoctonia solani but suppresses S. scabies and Verticillium alboatrum. Also when fertilizers are applied on forage directly, root exudation and in turn rhizosphere microflora are adversely affected (Rovira, 1965). Spectrum of microbial community is changed and their activity is increased when organic materials like crop residue, green manures etc. applied in field soil.

Baker and Nash (1965) noticed that disease of bean root caused by F.solani f. Phaseoli can be controlled with a high C: N ratio. Then Baker and Cook (1974) discussed that we can use crop residues as our strongest weapons for fighting soil-borne plant diseases without polluting the soil environment with pesticides which
cause many adverse effects in ecosystem. Inspite of these, they are being used freely to control pathogens on plants. Government has banned some pesticides for use in agriculture as they accumulate in the topmost trophic level of food chain (i.e. humans) during biological magnification process. Thus we have to reduce the use of these toxic chemicals in agricultural crops by utilizing the disease suppressive activities of some microorganisms which are antagonistic against targeted soil borne pathogens. The use of the pesticide and fumigant use and frequent appearance of fungicide resistant strains of Fusarium and V. dahliae are of increasing concern as they cause environmental pollution. (Safiyazov et al., 1995; Zhengjun et al., 1996; Berg et al., 2001; Tjamos et al., 2004; Çubukçu et al., 2007) Therefore, it becomes very difficult to manage the crop from the infection by fungi, which reduce the potential of crop rotation as a disease to control these pathogens. Nowadays researchers are very keen to find alternative control approaches for use in biological control strategies for crop diseases (Raupach and Kloeper, 1998). Regarding the environmental and health concerns, the extended use of some other control measures such as bio-control agents have been tried (Weller, 1988; O’Sullivan and O’Gara, 1992; Whipps, 1997; Emmert and Handelsman, 1999).

Over the past many years, research has constantly proved that bacteria and fungi have an intimate interaction with their host plants and they are able to promote plant growth as well as can suppress plant pathogens (Compant et al., 2005; Lugtenberg & Kamilova, 2009; Weller et al., 2002; Weller, 2007; Whipps, 2001). Many plant-associated fungi and bacteria have antagonistic activity against soil-borne pathogens and they could be utilized as biocontrol agents against wilt disease (Cook, 1993; Weller, 1988). Successful biocontrol application in form of pre-sowing treatments to control fusarium wilt has been reported in many ornamental plants (Hassan and Tawfik, 1996; Keinath, 1994; Postma and Rattink, 1992). Use of biocontrol agents leads to increase in plant growth and development (Baker et. al., 1984; Chang et al., 1986; Hassan, 1992; Linderman, 1994; Ousley et al. 1994). With technological advancements biocides industry that used biological control agents is currently developing that can establish a realistic alternative to chemicide used extensively in crop production. Biological control of plant pathogens has begun from 1900s when inhibitory activity of plant pathogens was
reported by Potter (1908). It was due to accumulation of its own metabolic compounds. Sanford (1926) suggested that diseases like potato scab can be controlled by using green manure. He proposed two concepts that can be used for controlling disease. (a) using saprophytic microorganisms for controlling plant pathogens and (b) by alteration of soil conditions that may lead to change in the microbial population of soil. He proposed that the activity and multiplication of saprophytes is promoted when fresh organic material is added in the soil. This happens due to competition for nutrients and oxygen that reduces the activity and multiplication of the pathogens. Millard and Taylor (1927) have also reported that scab of potatoes grown in sterilized soil can be controlled by inoculating with Streptomyces scabies along with simultaneous inoculation of the soil with saprophyte S. praecox. Then experimental evidence was provided by Sanford and Broadfoot (1931) in support of Sanford's original hypothesis in which he stated that infection of wheat seedlings by Ophiobolus graminis in sterilized soil could be completely suppressed by antagonistic action of various co-inoculated species of fungi and bacteria.

Researches based on biological control have been done with divergent approaches and practical uses in which different microorganisms like fungi, bacteria etc. have been tested for their disease suppressive capabilities. Most of the soil borne pathogen are fungi so biocontrol by fungi has been experimented on large scale. (Henis et al., 1979; Baker, 1987; Suarez et al., 2004). The terms “biological control” or “biocontrol” is used in different fields of biology. Such microorganisms that can be used as bio pesticides for controlling plant pathogens are called biocontrol agents. In the field of plant pathology, biocontrol is suppression of diseases by using microbial antagonists or host specific pathogens to control weed populations. Many definitions of biocontrol have been reported based on various parameters like target which is to be suppressed; type and source of biological agents; and the degree and timing of human intervention. Basic definition of Biological control is the suppression of harmful activities of one organism by another organisms referred to as natural enemies. Thus, its intentional utilization of introduced living organisms to suppress the activities and populations of one or more
plant pathogens. This cannot be used in disease resistant host plants. In this method of disease control, microbial inoculants can be used to suppress a single type or class of plant diseases or by soils that can be managed to promote the combined activities of native soil and plant-associated organisms that contribute to general suppression.

Many research studies on biological control against diseases and pests of agricultural crops has been done between 1973 and 2008. The published data that consists of a survey of the CAB Abstracts® database shows that there is a constant increase in the annual number of these publications. It has increased from 20 in 1973 to over 700 per year diseases since 2004, based on a survey of the CAB Abstracts® database. It has been known that there is a good interaction among plants and pathogens with a wide variety of organisms throughout their lifecycle which can significantly affect plant health in various ways. In order to understand biological control mechanism, it’s important to understand the different ways of their interactions like mutualism, protocooperation, commensalism, neutralism, competition, amensalism, parasitism etc. (Odum (1953). Beattie, 2006., stated those bacteria that can reduce the incidence or severity of plant diseases are known as biocontrol agents while those that exhibit antagonistic activity towards a pathogen are called antagonists. Some factors responsible for antagonistic activities in rhizosphere can be summarised as (1) by synthesis of hydrolytic enzymes, such as chitinases, etc. that can lyse pathogenic fungal cells (Neerja et al., 2010, Maksimow et al., 2011) (2) competition for nutrients and favourable colonization of niches at the root surface (Stephens et al., 1993). Kamilova et al., 2005), Validov S, 2007, PhD thesis, Leiden University, The Netherlands), (3) by regulation of plant ethylene levels through the ACC-deaminase enzyme that can modulate the level of ethylene in a plant in response to stress imposed by the infection (Glick and Bashan 1997., Van Loon 2007). Sedra and Malouhy (1994) characterized six antagonists against F. oxysporum f.sp. Albedinis from 420 samples which were obtained from conducive and suppressive soils. These antagonists suppressed the growth of F. oxysporum f. sp. albedinis in vitro by 24-47 per cent and its sporulation by 70-99 per cent. Gupta et al. (1999)
isolated *P. aeruginosa* from potato rhizosphere. It showed a strong antagonistic activity against fungal pathogens like *Macrophomina phaseolina* and *Fusarium oxysporum*.

**Dubey and Dwivedi (1988)** studied the fungi in the root region of soyabean at varied growth stages of the plant. It was reported that numbers of soil fungi increased both qualitatively and quantitatively when plant grew from seedling stage to flowering stage. After this their frequency reduced at the senescence stage. Nowadays, alteration of soil environment is being used as a tool for biological control of soil borne plant pathogens. Numerous attempts have been taken to control other formae specials of *F. oxysporum*, that infect commercial crops such as cumin, banana, chickpea and tomato (**Mohammed et al., 2011; Kaur et al., 2007; Haggag and Abo-Sedera, 2005; Chandel et al., 2010**).

Cumin is one of the oldest spice crop that is grown extensively in the arid and semi arid regions of India. It is mainly cultivated in the states of Rajasthan, Gujarat, Madhya Pradesh, Haryana, Punjab, Uttar Pradesh and Bihar. Rajasthan is the largest contributor in its production due to its cultivation in Jalore, Barmer, Nagaur, Jodhpur, Pali, Ajmer and Tonk districts (**Arora et al., 2004**). It is grown for production of the dry ripe fruits. Egyptians knew as a spice and medicinal plant. Besides being used as spice in our daily life, recent studies have indicated its pharmaceutical and medicinal importance (**Aruna and Sivaramakrishnan, 1996**). Its cultivated during winter season under irrigation of more than 300,000 ha (**Singhal, 1999**). Cumin has been used since ancient times. It has been originally cultivated in Iran and Mediterranean region. The name of cumin has been mentioned in the Bible in the Old Testament (Isaiah 28:27) and New Testament (**Matthew 23:23**).

The ancient Greeks used to keep cumin at the dining table in its own container and this practice was continued in Morocco. Cumin was also used heavily in ancient Roman cuisine. Then Spanish and Portuguese colonists introduced it to the America. Its production is however low due to two reasons viz. plant disease and improper soil fertilization. Most of the yield losses is due to wilt disease caused by *Fusarium*
oxysporum f sp. Cuminum (Lodha et al., 1986) which is decreasing cumin production worldwide like Argentina (Gaetan and Madia, 1993), Egypt (Arafa, 1985), Greece (Pappas and Elena, 1997) and India (Champawat and Pathak, 1990). The incidence of this disease is very frequent in soils so farmers are forced to stop cumin cultivation after three successive years of cropping AzzaTawfik et al. (2004) isolated twenty fungus and ten bacterium isolates from Fusarium wilt disease infected cumin plants. Till date no wilt resistant variety has been developed so one only uses physical and biological control of pathogens. (Lodha, 1995; Mawar and Lodha, 2002). In order to use these methods properly for controlling soilborne pathogens, one should have thorough knowledge of quantitative and qualitative aspects of inoculum dynamics.

Table 2.1: A list of fungal pathogens that have been reported to be suppressed by rhizobacteria:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of Bacteria</th>
<th>Pathogen suppressed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arthrobacter</td>
<td><em>Fusarium oxysporum f.sp. dianthi</em></td>
<td>Sneh et al. (1984)</td>
</tr>
<tr>
<td>2</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td><em>R. solani</em></td>
<td>Rosales et al. (1995)</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus coagulans</em></td>
<td><em>F. moniliformae</em></td>
<td>Pal et al. (1996)</td>
</tr>
<tr>
<td>4</td>
<td><em>P. glumae</em></td>
<td><em>P. ultimum</em></td>
<td>Pal et al. (1996)</td>
</tr>
<tr>
<td>S.No.</td>
<td>Name of Bacteria</td>
<td>Pathogen suppressed</td>
<td>Reference</td>
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</tr>
<tr>
<td>5</td>
<td><em>Bacillus subtilis</em></td>
<td><em>R. solani</em></td>
<td>Merriman et al. (1974)</td>
</tr>
<tr>
<td>6</td>
<td><em>B. subtilis</em></td>
<td><em>R. solani</em></td>
<td>Rosales et al., 1995</td>
</tr>
<tr>
<td>7</td>
<td><em>Rhizobium</em></td>
<td><em>M. phaseolina</em></td>
<td>Chakraborty et al., 1984</td>
</tr>
<tr>
<td>8</td>
<td><em>P. fluorescens</em></td>
<td><em>S. rolfsii</em></td>
<td>Jagadeesh et al. (1998)</td>
</tr>
<tr>
<td>9</td>
<td><em>Bacillus subtilis</em></td>
<td><em>F. oxysporum f. sp. cucumerinum</em></td>
<td>Bin et al., 2005</td>
</tr>
<tr>
<td>10</td>
<td><em>Bacillus cerius</em></td>
<td><em>Fusarium oxysporum</em></td>
<td>Becker et al., 1988</td>
</tr>
<tr>
<td>11</td>
<td><em>Bacillus subtilis</em></td>
<td><em>Pythium ultimum</em></td>
<td>Bashan et al., 2002</td>
</tr>
<tr>
<td>12</td>
<td><em>Pseudomonas putida</em></td>
<td><em>Fusarium oxysporum f. sp. raphani</em></td>
<td>Kloeppe et al., 1991</td>
</tr>
<tr>
<td>13</td>
<td><em>Pseudomonas fluorescens</em></td>
<td><em>Fusarium oxysporum f. sp. dianthi</em></td>
<td>Van Peer et al., 1991</td>
</tr>
<tr>
<td>14</td>
<td><em>Burkholderia cepacia</em></td>
<td><em>S. rolfsii</em></td>
<td>Jayaswal et al., 1992</td>
</tr>
<tr>
<td>15</td>
<td><em>P. fluorescens</em></td>
<td><em>Pyricularia</em></td>
<td>Chatterjee et al., 1996</td>
</tr>
<tr>
<td>16</td>
<td><em>P. aeruginosa</em></td>
<td><em>F. oxysporum f.sp. cucumerinum</em></td>
<td>Simeoni et al., 1987</td>
</tr>
<tr>
<td>17</td>
<td><em>Pseudomonas fluorescens</em></td>
<td><em>R. Solani,M.phaseolina</em></td>
<td>Indra et al., 2011</td>
</tr>
<tr>
<td>18</td>
<td><em>Bacillus safensis</em></td>
<td><em>Erwinia tracheiphila</em></td>
<td>Zehnder et al., 2000b</td>
</tr>
<tr>
<td>19</td>
<td><em>Bacillus subtilis</em></td>
<td><em>Ralstonia solanacearum</em></td>
<td>Ji et al., 2008</td>
</tr>
<tr>
<td>20</td>
<td><em>Bacillus subtilis</em></td>
<td><em>Phytophthora capsici R. solani</em></td>
<td>Ahmed et al., 2003</td>
</tr>
</tbody>
</table>

The first step towards the path of development of effective biological control is to identify effective antagonist strains. Then in order to implement it practically one has to check that the the antagonists must be ecologically fit to survive and easily establish and function within the particular conditions of the ecosystem. For this one has to collect much more information about the mechanisms of action, ecological fitness and interactions with the soil and rhizosphere microbial communities. After identification of organisms as potential antagonists, the specific mechanisms,
interactions, conditions and requirements responsible for effective biological control has to be determined. A thorough knowledge of these characteristics makes it possible to establish the limitations as well as the full potential of biocontrol to develop strategies for management and implementation. Slininger et al. (2003) developed such indices viz., relative performance index based on bioagents growth and their antagonistic activity under different conditions. Not only this but rapid identification of biocontrol agents can be done by the use of different markers viz., antibiotic production and the other regulatory mechanisms by bioagents. This type of screening and selection strategy is an excellent base for successful development and commercialization of potential biocontrol agents.

Extensive study has been done on biological control by antagonistic organisms and rhizobacterial strains have emerged as potential biocontrol agents for the control of root and foliar diseases (Anuratha and Gnanamanickam, 1990; Raupach and Kloepfer, 1998 and Ramamoorthy et al., 2002b). Measures taken for soil treatment with antagonist microbes have produced excellent results against various soil-borne pathogenic fungi.

A variety of soil microorganisms are present in the rhizosphere and rhizoplane regions of a plant. Their number remains many times more than the non-rhizosphere one. The German agronomist Hiltner has first defined the rhizosphere, in 1904. He defined it as the effect of the roots of legumes on the surrounding soil, in terms of higher microbial activity where a vast number of macroscopic organisms and microorganisms such as bacteria, fungi, protozoa and algae coexist. Bacteria are the leaders in terms of number among them Plants select those bacteria that give their maximum contribution to their fitness by releasing organic compounds through exudates (Lynch, 1990), thus creating a very selective environment (Lucas et al., 2001; Marilley and Arango, 1999).

Many studies have been done that demonstrate that soil-borne microbes interact with plant roots and soil constituents at the root–soil interface (Lynch, 1990; Linderman, 1992; Glick, 1995; Kennedy, 1998; Bowen and Rovira, 1999; Barea
et al., 2002b). This root–microbe interactions leads to development of a dynamic environment known as the rhizosphere where microbial communities also interact.

Rhizosphere is the immediate area around plant roots with increased microorganisms where the biology and chemistry of the soil are influenced by the root. In the rhizosphere, very important and intensive interactions take place between the plant, soil, microorganisms and soil microfauna which is greatly influenced by compounds exuded by the root and by microorganisms feeding on these compounds (Antoun and Prévost, 2006). All this activity makes the rhizosphere the most vibrant environment in the soil.

Gobat et al. (2004) have categorized rhizosphere inn three regions viz. (i) endorhizosphere which is the interior of the root; (ii) rhizoplane which is the surface of the root); and (iii) rhizospheric soil that adheres to the root when the root system is shaken manually. The soil that is not affected by the root is known as non-rhizospheric soil or bulk soil. The rhizosphere is considered to be the front-line of defense between plant roots and soil-borne pests. The increase in microbial number and their activity have been reported as 'rhizosphere effect'. Secretion of growth promoting substances like root exudates or casting of sloughed off root tissues by plants in soil during their growth phases are the prime reasons for this rhizospheric effect. (Baker and Sayder, 1965; Rovira, 1965; Whipps 1990; Morgan and Whipps, 2001). The Rhizosphere effect is more prominent in deserts than in other soils (Buyanovsky et al., 1982; Yechieli et al., 1995). There is lot of carbon substrate present in rhizosphere. This makes it a very significant place for different microbial activity. The deserts lack green plants and dont have much life but they possess ample microbes having extraordinary capabilities that are yet to be explored completely but unfortunately rhizosphere in desert plants has not been studied on large scale (Bhatnagar and Bhatnagar, 2005). It has been reported that the ratio of R: S (rhizosphere: soil) is high in arid soils for almost all metabolic types of bacteria and fungi in most plants studied (Khathuria et al., 1998, Mahmoud et al., 1964; Elwan et al., 1970). Diverse and dense microbial community consisting of plant growth-promoting rhizobacteria and biocontrol agents is present in potato rhizosphere, mycorrhizosphere which has been studied by (Diallo et al., 2011).
The rhizosphere zone has been defined as the volume of soil directly influenced by the presence of living plant roots or soil compartment influenced by the root (Hiltner 1904). Rhizosphere supports a large and active microbial population which are capable of exerting beneficial, neutral and detrimental effects on the plants. Rhizobacteria (root colonizing bacteria) that exert the beneficial effects on the growth of the host plant via direct or indirect mechanisms are termed as plant growth promoting rhizobacteria (PGPR) (Juanda, 2005). The plant-microbe interactions in the rhizosphere are responsible for increasing plant health and soil fertility (Khan, 2006; Hellriegel and Wilfarth, 1888) investigated the rhizosphere root colonization in grasses and legumes and suggested the ability of soil bacteria to convert atmospheric N2 into plant usable forms. Based on their experiments on radishes, Kloepper and Schroth (1978) introduced the term ‘rhizobacteria’ to the soil bacterial community that competitively colonized plant roots and stimulated growth and thereby reducing the incidence of plant diseases.

Kloepper and Schroth (1981) have termed the beneficial rhizobacteria as plant growth-promoting rhizobacteria (PGPR). A potential rhizobacteria is called as Plant Growth promoting Rhizobacteria (PGPR) when it causes positive and favourable effect on the plant upon inoculation. It is the most significant member of rhizosphere biota that can help in stimulation of growth of the host when grown in association with the host plants. PGPR are beneficial bacteria that colonize plant roots and enhance plant growth with a wide variety of mechanisms. They are looked upon as a successful rhizobacteria in getting established in soil ecosystem as they have better adaptability in a wide variety of environments, faster growth rate and biochemical versatility to metabolize a wide range of natural and xenobiotic compounds. Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, isolated from the rhizosphere, which, when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens (Kloepper et al., 1980). Bacteria that colonize roots effectively are termed “Rhizobacteria” (Schroth and Hancock, 1982). Root colonization is the process where bacteria survive on seeds, multiply in spermosphere in response to exudates released from seeds that are rich in carbohydrates and amino acids (Kloepper et al., 1989). They attach on to the
surface of roots (Suslow, 1982) and colonize around the developing root system. These are group of root associated bacteria that intimately interact with the plant roots and consequently influence plant health and soil fertility. Generally about 2-5% of rhizosphere bacteria are PGPR (Antoon and Prevost, 2005). They produce bioactive substances to promote plant growth and to protect them against pathogens (Harish et al., 2009). They have traits which are useful in disease control. Microbial activity in the rhizosphere indicates how metabolically active the microbial communities are using PGPR as inoculants in soil. Besides altering the structure of communities, it also influence microbial activity, and this could be related to the survival of the PGPR in the environment (Ramos et al., 2003). They help in uptake of nutrients from the soil through different direct mechanisms such as atmospheric nitrogen (N) fixation, solubilization of phosphate, and synthesis of siderophores for iron sequestration, thus making nutrients more available to plants (Glick et al., 2007).

Several rhizobacteria were isolated by Earnapalli et al. (2005). Potential strains were screened and selected that showed antagonistic activity against A. Solani. They worked on the biocontrol potential of ten efficient antagonistic bacteria against early blight of tomato in a glasshouse. Results revealed that seven strains possessed high biocontrol potential. Out of these, Pseudomonas strain B-25 was found to be the most promising strain with a disease control of 57.78 per cent and good plant growth promotion.

After such a exhaustive research of so many years we have not succeeded in identifying suitable rhizobacteria that participate in plant productivity completely. The rhizosphere and its association with the roots is very complex that cannot be understood easily. A small part of rhizospheric soil has millions of genomes so its very difficult to characterize all of them and to understand their functional role in increasing plant productivity. Past research has provided generalized approaches in which the first two is based on cultural techniques. In the first approach, isolation of many rhizobacteria is done through cultural means which are then screened for their plant growth promotion potential by various plant growth assays. This is the most common and generalized approach. This approach often is
dependent on cultural technique to But this approach has many problems as in this only small part of rhizosphere is sampled and all other interactions are neglected during assessment of whole rhizobacterial community.

In the second approach, screening of bacterial isolates is done functionally based on various functional assays for the identification of functional characteristics like production of antibiotics, evaluation for rhizosphere competence for general community combining ability, production of siderophores to chelate nutrients, production of hormones and release of compounds that improve soil properties that are thought to be connected to plant growth promotion and, at times, has difficulty correlating the functional assay results to plant productivity response.

The third approach is the metagenomic approach that is based on characterizing part of or the entire rhizosphere combined genome so that its contribution to productivity can be understood completely. Other molecular approaches are based on phylogenetic community structure using the 16S rRNA gene, a gene that is most often used for prokaryotic identification. The metagenomic is also called as molecular approach. Its very efficient than other approaches because it does not depend on cultural technique like other approaches. In this approach many strong and highly efficient advanced technologies and bioinformatic analytical tools are used to characterize the community in greater depth. The results obtained by this approach is quite helpful.

**Seint et al., 2012** screened antagonistic rhizobacteria against *Rhizoctonia solani, R. oryzae-sativa* and *Sclerotium hydrophilum* that are responsible for sheath diseases of rice. The objective of their study was to find out the potential antagonist microbes from the paddy field soil. They collected 10 different soil samples from the disease-prone area of paddy field soil. Then Antagonist microbes were identified by 16S rRNA sequence analysis. On furthur screening *B. subtilis* B37 and *P. aeruginosa* B258 emerged as the potential antagonist microbes to control sheath diseases of rice in Myanmar. *Bacillus subtilis* B37 and *P. aeruginosa* B258 could be used as control agents in the control strategy of causal agents of rice sheath diseases in Myanmar.

**Anith et al., 2003** isolated bacterial antagonists of *Phytophthora capsici* from underground shoot part of black pepper. First of all screening of isolates was done by
dual culture on potato dextrose agar and carrot agar. Finally, another screening was done on shoots of black pepper in order to observe suppression of lesion caused by the pathogen. They found that the level of antagonism shown by various antagonists was different in the dual culture and the shoot assay. This screening that involved the host, pathogen, and the antagonist, performed on black pepper shoot could be used as a quick and reliable method for the isolation of efficient bacterial antagonists of P. Capsici and other plants.

Garrett (1956) has discussed the significance of production of antibiotics by soil microbes. Their possible role in biological control by antibiosis and fungistasis, has been discussed (Lingappa and Lockwood (1961); Jackson (1965), and Dwivedi and Saravanamuthu (1985). In present decade, many research have been done that can lead to quick characterization of antagonists that can be applied in biological control of plant pathogens in different ways (Cook, 1977; Old, 1977; Chakraborty et al, 1983; Elad et al, 1984; Manocha, 1985; Dwivedi, 1986). Calvo et al., 2010 have also screened Bacillus strains from the rhizosphere of native potato varieties among which 91% showed antagonistic activity against Fusarium solani.

Ei-Mohamedy et al. (2011) isolated two fungal isolate (Trichoderma harzianum and Trichoderma viride) and two bacterial isolate (Bacillus subtilis and Pseudomonas fluorescens) from rhizospheric soil of healthy broccoli plants that were used as bio-control agents for controlling broccoli root rot disease caused by Pythium ultimum and Rhizoctonia solani pathogens.

Hyun et al. (1999) isolated Bacillus polymyxa strain KB-8 from the culture of an antagonist against Fusarium oxysporum f. sp. sesami. They tested for the control of Fusarium wilt of sesame in greenhouse conditions. Optimum conditions for culturing the antagonist to obtain the maximum antibiotic activity were determined using different culture media, initial medium acidity, and incubation periods, for
which yeast–malt extract agar with the initial acidity of pH 5 and over 13 days culture were the best

Now-a-days in most of the countries researches are being carried out to induce antagonistic potential and production of mutants adjustable to stress conditions. It is known that a suitable antagonist, present in rhizosphere, antagonise the pathogen and control the disease. But sometimes it fails perhaps due to its low inoculum potential. So, some of the methods have been developed which (i) can decrease pathogenicity of the pathogens, (ii) increase hosts resistance, and (iii) stimulate the antagonistic potentialities and intensify their activity. Also plant diseases can be managed by the use of resistant cultivars which is one of the most practical and cost efficient strategies. However, its efficiency is very low due to variability in pathogen populations, including the existence of pathogenic races and pathotypes (Jiménez-Gasco et al. 2004). By increasing the use of chemical inputs several negative effects are caused like the development of pesticide resistance to applied agents. Not only this but these chemicals also have an effect on non-targeted environmental impacts (Gerhardson 2002).

Pathak and Dwivedi (1981) have reported that population of antagonistic fungi, like Aspergillus terreus, Penicillium citrinum Cephalosporium roseogriseum, T. viride etc. increased in soil which are treated with fungicides, insecticides and herbicides etc. due to which the wilt of tomato caused by Fusarium oxysporum f. lycopersici decreased. However, pesticide-stimulated antagonists in soil may help in the integrated system of disease control in biocontrol systems, (Marois and Mitchel, Till date aproximately million tonnes of different chemical fertilizers have been used by farmers so that yield of crop could be enhanced.(Glick et al., 1999). Though its used extensivly but the potential negative effect of chemical fertilizers on the global environment is not unknown by common man. So nowadays research is being focussed on replacement of chemical fertilizers with biocontrol agents in any form. Those strains that show aggressive colonization, plant growth stimulation or biocontrol can be classified as PGPR. They can fulfill all of these or some characteristics.(Weller et al. 2002; Vessey 2003). These microbial strains are posing
to be a good substitute to chemical fertilizers, pesticides so are being used on large scale in agricultural practices. The association of Plant growth promoting rhizobacteria (PGPR) and the plant rhizosphere was studied by Siddiqui (2006). He found that they play a major role in the bio control of plant pathogens as they can suppress a many bacterial, fungal and nematode diseases.

Usha Rani et al. in 2012 isolated the PGPR from the rhizosphere soil of pigeon pea and characterized them for their ability to promote growth of pigeon pea. The antagonistic nature of selected strains against various pathogen were estimated by different methods. Then the best one was selected. These were further investigated to observe different PGPR traits like pigeon pea seedling emergence, increase of shoot length, root length, dry matter production of shoot, nodule number and nodule mass etc.

According to Whipps (2001) there are three basic categories of interactions that generally exists between the rhizobacteria and growing plants. These are neutral, negative or positive interactions. Most rhizobacteria associated with plants are commensals where no effect on growth or plant physiology is observed though there exists close interaction of bacteria with the host plants (Beattie 2006). In negative interactions, phytotoxic substances such as hydrogen cyanide or ethylene are given out by the phytopathogenic rhizobacteria due to which there is negative influence on the growth and physiology of the plants. Apart from these bacteria, there are some rhizobacteria that can exert a positive plant growth through direct mechanisms like solubilization of nutrients, nitrogen fixation, production of growth regulators, or by indirect mechanisms like stimulation of mycorrhizae development, exclusion of pathogens due to competition or removal of phytotoxic substances (Bashan and de-Bashan 2010).

Rhizosphere, phyllosphere and soil environment can be altered artificially by many methods which have been developed by researchers. This leads to increase in number of antagonistic microorganism. The methods used are (i) by artificial introduction of antagonists in soil or by spraying the antagonists on the aerial parts of plants, (ii) by modification of soil environment by organic amendments, (iii) through green manuring, changing soil pH, C: N ratios,
temperature, and (iv) by addition of selective chemicals or heat treatment of plant tissues (Garrett, 1965; Baker and Snyder, 1965; Baker and Cook, 1974). Biological control strategies can be used as an alternative management measures or integrated with other practices for combating various diseases.

Bacterial inoculants which help in plant growth are generally considered to be of two types a) symbiotic and b) free-living (Kloepper et al., 1988; Frommel et al. 1991). Beneficial free-living bacteria referred to as PGPR are found in the rhizosphere of the roots of many different plants (Kloepper et al., 1989). Breakthrough research in the field of PGPR occurred in the mid 1970s with studies demonstrating the ability of Pseudomonas strains capable of controlling soil-borne pathogens to indirectly enhance plant growth and increase the yield of potato and radish plants (Burr et al., 1978; Kloepper and Schroth, 1981; Kloepper et al., 1980; Howie and Echandi, 1983). Burr et al. (1978) and Kloepper et al. (1980) first reported that there is improved plant growth and biological control of root pathogens with rhizobacteria is possible due to seed bacterization. They reported the plant growth promoting effects of Pseudomonas strains that showed antagonistic activities against wide range of plant pathogens under in vitro conditions. Their studies gave first proof that the rhizosphere micro-organisms could be modified significantly with microbes introduced with the planting material. Many authors in the last two decades have done research on these and published their work with recent applications on trees (Bashan and Holguin, 1998; Enebak et al., 1998). Novel techniques for identification and characterisation of PGPR, and study of colonization pattern and molecular determinants of root colonization have been discussed (Lugtenberg et al., 1991, 2001; Rothballer et al., 2003; Espinosa-Urgel, 2004; Gamalero et al., 2004).

During 1983 and 1984 more than 4,000 bacterial strains have been isolated from the rhizosphere region of plants grown in the Canadian High Arctic and screened for the ability to fix nitrogen. Some strains demonstrated the ability to reduce acetylene and colonize roots of canola when grown at low temperatures. (Lifshitz et al. 1986).
Different biocontrol agents that consists of bacteria belonging to the genera Bacillus, Pseudomonas, and fungi has been discussed successfully. The results where pathogenic fungal growth is reduced in vitro and disease development in vivo are significant. Strains which exhibited the potential to be PGPRs were identified as *Pseudomonas putida*, *P. putida biovar B*, *P. fluorescens*, *Arthrobacter citreus* and *Serratia liquefaciens* (Lifshitz *et al.*, 1986; Klopper *et al.*, 1988). The ability of these strains to be used as bacterial inoculants in agriculture was tested in greenhouse and field trials with different formulations and they increased the yield of canola in both types of trial. *P. putida* solubilises Phosphate (P) in the rhizosphere (Richardson, 2001) and induces denitrification (Prescott *et al.*, 2002), due to which availability of nutrients increases to the host plants.

Pseudomonades are a major group of rhizobacteria found in agricultural soils which have great potential for biological control. Many studies have been done on characterization of root colonization by pseudomonads. The biotic and abiotic factors affecting colonization, contribution of bacterial traits and genes to rhizosphere competence, and the mechanisms of pathogen suppression has been studied widely. They have many characteristics due to which they can be suitably used as biocontrol and growth-promoting agents. They can grow rapidly in vitro so can be mass produced. They have ability to quickly utilize seed and root exudates. They can colonize and multiply in the rhizosphere and in the surrounding easily. Many bioactive metabolites like antibiotics, siderophores, volatiles, and growth-promoting substances are released by them. Above all they can adapt quickly to environmental stresses. They use combination of multiple mechanisms for effective biocontrol that includes direct antagonism and induction of plant resistance. Many metabolites are produced by them that have antimicrobial activity against other bacteria and fungi (Haas and Keel, 2003). Asha *et al.*, (2011) isolated Ten *P. fluorescens* from rhizosphere soil samples collected from various tomato-growing fields and evaluated for their efficacy in increasing seed quality variables of tomato and in inhibiting the mycelial growth of *Fusarium oxysporum*. Pseudomonas isolate produced effective results and was selected and mass multiplied.
Elicitation of induced systemic resistance (ISR) by plant-associated bacteria using Pseudomonas spp. was first of all reported by Kloeppler et al. (2004). Several reviews have discussed various aspects of the large volume of literature on Pseudomonas spp. as elicitors of ISR, showing that specific strains of the species B. amyloliquefaciens, B. subtilis; B. pasteurii, B. cereus, B. pumilus, B. mycoides, and B. sphaericus elicit significant reductions in the severity of various diseases on a diversity of hosts. Demonstration of elicitation of ISR by these strains has been done in greenhouse or field trials on tomato, bell pepper, muskmelon, watermelon, sugar beet, tobacco, Arabidopsis sp., cucumber, loblolly pine etc. Protection that results from ISR elicited by Bacillus spp. has been reported against fungal and bacterial pathogens, systemic viruses, a crown-rotting fungal pathogen, root-knot nematodes, and a stem-blight fungal pathogen as well as damping-off, blue mold, and late blight diseases. Generally, Bacillus spp. not only elicits ISR but also elicits plant growth promotion.

Zaghloul et al. (2008) reported that control of root-rot and wilting diseases of tomato can be done effectively by using bio-control agents like Trichoderma harzianum-I, Pseudomonas fluorescens-II and Bacillus subtilis-I which have been reported to show antagonistic activity against Rhizoctonia solani, Sclerotium rolfsii and Fusarium oxysporum f.sp. lycopersici. Not only this but also lowest records of disease severity of tomato can be achieved by the seed dressing of tomato and soil drenching with bio-control agents. The hazardous effects of fungicides can be reduced by the application of such inocula that protect the environment from pollution and also help in maintenance of the human health.

Antagonistic potentiality under laboratory and greenhouse conditions of Trichoderma harzianum, T. viride, Pseudomonas fluorescens and Bacillus subtilis against Fusarium oxysporum f.sp. Cumini was tested by Chawla and Gangopadhaya (2009). They studied the effect of organic amendments viz., farm yard manure, and vermicompost and mustard cake on disease control efficacy of four antagonists against Fusarium wilt. Maximum inhibition of mycelial growth of F. oxysporum f.
*sp. cumini* was recorded in presence of *P. fluorescens* which was closely followed by *T.harzianum*. These bioagents suppressed the pathogen population in soil and also increased the shoot and the root lengths and dry weight of cumin plants.

The first experimental demonstration that an antibiotic produced by bacteria could suppress plant disease in an ecosystem was demonstrated by **Tomashow and Weller (1988)**. They followed an elegant genetic approach in which they demonstrated the direct correlation between the production of a phenazine antibiotic by a fluorescent Pseudomonas sp. and its biocontrol activity against take-all disease of wheat. Competition is another important factor in the antagonistic properties of Pseudomonas spp. In addition to competition for substrates (**Couteaudier and Alaboubette, 1990**), research on the siderophores produced by Pseudomonas species (pyoverdine, pyochelin) has shown the involvement of siderophore-mediated competition for iron in the control of Fusarium and Pythium in soils (**Duijff et al., 1994; Raaijmakers et al., 1995**). Another example that illustrates a combination of mechanisms for successful antagonism of plant pathogens is observed by many filamentous fungus. The direct mechanisms involved in this protective effect include competition, antibiosis (**Howell, 1998**), and mycoparasitism (**Jeffries, 1997**). Many reports show the potential of combining different biocontrol agents with different disease-suppressive mechanisms in the field (**de Boer et al., 1999, 2003**). By developing appropriate combinations one can get a higher level of plant protection, a wider range of effectiveness and a reduction of variability in the results. Thus, by optimal use of the antagonistic properties of the microbiota, a more effective and reliable biocontrol of plant pathogens can be achieved which constitutes a very promising research area. Not only this but pseudomonads are responsible for the natural suppressiveness of some soils to soil borne pathogens. The major weakness of pseudomonads as biocontrol agents is that they cannot produce resting spores due to which their commercial use becomes difficult (**Kremer et al., 1998**).

Fluorescent Pseudomonas spp. has been studied for decades for their plant growth-promoting effects through effective suppression of soil borne plant diseases.
(Weller et al., 1988, 2002, 2007). Among various biocontrol agents, Fluorescent pseudomonads, equipped with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion, are being used widely. (Banasco et al., 1998, Dileep et al., 1998, Pierson et al., 1994, Yeole et al., 1997). Pseudomonas fluorescens isolates are effective bacterial antagonists for the management of soil borne and foliar diseases. Among the various isolates tested, P. fluorescens isolate Pf1 effectively inhibited mycelial growth of the pathogen in vitro conditions and decreased the fruit rot incidence under greenhouse conditions (Ramamoorthy and Samiyappan, 2001). Pseudomonas species were used successfully to control several diseases in crops (Hultberg et al., 2000; Pandey et al., 2001; Amein et al., 2008). They produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase, and can solubilize phosphorous (Kraus et al., 1995, Rodriguez et al., 1999).

Besides, some strains can also act by influencing on or by phytohormones (Mayak et al., 1999; Belimov et al., 2001). Bacillus is one of the most potential genera among PGPR cluster that suppress plant pathogens and insect pests by producing antibiotic metabolites (Van Loon, 2007). Thus Bacillus strains has received more attention than any other bacterial group (Powell et al., 1993; Merritt et al., 1989; Shoda, 2000; Israel et al., 2005; Dawar et al., 2010) for commercial and field applications (Liu and Sinclair, 1993). These species produce broad-spectrum antibiotics and are viable for a long time due to production of endospores (Emmert et al., 1999). Bacillus subtilis is a strong biocontrol agent that is frequently used by researchers. (Landa et al. 2001; Johri et al. 2003). Bacillus spp. are gram-positive bacteria isolated from the rhizosphere. They are found in large numbers in soil, produce heat resistant spores, have broad host range and produce various biological active compounds, so they have been exploited as potential biocontrol agents. (Milner et al., 1996, Nagorska et al., 2007). Bacillus megaterium from tea rhizosphere has the ability to solubilize phosphate, produce IAA, siderophore and antifungal metabolite and thus it helps in the plant growth promotion and reduction of disease (Chakraborty et al., 2006). Two strains Bacillus
*thuringiensis* and *Bacillus sphaericus* have the ability to solubilise inorganic phosphates and help in the control of the lepidopteron pests (*Sheshadri et al.*, 2007).

PGPR enter the tissues of living plants and cause unapparent and symptomatic infections (*Sturz and Nowak, 2000*) when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens (*Kloepper et al.*, 1980). PGPR are free living bacteria. *Somers et al.* (2004) classified PGPR as biofertilizers that increases the availability of nutrients to plants, phytostimulators that promotes plant growth generally by the production of phytohormones, rhizoremediators that can degrade organic pollutants and biopesticides that control diseases by the secretion of antibiotics and antifungal metabolites. PGPR causes symptoms of infections on entering the tissues of living plants (*Sturz and Nowak, 2000*). When applied to seeds or crops, they can either increase the growth of the plant or reduce the damage from soil-borne plant pathogens (*Kloepper et al.*, 1980). PGPR can protect plant parts against fungal, bacterial and viral diseases by induced systemic resistance (ISR). ISR by rhizobacteria is the induced state that develops when defense mechanism in plants is activated against primary infection by a pathogen. While systemic acquired resistance (SAR) creates a hypersensitive reaction due to which it is confined in a local necrotic lesion of desiccated tissue it is a general phenomenon that can enhance defensive capacity against all types of pathogens. (*Ryals et al.* 1996; *Sticher et al.* 1997) fig 2.1

*Kloepper et al.* (1992) reported that among the PGPR, fluorescent pseudomonads are the most studied bacteria that is used as biological control agents against plant pathogens. In the past few decades many strains of fluorescent pseudomonads have been isolated from the soil and plant roots by researchers and their biocontrol activity against various soil borne and foliar pathogens have been reported (Austin et al., 1997; Mew and Rosales, 1986; Rabindran and Vidyasekaran, 1996; Viswanathan and Samiyappan, 2001; Ramamoorthy et al., 2002). Salamone (2000) reported the growth-promoting effect of *P. fluorescens* strain on wheat and
radish plants by production of cytokinin phytohormones. As the effect of PGPR on plants was demonstrated, the concept of PGPR began to gain importance and a large number of bacterial strains have been isolated, screened (Cattelan et al., 1999; Bertand et al., 2001) and evaluated for plant growth promotion (Lifshitz et al., 1987; Abbas and Okon, 1993; Glick et al., 1997; Zhang et al., 1997; Mayak et al., 1999).

Bashan and Holguin (1998) proposed the division of PGPR into two classes: biocontrol-PGPR and PGPR. When the effect of PGPR on plants was demonstrated, the concept of PGPR began to gain importance. A large number of bacterial strains have been isolated, screened (Chanway and Holl, 1993; Bertrand et al., 2001), and were evaluated for plant growth promotion (Lifshtiz et al., 1987; Chanway et al., 1989; Glick et al., 1997; Bashan, 1998; Bent et al., 2001). Several research groups have randomly screened rhizosphere bacteria for antagonistic activity against nematodes (Becker et al., 1988; Oostendorp and Sikora, 1990 and Spiegel et al., 1991).

The application of biocontrol PGPR strains has given very good results in cereals, vegetables, fruit and ornamental plant production under glass house and field conditions (Raupach and Kloeppe, 1998). In greenhouse and field experiments, PGPR strain B. pumilus effectively protected pearl millet against downy mildew (Niranjan Raj et al., 2003). PGPR mediated resistance in mango trees infected with Colletotrichum gloesporiodes not only reduced the anthracnose infection but also enhanced fruit yield under field conditions (Vivekananthan et al., 2004). These studies clearly indicate the PGPR have diverse mechanism to operate to combat the pests and pathogens and work efficiently in both greenhouse and field conditions.

Rhizospheric bacteria can enhance plant growth and yield either directly or indirectly (Kloepper et al. 1989; Glick 1995). The direct mechanisms of plant growth promotion may be due to synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment (Glick et al. 1999). The indirect promotion of plant growth occurs when PGPR reduces the deleterious
effects of plant pathogens on plants by releasing inhibitory substances or by increasing the natural resistance of the host (Handelsman and Stabb, 1996; Nehl et al., 1996; Cartieux et al., 2003).

Fig 2.1: Systematic resistance against microbial pathogens induced locally by root colonization by non-pathogenic rhizobacteria (left ISR) or by limited pathogen infection (right SAR).

There are reports that microorganisms which are isolated from the root or rhizosphere of a specific crop have the better chances of adaptation to that crop and they can provide effective control of diseases than the organisms that are originally isolated from other plant species. They prove to be better biocontrol agents as they are already closely associated and adopted to the plant as well as to the particular environmental condition in which they are supposed to function. The screening of such locally adopted strains has yielded superior biocontrol strains in few cases (Cook and Baker, 1983). However, now-a-days microbial biodiversity studies have enhanced the identification of potential bio agents suited to different environmental
conditions. Identification of effective antagonists strains is the first step towards the
development of effective biological control. Practically, the antagonists must be
ecologically fit to survive, and should be established and function within the
particular conditions of the ecosystem. After identification of several organisms as
potential antagonists it is advised to select strains to determine the specific
mechanisms, interactions, conditions and requirements responsible for effective
biological control. Slininger et al. (2003) developed such indices, viz. relative
performance index based on bioagents growth and their antagonistic activity under
different conditions. In addition, use of different markers, viz. antibiotic production
and other regulatory mechanisms by bioagents gained the importance in rapid
identification of biocontrol agents. This kind of screening and selection offer the
system for successful development and commercialization of potential biocontrol
agents. Suri jit Sen et al. (2006) reported that in dual culture, significant growth
inhibition of Sclerotium rolfsii by Pseudomonas BRL-1 was observed. Mycelial
growth was restricted near bacterial streak. Increase in incubation period was
proportionate to growth inhibition of S. rolfsii upto six days. Microscopic study of
mycelia from interacting zone showed hyphal shriveling mycelial deformities like
swelling, fragmentation, short branching, and finally, lysis.

In the beginning of 1970s many researchers identified microbial populations
in the rhizosphere which act as the first barrier to pathogen infection. Nowadays, it
is well known that some soils have natural suppressive power to some soil-borne
plant pathogens like Fusarium, Gaeumannomyces, Rhizoctonia, Pythium, and
Phytophthora etc. Although this suppression is related to both physicochemical and
microbiological features of the soil yet in most systems the biological elements are
the primary factors in disease suppression .(Weller et al.,2002). These
microorganisms antagonistic against plant pathogens are numerous and consists of
various plant-associated prokaryotes and eukaryotes. A detailed interpretation of
mechanisms underlying their antagonism, and a compilation of organisms with
demonstrated antagonistic properties used in the biocontrol of pathogens has been
In prokaryotes, many bacteria such as Agrobacterium, Bacillus spp. Streptomyces, and Burkholderia have been reported to show efficient antagonistic properties against soil-borne pathogens. The most widely studied bacteria used as biocontrol is Pseudomonas spp., such as P. aeruginosa and P. fluorescens, which is one of the most effective root colonizing bacteria. Among the eukaryotes, there are number of non pathogenic fungal species like Pythium and Fusarium and other isolates like Trichoderma species that show antagonistic properties. Many scientists have done research in characterization of these antagonistic microorganisms. Tripathi and Johri (2002) studied the biocontrol potential of fluorescent pseudomonas in vitro and in vivo by isolating them from rhizosphere of pea and wheat against maize sheath blight caused by Rhizoctonia solani. They found that some isolates possessed disease control potential against several pathogens while some showed biocontrol potential against specific pathogens only. This proved that fluorescent pseudomonads are diverse in terms of their biocontrol potential. Many studies have been done on biological control of plant pathogens by antagonistic bacteria and fungi in recent years (Janisiewicz et al. 2000). Many stains of Bacillus subtilis have been reported have excellent characteristics like effective root colonization, versatile activity against multiple pathogens and promising ability to sporulate (Kloepper et al., 2004; Romero et al., 2004; Hassan et al., 2010)

Anjair et al. (2003) reported that Pseudomonas aeruginosa isolated from chickpea rhizosphere in India can protect pigeonpea and chickpea plants from fusarium wilt disease caused by Fusarium oxysporum f.sp. ciceri and Fusarium udum. When PNA1 rhizobacterial strain was inoculated in pigeonpea and chickpea, the incidence of fusarium wilt on both susceptible and moderately tolerant genotypes was reduced. It has been reported by Valverde et al., 2006 too that the growth and seed yield of chickpea under greenhouse and field conditions can be increased by co inoculating with Pseudomonas jessenii which is a phosphate-
solubilizing bacteria and \textit{Mesorhizobium ciceri}. Ahmadzadeh \textit{et al.} (2004) reported that antagonistic rhizobacteria mainly fluorescent pseudomonads and some Bacillus species have the ability to inhibit fungal and bacterial root diseases of agricultural crops.

Lemessa \textit{et al.}, 2007 collected 118 rhizobacteria from Ethiopia and screened them against an Ethiopian \textit{R. solanacearum} strain which is causal agent of wilt on potato and tomato in Ethiopia. By in vitro screening, six strains that showed good inhibitory effect were selected for further investigations. In a greenhouse soil and tomato seedlings were treated with the antagonists and their effects were studied. Their study showed that some strains significantly reduced disease incidence and increased dry weight of tomato plants. Thus there is a correlation in antagonist activity and dry weight of plants.

Ramesh \textit{et al.}, 2012, screened 48 endophytic bacteria and 101 rhizobacteria for their antibacterial activity against \textit{Ralstonia solanacearum} that causes wilt disease in egg plant. Among these 22 were antagonistic isolates in which 18 belonged to Pseudomonas spp. forming three groups based on biochemical characterization. Aliye \textit{et al.}, 2008, screened 120 rhizosphere bacterial isolates against virulent strain of \textit{Ralstonia solanacearum} which causes bacterial wilt in potato (\textit{Solanum tuberosum}). Their potential to suppress bacterial wilt disease development and their role as plant growth-promoting rhizobacteria (PGPR) was evaluated. Six antagonistic strains were selected after in vitro screening. In dual culture assay the strains whose inhibition diameter was more than 11 mm was studied further in the greenhouse, in vivo.

More than one mechanism may take place in the process of suppression of a pathogen by antagonistic microbes. It depends on the nature of antagonist involved. Direct effects on the pathogen may be due to competition for colonization or infection sites, competition for carbon and nitrogen sources as nutrients and signals, competition for iron through the production of iron-chelating compounds or siderophores, inhibition of the pathogen by antimicrobial compounds like antibiotics and HCN, Pathogenicity factors, and parasitism. Indirect mechanisms like
improvement of plant nutrition and damage compensation, changes in root system anatomy, microbial changes in the rhizosphere, and activation of plant defence mechanisms also are possible reasons of their antagonistic activities. This lead to enhanced plant resistance. An effective biocontrol agent often acts through the combination of several different mechanisms (Whipps, 2001).

Azza Tawfik et al., 2004 reported that 3 fungal isolates (Trichoderma harzianum, T. humatum, and T. viride) and one bacterial isolate (Bacillus subtilis) isolated from Cumin samples from different locations of Assiut Governorate showed antagonistic properties. On further screening a conclusion was derived that efficient bio-control agents may be developed through bioassay for microorganisms associated with local cumin cultures. This research is thus considered as a significant step towards finding an efficient environment friendly strategy for the management of Fusarium wilt disease in cumin.

Deepak et al., 2008, experimented to find suitable bio-agents for antagonistic fungi on growth of two cumin fungl pathogens under in vitro and field conditions. It was found that the radial growth of Fusarium oxysporum f. sp. cumini was inhibited maximum (82.86%) by Trichoderma harzianum strain I, while maximum inhibition (85.45%) of the mycelial growth of Alternaria burnsiï was observed in the presence of Trichoderma harzianum strain II under invitro conditions. These antagonists were used in field for further study. The lowest incidence of wilt disease was found when soil was treated with Trichoderma harzianum strain I. Blight disease incidence was lowest when T. harzianum strain II was applied to the soil. This study proved that Trichoderma sps can be used as biocontrol agent against blight diseases of cumin and they can be used a tool for sustainable management of crop diseases.

The technology to identify bacterial antagonism needs to be developed further. It's difficult to identify Bacillus subtilis like organisms as they cannot be distinguished from each other by simple phenotypic tests. The application of molecular methods has greatly changed the conventional taxonomic classification of
Bacilli. 16S rRNA sequencing is often used to define species (Wu et al., 2006) but due to the presence of highly conserved sequences in the 16S rRNA gene, the discrimination among some species and subspecies of this group becomes very difficult (Shaver et al., 2002).

Satyanaryana et al. (2005) reported the diversity of microorganisms that occur in stressed environments, their adaptations and potential biotechnological applications. Molecular methods that are based on both culture dependent and culture independent procedures have been used for understanding the diversity of microbes in these environments. They observed that these organisms have developed different structural and chemical adaptations due to which they are able to survive and grow in extreme environments. The enzymes of these microbes, which are functional in extreme environments known as extremozymes have various biotechnological applications.

Studies in the past have shown that competition for substrate may be the reason for antagonistic activities between bacteria and fungi (Mille-Lindblom et al., 2006). The actual mechanism responsible was not investigated in his study but the results suggested that there are some components in the culture filtrates that produced the antagonistic inhibition effect, on fungi spore germination of F. oxysporum f.sp.cumini.

Rhizobacteria produce different types of siderophores that chelate the Fe which is not abundant. In this way it can prevent pathogens from acquiring iron (Fig. 2.3). Marugg et al., 1985 obtained mutants of Pseudomonas putida PGPR strain using Tn5 transposon mutagenesis that could not biosynthesize siderophores. These were compared with the wild type strain. It was found that the wild-type strain increased potato root growth and tuber yield significantly in pot and field experiments while the mutants defective in siderophore biosynthesis had no such effect (Bakker et al., 1986, 1987). In these experiments with potato the increased plant growth was due to suppression of deleterious rhizosphere microorganisms (Schippers et al., 1987) The involvement of siderophore production in disease...
suppression by *Pseudomonas* strain was further studied on carnation, radish, and flax (*Linum usitatissimum*) against *Fusarium oxysporum* f.sp. *dianthi*, *F. oxysporum* f.sp. *raphani* and *F. Oxysporum* f.sp. *lini* as the pathogen. In all cases the siderophore mutant was less effective than the wild-type strain in suppression of disease (Duijff et al., 1993; Raaijmakers et al., 1995).

**Fig 2.2:** Competition for iron between microorganisms in the rhizosphere: a plant growth promoting rhizobacterium deprives a harmful microorganism (*HMO*) of iron by secreting siderophores (*SID*), which can (+) or cannot (−) also be used by plant roots. (source :Bakker 1989)

With the objective of understanding the relationship of plant species in determining the presence of bacterial antagonists in rhizosphere, Berg et al., in 2002 analysed antagonistic activity of rhizobacteria towards the soil borne pathogen *Verticillium dahliae*. He isolated them from potato, oilseed rape, and strawberry and from bulk soil in order to study their genetic diversity and other parameters. In 1998 and 1999, isolation of thousands of rhizobacteria was done five times over two growing seasons from a randomized field trial from rhizosphere and soil samples.
were taken and were screened. Then in vitro antagonistic activity towards Verticillium was determined using Dual testing. He observed that the antagonistic activity was highest for strawberry rhizosphere and characterized 331 Verticillium antagonists. Most of the antagonists from the strawberry rhizosphere was identified as *Pseudomonas putida* B (69%), while those of oilseed rape antagonists belonged to the Enterobacteriaceae (*Serratia* spp., *Pantoea agglomerans*).

Other scientist *Czaorla et al., 2007* also rhizoplane of healthy avocado trees and obtained 905 bacterial isolates out of which 277 were gram-positive isolates. Among these four strains were identified as *B. Subtilis*. Selection was done on the basis of their antifungal activity against various soil-borne phytopathogenic fungi. This is one of the research that was based on isolation and characterization of *B. subtilis* strains with biocontrol activity against the common soil-borne phytopathogenic fungi *F. oxysporum f.sp. radicis-lycopersici* and *R. Necatrix*. Similarly Plant growth promotory Pseudomonas strains were isolated from root nodules of five plant species, viz., *Trifolium pretense*, *Cicer arietinum*, *Amaranthus polygamus*, *Vigna mungo*, and *Trigonella foenum* by *Sakshi Issar et al., 2012*. 8 bacterial isolates was observed for their effect on growth promotion. Partial 16S rDNA sequencing was done in which it was found that these belonged to genus *Pseudomonas*. MEGA 4.0.2, software was used to construct a neighbor joining tree by employing boot strap method. Her results too revealed that there is significant diversity among recovered Pseudomonas strains.

The beneficial soil microorganisms can be used as agricultural inputs for improving crop production. For this selection of rhizosphere, competent microorganisms with plant growth-promoting attributes is required. *Hynes et al., 2008*, collected 563 bacteria from the roots of pea, lentil, and chickpea grown in Saskatchewan. They were then screened for plant growth-promoting traits, antagonistic activity against legume fungal pathogens, and plant growth promotion capability. 427 isolates were observed with siderophore production, 29 isolates in amino-cyclopropane-1-carboxylic acid (ACC) deaminase activity and 38 isolates with indole production. He observed that twenty-six isolates suppressed the growth of Pythium sp. strain. *Fusarium avenaceum* was suppressed by 40 isolates and 53
isolates inhibited the growth of *Rhizoctonia solani*. Most of the antagonistic strains belonged to Pseudomonadaceae and Enterobacteriaceae families. It was proved by Fatty acid profile analysis and 16S rRNA sequencing of smaller subsets of the isolates that were positive for the plant growth-promotion traits. Isolated strains were reported to have potential for development as biofertilizers or biopesticides for western Canadian legume crops.

**Nasraoui et al., 2008** collected soil samples from Tunisian and Missourian fields and bacterial isolates were collected from wheat rhizospheres in each soil. They were then tested in vitro for their antagonistic activity against *Gaeumannomyces graminis var. tritici* (Ggt). Twenty-three bacterial isolates were selected and tested in vitro against three Ggt strains using three different culture media. Dual cultures that proved that fungal inhibition depends on media and presence or absence of supplemental iron. A second assay based on detached wheat roots on potato dextrose agar revealed antagonistic activity in only half of the bacterial isolates classified as effective in vitro. The results of this experiment showed that rhizospheric bacteria can be used to control root wheat disease due to Ggt.

With advancement of Microbial technology in agriculture, new bacterial strains are being identified that are very effective in promoting growth of plants. Due to this its expansion is taking place very fast. It has been reported that the rhizobacteria promote the growth of plants and can have a positive effect on the productivity of crops. **Cherif et al., 2012** isolated a nitrogen-fixing bacterium from the wheat rhizosphere of an arid region. The strain was identified on the basis of different tests and 16S rRNA sequencing, as *Pantoea agglomerans* lma2. This strain showed many activities like it could degrade many sources of carbon, lipid, proteins, grew on KCN and could grow from pH 4 to 8 and had an optimum at pH 7. Its performance and activity was significantly better in the presence of salt. The Results revealed that *P. agglomerans* lma2 with its Plant Growth Promoting Rhizobacteria (PGPR) and halophilic properties could be used as a good fertilizer in arid and saline zone.

PGPR does not allow phytopathogen to multiply. Thus it improves plant growth and support plant growth. Some of them synthesize antifungal antibiotics. *P.*
fluorescens release 2,4-diacetyl phloroglucinol that inhibits growth of phytopathogenic fungi. Few PGPR can cause degradation of fusaric acid produced by Fusarium sp. which is the causal agent of wilt disease. Thus can help in prevention of pathogenesis. Enzymes are released by some PGPR that cause lysis of fungal cells. For example, Pseudomonas stutzeri produces extracellular chitinase and laminarinase enzyme that lyses the mycelia of Fusarium solani. Extensive research has revealed that fluorescent Pseudomonas has the potential to be used as as biological control agent as it can colonize rhizosphere and protect plants against a wide range of important agronomic fungal diseases like black root-rot of tobacco, root-rot of pea, root-rot of wheat, damping-off of sugar beet. There is also great prospects of genetically manipulating the producer organisms to improve the efficacy of these biocontrol agents. Pseudomonas shows biocontrol potential against Phytopathogenic fungi in vivo and in vitro conditions from chickpea rhizosphere. P. putida has potential for the biocontrol of root-rot disease complex of chickpea by showing antifungal activity against Macrophomina phaseolina.

Shyamala et al., 2012, evaluated antagonistic rhizobacteria Pseudomonas fluorescens in biological control of rice blast disease caused by Pyricularia oryzae. In trial tests done in pots it was observed that Pyricularia oryzae can be controlled with biocontrol agent P. fluorescens but less than chemical (like salicylic acid) used alone at the standard dose. But on combining chemical dose with antagonistic rhizobacteria the control was as effective as the standard chemical alone. Application of P.fluorescens along with salicylic acid significantly increased the disease resistance. Further, there were increases in activities of polyphenol oxidase and showed least activities of peroxidase and ascorbic acid oxidase treated with P.fluorescens plus salicylic acid. The results indicated that the combination of biological and chemical inoculation has a better response to fight against rice blast pathogen P.oryzae than the treatment alone. Furthermore, PGPR isolates remarkably increased seed germination of pigeon pea. Among the sixteen isolates, seven were found to be high IAA producing. Six were found to be efficient phosphate solubilizers, five isolates were found to be good antagonistic towards pathogen soil fungi and eight isolates were found to be better in enzyme productions, and thus, may enhance the mineralization efficiency of soils. Three isolates were shown to be
promising in IAA production, phosphate solubilisation, antagonism towards fungi, and mineralizing capacity.

Yap chin Ann 2012, made a primary selection through the antagonism test plates where the confluent growth of bacteria from the pepper rhizosphere inhibited the development of fungal mycelia. Five antagonistic bacteria were selected that could suppress the growth of *F. solani*, *C. capsici*, *C. gloeosporioides*, and *Septobacidium spp.* Based on 16S rDNA sequence analyses, all of this bioantagonistic bacterial isolates belonged to *Bacillus* spp. These results were consistent with the earlier raised hypothesis that this group of microorganisms is responsible for this kind of phenomenon in the soil such as antagonistic activity against *F. solani* (Jing et al., 2009); *C. gloeosporioides* (Havenga et al., 1999); *Meloidogyne incognit* (Ismail and Fadel, 1997), avocado post-harvest pathogens (Korsten and Jager, 1995) and peach brown rot (Pusey, 1988). The colonization of the pepper vine rhizosphere confirm reports on the interactions of Bacillus strains which supports their use as rhizosphere colonizers (Kang et al., 2006; Barea et al., 2005; Reva et al., 2004).

Gupta et al. (2002) reported Pseudomonas sp. acted as a potent phosphate solubilizer. Das et al. (2003) examined the tricalcium phosphate solubilizing activity of *P. fluorescens* and phosphate solubilizing pseudomonads are also reported to suppress damping-off disease in tomato caused by *Pythium* spp. (Srivastav et al., 2004). Edward et al., 2013, screened bacterial isolates for in vitro antagonistic activity against *F. oxysporum* through various in vitro tests like dual culture etc. 16S rDNA sequencing was used for further identification of five isolates that showed good antifungal activity. Isolates EB1 and EB2 showed highest antagonism against *F. oxysporum* mycelia with the percentage of inhibition up to 43% and 41%, respectively. The antifungal activities are probably due to the secretion of volatile and diffusible bioactive compounds. Analysis of the 16S rDNA sequences revealed the closest of the bacterial isolates as *Bacillus megaterium, Bacillus cereus, Enterobacter sp.*
All plant-associated microenvironments like the rhizosphere, are colonized by antagonistic microbes in high numbers (Berg et al., 2005a). Most of the microbial inhabitants showed antagonistic capacity to inhibit the growth of pathogens in vitro (Berg et al., 2002, 2006). The proportion of isolates, which express plant growth promoting traits is much higher. (Cattelan et al., 1999; Fürnkranz et al., 2009; Lottmann et al., 1999). Diverse microbial inoculants, which were selected from this promising indigenous potential, are already available in the market. In recent years, the popularity of use of microbial inoculants has increased, as extensive and systematic research has enhanced their effectiveness and consistency as an alternative to use of costly and hazardous chemicals (Berg, 2009).

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Methods based on cultivation techniques to analyse plant-associated rhizobacteria only represents the culturable fraction, which are only a small proportion (0.1 to 10%) of the total bacteria present in soil and in the rhizosphere (Amann et al., 1995). A larger number of microbes can be studied by the analysis of nucleic acids which are directly extracted from plant microenvironments. Most frequently ribosomal RNA gene fragments are amplified from total genomic DNA and is then analysed by various fingerprinting techniques like Terminal restriction fragment length polymorphism (T-RFLP), single-strand conformation polymorphism (SSCP), denaturing/temperature gradient gel electrophoresis (D/TGGE) using universal/specific primers (Schwieger & Tebbe, 1998; Smalla et al., 2007). These fingerprinting techniques have helped in understanding plant specific microbial
communities (Smalla et al., 2001) and the impact of cultivars on microbial communities (Milling et al., 2004). Briones et al. (2002) found cultivar-specific differences for ammonia-oxidizing bacteria (AOB) in rice rhizospheres by a multiphasic approach including DGGE of the amoA gene, analysis of libraries of cloned amoA, fluorescently tagged oligonucleotide probes targeting 16S rRNA of BCAs and pathogens Microbial communities.

New molecular and microscopic techniques is the foundation of path of progress in biocontrol research. These techniques have enhanced our knowledge about the plant and the rhizosphere as a microbial ecosystem. Due to this more effective screening strategies have been developed for bioactive microbes. These tools are helpful in studying the ecology of single plant growth promoting rhizobacteria (PGPR) or biological control agent (BCA) strains. It can be used to analyse the structure and function of the target microbial community. Molecular fingerprints that uses repetitive elements in the genome (Rademaker & de Bruijn, 1997) can be used at various levels of biocontrol research. While the functions of many of these repetitive sequence elements are still not known, they have proven to be useful. The repetitive, sequence-based PCR or rep-PCR, DNA fingerprint technique uses primers targeting these repetitive elements. PCR is used to generate unique DNA profiles or ‘fingerprints’ of individual microbial strains (Ishii & Sadowsky, 2009). These fingerprints can be applied to differentiate strains at population level in screening strategies, and help in selection of only unique isolates (Berg et al., 2006; Faltin et al., 2004). Later on these highly reproducible fingerprints can be used for identity check and quality control. Genome sequencing is a very crucial tool to study PGPRs in great detail. Strains of Pseudomonas fluorescens which is one of the dominant and cosmopolitan plant-associated species (Weller, 2007), were the first sequenced strains (Paulsen et al., 2005). Genomic information help in analysis of the mode of action, detailed investigations of interactions as well as optimisation of
fermentation and formulation processes (rev. in Gross & Loper, 2009). De Bruijn
et al. (2007) used genome mining to find unknown gene clusters and traits in P.
fluorescens SBW25. Proteomic and transcriptomic studies are interesting to study
the function of BCAs. For example, Garbeva et al. (2011) studied transcriptional
and antagonistic responses of Pseudomonas fluorescens Pf0-1 to phylogenetically
different bacterial competitors.

A new tool callede metabolism help in analysis of metabolites in situ. This
technique help in answering questions about the activity ad planta which is very
significant for registration procedures. Frimmersdorf et al. (2010) used a
metabolomic approach to reveal adaptation of Pseudomonas aeruginosa to various
environments. Not only this but by analysis of the mobilome of strains one can
obtain interesting findings for biocontrol research. Mavrodi et al. (2009) has shown
for P. fluorescens Pf-5 that mobile genetic elements contain determinants which
contribute to Pf-5’s ability to adapt to changing environmental conditions and in
colonizing new ecological niches. This type of studying the colonisation of
plants is greatly supported by the application of fluorescent proteins, used as vital
markers and reporter genes (rev. in Bloomberg, 2007). These novel insights have
changed our knowledge about Molecular Tools in order to Develop Effective and
Safe Biocontrol Strategies. (Chin-A-Woeng et al., 1997; Zachow et al., 2010).

Efficient characterization and selection process is required for development
of rhizobacteria for commercial applications. Most active organism can be selected
only when they are tested through various invitro and invivo tests. During this
screening procedure host plant specificity or adaptation to a particular soil,
ecosystem, climatic conditions or pathogens to be targeted should be considered.
(Nelson, 2004). Antagonistic microorganisms can selected by using any approach
like isolating them from those soils that are already suppressive to pathogens or can
be selected on the basis of their traits like root colonization or production of
antibiotics or siderophores. (Cattelan et al., 1999; Glick and Bashan, 1997;
Weller et al., 2002). Those microorganisms that can promote plant growth in absence of pathogen can be selected on the basis of traits that directly affect PGPR like nitrogen fixation, solubilization of phosphorus and iron, production of phytohormones such as auxins and cytokinins (Glick and Bashan, 1997).

Nakkeeran et al. (2005), reviewed that the selection of best antagonistic bacterial isolates is the foundation stone in commercialization of the isolates for disease management. Not only these but the best biocontrol and growth promoting isolates can be selected by analysing genetic stability, shelf life, growth rate and consistency of the isolates. Then commercial products can be developed. One should also consider the effect on non target organisms while developing a biocontrol agent into a commercial product (Nelson, 2004).

Stalstorm (1903) first observed that microorganisms are involved in solubilization of insoluble phosphate. Since then, a lot of work has been done on the isolation, enumeration, efficiency screening, mechanisms of solubilization and crop response to their inoculation. The biological process in which unavailable or fixed form of inorganic phosphorus is converted into primary orthophosphate and secondary orthophosphate has been termed as mineral phosphate solubilization (Goldstein, 1986). The solubilization of the precipitated calcium phosphate on agar medium has been used as the criterion for isolation and enumeration of MPS microorganisms (Sperber, 1957). Among the groups of mineral phosphate solubilizing bacteria (MPSB), Pseudomonas are the most important as they are the most common and frequency occurring group in the rhizosphere and are capable of utilizing a wide array of compounds as carbon and energy sources. They are also known to have wide range of plant growth promotional activity by virtue of nutrient mobilization, P-solubilization, production of plant hormones and biocontrol potential.

The occurrence of phosphate solubilizing bacteria in soils of Marthwada region of Maharashtra state was studied by Bilolikar et al. (1996), wherein they found predominance of Pseudomonas in soils of Aurangabad district. Nahas (1996) studied 31 bacteria for their ability to solubilize rock phosphate and calcium
phosphate in culture medium and reported that *Pseudomonas cepacia* had the highest solubilizing activity. **Illmer and Schinner (1992)** isolated *Pseudomonas sp.* and *Penicillium sp.* from forest soils and found them to solubilize high amounts of insoluble inorganic phosphates. **Neelam and Meenu (2003)** reported high tricalcium phosphate solubilizing ability of *Pseudomonas* sp. (TP2) isolated from rhizosphere of *Trigonella*. **Das et al. (2003)** have examined the tricalcium phosphate solubilizing activity of *Pseudomonas fluorescens* and their cold-tolerant mutants and reported that the cold-tolerant mutants were more efficient than their respective wild type counterparts for P-solubilization at low temperatures.

**Gupta et al. (2002)** described *Pseudomonas* species as a potent phosphate solubilizer while they were developing heavy metal resistant mutants of phosphate solubilizing *Pseudomonas* NBRI 4014. **Disimine et al. (1998)** recorded an interesting observation of solubilization of ZnPO4 by a phosphate solubilizing *Pseudomonas fluorescens* only in the presence of glucose as the carbon source.

Theoretical estimates have suggested that the accumulated phosphorus (P) in agricultural soils due to fixation is sufficient to sustain maximum crop yields worldwide for about 100 years (**Goldstein et al., 1993**). However, although P is abundant in soils in both inorganic form (originating mainly from applied P fertilizer) and organic form (derived from microorganisms, animals and plants) (**Paul & Clark, 1989**), it is still one of the major plant growth limiting nutrients. On average, most nutrients in the soil solution are present in millimolar amounts, but phosphorus is present only in micromolar or lesser quantities (**Ozanne, 1980**). Soluble P is highly reactive with Ca, Fe or Al due to which precipitation takes place and thus low amount of P is left. In Acidic soils, inorganic P is associated with Al and Fe compounds, while in calcareous soils, calcium phosphates are the predominant form of inorganic phosphates. Almost 75-90% of added P fertilizer is precipitated by Fe, Al and Ca complexes present in the soils, creating a demand for suitable alternatives to mobilize this fixed fraction of the important bioelement (**Stevenson, 1986**). Mineral phosphate is not soluble in soil so they are mobilised by soil microbes in a
more environmentally friendly and sustainable manner. This role of soil microorganisms in solubilization of inorganic phosphates has been reported as early as 1903 (Kucey et al., 1989). Researchers have reported that P solubilizing microorganisms are approximately 20 to 40% of the culturable population of soil microorganisms and they can be easily isolated rhizosphere soil (Kucey, 1983; Chabot et al., 1993). Numerous PSB have been isolated from the rhizosphere of different plants. They are observed to be metabolically more active than those isolated from sources other than rhizosphere (Baya et al., 1981). Rodríguez & Fraga, 1999 have reviewed on significant phosphate solubilizing microorganisms constituting bacteria and fungi. Generally number of P solubilizing bacteria is more than P solubilizing fungi (Kucey, 1983; Kucey et al., 1989).

But fungal isolates are superior in some qualities. Their P solubilizing ability is higher than bacteria in both liquid and solid media. Very interesting fact is that their P solubilizing ability is not lost upon repeated sub-culturing while P solubilizing bacteria loses its capability in such case (Kucey, 1983). Most of of the phosphate solubilizing microorganisms (PSMs) can mobilize Calcium phosphate complexes while only a few can solubilize Fe-P and Al-P complexes (Kucey et al., 1989). Availability of inorganic P can be enhanced by changing the pH of rhizosphere or by releasing organic anions. Plants and microorganisms help in this process by releasing exudates. They can mobilize P from organic pools and convert it to available inorganic forms by phosphatases. Microorganism release phytase enzyme that converts phytates to esters of phosphate which are broken down to inorganic phosphate by phosphatase enzyme. Plants can also increase the capacity to take up Phosphate by increasing their root surface area. This can be done by growing long and thin roots having many thin root hairs, and by altering the capacity or affinity of plasma membrane-embedded Phosphate transporters. The outline arrows in Fig 2.2 indicate P uptake. (Source: Rengel & Marschner, 2005).
Because variability in disease suppressive activity of introduced rhizobacterial inoculants depends on variations in biotic and abiotic environmental conditions, understanding of important soil properties that influence microbial communities and predominant disease-suppressive rhizobacteria in diverse soils must be improved. Soil conditions developed under certain management practices may increase naturally-occurring biocontrol microorganisms that suppress cereal root pathogens. For example, soil factors including organic matter content, pH, mineral concentrations, and clay type are linked to disease biocontrol activity. Hoitink and Boehm suggested that high levels of hydrolytic enzymes of microbial biomass in the soil with high organic matter is related to disease suppressive properties of a soil. Several farming practices that uses high soil organic matter can be used to manage soil microorganisms and microbial activity so as to optimize potential disease suppression.