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Fungi are a diverse group of organisms comprising both single celled and multicellular filamentous forms. It has been estimated that only a fraction of the diversity that exist is presently known. In the 20th century several species each with its own special advantages were introduced in research as simplest eukaryotic model systems that can be studied with the approaches of cell biology, genetics and biochemistry. The genome sequences of a few fungi are now known; those of several other species are underway. In the 21st century, fungi will not only be increasingly used for understanding their unique mode of life, but also for findings of general applicability to higher organisms such as assembly of intracellular organelles, adaptation to harsh environmental conditions, defense mechanism for protection from invasion by foreign DNA, biological rhythms, aging and death. Their ability to be transformed and the transgenic strains to be grown in relatively simple nutrient medium in industrial sized fermentors and their extracellular secretion of proteins is likely to be exploited for production of a variety of enzymes (proteins), including human vaccine (Maheshwari Ramesh, 2005).

The most important effect of fungi on human activities is the destruction of crops. A fungal epidemic which destroys the staple crop of a society depending on the subsistence farming is a human catastrophe with far reaching social and economic consequences. The rapid spreads which can occur with fungal pathogens is due to their abundant protection of both dispersal spores which are carried on the wind and survival spores and other structures which lie in the soil. The destructiveness of fungal pathogens


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results from their ability to break down plant tissues, to alter physiology so as to reduce yield and to produce toxins poisonous both to the plant and to animals eating it.

Fungi are non-photosynthetic eukaryotic organisms which grow as single cells (yeasts) or as multi-cellular filaments (moulds/fungi), acquiring nutrition by absorbing from their surroundings. There is no material of biological origin that remains free of fungi. Although commonly and unpleasantly thought of as causing spoilage of stored food and diseases in plants, the large majority of fungi decompose dead material and recycle essential mineral nutrients (particularly nitrogen, phosphorus, and potassium) required to build cytoplasm. Fungi thus contribute to the green cover on earth, (Terborgh, 1992).

Species of fungi are the only eukaryotic organisms that survive at a temperature range 45°C-60°C at which no plant or animal can survive (Maheshwari, 1998). Survival structures of pathogens fungi are in the soil, such as spores, sclerotia and hyphae associated with killed host plant remains, may make it difficult or impossible to grow susceptible crops in some area. Some fungi live in plants as endophytes (Redman et al., 2002) or as symbiotic partners with algae (lichens), enabling them to grow under harsh conditions which they could not do otherwise. Although their individual hyphae are hard to see by unaided eye, fungi are the largest living organisms (Smith et al., 1992).

1.1. Impact of fungi on agriculture

The fungal kingdom comprises an estimated 1.5 million species of which approximately 100,000 have been fully characterized and, of those about 8,000 are plant pathogens (Knogge, 1996; Agrios, 2005). A similar hierarchy in the plant kingdom teaches that out of approximately 50,000 edible plant species 29 species provide over 90% of mankind’s food requirements. Losses due to fungal attack still account for about...
30% of the total yield. Another important and often ignored problem associated with the fungal attack is the formation of mammalian toxic mycotoxins in infected plant tissue. Over the last few decades, there have been growing consumer concerns about food quality and safety. Other uneasiness about the short and long term susceptibility of the environment to pesticide usage has raised the question of the susceptibility of natural resources. (Geoffrey, 2004).

Agriculture has undergone tremendous changes, particularly since the introduction of industrialized practices. Agriculture is one example of what can be called a critical infrastructure, or a system without which our society can’t function (Norton, 2003). The contribution of agriculture to the overall economy is undeniable. It serves as the nation’s largest employer. India, out the country continues to suffer from annual crop losses due to export worth Rs. 1000 crores is rejected due to the phytosanitary requirements as per WTO norms an unaccountable amount is also being lost (Kheterpal 2006). Detection and quantification of pathogenic inoculum has considerable applicability and diagnosis and management of existing and emerging plant disease within agricultural crop producing systems (Kennedy and Wakeham, 2006).

1.2. Types of pathogens

According to Schaeffer (1991), plant pathogens are of three types-opportunistic pathogens, true pathogens and obligate pathogens. Opportunistic pathogens require weakened plants for colonization and enter plants through wounds. The true pathogens rely on living plants to grow and can survive outside their hosts. The obligate pathogens require a living plant to complete their life cycle.
1.3. Fungal identification and disease diagnosis

Phytopathology involves very specific interaction of two different organisms i.e., pathogen and host plant at molecular levels, which either results in disease resistance or susceptibility. This specific interaction between host and pathogen is affected by both external and internal environments and attracted attention of the plant pathologists worldwide to study host-pathogen interaction at molecular level. Recent advances in molecular biology and biotechnology have greatly influenced all the areas of plant pathology. Management of the plant disease starts with the identification of the plant pathogens. Accurate diagnosis and identification of plant pathogens is a prerequisite of disease management to sustain high yield potential of crops. Therefore, continuous efforts are being made to develop simple, reliable, rapid and safe methods for the disease diagnosis. Visual identification is hard to perform by inexperienced personal and is limited particularly to diseases affecting aerial parts of the plants. It cannot be performed effectively in case of soil and seed borne diseases where several species of pathogens may cause similar symptoms (Sharma, 2006). The preferred method of disease diagnosis is microscopic examination of diseased tissues and identification of pathogen on the basis of morphological characters, but it requires specialized taxonomists. Traditional methods using microscopy are time consuming and require expert knowledge and estimates are still prone to error (Kheterpal, 2006). Detecting target particles or spores of plant pathogens in air and soil samples offers several advantages in the early detection of important diseases. Additionally estimates in air samples can be used to forecast the likelihood of important diseases transmission events.
Identification of plant pathogenic fungi is generally carried out by morphological, biochemical and allozyme characteristics. However, these methods have proved to be insufficient and are limited particularly to diseases affecting aerial parts of the plants. It cannot be performed effectively in case of soil and seed borne diseases. Numerous pathogens are difficult to identify by morphological characteristics and require extensive, time consuming, work, pure cultures and pathogenicity tests. Selective media helps but in most cases they go only as far as genus selectively. In recent years, advances in molecular biology and biotechnology have greatly influenced all areas of management of the plant disease. Introduction of hybridization and PCR based DNA typing techniques has greatly facilitated characterization, identification and early diagnosis of pathogens, as well as studies on their epidemiology. Accurate diagnosis and identification of plant pathogens is a pre-requisite of disease management to sustain productivity and yield potential of crops. Therefore, continuous efforts should be made to develop simple, reliable, rapid and safe methods for disease diagnosis (Sharma, 2006).

1.4. Cereal crops and fungi

Cereals are grasses (members of the monocot family *Poaceae*, also known as *Gramineae*) cultivated for the edible components of their grain, composed of the endosperm, germ, and bran. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop; they are therefore staple crops. In their natural form (as in whole grain), they are a rich source of vitamins, minerals, carbohydrates, fats, oils, and protein. However, when refined by the removal of the bran and germ, the remaining endosperm is mostly carbohydrate and lacks the majority of the other nutrients. In some developing nations, grain in the form of wheat,
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maize, or rice constitutes a majority of daily sustenance. In developed nations, cereal consumption is moderate and varied but still substantial.

1.4.1. Wheat (*Triticum* spp.): Is a cereal grain, originally from the Levant region of the Near East and Ethiopian Highlands, but now cultivated worldwide. In 2007 world production of wheat was 607 million tons, making it the third most-produced cereal after maize (784 million tons) and rice (651 million tons). In 2009, world production of wheat was 682 million tons, making it the second most-produced cereal after maize (817 million tons) and with rice as close third (679 million tons).

Wheat is grown on more land area than any other commercial crop and is the most important staple food for humans. World trade in wheat is greater than for all other crops combined. Globally, wheat is the leading source of vegetable protein in human food, having higher protein content than either maize (corn) or rice, the other major cereals. In terms of total production tonnages used for food, it is currently second to rice as the main human food crop and ahead of maize, after allowing for maize's more extensive use in animal feeds. With rice, wheat is world's most favored staple food.

Wheat provides more nourishment for humans than any other food source. It is a major diet component because of the wheat plant’s agronomic adaptability with the ability to grow from near arctic regions to equator, from sea level to plains of Tibet (4000 meters above sea level). In addition to agronomic adaptability, wheat offers ease of grain storage and ease of converting grain into flour for making edible, palatable, interesting and satisfying foods. Wheat is the most important source of carbohydrate in a majority of countries. Wheat also contains a diversity of minerals, vitamins and fats (lipids). With a small amount of animal or legume protein added, a wheat-based meal is
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highly nutritious. A redominately wheat-based diet is higher in fiber than a meat-based diet.

There are many wheat diseases, mainly caused by fungi, bacteria, and viruses. Plant breeding to develop new disease-resistant varieties, and sound crop management practices is important for preventing disease. Fungicides, used to prevent the significant crop losses from fungal disease, can be a significant variable cost in wheat production. Estimates of the amount of wheat production lost owing to plant diseases vary between 10–25% in Missouri. A wide range of organisms infect wheat, of which the most important are viruses and fungi.

1.4.2. Maize (Zea mays L.): Is a grain domesticated by indigenous peoples in Mesoamerica in prehistoric times. The leafy stalk produces ears which contain seeds called kernels. Though technically a grain, maize kernels are used in cooking as a vegetable or starch. Maize spread to the entire world due to its ability to grow in diverse climates. Sugar-rich varieties called sweet corn are usually grown for human consumption, while field corn varieties are used for animal feed and as chemical feedstocks.

Maize and cornmeal (ground dried maize) constitute a staple food in many regions of the world. Maize meal is used as a replacement for wheat flour, to make cornbread and other baked products. Maize is a major source of starch. Cornstarch (maize flour) is a major ingredient in home cooking and in many industrialized food products. Maize is also a major source of cooking oil (corn oil) and of maize gluten. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup, and a sweetener and also fermented and distilled to produce grain alcohol. Maize is sometimes used as the starch source for beer. In the
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United States and Canada, maize is mostly grown to feed for livestock. Maize meal is also a significant ingredient of some commercial animal food products, such as dog food. Maize is also used as a fish bait, called "dough balls". It is particularly popular in Europe for coarse fishing.

There are many maize diseases, mainly caused by fungi, bacteria, nematodes and viruses. Fungicides, used to prevent the significant crop losses from diseases, can be a significant variable cost in maize production.

1.4.3. Rice: Is the seed of the monocot plants *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, rice is one of the three major food crops of the world. Being grown worldwide, it is the number one food crop which provides the majority of the daily nutrients to more than half of the world's total population (Shim et al., 2004). It is a nutritious cereal crop, provides 20 per cent of the calories and 15 per cent of protein consumed by world's population. Besides being the chief source of carbohydrate and protein in Asia, it also provides minerals and fibre. Rice straw and bran are important animal feed in many countries.

Rice is first mentioned in the Yajur Veda and then is frequently referred to in Sanskrit texts. In India, there is a saying that grains of rice should be like two brothers, close but not stuck together. Rice is often directly associated with prosperity and fertility; therefore there is the custom of throwing rice at weddings. Since a large portion of maize crops are grown for purposes other than human consumption, rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by the human species. Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor-intensive to cultivate and requires ample water. Rice can be grown
practically anywhere, even on a steep hill or mountain. Although its parent species are native to South Asia and certain parts of Africa, centuries of trade and exportation have made it common place in many cultures worldwide.

The traditional method for cultivating rice is flooding the fields while, or after, setting the young seedlings. This simple method requires sound planning and servicing of the water damming and channeling, but reduces the growth of less robust weed and pest plants that have no submerged growth state, and deters vermin. While flooding is not mandatory for the cultivation of rice, all other methods of irrigation require higher effort in weed and pest control during growth periods and a different approach for fertilizing the soil.

India is the largest rice growing country accounting for about one-third of the world acreage under the crop. It is grown in almost all the states of India, covering more than 30 per cent of the total cultivated area. Its cultivation is mostly concentrated in the river valleys, deltas and low lying coastal areas of northeastern and southern India, especially in the states of Andhra Pradesh, Assam, Bihar, Chhattisgarh, Karnataka, Kerala, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh and West Bengal, which together contribute about 97 per cent of the country’s rice production. Contributing about 42 per cent to country’s food grain production, rice not only forms the mainstay of diet for majority of its people (>55 per cent), but also is the livelihood for over 70 per cent of the population in the traditional rice growing regions.

World production of rice has risen steadily from about 200 million tonnes of paddy rice in 1960 to over 678 million tonnes in 2009. The most productive farms for rice, in 2009, were in China producing 6.59 tonnes per hectare. The three largest producers of rice in 2009 were China (197 million tonnes), India (131·MT), and
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Indonesia (64 MT). At 44 million hectares, India had the largest farm area under rice production in 2009. The rice farm productivity in India were about 45% of the rice farm productivity in China, and about 60% of the rice farm productivity in Indonesia. If India could adopt the farming knowledge and technology in use in China and Indonesia, India could produce an additional 100 million tonnes of rice, enough staple food for about 400 million people every year, and US$50 billion in additional annual income to its rice farmers (adjusted to 2010 dollars and global rice prices per tonne).

In addition to the gap in farming system technology and knowledge, many rice grain producing countries have significant losses post-harvest at the farm and because of poor roads, inadequate storage technologies, inefficient supply chains and farmer's inability to bring the produce into retail markets dominated by small shopkeepers. A World Bank – FAO study claims 8% to 26% of rice is lost in developing nations, on average, every year, because of post-harvest problems and poor infrastructure. Some sources claim the post-harvest losses to exceed 40%. Not only do these losses reduce food security in the world, the study claims that farmers in developing countries such as China, India and others lose approximately US $89 billion of income in preventable post-harvest farm losses, poor transport, the lack of proper storage and retail. One study claims that if these post-harvest grain losses could be eliminated with better infrastructure and retail network, in India alone enough food would be saved every year to feed 70 to 100 million people over a year.

The production advance in rice in the recent years has enabled self sufficiency despite increase in population. In India, rice is grown in 43 million hectares (m ha) in four major ecosystems: irrigated (19 m ha), rainfed lowlands (14 m ha), flood prone (3
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m ha) and rainfed upland (6 m ha). No other country in the world has such diversity in rice ecosystem. One of the important constraints in achieving higher rice yields is losses caused by pests. The common pests of rice are insects, nematodes, fungi, bacteria, viruses, phytoplasma etc. Among the fungal diseases, blast disease caused by the ascomycetes fungus Magnaporthe oryzae (Couch) [anamorph = Pyricularia oryzae Cavara] (Cavara, 1881) formally known as Pyricularia grisea (Cooke) Sacc. [teleomorph Magnaporthe grisea (Herbert) Barr; Rossman et al., 1990; Couch and Kohn, 2002], sheath blight disease caused by Rhizoctonia solani, aggregate sheath spot disease caused by Rhizoctonia oryzae-sativae [teleomorph: Ceratorhiza oryzae-sativae; Moore, 1989; Seint San et al.; 2009], bakanae disease caused by Fusarium moniliforme and wilt disease caused by Fusarium oxysporum, brown spot disease caused by Bipolaris oryzae, and false smut disease caused by Ustilaginoidea virens. The extent of losses due to these pests fluctuates widely depending upon the prevailing factors of abundance of these pests in a particular year / season and the local agroclimatic conditions. Losses of about 30 % average cumulative were reported. The Natural Resources Institute (NRI) London has developed a methodology for ranking different pests and diseases affecting agricultural crops. The knowledge of rice pests has been greatly expanded during the last few decades.

1.5. Management of fungal diseases

Outbreak of fungal diseases causes significant loss in many important plants. Many fungi are harmful as they are pathogens of plants, animals and human beings or produce metabolites that are toxic to plants and animals (Richard et al., 1993; Bowers and Locke, 2000). Generally, fungicide is used for control but despite their success, the
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use has not resulted in the complete eradication of pathogens. Moreover, indiscriminate use of fungicide has resulted in several adverse effects like development of resistance, resurgence of pathogens, toxic effects on beneficial microflora of the soil, residual toxicity to human beings, domestic animals, etc. and takes long time to degrade completely (Fawcett and Spencer, 1970). Therefore, there is a need for more effective and less toxic new antifungal agents (Himejima and Kubo, 1992; McCutcheon et al., 1992; Moossavi et al., 2001).

Control of fungal diseases depends mainly on fungicides (Minton 1986; DeVay et al., 1988). Fungicides are expensive, can cause environmental pollution and may cause the selection of pathogen resistance (Lumsden and Locke, 1989). The number of effective fungicides with no effect on environment is rare. The broad spectrum biocides used to fumigate soil before planting, particularly, methyl bromide, are environmentally damaging. The most cost effective, environmentally safe method of control is the use of resistant cultivars, when they are available (Fravel et al., 2003). For this reason, alternative methods of controlling the disease have been studied with emphasis on novel compounds derived from plant source and biological control.

Biological control is becoming an important component of plant disease management and offers solvations to many of the persistent problems in agriculture. The development of biological products based on beneficial micro-organisms can extend the range of options for maintaining the health and yield of crops. In the mid-1990s in the USA, Bacillus subtilis started to be used as a seed dressing, with registrations in more than seven crops and application to more than 2 million hectare. The genera Pseudomonas, Arthrobacter, Clostridium, Achromobacter, Micrococcus, Flavobacterium and Bacillus species are the most common types of bacteria isolated
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from soil samples. Many reports of rhizosphere colonization and root disease control with *Bacillus* spp. introduced as seed inoculants.

Microbial control of fungal and insect pests is in great demand in agriculture at present. The interest in microbial pesticides has expanded because of problems, such as insect pest resistance, emergence of secondary pests, and toxic residues, that have developed with use of the broad spectrum chemical pesticides. Moreover, recent successes of microbial pesticide have greatly encouraged their usage. Insect pests cause a wide variety of damage to plants. In the tropics it is virtually impossible to cultivate without resorting to pesticide application for controlling insect pests. Excessive use of chemical pesticides has posed serious problems of environmental contamination through the interference in the food chain. Thus there is a continuous need for safer, ecologically tenable pesticides. Bio-pesticides, as a component of integrated pest and disease management are cheaper and eco-friendly than synthetic pesticides and fungicides. So, it is the need of the hour that we must resort to safer agricultural practices for improved crop productivity. The interest towards organic agricultural produce is mounting to catch up benefits of present day market. Also the future agricultural exports face the risk of rejection if they are not free of pesticide residue. Use of naturally occurring microorganism which can suppress / control the growth of pathogen / disease will be a viable alternative choice.

Hence, nowadays interest has been shifted to environmentally safe and economically viable alternatives for crop improvement. Use of naturally occurring, free-living bacterial species, which can protect and promote plant growth by colonizing and multiplying along the root surface / root cortex of the introduced plant, is found to be a safe and suitable method to overcome this problem. Plant growth promotion by these
rhizobacteria, collectively referred as Plant Growth-Promoting Rhizobacteria (PGPR), can result in physiological and chemical stimulation of plant roots, resulting in faster emergence, higher chlorophyll content and enhanced stature (Reddy et al., 2003). Growth promotion can also depend on suppression of either deleterious microorganisms of the soil, that reduce plant growth and development, and/or of soil borne pathogens that cause diseases (Ogoshi et al., 1997).

In the present study, the pathogenicity tests have been conducted to confirm the pathogenicity of different phytopathogenic fungal strains of rice crop. The efficacy of PGPR and its bioactive metabolites on the growth promotion and development of induced resistance against these fungal diseases was studied. Also, in this research study molecular characterization of these fungi has been done using random amplified polymorphic DNA (RAPD) and MIF specific primer and the identification of these fungi has been done using D1/D2 region of LSU (Large SubUnit: 28S rRNA) based molecular technique.

1.6. Objectives

1) Screening and selection of bacterial strains through \textit{in-vitro} antagonism against potent phytopathogenic fungal strains.

2) Plant growth promotion and induction of disease resistance against potent phytopathogenic fungal strains under gnotobiotic and green house conditions.

3) PCR-RAPD Based genetic variation and DNA fingerprinting for phytopathogenic fungal strains collected from different parts of India.