CHAPTER 1

Introduction

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1.1 Iron: An essential element

Iron (Fe) is the fourth most abundant element in the earth's crust following oxygen, silicon, and aluminium (Storey, 2005). Iron is a vital element required by almost all living organisms, including bacteria, with the exception of only a few, including *Streptococcus sanguis,* some *Lactobacillus* spp. and *Borrelia burgdorferi* (Archibald 1983; Posey and Gherardini, 2000; Logeshwaran et al., 2009). So it is said that iron is universally required by all living cells. Iron is involved in many important cellular processes such as the electron transport chain and in deoxyribonucleotide biosynthesis and acts as a cofactor for many enzymes, such as ribonucleotide reductase, nitrogenase, peroxidase, catalase, and succinic dehydrogenase (Litwin and Calderwood, 1993). It also participates in other significant biological processes, such as photosynthesis, methanogenesis, H_2 production and consumption, respiration, the tricarboxylic acid cycle and gene regulation (Andrews et al., 2003). Iron is also essential in nitrogen fixation, in which the nitrogenase enzyme utilizes iron alone, or molybdenum or vanadium together with iron to reduce atmospheric nitrogen to ammonia. Iron plays a vital role in oxygen transport in both hemoglobin and myoglobin in which oxygen is bound to the Fe (II)-heme (Storey, 2005).

1.2. Siderophore: a carrier

The term siderophore is derived from the Greek word which means "iron carriers". While iron is widespread in the environment, but under aerobic conditions at nearly neutral pH it is present in an extremely insoluble form, such as hematite, goethite, and pyrite or as polymeric oxydehydrates, carbonates, and silicates which rigorously limit the bioavailability of this metal (Matzanke, 2005). It is considered biologically unavailable as it is often found only in the form of highly insoluble Fe(III) ion. In aerobic environment ferric ion (as free molecule) occurs in very low concentration due to the low solubility constant of $Fe(OH)_3$ ($K_{sol}=10^{-38}$). So iron is present as highly insoluble ferric hydroxide complexes which are forms that severely restrict the bioavailability of iron (Braun et al.1998). The solubility product of $Fe(OH)_3$ is approximately 10^{-38} so by calculation, the concentration of Fe^{3+} at neutral, aerobic conditions is 10^{-17} – 10^{-18} M in the absence of any external Fe(III) chelators. In response to this situation, one of the most common strategies for iron sequestration in an aerobic environment is through the synthesis and excretion of low molecular weight, high affinity chelators, with a very high and specific affinity for Fe(III), known as siderophores. Microbial metabolic products (mainly secondary metabolites) can be classified as siderophores, if

(i) they exhibit ability for iron chelation,

(ii) they participate in active transport across the cell membrane(s) and

(iii) their biosynthesis is regulated by the intracellular iron level.

These siderophores are able to solubilise iron prior to transport into the cell (Winkelmann, 2001). However, they also exhibit affinity to other metals. They are produced by bacteria, fungi and some monocotyledonous plants (Das et al., 2007). Over 500 different siderophores have been identified and are produced by various organisms (Butler and Martin, 2005). The secretion of siderophore in environment and entry of siderophores through cell walls or bacterial membranes is a highly specific process which is regulated by an array of proteins, up to eight in numbers, in most microbes (Matzanke, 2005). The advent of modern molecular biology has enriched us with various methods enabling high-yield production of specific gene products relevant to siderophore-synthesis and -transport, and analyses of structure-function relationships.

1.3 Biocontrol of plant pathogens

Almost all the cultivated crop plants on earth are attacked by plant pathogens which cause different types of diseases which may often lead to considerable damage and loss in yield. Plants provide us not only our food but also it provides us feed, fibre and presently fuel. Therefore, in order to avoid crop-loss and prevent socio-economic disaster especially in the developing countries, more efficient control of pests and diseases is of prime importance. Soil-borne pathogens including fungi, bacteria and nematodes have deleterious effects on agricultural field and conventional control measures like breeding of resistant varieties and crop rotation fail to reduce disease incidence. The pathogens survive by feeding on root exudates of host plant and reside there.

Chemical pesticides are in use for more than hundred years to combat the plant pathogens that pose a threat to the cultivated crops. It has been used by farmers worldwide as most effective tool in preventing economic losses. However, due to several reasons, use of chemical fungicides for addressing plant disease problems has become unpopular and even unacceptable in some cases. The reasons include negative impact on environment and modified safety regulations, effect on non-target organisms, development of pathogen resistance, increasing cost of pesticides and non-functionality of chemicals in particular cases.

Pathogenic microorganisms affecting plant health are severe threat to crop production and ecosystem sustenance worldwide. As agriculture is advancing and intensifying over past few decades, producers are becoming more and more dependent on chemicals as a relatively reliable method of crop protection. However, increasing use of chemical fungicides causes several deleterious effects, i.e., development of resistance strains of pathogen to that chemical and their non-target environmental impacts (Compant et al., 2005). Furthermore, the increasing cost of such fungicides, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food has led to a search for substitutes for these products. There are also a number of diseases for which chemical solutions are few or sometimes, ineffective.

With more strict regulations on chemical usage along with an increased pressure for minimizing chemical usage, the available number of usable compounds has considerably decreased. In order to meet the growing consumer demand for organic food, a lot of interest has developed on finding an alternative eco-friendly method in plant disease control.

In plant protection studies, the term 'biological control' is used for describing the utilization of living organisms with an aim to restrict the growth and proliferation of pests and pathogens. Biological control in plant pathology pertains to the use of antagonistic microbes for the purpose of disease suppression. These antagonistic microbes affect the growth of pathogens by a variety of mechanisms. These include antibiosis, parasitism, production of cell wall degrading enzymes, degradation of pathogenicity factor and competition for nutrients, space or infection sites (Pal and Gardener, 2006; Whipps, 2001).

Kloepper et al. (1980) were the first to demonstrate the importance of siderophores in the mechanism of biological control. Siderophores mediate the limited amount of iron in the rhizosphere, deprive pathogens of iron and suppress their growth. Many reports are available showing involvement of siderophore in the suppression of plant pathogenic fungi (Bakker et al., 1986; Kloepper et al., 1980; Loper and Buyer, 1991).

The microbial world is enormously rich in its diversity and is an infinite pool of organisms which may be utilized to fight plant pathogens (Emmert and Handelsman, 1999). Study of available literature shows that a wide spectrum of bacteria has been used as inoculums in disease management practices of various crops. They not only control or inhibit plant pathogens, but have often been found to induce resistance and stimulate plant growth (Huang and Wong, 1998; Ross et al., 2000; Berg et al., 2001; Zhang et al., 2002; Sabaratnam and Traquair, 2002; Collins and Jacobsen, 2003; Xue et al., 2009). Soil-borne, non-pathogenic bacteria with the ability to antagonise fungal phytopathogens and thus prevent plant disease represent a realistic alternative to chemical fungicides (Walsh et al., 2001). These bacteria are known by several generic names, including biological control agents (BCAs), plant growth promoting rhizobacteria (PGPR) and biopesticides.

India is the second largest vegetable producer after China with 11% production share in the world based on the information provided by the report of the Working Group on Horticulture, Plantation Crops and Organic Farming for the XI Five Year Plan (2007-12). West Bengal contributes a significant amount in making India the second largest vegetable producer. There is a wide diversity of horticultural crops grown in West Bengal. The sub-Himalayan region of West Bengal, commonly known as North Bengal, is an agriculturally developed area which cultivates various crops. Major crops include fruits and nuts, vegetables, spices, plantation crops, medicinal and aromatic plants, flowers and ornamentals. Common vegetables are: tomato, brinjal, chilli, cabbage, cauliflower, radish, carrot, pea, lady's finger (okra, bhendi), leafy vegetables, turnip, beet, tapioca etc (Source: National Horticulture Mission Action Plan for West Bengal, September 2005/ http://nhm.nic.in/actionplan/actionplan_wb.pdf). Being largely dependent on agriculture, the economy of North Bengal thrives on the well being of the agricultural system and seeks for more sustainable and eco-friendly way of plant disease control.

1.4. Objectives

A variety of biological control measures are available for application, but further development and effective adoption requires a greater understanding of the intricate interactions among pathogen, biocontrol agents and the environment. The research presented here aims towards a better utilization of soil microbes in limiting fungal diseases of crops. Siderophore production is a beneficial trait of antagonistic microbes as it can not only deprive the plant pathogen of iron but may also provide the plant with an additional iron acquiring pathway that may promote plant growth. But a literature study reveals that siderophore production trait in antagonists have not received due attention; particularly its capacity as a major contributor to biocontrol mechanism has not been emphasized. Hence, it was considered worthwhile to study the soil inhabiting siderophore producing bacteria with antagonistic potential against phytopathogens and characterize the type of siderophore they produce. Additionally a study on how far these bacteria can actually reduce disease occurrence in the plant is also necessary. Therefore the basic objectives of the present study are

1. To isolate siderophore producing bacteria from soil.

2. To study the antifungal activity of the isolated siderophore producing strains in suppressing some plant pathogens *in vitro.*

3. To characterize the selected siderophore-producing and antagonistic strains and their identification.

4. To partially purify and chemically characterize the siderophores produced by the selected strains.

5. To study the efficiency of siderophore producing bacteria in suppressing plant root pathogens *in vivo.*