CHAPTER 1

Lignin is a three dimensional natural plant biopolymer formed by radical coupling of hydroxycinnamyl subunits called monolignols mainly \( p \)-coumaryl, sinapyl, and coniferyl alcohols and creates together with hemicelluloses, a glueing matrix for cellulose microfibrils in tracheary elements and fibers of higher plants (Rastogi and Dwivedi 2008). Lignification occurs along the secondary cell wall formation which is initiated in xylem from cell corners and middle lamellae. It provides mechanical strength and resistance against pathogens and makes the cell walls impermeable to water. In addition, lignification also occurs as a stress response (Kim et al. 2008). The biosynthesis of monolignols occurs through general phenylpropanoid pathway where \( L \)-phenylalanine is an entry point. These monolignols are transported to cell wall from cytoplasm where after deglycosylation these are polymerized into lignin polymers.

Lignin and cellulose are both rather rigid organic polymers (Tuor et al. 1995), which have developed during evolution for construction and preservation purposes (Call and Mücke 1997). The degradation of lignin in the pulping and bleaching processes is essential for the manufacturing of paper products. These compounds have to be exposed to harsh physiochemical conditions to modify or degrade their structure for utilization in the pulp and paper industry (Coll et al. 1993). The problems caused by chemicals used in bleaching forced industry to consider alternative, more environmental friendly methods (Yang and Eriksson 1992). Such a biological alternative to traditional bleaching was provided through the discovery of oxidative enzymes (Poppius-Levlin et al. 1997). There are many types of lignin degrading enzymes such as lignin peroxidase, manganase peroxidase, laccase and glyoxal oxidase. While most of these enzymes are produced from fungi, it can also be produced by bacteria (Ramachandra et al. 1987).

A few years ago, applications of microbial technology in lignocellulose utilization were limited to the production of ethanol and microbial protein by yeast fermentation of spent liquor from sulphite pulping of wood, retting of flax and hemp, production of edible mushrooms, and various waste treatment processes. However, lignin has several
negative implications for effective agroindustrial utilization of plant biomass. It is considered as one of the greatest obstacle for the optimal utilization of the plant biomass for pulp and paper production, textile production, forage digestibility and biodegradation. As a result, the attention has been diverted to its removal or a decrease in its content for better utilization of cellulose. For example, in pulp and paper and textile industries, presently lignin is separated from cellulose by chemical processes. These processes are, however, energy-intensive, costly and exert environment-damaging effects. More recent research and development has resulted in bacterial treatment of stored logs to increase permeability to preservatives, the Ritter process for bacterial treatment to increase storage life and to de-pith bagasse, the use of mycelial fungi for producing fodder protein from spent sulphite liquor, and various improved waste treatment processes, e.g. the UNOX process. Very recent researches have been directed at exploring several other potential applications of the expanding repertoire of microbial technology in lignocellulose utilization. These include schemes for enzymatic or acid catalyzed hydrolysis followed by fermentation to a variety of chemicals; direct fermentation of waste cellulose to biological pulping of microbial protein or chemicals; bast fibres with pectinolytic bacteria; treatment of chemical wood pulping liquors to de foam, decolorize, and deodorize; direct conversion to food and feed; fungal delignification; and improved waste treatments.

Bioligninolytic systems are beneficial for various purposes, viz. partial delignification for the production of cellulosic products (biomechanical pulping, bio-bleaching), conversion of lignocellulosics into feed and food for improving ruminant digestibility, biodegradation of lignin (treatment of lignin-derived wastes i.e., decolorizing, removing BOD, COD) for environment restoration and biomodification of by-product lignins to yield valuable polymeric or low molecular weight chemicals (Blanchette 1995; Hatakka 2001; Scott and Akhtar 2001). Moreover, microbial degradation of lignin has several potential advantages over conventional chemical methods (Falcón et al. 1995; Kuhad et al. 1997; Hatakka 2001). These include greater substrate and reaction specificity, lower energy requirements, lower pollution generation and higher yields of desired products.
Thus knowledge of genetic variation of ligninolytic microbes is important for their efficient, economical, and environment-friendly use in above processes.

Molecular marker analysis, joined with phenotypic evaluation, is a powerful tool for grouping of genotypes based on genetic distance data and for selection of progenitors. As little is known about the diversity of lignin degrading microbes and their role in lignin degradation and nutrient cycling, the main impetus of the research should be on the development of PCR based techniques for the identification of microbial community structure in environmental samples. Thus, amplification of microbial ribosomal rRNA genes (16S rRNA for bacteria and 18S rRNA for fungi), RAPD analysis and amplification using gene-specific primers provide convenient and rapid identification and / or assessment of the differences in the genetic composition of related individuals and have been employed for the determination and assessment of genetic diversity, both within and between species of microbes. However, no single tool allows definitive assessment of the soil microbial community. Therefore, the use of a polyphasic approach involving a combination of molecular biology, microbiological, morphological and biochemical techniques is necessary to obtain a better understanding of the interaction between the microorganisms and their natural environment. The information obtained from these techniques is of practical use for mapping the bacterial/fungal genome. In the context of ex situ conservation, development of molecular markers may be very useful for the management of germplasm collections.

Thus, research on lignin biodegradation needs to be accelerated greatly, mainly because of the substantial potential applications of bioligninolytic systems in pulping, bleaching, production of biofiber, biofuel, enhance forage digestibility and for converting lignins to useful products, etc.

The main purpose of this work was to screen and characterize microorganisms from soil and effluent samples at morphological, biochemical and molecular levels.
Objectives of the study

The objectives of this study consists of the following four parts:

I. Isolation and screening of microorganisms from soils and effluent samples.

II. Morphological and biochemical (qualitative) characterization of lignin degrading microbial isolates.

III. Analysis of lignin degrading enzyme activities from various isolates.

IV. Molecular characterization of lignin degrading microbes.