SUMMARY

Lectins are nonenzyme, nonimmunoglobulin proteins that have at least one carbohydrate binding domain. Lectins comprise of a structurally diverse class of proteins. They are found in organisms ranging from viruses and plants to humans and serve to mediate biological recognition events. Microbial lectins are known for more than 80 years and still only a few bacterial lectins are fully characterized for their structure and functions. These lectins are very diverse in their molecular properties as well as in their sugar specificities. They are involved mostly in cellular adhesion, which may or may not exhibit cell agglutinating activity and may or may not be inhibited by simple monosaccharides.

Microbial lectins include bacterial, fungal, viral as well as protozoal lectins. Among bacteria, *E. coli* lectins are the most studied ones. However, many other bacteria also produce lectins most of which are in the form of pili which are filamentous rod like surface appendages. Nonpilus adhesins are also exhibited by some of the microorganisms. Microbial adhesion to the specific tissue through these lectins plays an important role in the infection (parasitism), symbiosis as well as commensalism.

In the last decade, interest in microbial lectins has increased tremendously. Studies on the structure and specificities and their biogenesis has led to a better understanding of the proteins as such, as well as their biological

functions.

Although much work has been done on human and animal pathogens and lectins produced by them, plant pathogens have not received much attention. Hence the present investigation was carried out to screen, purify and characterize lectins from plant pathogens.

Chapter 1 : General Introduction

This part comprises of literature survey with reference to 1. microbial lectins including bacterial, fungal and viral lectins and 2. plant pathogenic bacteria and their specific attachment to the hosts.

Chapter 2 : Purification and characterization of lectins from *Agrobacterium radiobacter* NCIM 2443 (ATCC 6466).

Crown gall tumor disease of dicotyledonous plants is known to be harmful and is produced by *Agrobacterium* strains. These are Gram negative short rods. The very first essential step in the disease production is the site specific attachment of the microbe to its host plant and this attachment is belived to be through lectin-carbohydrate interactions and hence the lectins produced by *Agrobacterium* were taken up in the present study.

Ele ven isolates of Agrobacteria were screened for extracellular lectin production and *Agrobacterium radiobacter* was selected because of its highest lectin producing capacity. *Agrobacterium radiobacter* NCIM 2443 (ATCC 6466) is the only known nonpathogenic microorganism of the genus. This microorganism produced 2 different kinds of lectins.

1. Extracellular lectin (Lectin I) : *Agrobacterium radiobacter* NCIM 2443 produced a lectin extracellularly within 48 hours of fermentation. The protein was a monomer ofrelative molecular mass M_r 37000. It is not a glycoprotein itself but is strongly associated with the polysaccharide produced by the organism just like the *Xanthomonas* strain. The lectin showed a pI of 4 and was very specific for the oligosaccharide glcNAc-glcNAc-Man. Tobacco plant extracts inhibited the lectin activity. The protein was purified by conventional chromatographies involving ion exchange (DEAE) and hydrophobic (phenyl Sepharose) chromatographies after a step of ammonium sulphate precipitation. The lectin was most stable at pH 5.0 and it retained the haemagglutinating activity as well as its monomeric form only in the presence of EDTA (1mM). The amino acid composition showed that it had a large proportion of acidic amino acids and no cysteine.

2. *Agrobacterium radiobacter* NCIM 2443 produced another lectin (Lectin II) which was situated on the surface. It could be the pilus protein. The extraction procedure involved shaking a (5 % w/v) suspension of *Agrobacterium radiobacter* cells at 60°C in presence of 6 M urea for 30 min. The cell debris was removed by centrifugation (9770 Xg, 20 min.). The extract was then subjected to 60 % ammonium sulphate precipitation and loaded onto a Sepharose 4B column in presence of 6 M urea. The first fraction just after the void volume showed lectin activity. The purified lectin was of relati ve molecular mass M_r 40000 and it formed aggregates after dialysis against plain buffer. The amino acid composition

showed that it had 44 % hydrophobic amino acids. It showed specificity for D- glucosamine and its pI was 9.15. Its activity also was inhibited by Tobacco plant tissue extracts. Among plant polysaccharides tested for haemagglutination inhibition of the lectin, Locust gum inhibited the lectin activity strongly.

Chapter 3 : Chemical modification studies on lectins from *Agrobacterium radiobacter* NCIM 2443 (ATCC 6466).

To determine the amino acid residues involved in sugar binding, both of the lectins from *Agrobacterium radiobacter* NCIM 2443 were subjected to chemical modification.

1. Extracellular lectin (Lectin I) : Chemical modification studies on purified extracellular lectin revealed that tryptophan and carboxyl groups are involved in sugar binding.

The protein is associated with polysaccharide produced by the microorganism and it could protect the protein from chemical modification reagents. To get rid of excess of polysaccharide, the purified lectin was passed through hydroxyapatite column, in presence of 5mM phosphate buffer pH 6.8. Further studies revealed that number of tryptophan residues involved in binding was 2 and modification of tryptophan caused more than 85 % loss of activity. Modification of carboxyl groups resulted loss of 60 % loss of haemagglutination activity.

2. Surface lectin (Lectin II): Chemical modification studies of surface lectin revealed that histidine and carboxyl groups were involved in sugar binding. With modification

of tryptophan 50 % loss of activity was obtained whereas modification of histidine residue caused 100 % loss of lectin activity and with Woodward's reagent K, the activity loss was 75 %.

Chapter 4 : Purification and characterization of an extracellular lectin from *Xathomonas campestris* NCIM 5028 (ATCC 29497).

In all 25 isolates of *Xanthomonas* were screened for extracellular lectin activity. Among them, culture No. 25 showed promising results and therefore was selected for further studies. The time course of the production showed that the lectin was produced always along with an acidic polysaccharide popularly known as xanthan gum. Reduced polysaccharide production hampered lectin production also.
A lectin from *Xanthomonas campestris* NCIM 5028 was isolated which was produced extracellularly by the organism within 48 hours of fermentation. The purification procedure of the lectin was very simple and it involved only one chromatographic step (hydrophobic chromatography with phenyl Sepharose) after ammonium sulphate fractionation step. The lectin was a protein of relative molecular mass 70000; a dimer consisting of subunits of M_r 28000 and 30000. It showed specificity only towards fetuin glycoprotein and its o-linked glycopeptide but not with other mono or oligosaccharides. Isoelectric focussing revealed that the strong association between polysaccharide and protein did not involve covalent bonding. All of the polysaccharide remained on the acidic side whereas the lectin concentrated itself at pH 7.2.

The lectin itself was not a glycoprotein and it must be attached to the polysaccharide through hydrophobic and ionic interactions. Lectin molecule alone (without any attached polysaccharide) remained stable only for a few hours only. *Xathomonas campestris* var. *campestris* NCIM 5028 used in this study was pathogenic for the *Brassica oleracea* plant. *Brassica oleracea* tissue extracts inhibited the lectin activity strongly. Inhibition of lectin activity by host plant polysaccharides is supposed to be a clue for its specific attachment to the host plant tissue.

Chapter 5 : General Discussion.

In this part of the thesis, lectins produced by plant pathogenic microorganisms, their role in pathogenicity and disease production is discussed.