CHAPTER 5

General Discussion

5.1 Plant - bacterial interactions

The attachment of microorganisms such as bacteria to human, animal or plant host tissues requires adhesion factors (1,2). Interaction of bacteria with eukaryotic structures leads to colonization. The adhesion factors or adhesins can be considered as virulence factors contributing to the pathogenicity of the bacteria since they play a major role in the colonization of ecosystems where bacteria lacking adhesins would not survive. The majority of adhesion factors are of fimbrial nature (3) and since many of the fimbrial as well as nonfimbrial adhesins recognize glycoconjugates on other cells, they are classified as lectins.

Plants coexist with a great number of microorganisms. Plant microbe interaction can be beneficial, as in symbiotic *Rhizobium* - legume and mycorrhizal root associations, but in other cases microbes represent potential pathogens with the ability to parasitize the plants and cause disease. Early in the interactions between plants and microbes, signals are produced that elicit discrete responses in the respective partners, the first step in the cascade that determines the outcome of the symbiosis or pathogenesis. In most cases however, the receptors and signaling mechanisms mediating these responses are ill defined. Signal generation and perception in many of the microbes that interact with plants are understood in greater detail by parallel findings from other prokaryotic and eukaryotic microbes.

In symbiotic bacteria, a relationship has been estab-

lished between *Bradyrhizobium japonicum* and soybean plant. Host plant lectin was proposed to involve in the specific attachment of the homologous *Rhizobium* system to host roots and the initiation of the infection process (4). The same mechanism involved in the attachment of *Rhizobium leguminosarum* pv. *Trifolii* to the white clover root surface has been reviewed (35,36). But in case of *Bradyrhizobium* the available evidence fails to provide a compelling reason to accept the notion that soybean agglutinin plays specific role in surface recognition events. Instead it has been shown that *Bradyrhizobium* produces a lectin which plays a significant role in host surface attachment and symbiosis (5,6).

During their initial association with plant hosts, pathogenic bacteria interact with plant cell walls. The results of this interaction appear to determine whether bacterial multiplication will take place. With one group of bacterial plant pathogens *viz.* - *Erwinia*, attachment to host surface appears to be essential for pathogenesis and it has been shown that it produces a fimbrial lectin which might be responsible for attachment (37,38). With another group of microbes, it is the other way round. Among Pseudomonads, only those strains that do not attach to the host cell wall are able to multiply in the intercellular spaces and infect the host successfully e.g. *Pseudomonas solanacearum* (8). Attachment of *Pseudomonas* strains to tobacco mesophyll cell wall leads to a rapid hypersensitive response (HR) and a drastic reduction in bacterial multiplication.

5.2 Pathogenicity factors of Agrobacterial infection

Site specific attachment of *Agrobacterium tumefaciens* to the plant cell surface is an essential step in tumorigenicity. This statement was based on the observation that avirulent *Agrobacterium tumefaciens* strains which were still able to attach and inhibit initiation of tumor formation by virulent strains, presumably by occupying all available binding sites on the plant cell surface. In addition, specific receptors on the *Agrobacterium tumefaciens* cell surface were supposed to be involved since tumorigenesis could not be blocked by inoculation with heterologous bacteria such as *Rhizobium meliloti or Pseudomonas aeruginosa* (7).

During the last two decades evidence has accumulated that attachment of *Agrobacterium tumefaciens* to plant cells is a two step process (9). In the first step *Agrobacterium tumefaciens* adheres to plant cell surface as a single cell. In the second phase, in response to plant factors, *Agrobacterium tumefaciens* elaborates cellulose fibrils that entrap bacteria resulting in the formation of bacterial aggregates. These fibrils also cause the bacteria to bind very tightly to the plant cell surface (10).

There are several molecules which are claimed to be involved in the early stages of *Agrobacterium* attachment and infection.

1. Low molecular weight polysaccharide β 1-2 glucan : β 1-2 glucan is claimed to be a mediator molecule between plant cell surface and bacterium. *chvA* gene codes for a protein

that is necessary for the transport of β 1-2 glucan into the periplasm. The *chvB* gene codes for 235 kDa protein which converts glucose into cyclic β 1-2 glucan (11, 13) and both these genes have been shown to be involved in the bacterial interaction with the plant host.

2. **Lipopolysaccharides** : Lipopolysaccharides produced by Agrobacteria have also been reported to be involved in attachment (14).

3. **Chemotaxis** : Agrobacteria are peritrichous, motile bacteria and possess a highly sensitive chemotaxis system which responds to a wide range of amino acids and sugars. Some of these sugars like galactose, glucose, arabinose, fucose and xylose have been identified as *vir* gene inducers (15). The genes involved in the general chemotactic response towards amino acids and sugars are located on the chromosome. In addition, Ashby (16) reported that *Agrobacterium tumefaciens* C 58 also exhibits chemotaxis towards acetosyringone, one of the major plant phenolic *vir* gene inducers. Broek and Vanderleyden (17) have suggested that the migration towards acetosyringone may constitute the first step in the recognition between *Agrobacterium tumefaciens* and its host plant in soil. Therefore specific chemotactic attraction may guide the bacteria towards plant wounds where the concentration of the inducer is sufficiently high to switch on expression of *vir* genes. However this hypothesis is still in dispute.

4. **Lectins** : Depierreux *et al* (12) have claimed that lectin resides on the surface of the bacterium while plant cell

surface represents the carbohydrate ligand. Lectin produced by *Agrobacterium tumefaciens* has been shown to be an important determinant of infection at very early stage.

As the attachment of *Agrobacterium tumefaciens* to host cells is saturable, presence of specific receptor molecules on the plant cell surface has been postulated for a long time. It is supported by the observation that avirulent strains like *Agrobacterium radiobacter* biologically control the crown gall disease. This may be because both (virulent as well as avirulent strains) of them bind to the same specific receptor molecule on the plant surface (19,20).

It has been shown recently that *Agrobacterium tumefaciens* produces pili which are essential for T DNA transfer from bacterial cell to plant cell. Thus for attachment and channel formation between bacterial and host cell pili are required which may represent lectin-carbohydrate interaction (18).

Two different lectins have been isolated from avirulent strain *Agrobacterium radiobacter* ATCC 6466 in the present study. The lectins show different sugar specificities. One of them is produced extracellularly (Lectin I) whereas the other one is cell bound (Lectin II). The cell bound Lectin II was specific for acidic plant polysaccharides whereas the extracellularly produced Lectin I was specific for high mannose type glycosyl moieties of glycoproteins i.e. invertase or ovalbumin. The core structure for which the lectin I is specific, is probably glcNAc-glcNAc-man3. These lectins, produced by avirulent strain of *Agrobacterium*, are

indicative of involvement of a lectin carbohydrate interaction in the adhesion of the microorganism to the host plant.

5.3 Pathogenicity factors of *Xanthomonas*

Many laboratories have used genetic methods to analyze aspects of pathogenicity of bacteria to plants. Many similarities have emerged between sets of "pathogenicity " genes discovered in various pathogens. Daniels *et al.* (21) cloned pathogenicity genes from *Xanthomonas campestris pv. campestris* causing black rot of crucifers. They include *hrp* genes, genes for extracellular enzymes and polysaccharide genes.

1. ***hrp* genes** : *hrp* mutants are defective in their ability to incite hypersensitive response and they have been isolated from *Erwinia*, *Pseudomonas* and *Xanthomonas* (22). The mutations fall in clusters of genes covering at least 20 kb. *hrp* mutants of *X.campestris pv. campestris* were not affected in any enzyme or extracellular polysaccharide production and pathogenicity defect is not caused by ability to produce any of these factors. Starvation promotes *hrp* genes expression. Recent sequence data on *hrp* genes of *Pseudomonas solanacearum* and *Xanthomonas campestris pv. vesicatoria* indicate that some of the gene products are related to some pathogenicity determinants of animal pathogens, particularly *Yersinia* (C. Boucher and U. Bonas *et al.* , Abstracts of the sixth international symposium on molecular plant-microbe interactions 1992).

2. **Extracellular enzymes** : *Xanthomonas campestris pv. campestris* produces a number of extracellular enzymes including protease, endoglucanase, polygalacturonate lyase, lipase

and amylase. Since these enzymes have the capacity to degrade plant cell compounds, they are the obvious candidates as pathogenicity factors.

Proteases : Work from several laboratories has suggested that extracellular proteases of phytopathogenic bacteria have a role in disease development. Protease deficient mutants of a number of pathovars of *X.campestris* have been shown to give less severe disease symptoms and lower bacterial numbers in their respective hosts (23,24,25). *X.campestris* produces 2 major proteases. PRT 1 is a serine protease and PRT 2 is a metalloprotease (26). Serine protease is an essential pathogenicity factor at early stages of the disease process, but once infection is well advanced and the bacteria have begun to break out from veins into mesophyll tissue, this enzyme is less significant.

Endoglucanase : It is a major extracellular protein. Mutants lacking endoglucanase however show no reduction in virulence (27). The role of the enzyme is not understood but one possibility is that it contributes to bacterial nutrition during the saprophytic phase of the life cycle.

Pectic lyases : 3 types of lyases are produced, however there is no effect on virulence (28).

3. Extracellular polysaccharide : *X.campestris pv. campestris* produces acidic polysaccharide in large amounts popular as xanthan gum. EPS^- mutants have been shown to have reduced virulence (29). However, in some cases, certain strains retained partial or full virulence. The polysaccharide pre-

vents dessication and thereby helps the microorganism in survival in the vicinity of plant and it also helps in rapid spread and multiplication of the bacteria.

4. Bacterial pilus : Pilus is a structure that is able to bridge the distance between a bacterial cell and a solid surface such as a plant leaf. These filaments are in many cases hydrophobic, suggesting a rather nonspecific hydrophobic interaction between the fimbriae and plant surface. On the other hand, specific interactions are also known in several cases (30-32). *Xanthomonas campestris pv. campestris* can be found growing epiphytically without causing visible symptoms (33). In suitable conditions, bacteria invade the plant tissue through stomata, hydathodes as well as wounds. van Doorn *et al.* (34) have isolated pili from strains of *Xanthomonas campestris pv. hyacinthi* and *Xanthomonas campestris pv. vesicatoria*. Expression of pili is constitutive and these long flexible pili form bundles. It has been shown that the pili show homology with type 4 pili expressed by *Pseudomonas aeruginosa*. It has been suggested that these pili have a significant role in plant surface attachment and infection, although it has not been confirmed.

A lectin from *Xanthomonas campestris pv. campestris* ATCC 29497 was isolated in this study which was strongly associated with the extracellular polysaccharide produced by the organism. The lectin could be a pilus protein which may have been shed in the medium during growth or it could have been produced extracellularly. The lectin was host plant

tissue specific as its haemagglutination activity could be inhibited by *Brassica* plant tissue extract but not by rice plant tissue extract. This indicates a probable role for lectin - carbohydrate interaction in adherence of *Xanthomonas campestris* to its host.

Although much more work in this area is needed to say anything conclusively, it can be said that lectin carbohydrate interactions could be one of the possible ways for bacterial attachment to plant surfaces where lectin resides on the bacteria and plants present the carbohydrate receptors. More work in this area can put some light on exact mechanisms of pathogenicity of plant pathogenic bacteria and their strategies for infection. Such studies would be very helpful in designing preventive measures against these phytopathogens.

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