A century has elapsed since Burrill (1882), Wakker (1883) and Savastano (1886) unequivocally and unmistakably demonstrated the bacterial etiology of plant diseases, but (alas!) phytobacteriology even today compares poorly with other areas of bacteriology e.g. medical and animal bacteriology. To quote J.F. Bradbury (1982), "Although fungi are often isolated, examined and tentatively identified by plant pathologists, bacteria are usually either left to specialists, or recognized only by the symptoms they produce. Sometimes they are even ignored. This neglect of bacteria is usually due to lack of knowledge of bacteriology rather than to the difficulties that may be encountered." The causes are not far to see. M.P. Starr (1983) has ascribed it to disciplinal insularity. The phytopathogenic bacteria were studied exclusively by plant pathologists usually in facilities physically quite separate from those devoted to other areas of bacteriology. There was little intellectual contact between bacterial phytopathologists and other bacteriologists. Their papers were published in different periodicals; they attended different scientific meetings; the paths of phytopathologists, animal pathologists and bacteriologists rarely crossed. Although the barriers have been breached somewhat in recent years, they have by no means disappeared. This disciplinal insularity (Starr, 1979) has had important methodological
consequences, such that the phytopathogenic bacteria were usually studied by methods quite different, for example, from those used by then dominant (medical) bacteriologists.

It is not surprising, given these circumstances of disciplinal insularity, that the early bacterial taxonomists practised a derivative nomenclatural insularity by erecting separate taxa for the phytopathogenic bacteria, segregating them from all other bacteria solely on the basis of plant pathogenicity. For example, the first edition of Bergey's Manual of Determinative Bacteriology handled the phytopathogenic bacteria by placing all of them in a tribe Erwiniae, defined simply as plant pathogens (Bergey et al. 1923). (This held sway for half a century). The effect of this "Erwinieae notion remain with us today".

The situations are changing fast. Phytobacteriology is now poised for a big leap, with the stimulus generated by the publications of some invaluable laboratory manuals (Schaad, 1980; Bradbury, 1982) and books and treatises like "Phytopathogenic Prokaryotes" (Ed. Mount and Lacy, 1982), "Phytopathogenic Bacteria" (ed. M. P. Starr, 1983), Plant Pathogens (ed. Lovelock, 1979).

There is great scope for microbiologists to contribute in this new area of recognizing the bacteria that cause plant disease and the bizarre aspects that fall within the
big expanse called "Plant-bacterial interactions". It may not be out of place to mention here that taxonomy of bacteria, like any other taxonomy, is at a discount. But other organisms like fungi, for example, have had a long record of taxonomic studies, which is not the case with bacterial pathogens. Morphological parameters provide little scope for identification of bacteria and, therefore, search has to be made for stable characters which also at the same time are easily workable. The practical identification is the basis of taxonomy (Sneath, 1972). It includes the production of keys and diagnostic tables, together with methods of discriminating between forms that could easily be confused. The identification of a pathogen to a particular taxonomic entity can pretend considerable information since related organisms tend to have similarities in characteristics which relate to their ecology and disease-causing potentials.

In the recent years studies with plant pathogenic bacteria have been extended to also examining the bacteria that are not causing diseases but are in juxataposition with them; those that be on the surface of plants or in their vicinity. What is their role in the life of phytopathogenic bacteria and in the infection process? We know much about bacteria that share the rhizosphere with plant pathogens during their saprophytic phase but the
realization of the importance of those that are widespread and living on the plant surface as epiphytes. According to Sands (1982) phytopathogenic bacteria are those epiphytes that have escaped from microbial competition on plant surface! Is the abode of these bacteria inside the susceptible a survival mechanism, in absence of resting structure found abundantly in fungi? It will be highly rewarding to discover the relationship (not interaction as this term is preferably used between unrelated organisms) between the epiphytic/saprophytic/parasitic bacterial flora on the aerosphere (aerial plant surfaces). Since Last and Deighton (1965) conclusively demonstrated the existence of saproptic, usually pigmented bacteria, which were significantly more on leaves (phyloplane) than in soil, and that they constituted a characteristic ecological group, a wide and interesting variety of microorganisms are now known to occur on surfaces of plants (Rüinen, 1961; Leben, 1961, 1965, 1974; Blakeman and Brochie, 1976; Verma and Singh, 1976). Majority of these bacteria were producers of extracellular polysaccharide (Sztejnberg and Blakeman, 1973). The bacteria include predominantly gram negative (-ve) genera like Erwinia, Flavobacterium, Pseudomonas and Xanthomonas. The gram positive (+ve) are Bacillus, Corynebacterium and Lactobacillus. These bacteria may react with each other and the effect may have a bearing on the pathogenicity of the plant pathogenic organisms. Some of the bacteria may occur inside the host and react
with the plant to elicit responses which may have a bearing on the host-pathogen relationship. The two most common species are *Erwinia herbicola* (yellow) and *Pseudomonas fluorescens* (green fluorescence under UV light and oxidase positive). Similarly, the epiphytic spiroplasmas have no known plant-pathogenic potential and, their association with plants, are known only from surfaces of flowers (Davis, 1979, 1981). Among the intriguing questions provoked by the discovery of spiroplasmas in this specialized ecological niche are those related to the origins of these spiroplasmas, their relationships with spiroplasmas from other habitats, the nature of their vectors and/or hosts, their possible specificities for flowers and orthopods, possible pathogenicity in vector and non-vector hosts including vertebrates as well as orthopods, and the nature of their associations with vector and plant tissue, and with other microorganisms in the flower site. Davis (1981) has discussed these questions in his article entitled "The enigma of the flower spiroplasmas".

Verma et al. (1980) have reviewed several important aspects in this area viz. the physiological relationship, bacteriostasis, resident phase, over wintering population levels, synergism and antagonism, cross protection and the role of bacteriocin, Bdellovibrio and phage.
It appears that the resident epiphytic bacteria occupying the phylloplane contribute towards protection against disease. When the population is large, some non-pathogenic bacteria (along with the pathogens) enter the plant. Some enter actively before hand and provide "protection". The entry includes host response akin to phytoalexin production. May be bacteria block the attachment or multiplication sites of the pathogenic bacteria. Nature may be operating biological control through them but antagonism can play much greater role in biological control (Baker and Cook, 1974). The line between pathogenic and saprophytic bacterial pathogens seems thinning down. The genus *Erwinia* includes many saprophytes (*Herbicola* group) and *Escherichia coli*, a saprophyte has been found related to *E. amylovora*, *E. chrysanthemi* and *E. herbicola*.

Some strains of *E. herbicola* when incubated at a temperature slightly above optimum for growth (34°C), changed irreversibly to non-pigmented, auxotrophic (for thiamine) variant having closer serological relationship with pathogenic *E. amylovora* strains (Chatterjee and Gibbons, 1971). Such transformations can thus surely occur under field conditions. If so, then the epiphytic bacteria will need a much closer and more serious look with regard to their role in bacterial diseases.

The situation has to change for isolating a bacterium from diseased specimen and name it based on the host.
Pathogenicity has rightly been challenged by M.P. Starr (1981) as the reason to segregate plant pathogenic bacteria. It is not "head" or "tail" affair. Pathogenicity is a very stable affair under influence of great many factors and events.

The practice of naming unreported disease-causing isolates of bacteria as "new" on few characters mostly on the host, and without making vigorous comparisons with other bacteria has to go. Similarly, the usual emphasis on "proving" the uniqueness of the "new" species has resulted in a chaos of probable synonymy.

The efforts made to determine the phytopathogenic specialization (host range) of the "new" bacterial species was non-existent or ineffectual. The common errors and omissions included: absence of direct comparisons with previously named bacteria, failure to control the choice of horticultural varieties used in testing pathogenicity, unconcern over the preparation and condition of inoculum, indifference to the site and mode of inoculation, inattention to the physiological and environmental situation of the host plant during the ineffective incubation period, and bias in the reading and evaluation of the pathological response. "New-host - new species" cliche has been rejected. Many plant pathogenic bacteria, including most pseudomonads and xanthomonads, were relegated to pathovars (pv) from species level (See Dye et al. 1980).
"patho-species" concept so firmly entrenched has been accommodated under this intraspecific designation (p.v.).

Thus, many species have been proposed with disregard or inorgance of available taxonomic information. Plant Pathologists, it must be admitted, despite their much commendable efforts under existing insularity of plant bacterial taxonomy, have been deprived of a sound reference system to which unknown isolates could be compared. They have been denied a taxonomy which is ideally based on a classification which groups together closely related strains, a logical nomenclature and a realistic identification system as envisaged by Williams (1985). He has defined the ideals of microbial taxonomy as given in table (1.1).

Table (1.1): The Ideals of Microbial Taxonomy.

<table>
<thead>
<tr>
<th>Classification</th>
<th>a) High information content</th>
<th>b) Groups should be &quot;natural&quot;</th>
<th>c) Taxospecies should approximate to genospecies</th>
<th>d) Useful to wide range of scientists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nomenclature</td>
<td>a) Names should conform the rules of nomenclature</td>
<td>b) New names should not be applied for trivial reasons</td>
<td>c) Name should predict properties of microbe</td>
<td></td>
</tr>
</tbody>
</table>
Identification

a) Use minimum number of characters needed for accuracy

b) No over-weighting of single characters

c) Result should predict other characters of the microbe.

At a meeting of the Judicial Commission of the ICSB (International Commission of Systematic Bacteriology) in 1973, it was decided to produce a review of the recently valid names of bacteria, with the object of retaining only those taxa which were adequately described and for which there was a type, neotype or reference strain available. After consultation with all the ICSB subcommittees on Taxonomy and experts, the Approved Lists of Bacterial Names was produced, (Skerman, et al., 1980), the aims and consequences of which are summarized in the table (1.2).

Table 1.2: The Aims and Consequences of the Approved Lists of Bacterial Names (Skerman et al., 1980; Sneath, 1984).

1) Contains the names of bacterial taxa that are validly published and in current use.

2) Names excluded from the list lost their standing in nomenclature from Jan. 1st, 1980.

3) Excluded taxa can be re-established in an official publication.
4) To avoid the need to search a wide range of scientific literature, names of new taxa should be published or validated in an official publication.

5) At present, the only official publication is the *International Journal of Systematic Bacteriology*.

This was an extremely useful and timely project which resolved many existing nomenclatural anomalies and provided a baseline for future designations of new taxa. Skerman *et al.* (1980) have listed the names of the plant pathogenic bacteria approved by the Judicial Commission of the ICSB. It is given in the following table (1.3).

The new list is on lines of the eighth edition of the *Bergey's Manual* (1974). In absence of fuller descriptions of the species many of them (unfortunately) were reduced to pathovars (p.v.) and several were totally eliminated, such as *Streptomyces scabies sensu* (Thaxter) Waksman and Henrici-Schaad (1982) feels that this was "unfortunate", because many of these pathovars are legitimate species. The current list of approved names contains only three phytopathogenic fluorescent pseudomonads, *Pseudomonas syringae* van Hall, *P. cichorii* (Swingle) Stapp, and *P. viridiflava* (Burkholder) Dowson. All other such fluorescent pseudomonads listed in *Bergey's* eighth edition (Buchanan and Gibbons, 1974) are currently considered invalid, due to lack of data and,
<table>
<thead>
<tr>
<th>Group</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative</td>
<td>Pseudomonadaceae</td>
<td>Pseudomonas</td>
<td>agarici, andropogonis, <em>avenae</em>, caricapapayae, cariophylli, cichorii,</td>
</tr>
<tr>
<td>aerobic rods</td>
<td></td>
<td></td>
<td>cissicola, gladioli, glumae, marginalis, <em>pseudoalcaligenes</em> subsp.</td>
</tr>
<tr>
<td>and cocci</td>
<td></td>
<td></td>
<td><em>citrulli</em>, <em>rubrilineans</em>, <em>rubrisubalbicans</em>, solanacearum, syringae,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>tolaasi</em>, <em>viridiflava</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xanthomonas</td>
<td>albilineans, <em>ampelina</em>, <em>axonopodis</em>, <em>campestris</em>, <em>fragariae</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agrobacterium</td>
<td>rhizogenes, <em>rubi</em>, <em>tumefaciens</em></td>
</tr>
<tr>
<td>Gram-negative</td>
<td>Enterobacteriaceae</td>
<td>Erwinia</td>
<td>amylovora, <em>ananas</em>, <em>cancerogenae</em>, <em>carnegieana</em>, <em>carotovora</em> subsp.</td>
</tr>
<tr>
<td>facultatively</td>
<td></td>
<td></td>
<td><em>atroseptica</em>, carotovora shubsp. <em>carotovora</em>, <em>chrysanthemi</em>,</td>
</tr>
<tr>
<td>anaerobic rods</td>
<td></td>
<td></td>
<td><em>cypripedii</em>, <em>dissolvens</em>, <em>herbicola</em>, <em>mallotivora</em>, <em>milletiae</em>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>migrifluens</em>, <em>nimipressuralis</em>, <em>quercina</em>, <em>rhapontici</em>, <em>rubrificiens</em>,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>salicis</em>, <em>stewartii</em>, <em>tracheiphila</em>, <em>uredovora</em></td>
</tr>
<tr>
<td>Gram-positive</td>
<td>None</td>
<td>Corynebacterium</td>
<td><em>betae</em>, <em>beticola</em>, <em>facians</em>, <em>flacumfaciens</em>, <em>ilicis</em>, <em>insidiosum</em>,</td>
</tr>
<tr>
<td>actinomycetes and</td>
<td></td>
<td></td>
<td><em>michiganense</em>, <em>nebrakense</em>, <em>cortii</em>, <em>poinsettiae</em>, <em>sepedonicum</em></td>
</tr>
<tr>
<td>related organisms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycetaceae</td>
<td>Streptomycetes</td>
<td></td>
<td><em>ipomeae</em></td>
</tr>
<tr>
<td>Nocardiaceae</td>
<td>Nocardia</td>
<td></td>
<td><em>vaccini</em></td>
</tr>
<tr>
<td>Mycoplasmas and</td>
<td>Spiroplasma</td>
<td></td>
<td><em>citri</em></td>
</tr>
<tr>
<td>genera of uncertain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>affiliation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.3: Approved list of Plant Pathogenic Bacteria (Skerman, et al., 1980).
therefore, considered as pathogens of *P. syringae*. On the contrary, DNA homology data is available that indicates differences among phytopathogenic pseudomonads and does not support the "pathovar" taxonomy. Schaad (ibid) says: "Accepting the indiscriminate use of pathovar for many valid species is a mistake. This is not only a rejection of pathogenicity as an important character, but a rejection of other important data. Certainly many plant pathogenic bacteria have been poorly characterized and do not deserve species ranking, but many currently designated as pathovars have been well characterized. Furthermore, many xanthomonads and fluorescent pseudomonads are easily differentiated not by traditional physiological tests, but by such methods as DNA homology, serology - PAGE, and nutrition. The use of "pathovar" certainly has its place in the nomenclature of plant pathogenic bacteria. On the other hand, its use should be restricted to those organisms which vary only in their pathogenicity. Organisms that can be differentiated by other traits should be designated as species or subspecies".

The Soil-inhabiting bacteria.

The soil-inhabiting bacteria provide the stock from which the pathogens emerge and make their career in the new environment inside the plant. Their study is indispensable in any plan of work with plant associated bacteria, because roots of plants are fixed in soil.
Crosse (1968) placed plant pathogenic bacteria into four groups:

1) with permanent soil phase,
2) with protracted soil phase,
3) with transitory soil phase, and
4) with no soil phase

like any other grouping here also organisms transcend the boundaries. That the phytopathogenic bacteria probably have a greater soil phase than presently understood, has been suggested by Schroth et al. (1979).

It is difficult to know the complete roster of microorganisms or even fungi or bacteria of any soil sample and to study all the possible interrelations in such a field is, to use Kenneth, F. Baker's phrase, "akin to contemplation of infinity".

The Rhizosphere.

Work on soil-inhibiting plant pathogenic bacteria has been confined to the zone around the root called "rhizosphere" a term coined by Hiltner (1904). It is increasingly clear that mastery of the soil microflora will come only when we understand the non-pathogenic organisms even though the pathogens have seemed the logical point of attack.

Certainly there are many more microorganisms injuring
roots than we now recognize. The "despotism of Koch's Postulates", coupled with the failure to realize that a senescent transient root or weakened by a changed environment may be the natural point of entry, have undoubtedly helped to keep our list of root pathogens shorter than that facing the plant.

In a monumental series of papers, Lochhead and his associates, commendably without waiting for the microflora to be catalogued, studied its dynamics and set about systematizing these undescribed legions en masse on the basis of their nutritional requirements. They found that the rhizosphere flora generally requires the amino acids excreted from plant roots but is able to synthesize growth substances and vitamins whereas the soil microflora is less stimulated by amino acids and is somewhat less able to synthesize growth substances. These studies have done much to give character and reality to the rhizosphere concept. In the few root diseases investigated, the severity of attack tended to be correlated in the rhizosphere with a decreased number of bacteria that require amino acids. Exploration of the relation for specific root pathogens, of antibiotics produced by these rhizosphere populations is of obvious promise, but the significance of the production of growth-promoting substances should be equally studied. The relationship of the rhizosphere flora to root-rot resistance in plants, and the effect of this
flora on root excretion are promising areas for investigation. It is now clear that the nature of root exudations, and the conditions which favor such leakage, are of paramount importance.

The root-soil interface, consist of two connected components - the rhizoplane (the actual two dimensional surface) and the rhizosphere (an adjacent volume of soil under the influence of the plant root). As Foster and Bowen (1982) feel "There is a serious lack of information on the distribution of individuals of known identity on roots grown in natural environments infected with the natural microflora". Traditional microbial taxonomy requires the removal of microorganisms from surface for identification so that although we know that species were present, we have no idea where the various forms were located originally and how they may be interacting with the host and with each other. Conversely, except where known microorganisms of distinctive morphology occur, these techniques that tell us the precise location of bacteria (Transmission and Scanning electron microscopy) are unable to reveal the identity of particular individuals, even though different colonies and individual cells can be easily distinguished cytologically (Foster and Rovira, 1978). At present therefore, we are unable to take full advantage of the high resolution of electron microscopes in studies of microbial ecology.
Fortunately, techniques are emerging which may resolve this problem. Of these, immunohistological methods seem most promising. Unfortunately soil fabrics are filled with remnants of bacterial capsules (Foster, 1981b; Foster and Martin, 1981), so capsule materials cannot be used for this purpose at present.

All samplings from rhizosphere of different plants have shown that the bacteria constitute a significant portion of microbial life in soil-root and even soil-free root zones. The out-numbering fungi, algae, protozoa by bacteria could be due to their high resistance to extreme stresses of temperature, soil type, soil-pH, for different ecological interactions, to toxic compounds and most importantly, the nutritional requirements.

Isolation and identification of the bacteria.

Isolation and identification, are the basics in plant bacteriology. As is obvious a clear picture of plant pathogens will emerge only when bacteria can be identified easily and correctly even when they have not been isolated directly from diseased specimens. Since the populations of the pathogens are more than that of the non-pathogens (both absolute and relatively) in fresh, active lesions they can easily be isolated. One must be alert to those less well-known phytopathogenic bacteria that require special culture media or unusual conditions (e.g. anaerobic
incubation), and even the existence of phytopathogenic bacteria that can not be cultured axenically (mycoplasma-like organisms). The situation changes when the diseased specimen is not freshly-infected one overgrown by contaminating microbes. The "art" of isolation has not progressed enough to resolve the hitherto microbial mixtures. However, rational procedures of enrichment and/or selection are known which help in isolating the pathogens from over-grown lesions or from plant habitats.

Qualitative Identification of Plant Pathogenic Bacteria

Schaad (1980) has mentioned the characters that are useful in the characterization of the common phytopathogenic bacteria up to generic level (table 1.4).

Differentiation of the commonly isolated genera of plant pathogenic bacteria that can not be cultured axenically (mycoplasma-like organisms) is easy on the basis of colony characteristics on certain agar media and several key tests such as gram stain and anaerobic growth. Gram staining serves as a very basic and rapid step in the identification flow chart given by Schaad (1980) serves a quick initiation of any detailed examination of a bacteria. Various biochemical tests provide further guides towards the identification of the species. The purity of the cultures must be confirmed. Sex
### Table 1.4: Characters used to differentiate commonly isolated plant pathogenic prokaryotes.

<table>
<thead>
<tr>
<th>Character</th>
<th>Common Genera</th>
<th>Uncertain Affiliated Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corynebacterium</td>
<td>Agrobacterium</td>
</tr>
<tr>
<td>Growth on common media</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gram reaction</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Yellow or orange colonies on NA, YDC, or MBY media + c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluorescent pigment on KB</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Grows anaerobically</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>More than four peritrichous flagella</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Growth on D-1 agar</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Serum-containing medium required for growth</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Possess cell wall</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Helical morphology</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Several bacteria of uncertain affiliations or of minor importance as plant pathogens such as *Acetobacter*, *Bacillus*, *Clostridium*, *Flavobacterium*, *Nocardiia* and *Streptomyces* are not included.

b Only the bacterium associated with Pierce's disease of grape, almond leaf scorch, and alfalfa dwarf has been cultivated *in vitro*.

c Colonies of *Corynebacterium sepedonicum* are generally colorless.
remove persistently contaminating organisms. Media commonly employed in the isolation of plant-pathogenic bacteria from diseased material and from soil are nutrient-agar and media containing tryptone, peptone, beef extract etc., supplemented with one or more sugars. These media were designed initially for bacterial whose ecological niche involves animals and are used for isolating and identifying bacteria primarily of medical interests. It has been difficult to design media for selectively supporting plant pathogens. Some media have been designed for bacteria that develop distinctive colony characteristics so that the plant pathogens may be distinguished in a population of saprophytes. Other media have been more or less selective for certain bacteria i.e. Agrobacterium tumefaciens (Patel, 1926; Riker et al., 1930; Schroth et al., 1930 and Stapp and Ruschmann, 1924); Erwinia carotovora (Noble and Graham, 1956; and Segall, 1969); Corynebacterium species (Crosse and Pitcuer, 1952; Dowson, 1957; Martén et al., 1943; Mohanty, 1951; and Snieszko and Bonde, 1943); and E. stewarti (Ivanofe, 1935); E. rubrifaciens (Schaad and Wilson, 1967) and Pseudomonas mors-prunorum (Crosse and Bennett, 1955).

A medium containing te-trazolium salts used for differentiating mutants of E. coli has been modified and used to differentiate pathogenic isolates of P. solanacearum and E. carotovora from E. atroseptica and E. aroideae.
Pseudomonas species were isolated with a medium containing taurocholate or crystal violet as the selective agent.

Few media have been designed for Xanthomonas; pathogenic cultures of Xanthomonas are maintained on a medium containing commercial carrot juice in glucose-yeast extract calcium carbonate agar, and also on the same medium without carrot juice. The selectivity and differentiating abilities of most media are based on preventing or retarding the growth of cells by using inhibitors of metabolic pathways (e.g. heavy metals, crystal violet) and antibiotics that suppress protein and nucleic acid synthesis (e.g. streptomycin, neomycin, novobiocin, actinomycin D).

Five selective plating media (designated as D-Series media) were developed by Kado and Heskett (1970) for plant pathogenic bacteria in the genera Agrobacterium, Corynebacterium (including species pathogenic for animals and men), Erwinia, Pseudomonas and Xanthomonas. The active constituents of these media were lithium chloride, sodium dodecyl sulphate, polymyxin, and glycine, all of which are known to affect the permeability of bacterial membranes. These selective media were designated using one or a combination of these compounds to permit the growth of bacteria of one genus and restrict the growth of all others. Using inoculum containing a mixture of different bacteria, the efficiencies of these media are as follows: Plating
efficiencies ( [Colony numbers on selective medium: colony numbers on non-selective medium] \times 10^2 ) were 90.3% for \textit{A. tumefaciens} on medium \textit{D}_1; 27.1% for \textit{C. michiganense} on medium \textit{D}_2; 77.7% for \textit{E. amylovora} on medium \textit{D}_3; 6.5% for \textit{P. syringae} on medium \textit{D}_4; and 78.4% for \textit{X. campestris} on medium \textit{D}_5. Recovery efficiencies ( [colony numbers from soil on selective medium] \times 10^2 ) of the respective bacteria from soil artificially inoculated with a mixture of bacteria were 48.1% on \textit{D}_1, 79.8% on \textit{D}_2, 57.0% on \textit{D}_3, 26.9% on \textit{D}_4 and 79.9% on \textit{D}_5. Recovery of the pathogenic bacteria from diseased tissues was facilitated by these media.

Kado and Heskett's media (designated as D-series media); were designed principally on the basis of altering the surface components and membrane of the bacterial cell as proposed earlier. Chosen as constituents, therefore, were compounds such as sodium dodecyl sulfate, lithium chloride, glycine and polymyxin. The advantages of using such compounds are that they eliminate the necessity of using inhibitors of protein and nucleic acid synthesis and allow ordinary constituents in the medium to control growth. Also, there are other advantages of the media e.g. the primary purpose of diagnostic work is the isolation of the pathogen, but efficient use of labor, material, and time becomes increasingly important as the number of diseased specimens in the laboratory increases. The use
of medium that is specifically designed to isolate species of a given genus greatly increases the efficiency and provides a minimum of further investigation. Furthermore, rarely has there been data on the plating and recovery efficiencies of useful media. Kado and Heskett (1970) have felt a need for such media as well as a general medium designed specifically for plant-pathogenic bacteria. Thus, the D-series media were developed avoiding strictly empirical methods by employing multiple titrations of the active constituents of each medium, by replica plating and based on selectively altering membranes of bacteria by the above compounds.

However, there are some puzzling features about the claim made by Kado and Heskett and several of the media (D₂, D₃, D₄ and D₅) have not found favourable response from several workers (Gross and Vidaver, 1979; Schrotth et al., 1981; Starr, 1981). Schaad (1980) has also not made use of all the media in his identification scheme.

The Plant Pathogenic Bacteria.

The plant pathogenic bacteria are placed in the Bergey's Manual (8th edition, 1974) in different groups.

Part 7: Pseudomonas, Xanthomonas, Agrobacterium
Part 8: Erwinia
Part 15: Bacillus, Clostridium
Part 17: Corynebacterium
Part 18: *Rickettsias*-like organisms

Part 19: *Spiroplasma* (= plant mycoplasmas)

The genus *Pseudomonas* is most versatile bacterium having members colonizing all sorts of habitats. Some are plant-pathogenic. There are suggestions that at least five genera may be made out of the present species of *Pseudomonas*. *Pseudomonas* usually fall into two broad categories of fluorescent and non-fluorescent members. The former don't accumulate PBHB (poly-β-hydroxybutyrate) while the latter accumulate this bacterial reserve product. (Exceptional cases of latter accumulating PBHB are known). Not that only PBHB accumulation is not reliable, the production of fluorescent pigment too is not fool-proof. Some non-fluorescent pseudomonads produce pigments similar in appearance to the fluorescent pigments but that they do not fluoresce. We have to remember Starr's (1983) comment "The vagaries of *Pseudomonas* taxonomy have led some to conclude that identification is an ill-defined, some what chaotic mystical art".

The phytopathogenic fluorescent pseudomonads in the current list (Skerman et al., 1980) include only three species: *P. syringae*, *P. cichorii* and *P. viridiflava*. All the other fluorescent pseudomonads listed in Bergey's Manual (8th edition) now stand invalid and are condensed as pathovars of *P. syringae*. *P. syringae* now includes
many pathovars which were previously recognised as species. Starr (1981) has included nineteen nomenspecies in *P. syringae*. *P. savistoni*, a nomenspecies of *P. syringae* incites tumors on stems and leaves of oleander (*Nerium oleander*) and olive (*Olea europaea*) while several other nomenspecies cause chlorosis. Several toxins have been isolated from these chlorosis-inducing nomen species. *P. phaseolicola*, *P. coronofaciens* var. *atropurex*, *P. glycinea*, *P. morsprunorum* are important nomenspecies of *P. syringae* causing chlorosis. Majority of the *Pseudomonas* species belong to the non-fluorescent group. *P. solanacearum* is the most important species which causes wilts, especially in tropical countries. *P. gladioli* (= *P. marginata*), originally described as causing rot of gladiolus now includes also *P. allicola*, the onion-rot pathogen. *P. caryophyllii* is also associated with rots.

The genus *Xanthomonas*.

This is a genus comprising exclusively of plant pathogens and is unmistakable by the typical dome-shaped, yellow and highly-mucoid colonies on sugar-containing media. YDC (yeast extract-dextrose-calcium carbonate) is the best known selective medium on which essentially all xanthomonads grow at 25-30°C. These are Gram-ve, polarly flagellated rods (monotrichous, aerobic, oxidative and catalase positive, intolerent of TTC (triphenyl tetrazolium chloride) medium and NaCl.
The significance of the two diagnostic traits (viz. the yellow pigment and slime) is enhanced by the fact that the pigment and the slime both are entirely different and unique. The yellow pigment is not a carotenoid as are the yellow pigments of *Erwinia, Pseudomonas, Corynebacterium* etc. It is an unusual, hitherto unknown mixture of chemicals collectively called xanthomonadins. Chemically, these are brominated aryl-polyene esters. Different species carry a varying assortment of the constituents of xanthomonadins. Crude extracts of the pigments show typical absorption spectra showing peak at 445 nm. This provides a fool-proof identification of xanthomonadins and thus of *Xanthomonas*.

The slime responsible for the mucoid nature of the colonies is a heteropolysaccharide which is called xanthan gum. Since it is economically valuable it is produced at industrial scale from *X. campestris*. Xanthan gum is made up of a backbone of β-1,4-linked glucose units with short side chains comprising of mannose and galacturonic acid residues, attached to the alternate glucose units.

Only four species of *Xanthomonas*, which can be differentiated by physiological tests, are now valid. All other species of viz. *X. oryzae, X. malvacearum, X. translucens, X. vesicatoria* etc. are now pathovars of *X. campestris*. According to Dye and Lelliott (1974) most of the
nomenspecies listed can be distinguished with certainty only by plant host reactions, but Schaad (1982) contends that these can be distinguished by serological reactions, PAGE (polyacrilamide gel electrophoresis) membrane protein profiles, phase-susceptibility and growth on selective media. Such methods clearly show that many xanthomonads are not simply pathovars of X. campestris. Phytobacteriology has come of age!

Certain plant-pathogenic Erwinia and Corynebacterium species also produce yellow, mucoid colonies but they can easily be known. Yellow-pigmented erwinias are fermentative (not oxidative), peritrichously flagellate (not polar and monotrichous) and, above all, their yellow pigment is carotenoid (not xanthomonadin). Yellow-pigmented corynebacteria are gram +ve (not gram-ve), non-motile, different is shape, and the pigment is carotenoid. Xanthomonas can be easily distinguished from Pseudomonas by its failure to grow in presence of 0.02% TTC or 1% NaCl.

genus Agrobacterium.

The agrobacteria are common soil-inhabitants and constitute a common component of soil microflora, especially in the rhizosphere. These have long survival (2-5 years). The non-phytopathogenic forms predominate in soil and even in the vicinity of diseased plants.
Only about 1% of the isolates from soil are phytopathogenic. In culture, these produce copious slime on carbohydrate-containing media. Colonies are non-pigmented and smooth though some strains do produce rough colonies. The four species mentioned above can be delimited into two groups as indicated below, on the basis of numerical studies, genotypic studies and physiological tests.

Group I: Produce 3-ketolactose, grow on salts plus carbohydrate media, give a neutral or alkaline litmus-milk reaction and use amino acids, nitrites and ammonium salts as sole nitrogen source.
1. Produce galls — A. tumefaciens
2. Don't infect plants — A. radiobacter

Group II: Do not produce 3-ketolactose, slow, weak and no growth on salts plus carbohydrate media, give an acid litmus-milk reaction and do not utilize amino acids, nitrites or ammonium salts as sole source of nitrogen.
1. Produce hairy-rot of nursery stock — A. rhizogenes.
2. Produce galls on raspberries — A. rubi.

Three phytopathogenic species of Agrobacterium are recognized viz. A. rhizogenes, A. rubi and A. tumefaciens.
All induce tumorous outgrowths on their hosts. A. rhizogenes, the "hairy-root" bacterium, invades the primary root of its host and causes a proliferation of secondary roots. A. rubi caused galls on aerial parts of canes, i.e. Rubus, (raspberries and black berries) while A. tumefaciens forms "Crown" galls (at the junction of the stem and root) almost exclusively on dicotyledonous plants (some 140 genera belonging to over 60 families). A. radiobacter which is not plant pathogenic is used to control Agrobacterium infections. Seed inoculation with the K 84 strain of A. radiobacter has effectively reduced crown gall incidence. This strain produces agrocin (a bacteriocin) which inhibits the growth of A. tumefaciens.

**genus Erwinia.**

The genus comprises of Gram-ve, facultatively-anaerobic, motile (peritrichously-flagellate) rods. It includes important plant pathogens as well as non-phypathogenic epiphytic flora of plant surfaces and some opportunistic pathogens of man and animals. Erwinia belongs to family Enterbacteriaceae along with enteric bacteria like Escherichia, Shigella, Salmonella, Proteus and Serratia with whom its close resemblance is accepted since beginning of this century. Erwinia is heterogenous in terms of pathogenic capacity and phenotypic characters. The species are placed in three natural groups, viz. carotovora, amylovora and herbicola group.
Carotovora group.

The carotovora group (soft-rot group, pectolytic group; also referred earlier as genus *Pectobacterium*) consists of species having strong pectolytic activity that enables them to cause soft-rots. *E. carotovora* subsp. *atroseptica* (Eca), and *E. carotovora* subsp. *carotovora* (Ecc) are the causal agents of soft-rot diseases of diverse plants and plant parts. Within each subspecies there are numerous strains, many of which are identifiable serologically. The "black-leg" of potato is caused by Eca while soft-rot of carrot is caused by Ecc. (However, now it is generally believed that both Ecc and Eca can cause black leg of potato, see Stanghellini, 1982).

Amylovora group.

Amylovora group, also called as true erwinias, or *Erwinia* sensu stricto, is typified by *E. amylovora* which is the causal agent of fire blight of apples and pears (first bacterial plant disease reported by Burrill in 1876 and Arthur in 1878). The species placed under this groups are non-pigmented and non-pectolytic, and cause dry necrosis or wilt symptoms. *E. quercina*, *E. rubrifaciens* (Canker of persian walnuts), *E. salicis* (water mark disease or vascular wilt of willow (*Salix alba*, the commercial source for cricket bat) and *E. tracheopnila* (causing wilt of cucumbers) belong to amylovora group.
Herbicola group. (Yellow-pigmented erwinias).

The members are characterized by their yellow colonies. The extracellular yellow, water insoluble pigments are carotenoid in nature. The members are common on leaves and buds of plants as non-pathogenic epiphytes. All the epiphytic erwinias are put under E. herbicola, while those isolated from diseased plants, with claimed or dubious pathogenicity, are placed under E. milletiae, E. anans, E. lathyri, E. stewartii etc. Erwinias isolated from man and animals are referred as "human clinical erwinias" or E. herbicola group from human sources, which are called Enterbacter agglomerans by some bacteriologists (again due to disciplinal insularity). The non-phytopathogenic (epiphytic) erwinias have been found to reduce the severity of fire-blight disease of pears and apples and the principle has been applied for "biological control" of infection of pea flowers by E. amylovora. Some species of herbicola groups have been reported to fix nitrogen which explains their ability to colonise a nitrogen-poor habitats like the leaf surface.

genus Corynebacterium.

Unlike above organisms (which were all Gram-ve bacteria), Corynebacterium is Gram +ve. It belongs to part 17 of the Bergey's Manual, "Gram positive actinomycetes and related organisms". It is a highly heterogenous assemblage. Nevertheless, they are similar in that all are
Gram +ve, non-spore forming rods, showing tendency towards filamentous growth or are filamentous. These bizarre organisms are segregated into three units: (1) Coryneform-group of bacteria, (2) family Propionibacteriaceae and (3) order Actinomycetates. The "Coryneform-group of bacteria" include four genera out of which Corynebacterium is one. It is pleomorphic rod-shaped (Straight or slightly curved) and frequently coryneform i.e., club-shaped. The cells show abundant metachromatic granules and are aerobic. Due to "Snapping" cell divisions the cells are arranged in angular (V or Y shaped) or palisade (in rows) fashion. But this character is less distinct in plant pathogenic corynebacteria compared to human or animal pathogenic corynebacteria. The genus is split into three sections to include the human and animal corynebacteria (Section-I), plant-pathogenic corynebacteria (Section-II) and non-pathogenic corynebacteria (Section-III).

Most of the species of Corynebacterium (except C. fasciens; C. michiganense have been isolated principally from single host and named after the major symptoms they induce. Dye (1974) has placed the species in five groups viz. (I) C. fascians (II) C. raythayi, C. agropyi, C. iranicum, (III) C. sepedonicum, (IV) C. insidiosum, C. michiganense, and (V) C. flacumfaciens, C. poinsettiae, C. betae and C. oorti.
A perusal of the Approved list of Plant Pathogenic Bacteria will indicate that many of the species have been relegated to pathovar status due to their insufficient descriptions.

*genus Streptomyces.*

It is gram +ve, aerobic, filamentous bacterium (resembling fungi), forming non-motile spores in long chains which are of characteristic shapes in various species. The colonies are small, leathery, initially smooth-surfaced but later form abundant aerial mycelium, which appear granular or powdery. The organism produces variety of melanoid pigments which along with spore chain morphology (Straight, flexuous, spiral etc.), are helpful in identifying the species. Species of *Streptomyces* are the most abundant bacteria in soil and are the source of commercial production of antibiotics like chloramphenicol, streptomycin, erythromycin, tetracyclines, cycloheximide, griseofulvin, to name only some. Quite a few of these are used in control of plant diseases also. Then, the typical fragrant smell of wet soil, so pleasant after a shower, is due to a volatile compound produced by *Streptomyces*!

*Streptomyces* causes scab and rots in a variety of root crops, the best-known being the potato-scab which is caused by several species of the genus, though *S. scabies* is traditionally regarded as the causal agent. This
species is no more accepted as a valid species (Skerman et al. 1980) although it is recognised by the International Streptomyces Project. So, the status of *S. scabies* is left in doubt. Only three species viz. *S. intermedius* (causal agent of sugar beet scab), *S. ipomeae* (sweet potato scab), and *S. setori* (one of the causal agents of potato scab) were recognised in the 8th edition of Bergey's Manual (1984) and now in the Approved list of Plant Pathogenic Bacteria (Skerman, 1980) only one species viz. *S. ipomeae* is recognised; others are regarded as pathovars. The non-cultivatable organisms RLOs and MLOs have not been examined in this work.

Biological Control & Plant Growth Promoting Rhizobacteria (PGP)

Biological control is becoming one of the most fascinating and challenging area of study with great commercial promise. The first experiments on biological control of plant pathogens with antagonists were conducted by G.B. Sanford (1926). Later, Sanford and W.C. Brogfoot (1931) published on biological control of the wheat "take-all" fungus—which was the first usage of the term, "biological control" in plant pathology (Cook, 1985). After a long gap in serious research in this field, again it has drawn the attention of researchers from many other fields like bacteriology, virology, nematodology, ecology and so on. No doubt all this is due to some positive and promising results which have been obtained recently. The definition
for biological control which is also recommended by Vidaver (1982), is the one given by Singleton and Sainsbury (1978). "The deliberate use of one species of organism to control or eliminate another is biological control". Despite the different strategies for future of biological control, at present two developments are leading this aspect of science. The first development has been to use a biological agent's with specific antibiotic activity against pathogenic prokaryotes, for example, *A. radiobacter* strains effective against *A. tumefaciens*. The second development in biological control is the use of plant growth-promoting rhizobacteria (PGPR) to enhance plant productivity (Kennedy and Lacy, 1982).

The discovery of PGPR was accidental. During search for bacteria antagonistic to *Erwinia* soft-rot and blackleg pathogens (*E. carotovora* subsp. *carotovora* (Jones) Dye and *E. carotovora* subsp. *atroseptica* (Van Hall) Dye on potato, some oxidase-positive, fluorescent *Pseudomonas* sp. appeared in the rhizosphere microflora which were able to colonize roots when added back to soil. These organisms failed to cause antibiosis and control disease but, interestingly, their association invariably resulted in enhanced plant growth. This prompted research in the area of PGPR (Suslow, 1982) and has generated interest in planing additional strategies for isolation and identification of PGPR. The development of PGPR, inducing better plant growth, is
becoming an attractive alternative to the traditional chemical treatments from which not only have arisen many problems like pollution but also they have failed to control diseases particularly the bacterial diseases. In some cases the frequent application of fungicides and bactericides has resulted in the resistance by the pathogens. Whereas the biological agents may benefit the plants directly by supplying essential metabolites or growth hormones and indirectly by suppressing phytopathogens (Suslow, 1982).

Among plant growth promoting rhizobacteria, *Pseudomonas fluorescence*—putida group has been known as most effective plant growth enhancers. The mechanisms by which PGPR induce plant growth and increase yields of plants were described by Kloepper et al. (1980 a & b). According to them, PGPR by producing iron-chelating compounds, called as siderophores, deprive the complex environmental iron, making it less available to certain native microflora. In this way the siderophores not only inhibit the growth of other bacteria including pathogenic microorganisms but also exert beneficial effect on plant growth. Many siderophores have been identified and characterized like the siderophores from *Echerichia coli* (Braun, 1981), *Pseudomonas* spp. (Cox and Graham, 1979; Meyer and Abdallah, 1978, 1980) *Bacillus megaterium* (Byers et al., 1967), *Actinomyces* sp. (Blckel et al., 1960), *Mycobacterium* sp. (Snow, 1970), *Agrobacterium*
tumefaciens (Leong and Neilands, 1981), and Azotobacter
vinelandii (Page and Dale, 1986). But the various sidero-
phore molecules are different from one another in their
structure and ability to bind iron. Only those bacteria
which possess root colonizing ability, and hydroxamate
siderophore molecules have been shown to be promising
biocontrol agents. Pseudomonas fluorescens with a linear
hexapeptide siderophore pseudobactin, has been demonstrated
to have the ability of inhibiting a variety of phytopathoge-
nic fungi (Vandenberg, et al. 1983). Some of these plant
enhancers have been demonstrated to cause considerable
plant growth responses in green house and field tests. In
Texas, Strain pf-5 P. fluorescens, isolated from the rhizo-
sphere of cotton seedlings and used as a seed treatment for
cotton gave 30 to 79 per cent (Howell and Stipanovic, 1979)
and 28 to 71 per cent (Howell and Stipovanic, 1980) better
survival of seedlings in soil infested with R. solani and
P. ultimum, respectively. In California, strains of the
P. fluorescens Migula putida (Trevisan) Migula group intro-
duced on the seed or seed pieces at the time of sowing re-
sulted in 60 to 144 per cent increase in yield of raddish
and 50 to 30 per cent increase in sugarbeets and potatoes
(Suslow et al. 1979). Kloepper and Schroth (1979) and
treated seed pieces of potatoes with P. fluorescens and
P. putida which increased the potato yield by 59 per cent.

Seed treatments by antagonists for biological control
of soil-borne diseases have also been extensively studied
by many workers. Soil-borne pathogens are the main target since soil inhabitants, by their very persistence in the soil, are difficult to eliminate or control (De Boer, 1982).

Apart from many interrelating factors, individual or general population of antagonistic microflora may be considered as an important factor in the reduced incidence of some well-known diseases. They may be used as a means of protecting roots. *e.g.* *A. radiobacter* strain 84, producing a bacteriocin, has been found effective for biological control of crown gall caused by *Agrobacterium tumefaciens* (Kerr, 1980; Moore, 1981). *Phytophthora cinnamoni* Rands which has been controlled by protecting of pine roots with ectomycorrhizae (Marx, 1975). But, as stated by Baker and Cook (1974), the problem of establishing antagonists in soil for biological control has not been resolved so far, though there are many examples for its feasibility in natural conditions. Therefore, further intensive research is needed particularly on understanding the negative results obtained during the course of studies. These may probably lead to a better understanding on why there have been frequent failures in the use of introduced antagonists for biological control.