


Phytopathogenic Bacteria and Plant Diseases

B.S. THIND


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Dedication

Dedicated to the memory of my mentor and teacher late Dr. M. M. Payak, Ex-Head, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, who guided and trained me in the discipline of Plant Pathology and Plant Bacteriology.

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Contents

SECTION I General Aspects

SECTION II Specific Plant Diseases

Abbreviations of Bacterial Genera Used in This Book

Genus	Abbreviation	Genus	Abbreviation
Acetobacter	*Ace.*	*Listeria*	*Li.*
Acidovorax	*Aci.*	*Lonsdalea*	*Lo.*
Acinetobacter	*Acin.*	*Neisseria*	*Ne.*
Agrobacterium	*Ag.*	*Nocardia*	*No.*
Arthrobacter	*Ar.*	*Paenibacillus*	*Pae.*
Bacillus	*Ba.*	*Pantoea*	*Pa.*
Bacterium	*Bac.*	*Paraburkholderia*	*Par.*
Bradyrhizobium	*Bra.*	*Pectobacterium*	*Pe.*
Brenneria	*Br.*	*Phytomonas*	*Ph.*
Burkholderia	*Bu.*	*Pseudoalteromonas*	*Psa.*
Campylobacter	*Cam.*	*Pseudomonas*	*Psm.*
‘*Candidatus* Liberibacter’	‘*Ca.* Li.’	*Ralstonia*	*Ral.*
‘*Candidatus* Phlomobacter’	‘*Ca.* Phl.’	*Rathayibacter*	*Rat.*
‘*Candidatus* Phytoplasma’	‘*Ca.* Phy.’	*Rhizobacter*	*Rhba.*
Citrobacter	*Cit.*	*Rhizobium*	*Rhbi.*
Clavibacter	*Cla.*	*Rhizorhapis*	*Rhr.*
Clostridium	*Clo.*	*Rhodococcus*	*Rho.*
Corynebacterium	*Cor.*	*Salmonella*	*Sal.*
Curtobacterium	*Cur.*	*Samsonia*	*Sam.*
Dickeya	*D.*	*Serratia*	*Se.*
Enterobacter	*En.*	*Shigella*	*Sh.*
Enterococcus	*Enc.*	*Sphingomonas*	*Sph.*
Erwinia	*Er.*	*Spiroplasma*	*Sp.*
Escherichia	*Es.*	*Staphylococcus*	*Sta.*
Gibbsiella	*Gi.*	*Streptococcus*	*Stc.*
Gluconobacter	*Gl.*	*Streptomyces*	*St.*
Herbaspirillum	*He.*	*Tatumella*	*Ta.*
Janibacter	*Jani.*	*Thermus*	*Th.*
Janthinobacterium	*Jant.*	*Vibrio*	*V.*
Lactobacillus	*Lactob.*	*Xanthomonas*	*Xa.*
Lactococcus	*Lactoc.*	*Xylella*	*Xyl.*
Leifsonia	*L.*	*Xylophilus*	*Xyp.*
Leucobacter	*Leb.*	*Yersinia*	*Y.*
Leuconostoc	*Len.*		

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Preface

The study of phytopathogenic bacteria and the plant diseases caused by them is of utmost importance to mankind as these diseases cause huge economic losses. Some of these diseases, such as bacterial wilt of solanaceous plants, soft rot of fleshy vegetables and fruits, crown gall of plants, citrus canker, citrus huanglongbing, fire blight of pome fruit trees, and bacterial blight of rice, are of international importance. Plant diseases caused by phytopathogenic bacteria are also disastrous because many of these diseases cannot be effectively controlled due to the lack of effective chemicals. Moreover, the secondary spread of these diseases is very fast compared to fungal diseases.

The classification and nomenclature of bacteria has undergone a sea change during the last three decades. The sequence analysis of highly conserved regions of the bacterial genome such as small sub unit rRNA gene has provided very valuable information concerning numerous taxonomic changes and realignment of different taxonomic groups. The sequencing of genomes of many phytopathogenic bacteria has certainly stabilized their ever fluctuating nomenclature and taxonomy. Since the 1st edition of *Bergey's Manual of Systematic Bacteriology* was published in 1984, the number of published bacterial species has more than tripled, i.e., 390 new genera and 2,200 new species have been described. Many new genera containing plant pathogenic bacteria have been recognized, and the rearrangement of the species among the genera has also occurred. During 1970s, there were only 6 genera of bacteria that contained plant pathogenic bacteria, but now this number is 53, including 3 candidatus categories.

In contrast to direct monetary losses and other harmful effects of phytopathogenic bacteria, they have a great deal of commercial value. They are used for the commercial production of many industrial products as well as tools for various scientific studies. Their use in industrial fermentation, production of antibiotics, xanthan gums, and polysaccharides such as curdlans are the important applications. Production of pectin-degrading enzymes produced by soft-rotting erwinias and restriction nucleases produced by *Agrobacterium* and *Xanthomonas* species are other notable examples of their commercial use. *Gluconobacter* species have various biotechnological applications. Their biotransformed strains are used for the production of rare and special sugars like L-ribose and miglitol, both very promising pharmaceutical lead molecules. *Ag. tumefaciens* (the causal agent of crown gall of many plants), apart being a plant pathogen, is one of the best known vehicles for gene transfer and most useful genetic engineering tool for developing transgenics.

Our knowledge of phytopathogenic bacteria, plant diseases caused by them, host-pathogen interactions, molecular mechanisms involved in virulence and pathogenesis, and disease management strategies developed has now reached a stage where it would be useful to integrate the concepts and ideas into a book. Therefore, the book entitled, *Phytopathogenic Bacteria and Plant Diseases* is proposed.

There are 7 chapters included in the book. Chapter 1 includes economic importance of phytopathogenic bacteria and historical review of phytobacteriology. Chapter 2 contains basic information about the bacterial cell. Chapter 3 deals with diagnosis of bacterial diseases of plants. The up-to-date classification of plant pathogenic bacteria is given in Chapter 4. The bacterial names included are based on the information published in *International Journal of Systematic Bacteriology* (*IJSB*), *International Journal of Systematic and Evolutionary Microbiology* (*IJSEM*), and related journals up to December issues of 2018, and supplemented with the information given in *Names of Plant Pathogenic Bacteria*, *1864–2004* prepared by Young et al. (2004), *Comprehensive List of Names of Plant Pathogenic Bacteria, 1980–2007* prepared by Bull et al. (2010), *List of New Names of Plant Pathogenic Bacteria (2008–2010*) prepared by Bull et al. (2012), and *List of New Names of Plant Pathogenic Bacteria (2011–2012*) prepared by Bull et al. (2014). Chapter 5 deals with information on molecular mechanisms of phytopathogenic bacteria involved in virulence and pathogenesis. In Chapter 6, an altogether a new topic, i.e., plants acting as carriers of human enteric bacterial pathogens has been included. It needs an immediate attention of the scientists to decontaminate plant and plant products used for human consumption.

Chapter 7 contains descriptions of specific bacterial plant diseases. Although it was not possible to include all the bacterial plant diseases, 70 specific diseases, including economically important diseases of international importance and representative diseases of different crops and regions, have been described. In order to make discussion of phytobacteriology even more effective, full-color photographs of symptoms of most of these diseases are given. Information published in plant pathological and related biological journals until December issues of 2018 have been included in the book.

The abbreviations of names of bacterial genera, included in the book, are given on page xix. Because a majority of the genera included in this book start with the same letter, a deviation has been made from the tradition of abbreviating the genus name by using only the first letter of the genus. In Chapter 7, the names of causal agents of different diseases are given along with author citations. In author citations, when there are more than two authors, the name of the first author is given followed by "et al." One or two and, in a few cases, up to three recent synonyms of each causal organism are also given. In the corresponding references, the names of up to five authors are given, followed by "et al." when there are additional authors. The names of all the authors/editors are given for book references.

At the undergraduate level, students have limited exposure to phytobacteriology. However, at the post-graduate level, students are taught phytobacteriology in detail. State agricultural universities and the universities teaching biological sciences have courses dealing exclusively with phytobacteriology. PhD students specializing in phytobacteriology are taught advanced courses. This book will serve as a textbook for advanced undergraduate and post-graduate students and also as a reference book for research workers engaged in the discipline of plant pathology and phytobacteriology.

I am indebted to numerous colleagues and friends for suggestions made during the preparation of the manuscript. Also, in the preparation of this manuscript, I have referred to a number of publications, and I would like to acknowledge all the authors of these excellent and valuable publications/articles.

There might be some errors, mistakes, and shortcomings in this publication. I will greatly appreciate the healthy criticism and suggestions for the improvement of this publication in future.

B.S. Thind

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It is my pleasure to acknowledge the help of all those who contributed in the preparation of this publication. At the outset, I am highly thankful to my friend and ex-colleague, Dr. G.S. Miglani, formerly Professor of Genetics, Punjab Agricultural University (PAU), Ludhiana, for his frequent counseling and help. I am also grateful to my friend and ex-colleague, Dr. R.S. Kahlon, Ex-Prof.-cum-Head, Department of Microbiology, PAU, Ludhiana, for reviewing Chapter 2, “Bacterial cell,” and making critical suggestions.

I owe my special thanks to Dr. Parminder Singh, Assistant Plant Pathologist, PAU, Ludhiana, who helped me immensely in downloading a large number of pictures and photographs from the Internet and also for editing and arranging these photographs. His help in typing and setting the text material is also acknowledged. I am also indebted to Dr. M.S. Hujan, Assistant Professor of Plant Pathology, PAU, Ludhiana, for going through Chapter 6, “Plants as carriers of human enteric bacterial pathogens,” and making useful suggestions.

The generous help rendered by my ex-colleagues and friends, namely, Dr. Bipin Kumar Sharma, Ex-Director, PAU Regional Research Station, Kapurthalla; Dr. Surinder Kumar Thind, Prof. of Plant Protection, PAU, Ludhiana; Dr. Kuldip Singh, Ex-Senior Molecular Geneticist, PAU, Ludhiana; Dr. R.P. Maharshi, Ex-Head, Department of Plant Pathology, SKN College of Agriculture, Jobner, Rajasthan; and the late Dr. Ram Kishun, ex-Principal Scientist and Head, Crop Protection Division, Central Institute for Subtropical Horticulture, Lucknow, for lending me the photographs of the symptoms of different plant diseases.

I am also indebted to Dr. N.W. Schaad, Agricultural Research Service-United States Department of Agriculture, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD, USA, for his expert advice and frequent counseling. My sincere thanks are also due to Professor Ian K. Toth, The James Hutton Institute Invergowrie, Dundee, DD2 5DA, UK; Dr. N.W. Schaad; Dr. M.K. Mondal, Sr. Scientist, Division of Plant Pathology, I. A. R. I., New Delhi; Dr. R. Sridhar, ex-Head, Department of Plant Pathology, Central Rice Research Institute, Cuttack; and Dr. R.P. Maharshi for agreeing to act as referees/reviewers for this book. The frequent counseling and help rendered by Dr. Paramjit Singh Randhawa, President, California Plant and Seed Labs, USA, is thankfully acknowledged.

I owe a special gratitude to my wife, Mrs. Ravinder Kaur Thind for her continuous and untiring encouragement during this entire period and also for her help in the preparation of subject index. My sincere thanks also go to my ex-colleagues and friends who helped me directly or indirectly in the preparation of this manuscript, but whose names have not been mentioned here.

B.S. Thind

Author

Dr. B.S. Thind retired as Professor-cum-Head, Department of Plant Pathology, Punjab Agricultural University (PAU), Ludhiana, India after serving the university for 32 years. After retirement, he became an Emeritus Scientist (Indian Council of Agricultural Research [ICAR], New Delhi) in the Department of Plant Pathology, PAU, Ludhiana from July 2003 to July 2005 on the scheme entitled, "Development, mass culturing, and formulation of effective and economical biocontrol agents for the control of *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight of rice."

Dr. Thind completed his PhD in Plant Pathology from Indian Agricultural Research Institute, New Delhi in 1970. His first encounter with plant bacteriology or phytobacteriology sub discipline occurred during his PhD program when he worked on bacterial stalk rot of maize caused by *Dickeya zeae*. To this day, he continues to work on bacterial diseases of plants and has made significant contributions in this field.

He has been a principal investigator of an Indian Council of Agricultural Research (ICAR)-funded research scheme entitled, "Biological control of bacterial blight, sheath blight, sheath rot, and brown leaf spot of rice," two ICAR-funded research schemes entitled, "Perpetuation, variability, and control of *X. oryzae* pv. *oryzae*, the causal agent of bacterial blight of rice," and "Detection and control of phytopathogenic bacteria from cowpea and mung bean seeds."

He has also taught many courses of plant pathology to undergraduate and post-graduate students, including 600 series advanced courses of plant bacteriology to PhD students and has guided 10 PhD and 13 MSc students. All these students conducted the research work on bacterial diseases of different crops.

Dr. Thind attended the 7th, 8th, and 9th International Conferences on Plant Pathogenic Bacteria held in Budapest in 1989, in Versailles in 1992, and in Chennai in 1996, respectively. As member of the National Organizing Committee, he organized the 9th International Conference on Plant Pathogenic Bacteria held in Chennai, India during August 26–29, 1996 and also chaired a session. He also attended I, II, and III International Symposia of Plant Pathology and International Conference in New Delhi in 1967, 1971, 1981, and 1997, respectively; the Global Conference in Plant Pathology in Udaipur in 1995 and Asian Biotechnology Rice Network Workshop at Ludhiana in 1998; and 25 national and eight zonal meetings of different professional societies.

Dr. Thind has been a member of Indian Phytopathological Society, New Delhi, since 1966 and is now designated as a life member. He has been a member of the Indian Society of Mycology and Plant Pathology, Udaipur, since 1976, of the Indian Society of Plant Pathologists, Ludhiana, since 1984, and of the Indian Science Congress Association, Kolkata, since 1992.

Dr. Thind has served as paper setter and external examiner of four universities and Agricultural Scientists' Recruitment Board, New Delhi; as an expert of selection committees for the post of Associate Professors/Professors and as an external examiner of PhD and MSc students of different universities; and as reviewer/referee of various national and international research journals.

He has also published 127 research papers in scientific journals of international and national repute, has authored one manual entitled, *Plant Bacteriology* and one textbook entitled, *Phytopathogenic Procaryotes and Plant Diseases* published by Scientific Publishers (India).

Section I

General Aspects

1 Introduction

1.1 PHYTOBACTERIOLOGY

Phytobacteriology or plant bacteriology is a subdiscipline of plant pathology, which deals with plant-associated bacteria and their interactions with each other and with their hosts (Dumenyo et al. 2001). The subdiscipline covers all aspects of plant-associated bacteria including basic as well as of applied nature. The morphology, physiology, taxonomy, genetics, and serology of the bacteria comprise the basic aspects. Host-pathogen interactions, including pathogenesis and infection process, epidemiology, effect of environment on disease development, and disease management are the applied aspects dealt with in this subdiscipline. In the past, the emphasis has been mainly on the investigation of plant diseases caused by bacteria due to heavy losses caused by these diseases. However, lately, the field of phytobacteriology has expanded considerably to include beneficial bacterial-plant interactions such as nodulation and nitrogen fixation; promotion of plant growth by phyllosphere, rhizosphere, and soil-inhabiting bacteria, and control of ice-nucleation active bacteria by phyllosphere bacteria. Phytobacteriology also includes the management and control of bacterial plant diseases using various methods such as cultural practices, resistant cultivars, chemical and biological control, and molecular genetics. A basic knowledge of various branches of science like microbiology, genetics, chemistry, biochemistry, plant physiology, agronomy, soil science, and molecular biology is essential for better understanding of plant diseases caused by microorganisms. The molecular biology is now becoming increasingly important in every biological science including phytobacteriology.

1.2 PROCARYOTES

On the basis of cellular organization, the living organisms can be divided into two groups, namely, procaryotes and eucaryotes. The procaryotes have a set of characteristics, which differentiate them from eucaryotes. The main characteristic of the procaryotes is that with a few exceptions, nucleoplasm or genophore (earlier called nucleoid) is not separated from the cytoplasm by a unit-membrane system, i.e., the nuclear membrane is absent. The other characteristic features are the presence of 70S ribosomes (with the exception of archaea, whose ribosomes have a slightly higher S value) dispersed in the cytoplasm, no cytoplasmic streaming, and absence of endoplasmic reticulum with attached ribosomes. They take the nutrients in liquid form. Most of them possess a rigid cell wall except one group, i.e., **mollicutes**. They occur as single cells or simple association of similar cells. The cells may be motile or non-motile; in case of motile, the motility is mediated by bacterial flagella or gliding motility on solid surface.

The procaryotes are inhabitants of moist environments and predominantly occur as unicellular microorganisms. However, they also occur in other forms such as, filaments, mycelia, or colonies. In some cases, differentiating structures like holdfasts and resting cells are formed. The gene transfer and recombinations occur, but in these processes gametogenesis and zygote formation does not ever occur.

The above given characteristics of procaryotes are sufficient to distinguish them from eucaryotic microorganisms, but some common features are found in some procaryotes and eucaryotes. For example, the hyphae formed in actinomycetes look similar to those of molds, and ability of spirochetes to twist and contort their shape matches the flexibility shown by certain protozoa. Some large bacteria may approach the size of eucaryotic cells, while some eucaryotic cells are as small as bacteria. However, procaryotic and eucaryotic cells can be easily differentiated on the basis of fluorescent-labeled genes.

The procaryotes comprise two different groups, namely, the ***Bacteria*** and the ***Archaea***, which have been recognized as different domains for the purpose of classification (Krieg 2001).

1.2.1 Bacteria

On the basis of Gram stain reaction and presence or absence of cell wall, the bacteria have been divided into the following three phenotypic subgroups:

1. Gram-stain-negative bacteria having cell walls
2. Gram-stain-positive bacteria having cell walls
3. Bacteria lacing cell walls.

1.2.1.1 Gram-Stain-Negative Bacteria Having Cell Walls

These bacteria possess a Gram-stain-negative type of cell wall, which is composed of an outer membrane, and an inner, relatively thin peptidoglycan layer containing muramic acid; the latter may be absent in a few organisms. Reproduction is usually by binary fission, some groups show budding and a very few divide by multiple fission. The cells may occur in the form of spheres, ovals, straight or curved rods, helicals, or filaments. The cells may be non-motile or motile showing swimming or gliding motility. In relation to oxygen requirement, the members may be aerobic, anaerobic, facultatively anaerobic, or microaerophilic.

1.2.1.2 Gram-Stain-Positive Bacteria Having Cell Walls

These bacteria have a Gram-stain-positive type of cell wall, in which there is no outer membrane and the peptidoglycan layer is relatively thicker than that of Gram-stain-negative type of cell wall. The cell shapes include rods, spheres, or filaments; the rods and filaments may show true branching.

The reproduction is mostly by binary fission, but actinomycetes and their relatives produce sporogenous hyphae. Some members produce spores, also called **endospores**, which help to tide over the adverse conditions, but are not a mean of multiplication. The oxygen requirement of the members is the same as that of Gram-stain-negative bacteria.

1.2.1.3 Bacteria Lacking Cell Walls

They are commonly called the "**mycoplasmas**" (also called mollicutes or soft-skin microorganisms). They lack the cell wall, but are enclosed by a unit membrane, plasma membrane. As they lack the cell wall (peptidoglycan layer), they are not sensitive to β-lactam antibiotics or other antibiotics, which inhibit cell wall synthesis. They are highly pleomorphic, have different cell forms varying in size and some of these forms are filterable. Cells stain Gram-stain-negative, no resting forms are produced, and usually, they are non-motile, but some members show gliding motility. Different modes of reproduction such as, budding, fragmentation, and/or binary fission occur. Most members require complex media and all require cholesterol or related sterols for growth. The typical colony under adequate growth conditions is biphasic having **fried-egg appearance**, i.e., consisting of an opaque, granular central area which grows down into the medium and a flat, translucent peripheral zone. The mol% G + C contents of rRNA (43–48) and of DNA (23–46) are lower than that of walled bacteria.

1.2.2 ARCHAEA

Archaea, predominantly inhabitants of terrestrial and aquatic habitats, are found in anaerobic, hypersaline, or hydrothermally and geothermally heated environments. Some members also live symbiotically in the digestive tracts of animals. Archaea grow as mesophiles and thermophiles, but some species grow at very high temperatures, i.e., up to 110°C. They grow as aerobes, anaerobes, and facultative anaerobes. Archaea are not sensitive to β-lactam antibiotics as their cell walls do not contain murein, peptidoglycan containing muramic acid. All archaea contain glycerol isopranyl ether lipids, which is their unique biochemical property.

Gram-stain-reaction varies depending on the composition of cell envelopes. It may be Gram-stain-positive or -negative within the same group. The cells are of different shapes, which include spherical, spiral, plate, or rod. Unicellular or multicellular forms also occur in filaments or aggregates. The modes of reproduction include binary fission, budding, fragmentation, constriction, or mechanisms not yet known. The cell masses exhibit a variety of colors including red, pink, purple, green, yellow, grey, white, etc. Five major groups recognized in archaea are given below.

1. Methanogenic archaea
2. Sulphate-reducing archaea
3. Extremely halophilic archaea
4. Cell wall-lacking archaea
5. Extremely thermophilic S°-metabolizing archaea.

1.3 PHYTOPATHOGENIC BACTERIA

Phytopathogenic bacteria include cell-walled bacteria, including both Gram-stain-negative and Gram-stain-positive, and cell wall-lacking bacteria (mollicutes). The general characteristics of these phytopathogens are that majority of them are non-spore-forming, rod-shaped organisms. Only *Streptomyces* species produce spores and are filamentous. Most plant pathogenic bacteria are nutritionally non-fastidious. Their optimal growth temperature is around 23°C–28°C. However, a few of them, such as *Ralstonia solanacearum* and *Burkholderia cepacia*, can grow at 42°C. Plant pathogenic bacteria can be either strictly aerobic or facultatively anaerobic.

Phytopathogenic and photosymbiotic procaryotes have one unique feature, i.e., their ability to multiply in the plants that separates them from all other procaryotes. Pathogens can increase from an initial low number of cells to a higher number in a short period of time within a host plant. No other feature clearly distinguishes them from the myriad of saprophytes that occupy every other plant-associated habitat (Kennedy and Lacy 1982). They have evolved to grow well in their plant hosts, often in specific hosts as well as in a specific plant part, such as blossoms, leaves, roots, tubers, or corms. Inside the plant hosts, phytopathogenic procaryotes multiply in the intercellular spaces and penetration of the cells generally occurs after the disruption and disorganization of the cell walls. Genetic studies have shown the involvement of numerous genes in virulence and pathogenesis. A number of plant pathogenic bacteria harbor virulence and pathogenicity genes on plasmid (an extrachromosomal element), whereas other pathogens bear virulence and pathogenicity genes in their chromosomes. The conservation of virulence and pathogenicity genes in pathogenicity islands strongly suggests interchangeable movement of these genes across species boundaries via lateral gene transfer flow (Hacker and Kaper 2000; Syvanen and Kado 2002). Many of these genes provide an inherent ability to the pathogen to grow in its host and hence, are essential for successful colonization of the host.

Phytopathogenic procaryotes cause plant diseases on a wide range of host plants. Some of these diseases are highly devastating and cause serious economic losses. Bacterial plant pathogens develop new survival strategies to thwart efforts to control and eliminate plant diseases caused by them. Kado (2009) reported that acquisition of novel genes through lateral gene transfer is a powerful means for pathogen development. The generation, through selective mutations, of novel and functional genes whose products enhance successful colonization can also facilitate pathogen fitness. Irrespective of the mechanism, genetic changes that are beneficial to pathogen survival occur due to selection pressure put on the pathogen by various control measures adopted in farming practices.

As the procaryotes include both bacteria and archaea, and among them only bacteria are pathogenic to plants, the terms "**phytopathogenic prokaryotes**" and "**phytopathogenic bacteria**" have been used interchangeably in this book.

1.3.1 ECONOMIC IMPORTANCE OF PHYTOPATHOGENIC BACTERIA

There is not enough data available on losses due to plant diseases caused by phytopathogenic bacteria in spite of the fact that some of these diseases are of international importance. The loss caused by a plant disease varies from region to region due to variation in environmental factors, which influence the disease development and also variation in susceptibility of the varieties under cultivation. For example, bacterial blight of rice is severe mainly in Southeast Asia. Moreover, the importance of a crop also varies in a particular region or country. Chemical control of bacterial plant diseases is less effective than those of caused by fungi due to the availability of less number of effective chemicals and low efficacy of the available chemicals. Moreover, many antibiotics, which are effective against many plant pathogenic bacteria, are also used for the control of human and animal bacterial pathogens. Hence, their use in plant disease control in many countries is not permitted due to the risk of transfer of resistance to human and animal pathogens. The crop losses due to plant pathogenic procaryotes in the United States in 1976 are given in Table 1.1 (Kennedy and Alcorn 1980).

Bacterial wilt of solanaceous plants is considered the world's single most destructive bacterial plant disease. According to Champoiseau and Allen (2009), brown rot bacterium is responsible for an estimated loss of one billion US dollars worldwide every year, mostly to poor small-scale farmers in tropical highlands. Bacterial wilt of potato is also considered the second most important disease of potato worldwide after late blight.

Bove (2006) has rightly stated that citrus Huanglongbing (greening) is the most important, serious, severe, destructive, and devastating disease of citrus in the world. The reduction in yield can range from 30% to 100% depending on proportion of affected canopy. The affected orchards become economically unviable in 7–10 years after planting. About 100 million trees have been destroyed in many countries of South and Southeast Asia, Indonesia, the Philippines, India, Arabian Peninsula, and South Africa. Since 2004, more than 500 thousand trees have been officially destroyed in Brazil due to this disease and around 300–400 thousand trees unofficially destroyed by commercial citrus growers (Gottwald et al. 2007). Catastrophic losses due to the disease from India during 1960s were reported by Fraser et al. (1966).

Incomplete eradication of citrus canker from the United States, particularly from the state of Florida, has taken a very heavy toll of citrus trees. After its first introduction in Florida in 1912, it took about 20 years to eradicate the disease from Florida by destroying and burning more than three million nursery and a quarter million fruit bearing trees. The second introduction of disease in Florida in 1986 resulted in the destruction of more than 20 million nursery and orchard trees, and the eradication program continued until 1992. The third introduction of the disease in Florida in 1995 and subsequent years led to the destruction of 1.56 million commercial trees and 6,000,000 dooryard trees leaving the eradication target incomplete. The successful eradication of the disease from Australia, South Africa, and New Zealand and partially successful eradication (incidence below 0.22% in São Paulo) from Brazil was achieved after destroying citrus trees on a large scale.

Pierce's disease of grapevine caused by *Xyl. fastidiosa* subsp. *fastidiosa* was noticed in California State of the United States in the 1880s, but the lack of appropriate vector of casual agent and other non-favoring factors kept the disease

TABLE 1.1

Estimates of US Crop Losses Caused by Phytopathogenic Procaryotes in 1976[a]

Pathogen[b]	Disease	Loss (millions of US dollars)
Ralstonia solanacearum	Bacterial wilt of tomato and tobacco	9.4
Agrobacterium tumefaciens	Crown gall of fruits and nuts	23.0
Erwinia amylovora	Fire blight of pear	4.7
Er. tracheiphila	Cucumber wilt	0.9
Pantoea stewartii subsp. *stewartii*	Stewart's wilt of corn	0.2
Pectobacterium atrosepticum and/or *Pe. carotovorum* subsp. *carotovorum*	Blackleg and/or soft rot of potato	14.0
Clavibacter michiganensis	Bacterial wilt of alfalfa	17.0
Pseudomonas savastanoi pv. *glycinea*	Bacterial blight of soybean	65.0
Psm. syringae pv. *syringae*	Bacterial leaf blight of wheat	18.0
Xanthomonas citri subsp. *malvacearum*	Angular leaf spot of cotton	15.0
Xa. axonopodis pv. *phaseoli*	Common bacterial blight of bean	5.0
Leifsonia xyli subsp. *xyli*	Ratoon stunting disease of sugarcane	10.0
Xylella fastidiosa subsp. *fastidiosa*	Pierce's disease of grapevine	3.0
Spiroplasma citri	Citrus stubborn disease	1.0
'*Candidatus* Phytoplasma spp.'	Pear decline	1.6
	Lethal yellowing of coconut	3.0

[a] Based on data summarized from Kennedy and Alcorn (1980).

[b] Recent names of the pathogens are given.

under check. The introduction of glassy-winged sharpshooter (*Homalodisca vitripennis*), a highly efficient vector of the bacterium, in Southern California has changed the status of the disease from minor to severe with enormous increase in losses. It has threatened not only the cultivation of the European grape (*Vitis vinifera*), but also of the many ornamental crops in the southeastern United States.

In 1918, 75% of the bean fields in New York State of the United States were affected with common blight (*Xa. axonopodis* pv. *phaseoli*), and the disease caused heavy losses. In the subsequent years, losses ranged from 20% to 50%. In 1953, the disease was widespread in Nebraska (USA), and it caused an estimated loss of over one million US dollars. In 1976, in the United States, common blight was economically the most important disease among the bacterial diseases of beans, and it caused an estimated loss of four million US dollars. According to Gilbertson and Maxwell (1992), in 1967, the disease caused yield losses up to 38% in dry bean in Ontario (Canada) and 10%–20% in navy bean in Michigan (United States). Yield losses ranging from 17% to 22% were also reported from Colombia. Besides loss in yield, the disease causes reduction in quality of seeds through discoloration of infected seeds.

Although the bacterial blight of rice, caused by *Xa. oryzae* pv. *oryzae,* occurs in many countries, it is most destructive in Southeast Asia. The losses are generally high in an early-infected crop and occurrence of kresek phase may lead to the total failure of the crop under certain situations. In Japan, prior to introduction of resistant varieties, losses generally ranged from 20% to 30%, but in some cases went as high as 50% (Ou 1972). Reports from Philippines, India, and Indonesia estimate that losses due to kresek phase have reached 60%–75% depending on weather conditions, location, and rice variety (Reddy et al. 1979; Ou 1985). In addition to reduction in yield, the disease may also affect grain quality by interfering with maturation.

Bacterial canker (black spot) of mango causes heavy losses because most of the commercial varieties of mango are highly susceptible to the pathogen, and the infection results in drastic yield losses. The fruit infection ranging from 50% to 80% is common on highly susceptible varieties. In 1966 and 1967, severe epiphytotics of the disease caused almost 100% fruit loss on most susceptible cultivars in most of the mango growing areas of South Africa. The fruit loss was mainly due to the fruit drop and non-marketability of the harvested fruit. The estimated loss alone in 1966 was approximately one million US dollar (Gagnevin and Pruvost 2001). Kishun (1981) observed 20%–80%, 10%–70%, 10%–40%, and 10%–55% fruit drop in Alphonso, Pairi, Totapuri, and local cultivars grown in India, respectively, and the maximum fruit drop in all the cultivars occurred 30–60 days after fruit set. He further observed that the rot in storage in these varieties was 5%–80%, 11%–67%, 10%–100%, and 5%–80%, respectively.

Lethal yellowing of coconut palm trees has killed several palm trees in Florida State of the United States and some other countries. First observed in Florida in 1971, it killed 40,000 trees by 1974, and by 1975, 75% of palm trees in Dade County of the state were either dead or dying due to this disease. In Jamaica, 5.4 million trees were killed during a span of 20 years starting from 1961. In Mexico and Tanzania, palm trees in thousands of hectares and in Ghana over a million coconut palm trees were killed within 30 years. The disease also killed more than 60 thousand palm trees in Togo by 1964 (Agrios 2004).

Common scab of potato is one of the most economically important diseases of the crop. It costs the Tasmanian potato industry in excess of 3.6 million Australian dollars per annum (Wilson 2004).

An estimated loss of $4.7 million due to fire blight occurred in California in 1976, while in Southwest Michigan alone, the loss in 1991 was $3.8 million. Again in 2000, a severe outbreak of the disease occurred on apples in Michigan, resulting into removal and destruction of 35,000–45,000 apple trees from approximately 1550–2300 acres and amounting to a loss of $42 million. In 1998, apple and pear growers in Washington and northern Oregon suffered a loss of $68 million due to fire blight damage.

Stewart's wilt of corn, caused by *Pa. stewartii* subsp. *stewartii*, is a good example of a disease that continues to have enormous implications on trade and commerce. The disease has been a major threat to corn producers in the eastern corn belt of the United States for over 100 years. Severe outbreaks of the disease occurred in Nebraska in 1999 and 2000. In 1999, it was found to occur in 762 out of 1317 fields in Iowa (58% prevalence). The impact of the disease was that the seed produced from 58% of the seed corn fields in Iowa in 1999 could not be exported to the countries having phytosanitary (quarantine) restrictions for this pathogen.

In addition to causing direct loss in crop yield and quality, phytopathogenic bacteria cause other harmful effects to mankind. *Rathayibacter toxicus* causes gumming disease in ryegrass and other fodder grasses, and produces toxins, which when consumed often result in fatal poisoning of livestock. Various species of *Anguina* (nematode) act as vector of this bacterium. Glycolipid toxins, known as corynetoxins, are produced by the bacterium as the infected grass matures and becomes senescent. In Australia, *Rat. toxicus* is found most commonly in *Lolium rigidum* (annual ryegrass) with *Anguina funesta* as a vector and in *Polypogon monspeliensis* (rabbit-foot grass) and *Lachnagrostis filiformis* (syn. *Agrotis avenacea*, annual blown grass) with an undescribed *Anguina* vector. In Australia, more than 100,000 sheep and thousands of cattle have died from this disease in certain years (Edgar 2004). Besides affecting farmers or growers, bacterial diseases also affect transport and other industries dependent on the affected crops.

In contrast to direct monetary losses and other harmful effects caused by phytopathogenic bacteria, they have a great deal of commercial value (Starr 1984). The studies of these bacteria have yielded very useful products and tools. Several phytopathogenic bacteria are used in industrial fermentation. The most prominent case is the large-scale use of *Xa. campestris* to make xanthan gums, extracellular polysaccharides of considerable economic importance. Xanthan gums are used for various purposes including oil-well drilling muds, tertiary recovery of petroleum from spent wells, ceramic glazes, thickening agents in foods, and antidrip agents in paints. Another class of economically important polysaccharides produced by phytopathogenic bacteria includes curdlans, which are produced by various species of *Agrobacterium* and other bacteria. On heating, the curdlans form very elastic and resilient gums. The curdlans have a potential use in various food products as non-caloric gelling, thickening, binding, and stabilizing additives. A pseudomonad isolated from an aquatic plant produces an agar-like polysaccharide that has the potential to be used as a substitute for agar in bacteriological media.

Besides vinegar production, *Gluconobacter* species have many biotechnological applications. In the last decades, many bioconversion routes for rare and special sugars involving *Gluconobacter* spp. have been developed. Among the recent ones, are the biotransformations involved in the production of L-ribose and miglitol, both very promising pharmaceutical lead molecules. Most of these processes make use of *Gluconobacter*'s membrane-bound polyol dehydrogenases.

Various enzymes produced by phytopathogenic bacteria have various applications and are produced commercially. Pectin-degrading enzymes have found an important application in the food industry, where they are used in the clarification of fruit juices. An enzyme produced by an *Agrobacterium* strain is used for converting super coiled, covalently closed, circular DNA into a simple, covalently closed, circular form. Various *Xanthomonas* species and other phytopathogenic bacteria produce restriction endonucleases having much utility in genetic engineering and basic research. An L-asparaginase (protein synthesis inhibitor drug) produced by soft rot bacterium, *Pe. chrysanthemi* has a great potential for use as an antileukemic agent. The potential of Ti-plasmid of *Ag. tumefaciens* to move genetic material from bacteria to plants has made it an important tool in plant genetic engineering and it is widely used for this purpose. This technology is largely responsible for the development of plant biotechnology industry and the latter is revolutionizing agriculture and health care. The production of agrocin by an avirulent bacterium, *Ag. radiobacter* (strains K84 and K1026) and its role in controlling crown gall disease on commercial scale are well documented. At present, there are more than 16 commercial biocontrol products based on different bacteria including *Ag. radiobacter*, which are used to control plant diseases caused by different pathogens. The detailed studies of the nodulation process by *Rhizobium and Bradyrhizobium* species have led to the preparations of bacterial inoculum for application to legume crops (Dumenyo et al. 2001).

1.4 HISTORICAL REVIEW OF PHYTOBACTERIOLOGY

Antony van Leeuwenhoek discovered the bacteria for the first time in 1676 and called them animalcules, i.e., small animals. In 1876, Robert Koch showed that anthrax of animals is caused by a bacterium, *Bacillus anthracis*. Robert Koch also devised pour plate method for the purification of bacteria and gave Koch's postulates to prove the pathogenicity of causal agents of pathogenic diseases.

Woronin, a Russian worker, was the first person to show association and multiplication of bacteria in root nodules of leguminous plants in 1866. However, the multiplication of bacteria in root nodules is considered a symbiotic relationship and not a strictly pathogenic action. Professor T.J. Burrill of the University of Illinois was the first to show a bacterium as a causal agent of a plant disease. In a series of experiments starting from 1878 till 1884, he showed that the fire blight of pear was caused by a bacterium, which he identified as *Micrococcus amylovorus*. As the pure culture technique was in early stage of development in the laboratory of Robert Koch in Germany, Burrill was unaware of this modern microbiological method. Therefore, he did not use the pure culture of the bacterium in his work. J.C. Arthur working at New York Experiment Station confirmed Burrill's work during 1885–1887 using pure culture of the bacterium. In 1883, J.H. Wakker from Holland showed that yellow-slime disease of hyacinth was caused by a bacterium. Luigi Savastano, an Italian, demonstrated in 1887 that a bacterium incited the knot disease of olive. In 1893, L.H. Pammel found that bacteriosis of cabbage is caused by a yellow bacterium, *Bacillus campestris*, a name that was later changed to *Xa. campestris* pv. *campestris*.

Frediano Cavara (1857–1929), an Italian botanist who was the director of the Royal Botanical Gardens at the University of Naples, had for some time keenly observed galls on grapevines. He made the successful isolations of a bacterium from the galls and demonstrated that it caused an identical gall disease on healthy grapevines (Cavara 1897). Later on, Erwin F. Smith identified this bacterium and named it as *Bacterium tumefaciens* (Smith and Townsend 1907), which was changed to *Phytomonas tumefaciens* and then again changed to *Agrobacterium tumefaciens*. In the reports by Cavara (1897), it is clear that Smith, who visited Cavara in 1904, was shown the bacterial cause of the crown gall disease. Although Erwin F. Smith and C.O. Townsend (Smith and Townsend 1907) are cited as the discoverers of the bacterial nature of crown gall, credit should also be given to Cavara for the initial discovery and demonstration of the bacterial etiology of crown gall. In fact, Smith and Townsend confirmed the earlier findings of Cavara. Smith became intrigued with this disease and used it as a paradigm of cancer in animals and humans (Smith 1911). In 1901, L.R. Jones showed that the soft rot of vegetables is caused by *Bacillus carotovorus*.

Soon after 1890, Erwin F. Smith started work on bacterial diseases and the first disease investigated by him was bacterial wilt of cucurbits. Later on, he worked on many bacterial diseases of plants including bacterial wilt of solanaceous plants and black rot of crucifers. Erwin F. Smith's monumental contributions are included in his monograph entitled, *Bacteria in Relation to Plant Diseases* published in three volumes from 1905 to 1914. In 1896, Smith postulated that there are in all probability as many bacterial diseases of plants as there are of animals (Smith 1896). As late as 1920, Smith predicted that there will be bacterial diseases found on plants of every plant family.

A number of plant pathologists and authors of many books have given credit to T.J. Burrill for providing first credible proof that bacteria could cause plant diseases. However, Kennedy et al. (1979), in their critical appraisal of research work on bacterial diseases of plants carried out by T.J. Burrill and others, have opined that the credit for this discovery should be shared by E. Mitscherlich, a German chemist, T.J. Burrill, and J.C. Arthur and not given to Burrill alone. They have further stated that Burrill's place in history with respect to these studies is perhaps based on the fact that he was proved by others to have been right, and that he had a strong advocate in Erwin F. Smith.

Most of the work on bacterial diseases of plants from 1878 to 1900 and even later was carried out in the United States because there was a distinct air of disbelief on the part of European botanists regarding the role of bacteria in causing plant diseases. This was mainly due to the influence of De Bary, who considered bacteria as of minor importance as pathogens of plants. De Bary was aware of Wakker's work on the hyacinth disease, but not of Burrill's work on fire blight when he published his *Comparative Morphology and Biology of Fungi*, *Mycetozoa and Bacteria* in 1884. In this publication in the section of bacteria, he devoted just one short paragraph to those parasitic on plants and also pointed out that they had scarcely ever been observed. De Bary in his *Lectures on Bacteria* published in 1887 devoted only 2 out of 146 pages to bacteria parasitic to plants and stated that saprophytic bacteria may also under special conditions, attack the tissues of living plants as facultative parasites, produce disease in them, and destroy them.

Alfred Fischer, a professor of botany at Leipzig, Germany, who was a student of De Bary in 1881, challenged the work of Erwin F. Smith and others concerning the role of bacteria in causing plant diseases in 1897. A. Fischer in his book entitled, *Lectures on Bacteria* refused to accept the findings of Smith and others terming them unreliable. Smith replied to Fischer in 1899 pointing out his complete ignorance of the subject. Fischer wrote back the same year, and Smith replied in two rebuttal papers, which seemed to end the controversy. Smith was the clear winner of the debate. This debate has been duly recorded in one of the Phytopathological Classics published by APS Press (Campbell 1981). Walker (1969) has rightly commented on the controversy created by Alfred Fischer, stating that while this debate is now chiefly of historical importance, it is significant that more than 20 years after the work of Robert Koch and of T.J. Burrill; a rather prominent botanist still challenged the existence of bacterial plant pathogens. By 1900, the concept that bacteria could cause plant diseases was solidly established (Starr 1984), thereby creating **phytobacteriology** as a new subdiscipline of plant pathology. Erwin F. Smith's contributions to the field of plant bacteriology and his efforts to put it on sound footing earned him the title of "**father of plant bacteriology**." Goto (1992) has given a brief account of research work done on bacterial diseases of plants in Japan from the late 1880s to 1970s.

Although Erwin F. Smith made highly significant contributions to the field of plant bacteriology, he did not establish a dynasty or a school where young scientists could nurture their enthusiasm and interest in the newly discovered science of phytobacteriology as remarked by Sequeira (2000) in his review article. Therefore, after his death in 1927, phytobacteriology began to wane. Around the middle of twentieth century, plant pathology was dominated by mycology and virology.

In the initial stage of plant bacteriology, the emphasis was on reporting new bacterial diseases of plants and description and identification of their causal bacteria. Then the emphasis shifted to the investigations on the physiology of diseased plants, and during 1940s to 1960s, the significant contributions were made in this field along with ecology of plant pathogenic bacteria. The work of R.M. Klein (1954) and of A.C. Braun and Ross Pringle (1959) provided the evidence that the crown gall bacterium, *Ag. tumefaciens* caused permanent transformation of host cells leading to their autonomous and rapid growth in culture. This work along with pioneer work of A.C. Braun and his colleagues on the structure and mode of action of wildfire toxin produced by *Psm. syringae* pv. *tabaci* greatly impacted many plant bacteriologists to determine the pathogenicity determinants in other diseases (Sequeira 2000).

The discovery of mycoplasma-like organisms by Doi et al. (1967) gave the correct etiology of yellow-type diseases, which were earlier thought to be caused by viruses. The discovery of mycoplasma-like organism also resulted in the subsequent discovery of spiroplasmas and fastidious procaryotes. The mycoplasma-like organisms are now called phytoplasmas. The demonstration of conjugative transfer of bacterial genes in *Erwinia* by Chatterjee and Starr (1972) shifted the emphasis of research to molecular genetics of bacterial plant pathogens. Another notable contribution during 1970s was the success achieved in the biological control of crown gall with *Ag. radiobacter* strain K84 (New and Kerr 1972). Subsequently, an effective and viable biological control of fire blight has also been achieved. The introduction of pathovar system by Young et al. (1978) has greatly streamlined the taxonomy of plant pathogenic bacteria. Rapid advances in DNA sequencing technology and sequence analysis of highly conserved regions of the bacterial genome, such as the small subunit rRNA gene have led to the natural classification of procaryotes that reflects the evolutionary history of bacteria and archaea. The widespread application of new methods of classifying procaryotes has led to an explosive growth in the number of validly published species and higher taxa. Since the completion of first edition of *Bergey's Manual of Systematic Bacteriology* in 1989, the number of published species has more than tripled and accompanied with numerous taxonomic rearrangements and changes in nomenclature. The number of bacterial genera containing phytopathogenic bacteria, which was only six during 1970s, now stands at 53 including three '*Candidatus* genera'.

The first avirulence gene (*avrA*) characterized was cloned from a race 6 strain of the soybean pathogen, *Psm. syringae* pv. *glycinea* (recent name, *Psm. savastanoi* pv. *glycinea*) by Staskawicz et al. (1984). It elicits a resistant reaction on soybean cultivars having resistant gene *Rpg2*. Avirulence genes determine the race specificity of pathogen in a compatible host pathogen interaction. The products of *avr* genes apparently function as recognition factors in resistant hosts (i.e., carrying the *R* gene) and virulence determinants in susceptible hosts (i.e., carrying the *r* allele). In most cases, bacterial avirulence gene function is dependent on interactions with hypersensitive reaction and pathogenicity (*hrp*) genes. The *hrp* genes which control the ability of phytopathogenic bacteria to cause disease on susceptible plants and elicit hypersensitive reaction on resistant or non-host plants were first discovered for *Psm. syringae* pv. *phaseolicola* by Lindgren and associates in 1986. The role of avirulence genes and of *hrp* genes in host parasite interaction have been reviewed by Leach and White (1996) and Lindgren (1997), respectively.

Investigations carried on *Ag. tumefaciens*, the causal agent of crown gall, during the last 100 years (since its report by Smith and Townsend in 1907) have made this bacterium from merely a plant pathogen to a house-hold name in plant genetic engineering and plant molecular genetics (Nester 2008). The basic research carried out with this bacterium has provided very useful insights into host-parasite interactions and the mechanism of gene transfer from a procaryote to a eucaryote. The main problem of using Ti-plasmid as a gene vector, due to its larger size, has been solved by separating it into two parts; the 25 bp borders flanking the T-DNA between which the genes of interest are inserted and the vir region that is necessary for the processing and transfer of the T-DNA. This binary vector system is used in most laboratories. *Agrobacterium* is truly a natural genetic engineer.

Lately, atmospheric pressure plasmas have received much attention as a promising physical tool for biological decontamination and sterilization. Several types of plasma devices have been described for sterilization of animal and human tissues, and the system can also be used for reducing the population of various plant microbes that cause infectious diseases. Mráz et al. (2014) used *Cla. michiganensis* subsp. *michiganensis*, *Er. amylovora*, and *Escherichia coli* (as a control) to study the bacterial growth mechanism after exposure to the atmospheric pressure GlidArc plasma. It was found that low-temperature plasma treatment affected all the three bacterial species by slowing down growth and reproduction rate. After plasma treatment, both *Es. coli* and *Er. amylovora* reached their maximum growth sooner than the *Cla. michiganensis* subsp. *michiganensis*. This is, however, caused by a longer cultivation period of *Clavibacter* genus. The application of GlidArc plasma is a suitable treatment that can be used in agriculture for plant protection. Further studies of low-temperature atmospheric pressure plasmas' effect on bacteria could be focused on its practical use in seed treatment of various crops.

A brief account of development of phytobacteriology in India is given below. In India, Cappel (1892) reported bangle blight or bungdi disease of potato from Pune, Maharashtra. Butler in 1903 also reported this disease and stated that it was similar to brown rot of potato caused by *Psm. solanacearum*. Coleman in 1909 proved the bacterial nature of the disease. Later on, Dr. M.K. Patel started work on bacterial diseases in 1948. He along with his associates worked for nearly 15 years and reported nearly 40 bacterial diseases, most of which were new records. Based on his contribution to the field of plant bacteriology in India, Dr. M.K. Patel is regarded as the "**father of plant bacteriology**" in India. A review of bacterial plant disease investigation published by Patel and Kulkarni (1953) included 27 species of bacterial pathogens reported from India. The first book on bacterial plant diseases in India was written by G. Rangaswami in 1962, in which he listed 73 bacterial plant pathogens. In 1965, Mathur and associates published a list of bacterial plant pathogens found in India. Phytopathogenic bacteria of India and bibliography published by Chakravarti et al. (1973) contains a list of 483 references on plant pathogenic bacteria and 148 bacterial plant pathogens belonging to six genera, namely, *Agrobacterium*, *Bacillus*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. A summer institute on Plant Bacteriology under the directorship of Dr. P.N. Patel was held in the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi in 1972, and its proceedings were published in three volumes edited by Dr. P.N. Patel. First National Symposium on Plant Bacterial Diseases was held at Sri Venkateswara University, Tirupati in 1980 under the directorship of Dr. M.V. Nayudu and several important recommendations were made. Ninth International Conference on Plant Pathogenic Bacteria was held in 1996 at University of Madras, Chennai under the chairmanship of Dr. A. Mahadevan.

At present, phytobacteriology is one of the leading areas in plant pathology and has attracted a large number of scientists from areas other than plant pathology. It is evident from the large number of papers on phytobacteriology published in *Molecular Plant-Microbe Interactions*, *The Plant Cell*, and *Applied and Environmental Microbiology*.

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2 Bacterial Cell

On the basis of cellular organization, the living organisms are divided into two groups, namely, procaryotes and eucaryotes. The members of the procaryotic world comprise a vast heterogeneous group of very small, mostly unicellular organisms. Stanier and van Niel (1962) proposed that bacteria are distinguished from other forms of life, including viruses, protists, fungi, algae, plants, and animals, by their procaryotic cell structure. They defined the procaryotic cell by three major criteria: the absence of internal membranes that compartmentalize the nuclear material and the enzymatic machinery for respiration and photosynthesis, nuclear division that occurs by fission and not mitosis, and the presence of peptidoglycan in the cell wall.

During the last 50 years, a wealth of new information has been produced that greatly enriches our understanding of procaryotes. These organisms have proven to be of enormous abundance and diversity, the product of complex evolutionary processes over billions of years. They dominated life on the earth prior to the appearance of eucaryotes. Their distant past implies that many of the most salient features of modern life evolved in an entirely procaryotic world, including most of the organizing principles of the cell; the basic mechanisms of replication, transcription, and translation; the major catabolic and anabolic pathways; and the biogeochemical cycles that maintain the biosphere (Whitman 2009). Although Stanier and van Niel (1962) precisely defined the term procaryote, it has occasionally been misused, usually as synonymous with bacteria.

Procaryotes include two types of organisms, namely, bacteria and archaea. The majority of procaryotes, including the photosynthetic cyanobacteria are included in the bacteria. The bacteria look quite simple when we look at size, shape, and arrangement of their cells with an ordinary light microscope. However, the modern electron microscopy has revealed an amazing complexity and details of external and internal cell structures, which were not possible with an ordinary light microscope.

2.1 SIZE

Being microscopic, the bacteria are measured in micrometers (µm), which are equivalent to 1/1000 mm (10^{-3} mm). The cell size of bacteria varies with the species, but most cells are approximately 0.5–1.0 µm in diameter or width and 2.0–5.0 µm in length. The cells of *Staphylococcus* and *Streptococcus* species show a slightly larger diameter measuring 0.75–1.25 µm and some filamentous forms may be as long as 100 µm.

A few cases of bacteria having extremely large size have come to the knowledge of bacteriologists. *Epulopiscium fishelsoni* found in the gut of surgeon-fish measures 50–100 µm in width and 0.5 mm in length. Another bacterium, *Thiomargarita nomibiensis* measures 100–300 µm in diameter. This appears to be the largest bacteria discovered so far. The eucaryotic cells on an average measure from 2 to 200 µm in diameters.

The size of the bacterial cells is influenced by age and cultural conditions. Actively growing cells are slightly larger than the cells in their stationary phase. In stained preparations, the cell size is also reduced due to shrinkage of cells.

The extent of small size of a bacterium is evident from the fact that approximately 1 trillion (1,000,000,000,000 or 10^{12}) bacterial cells weigh a mere 1 g.

The importance of extremely minute size of a bacterial cell is reflected due to the very high ratio of its surface area to the volume. This highlights the fact that there is a large surface through which nutrients can enter for a relatively small volume of cell substance to be nourished. This is partly responsible for the high rate of metabolism and growth of bacteria. Due to their rapid growth and multiplication, the bacteria are used more frequently in biological research.

2.2 SHAPE

Every bacterial species is associated with a constant shape. There are three basic shapes of bacteria, namely, spherical, cylindrical, or spiral. Most commonly found bacteria are either cocci or bacilli.

2.2.1 Spherical

Spherical cells are called **cocci** (singular, **coccus**). They are usually round, but they can be ovoid or ellipsoidal (Figure 2.1a). Their size is measured in diameter.

2.2.2 Cylindrical

They are rod-shaped and have length and breadth. They are called **bacilli** (singular, **bacillus**). The ends of the cells may be flat, rounded, pointed, or tapered (Figure 2.1b). Their size is measured in length and breadth. There is not much difference in the width of different species, but differences in length are considerable.

2.2.3 Spiral

The cells are helical in shape and are called **spirilla** (singular, **spirillum**). Spiral bacteria have one or more twists, and they are never straight (Figure 2.1c). Certain spiral bacteria are curved short rods and resemble distinctive commas. These are called **vibrios**. *Vibrio cholerae*, the causal agent of cholera, is a typical example of vibrios. The other spiral bacteria are long rods

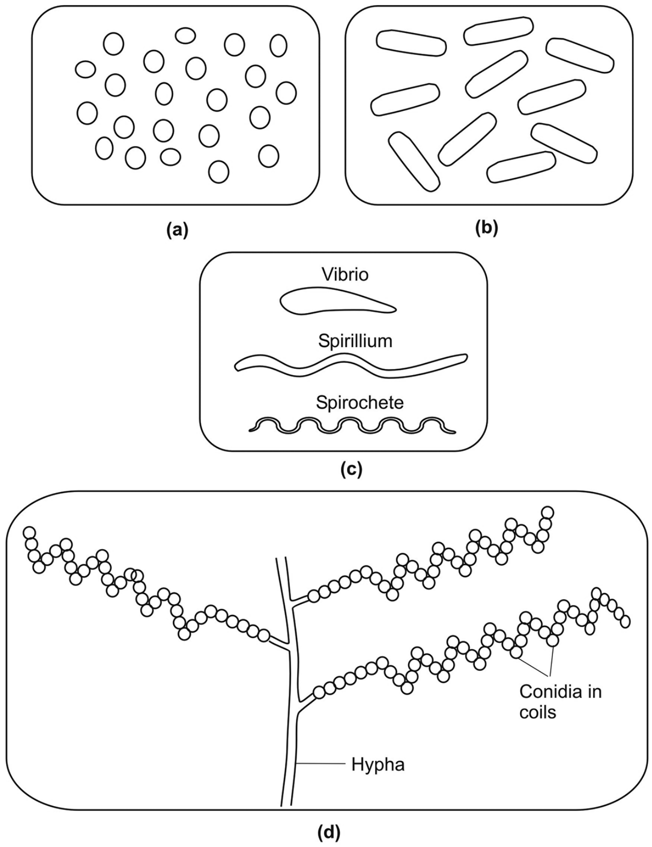

FIGURE 2.1 Shapes of bacterial cells: (a) cocci, (b) bacilli, (c) spirals, and (d) filamentous hyphae.

twisted into spirals; if the rods are rigid having thick cell walls, they are called **spirilla**, and **spirochetes** when cells have thin flexible cell walls (Tortora et al. 2007). *Treponema pallidum*, the causal agent of syphilis, is a typical example of a spirochete.

There are many modifications of these three basic shapes. The cells of *Pasteuria* are pear-shaped, while those of *Caryophanon* are disc-shaped. Square and star-shaped forms also occur. The genus *Caulobacter* contains appendaged bacteria.

The members of Actinomycetes produce long **filamentous hyphae** that may branch to produce a mass of hyphae called **mycelium** (Figure 2.1d). Although they appear like fungi, but the cells are typically procaryotic, hence classed with eubacteria.

Although the shape of cells of most bacterial species is constant under a definite set of environmental conditions, a few species have a variety of cell shapes and are called **pleomorphic**. Mycoplasmas are pleomorphic.

2.3 ARRANGEMENT

The majority of bacteria exist in unicellular forms; however, some species show arrangements or attachment of cells. This arrangement is usually found in spherical and cylindrical forms. In spherical cells, the following five types of arrangement of cells are found.

2.3.1 Diplococci

A coccus cell divides in one plane forming a pair of two cells, e.g., some spp. of *Neisseria* (Figure 2.2a).

2.3.2 Streptococci

The cells divide in one plane forming a chain of cells, e.g., *Streptococcus* and *Lactococcus* spp. (Figure 2.2b).

2.3.3 Tetrads

The cocci divide at a right angle to first plane of division forming a group of four cells in the shape of a square, e.g., *Pediococcus* and *Micrococcus* spp. (Figure 2.2c).

2.3.4 Sarcinae

In this case, the third division occurs in a plane different to the first two divisions resulting in cuboidal or packet arrangement of eight cells, e.g., *Sarcina* spp. (Figure 2.2d).

2.3.5 Staphylococci

When the divisions in the three planes occur in an irregular pattern, the arrangement of cells look like a bunch of grapes, e.g., *Staphylococcus* spp. (Figure 2.2e).

In cylindrical bacteria, only the following two types of cell arrangement are found.

2.3.6 Diplobacilli

Two cells are arranged from end to end (Figure 2.2f).

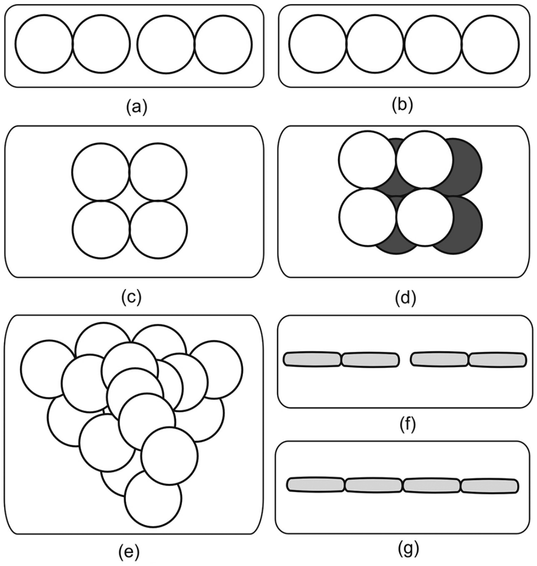

FIGURE 2.2 Arrangement of cells: (a) diplococci, (b) streptococci, (c) tetrads, (d) sarcina, (e) staphylococci, (f) diplobacilli, and (g) streptobacilli.

2.3.7 STREPTOBACILLI

Three or more cells are arranged from end to end forming a chain, e.g., *Bacillus* spp. (Figure 2.2g).

Each of these arrangements is typical of a particular species and is helpful in its identification. However, rarely all the cells of a given species are arranged exactly in the same pattern. It is the predominant arrangement that is taken into account while studying bacteria. The size, shape, and arrangement of bacteria are important parameters of their gross morphology and are used in their identification.

2.4 CELL STRUCTURE

The cell structures described below are not common to all the bacteria. Some of these structures such as cytoplasmic membrane, cytoplasm, ribosomes, and nucleoid, region containing all or most of genophore, are found in all the bacteria, while the others are associated with certain particular species. Some of these structures are present inside the cell, while others are present outside the cell wall (Figure 2.3).

2.4.1 FLAGELLA AND PILI

Many bacteria are motile, and this motility is due to the presence of flagella (singular, flagellum). Many species of bacilli and spirilla possess flagella, while cocci rarely have these organelles. Flagella are thin hair-like structures with a helical shape. These originate from the cytoplasmic membrane and extend to the surface after penetrating the cell wall. Flagella are many times longer than the cells, measuring 15–20 µm in length. However, the diameter of a flagellum is only a fraction of width of the cells, i.e., 12–20 nanometers (nm). Being too thin in width, unstained flagella cannot be seen with an ordinary light microscope. The staining procedures using a mordant make flagella thicker due to the deposition of dye and thus making them visible by light microscopy.

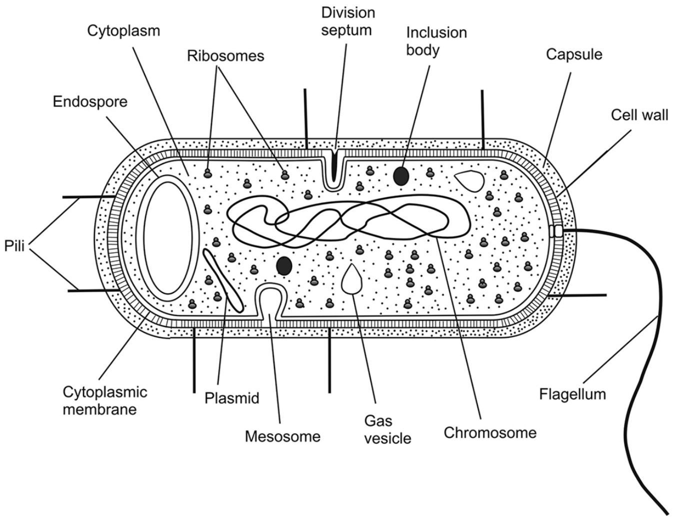

FIGURE 2.3 Bacterial cell.

The number of flagella and the pattern of flagellar attachment on a bacterial cell are used to classify bacteria. Four types of flagellar attachment found in bacteria are given below:

Monotrichous: One flagellum at one pole of the cell, e.g., *Xanthomonas* spp. (Figure 2.4a).
Lophotrichous: A cluster of flagella at one pole of the cell, e.g., *Pseudomonas* spp. (Figure 2.4b).
Amphitrichous: One flagellum or cluster of flagella at both poles of the cell, e.g., *Spirillum* spp. (Figure 2.4c).
Peritrichous: The flagella scattered over the entire surface of the cell, e.g., *Erwinia* and *Escherichia* spp. (Figure 2.4d).

Some bacteria, e.g., spirochetes have specialized flagella called **periplasmic flagella** (also called **axial filaments**). The helical filaments of these flagella are not free and loose like that of normal flagella. These arise at the poles and twine around the protoplasmic cylinder beneath the outer membrane of the cell wall. The spirochetes move in corkscrew-like manner with the help of these flagella.

A flagellum consists of three parts, the basal body, hook, and a long filament. The basal body is embedded in the cell and consists of a central rod surrounded by rings. Gram-stain-positive bacteria have only one pair of rings surrounding the central rod, one ring present in each of cytoplasmic membrane and cell wall. In Gram-stain-negative bacteria, two pairs of rings are present,

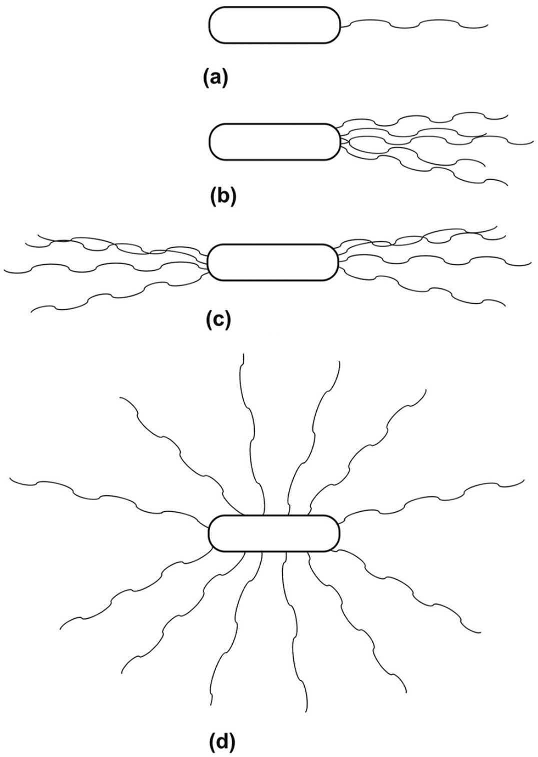

FIGURE 2.4 Arrangement of flagella: (a) monotrichous, (b) lophotrichous, (c) amphitrichous, and (d) peritrichous.