Frédérique Van Gijsegem
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Foreword

This book covers a wide range of aspects relating to the biology of the most common soft rot bacteria, currently classified in the genera *Pectobacterium* and *Dickeya* within the family *Pectobacteriaceae* (SRP). It pays particular attention to the taxonomy, epidemiology and pathogenicity, including molecular aspects of the diseases they cause on plants, as well as their economic importance. It is the first comprehensive compilation of research on these bacteria.

With the experience of three decades of research on this subject, I will attempt here to place recent research on SRP in its historical context. Unavoidably, attention will focus on relevant potato diseases, which have been studied the most for over a century.

Interest in the Soft Rot *Pectobacteriaceae* (SRP) arose in the late nineteenth century following the groundbreaking work of Pasteur and Koch on bacteria. It was soon realised that plant diseases can also be caused by micro-organisms, including bacteria. Thus, L. R. Jones was the first to isolate and describe a bacterium from soft rotting vegetables which he named *Bacillus carotovorus* (now *Pectobacterium carotovorum*) in 1901. C. J. J. van Hall in 1902 then described another bacterium isolated from potato which he named *Bacillus atrosepticus* (now *Pectobacterium atrosepticum*). Interest soon focussed on the diseases they were associated with, especially on potato which is widely grown in temperate regions and at the time an even more important staple food crop than presently. Subsequently, several other SRP were isolated on other plants, mostly ornamentals, and in the 70s, one now known as *D. chysanthemi* (now *Dickeya* species) generated some interest because of its pathogenicity on potatoes grown in hot dry conditions.

There was considerable confusion concerning the two closely related bacteria, *P. carotovorum* and *P. atrosepticum*, both causing soft rot on potato tubers and other parenchymatous plant tissues but only the latter apparently able to cause blackleg or soft rot of potato stems. The inability to distinguish between them easily using the differentiating biochemical tests of the time did not help. It was only when D. W. Dye in 1969 published his comprehensive taxonomic studies that there was some clarity. The genus name evolved over time from *Bacillus* to *Erwinia* to *Pectobacterium* back to *Erwinia* and then back to *Pectobacterium.* More recently, with the advent of molecular techniques, it was realised that some confusion also arose from the fact

that some of the strains within *Pectobacterium* spp. should be attributed to different species or genera.

Because of the importance of SRP in potato cultivation, where they cause losses both in the field (seed tuber rots before emergence and blackleg) and tuber rots in stores, increasing attention was paid to their study. Although potato was and is still grown widely in Europe, this was first done in the USA by J. G. Leach, W. J. Morse and others, especially in Maine before 1940. One likely explanation is the larger scale of the operation relative to Europe and the greater scale of losses due to the fact that cut seed was, and remains, a common practice—hence more susceptible to rotting and failure to emerge under wet conditions. Over irrigation often led to blackleg development and bulk storage of wet tubers, which are highly susceptible to massive tuber break down in stores when adequate ventilation is lacking. The general consensus was that the pathogen(s) was soil borne and hence inclusion in a seed certification system would be of little value. The advocated control measures were based on avoiding over irrigation and improving storage facilities, especially insulation essential under the severe prevailing winter conditions.

In Europe in contrast, research on SRP came later and by the 50s attention has focussed mostly on blackleg, with disease control relying mainly on seed certification, with crops inspected and discarded if they had an infection level higher than set limits. However, this approach essentially failed to achieve its objective. Harvested crops were often stored in the open in heaps with a cover of straw and soil. Not surprisingly, soft rotting was not infrequent. It became clear by the 60s that a reappraisal of the epidemiology of the disease and better storage conditions were needed. Studies of the problem were undertaken mainly at three centres, Wageningen, Edinburgh and Dundee.

Improved storage conditions for potatoes were achieved by bulk indoor storage with under floor forced ventilation, or in one or half tonne stacked boxes in doors. For field diseases, the findings were not as hopeful. The consensus was that the bacteria do not survive in soil and are tuber (seed) borne. Blackleg development was weather dependent, greater under wet conditions and related to the level of seed tuber contamination. However, blackleg incidence was, and remains, an unreliable guide to the health status of the harvested crop, as most tubers were by that time contaminated often by more than one species. The conclusion was that since the seed tuber is the main source of the disease, seed stocks raised from stem cuttings or axenic plants would produce pathogen-free progeny tubers. When put into practice, it was evident that recontamination of the subsequent crop generations occurred early and within a few years of multiplication the stocks were as contaminated as before.

What followed was an intensive study of alternative sources of the bacteria including surface water, aerosols scrubbed down by rain and, importantly, contaminated machinery at harvest and tuber size grading stage. Sophisticated detection and quantification techniques were developed in an attempt to identify stocks with low levels of seed contamination but, overall, they were not widely used for practical reasons. One partially successful measure was limiting the number of years of seed

stock multiplication regardless of its health status. Attempts to control contamination by chemicals were not successful and thermotherapy (hot water treatment) fared better but was not a practical proposition.

There has always been the possibility of achieving disease control by breeding for resistance but attempts to do so have failed for different reasons, mainly because of the narrow range of genetic variation among potato cultivars but also the relative low importance given to the disease by the industry.

In the last two decades there has been an explosion of interest in molecular biology of SRP in Europe, the USA and elsewhere, focussing primarily on the regulation and expression of perceived pathogenicity genes. These studies have not always taken into account the fact that SRP are basically facultative pathogens, causing tuber infection only under favourable environmental conditions. For example, the presence of excess water, which induces anaerobiosis in tubers allowing bacterial multiplication and production of pectolytic enzymes that results in tissue maceration. A better understanding of the molecular biology of SRP would be useful in developing resistance in cultivars in trans- or cis- breeding systems utilising resistance genes present in wild, even in non-tuber bearing *Solanum* spp. However, one should bear in mind that the infection process, hence the nature of resistance, is not necessarily similar in tuber soft rot and in blackleg.

It is clear that future research must take a comprehensive outlook to encompass and integrate all the different disciplines as they complement each other. This book is a first step to that end. It will prove to be of value not only to current workers but also, importantly, to newcomers in the field.

Dundee, Scotland
July 2020

Michel C. M. Pérombelon

Acknowledgements

We would like to thank all those scientists, extension specialists, growers and policymakers who have studied and sought to control these pathogens and their diseases over the last 100 years and beyond. The authorship of the book shows the current global reach of this research area and we thank all authors for their valuable contributions. We would also like to thank Dr Michel Pérombelon for his forward to this book but also for his many years of dedicated research on these pathogens, much of which remains relevant today. We believe that the first book to contain a description of the SRP was published in 1920 by Erwin Frink Smith entitled 'An introduction to bacterial diseases of plants' (Philadelphia and London W. B., Saunders Company). In the same year, to honour his contribution to this field of study, the genus *Erwinia* (encompassing all SRP) was named and used for the remainder of the twentieth century. In memory of a scientist who has made such an important contribution to the early study of the erwinias, and on the 100 year anniversary of his book, we would like to dedicate our book to him.

Erwin Frink Smith was an America plant pathologist (1854–1927) with the United States Department of Agriculture (USDA) and played a major role in the early work on bacterial plant diseases. For further reading see Campbell CL (1983) Erwin Frink Smith—a pioneer plant pathologist. Annual Review of Phytopathology 21: 21–27.

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Chapter 1
Soft Rot *Pectobacteriaceae*: A Brief Overview

Frédérique Van Gijsegem, Ian K. Toth, and Jan M. van der Wolf

Abstract Bacterial soft rot diseases devastate a wide range of crops, vegetables and ornamental plants worldwide. Amongst the most damaging agents of these diseases are members of the *Pectobacterium* and *Dickeya* genera belonging to the family *Pectobacteriaceae* in the order Enterobacterales. As an introduction to the topics of this book, this chapter presents a brief overview on taxonomy history, presence in multiple environments, disease characteristics, population dynamics, management and economic impact of these bacteria.

Bacterial soft rot diseases devastate a wide range of crops, vegetables and ornamental plants worldwide and are caused by species from genera including *Pseudomonas*, *Bacillus*, *Burkholderia*, *Pantoea*, *Enterobacter*, *Klebsiella*, *Leuconostoc* and *Clostridium* (Charkowski 2018). In addition, and amongst the most damaging of these, are members of the *Pectobacterium* and *Dickeya* genera belonging to the family *Pectobacteriaceae* in the order *Enterobacterales* (Adeolu et al. 2016).

The present book focuses exclusively on the Soft Rot *Pectobacteriaceae* (SRP), *Pectobacterium* and *Dickeya,* and to our knowledge this is the first book to do so. It covers a wide range of topics in relation to these organisms in a series of chapters introduced below. To avoid repeating ourselves where information between chapters inevitably overlaps, we have chosen to focus information in one chapter while briefly mentioning it in others and cross-referencing to the main text. The reader will see time and again reference to potato in this and other chapters. This is also inevitable as there is so much more research carried out on this crop than any other. However, we know that many of the environments, infection pathways and modes of disease are

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similar in most plants and so we hope that you will be able to make direct comparisons to your own plants of interest.

1.1 A Brief History of Taxonomy

Soft rot bacteria have been known for more than a century. Indeed, the first report on the association of a non-fungal microorganism with soft rot of diverse plants, and the transmissibility of this disease via rotted plant material, dates to 1868 (Paulin et al. 2001). From then on, these bacteria have been renamed several times often making it difficult to relate findings from one named species/subspecies to those of another. The first isolation on carrot and other vegetables of what was then called *Bacillus carotovorus* was reported in 1900 (Jones 1900). The name *Bacillus atrosepticus* was then created to designate bacterial pathogens causing potato blackleg disease (van Hall 1902). Jones showed the importance of a pectinolytic enzyme produced by these bacteria that dissolved the middle lamella and broke apart cells during rapid bacterial progression through storage organs of plants including tubers, bulbs or rhizomes (Jones 1909).

In 1920, the Committee of the Society of American Bacteriologists on characterization and classification of bacterial types united all Gram-negative, fermentative, non-sporulating, peritrichous flagellated plant pathogenic bacteria into one 'tribe' named erwiniae in honour of the American phytopathologist Erwin F. Smith. In this tribe, which still includes other species such as *E. amylovora* and *E. stewartii,* pectinolytic bacteria were named *Erwinia carotovora* and were classified into two subspecies: *E. carotovora* subsp. *atroseptica* for potato blackleg causing pathogens and *E. carotovora* subsp. *carotovora* (Winslow et al. 1920). Pathogens isolated from several hosts were also further classified into the *E. carotovora* species based on the exhaustive study of Dye (1969), who concluded that all such pathogens represent a single species based on their common biochemical characteristics. Three other pathogens were also more recently described as subspecies of *E. carotovora*: subsp. *betavasculorum*, responsible for vascular necrosis of sugar beet (Thomson et al. 1981), subsp. *wasabiae*, responsible for internal discoloration of rhizomes of wasabi (Goto and Matsumoto 1987), and subsp. *odorifera*, responsible for slimy rot of witloof chicory (Gallois et al. 1992).

A second species of soft rot-causing pathogens was created in 1953; *Erwinia chrysanthemi* so named because of its first isolation on chrysanthemum (Burkholder et al. 1953). The species was found to be diverse in phenotypic properties including host range. However, such a broad classification in species did not always meet the needs of phytobacteriologists, who preferred to have bacterial names that clearly indicated differences in pathogenicity and plant hosts. For this reason, the epithet "pathovar" was proposed to deal with the differences in pathogenicity between groups within the same species. *E. chrysanthemi* was thus subdivided in six pathovars (pv. *chrysanthem*i, pv. *dianthicola*, pv. *dieffenbachiae*, pv. *parthenii*, pv. *zeae* and pv. *paradisiaca*) (Dye et al. 1980; Young et al. 1996). The practice of using pathogenicity

to determine the pathovar, however, proved difficult to implement due to overlapping host range and the lack of reproducibility of the bioassays. Alternatives were proposed to characterize all strains of the soft rot *Erwinia* complex including the classification of strains into serovars by serological tests (Samson 1973; De Boer et al. 1979) or into biovars using batteries of differential biochemical tests (Dye 1969). The classifications in pathovars, serovars and/or biovars were unfortunately often not in concordance.

The advent of DNA sequencing techniques allowed the relationships between *Erwinia* species and subspecies to be studied based on genetic evolutionary trees. The first studies were based on 16S rDNA sequence. Using such methods, Hauben et al. (1998) united the members of the soft rot erwiniae, including *Erwinia carotovora*, *Erwinia cacticida*, *Erwinia chrysanthemi* and *Erwinia cypripedi* into the genus *Pectobacterium*, adopting an earlier proposition by Waldee (1945) who proposed the inclusion of all pectinolytic enterobacteria into a single genus. Samson et al. (2005) then renamed *E. chrysanthemi* as a new genus, *Dickeya,* after the famous American phytobacteriologist Robert S. Dickey and defined six *Dickeya* species that largely fitted with the previous classification in pathovars. The accumulation of genomic sequences now available in databases, thanks to increasingly cost-effective, high-throughput DNA sequencing technologies, allowed whole genome comparisons that resulted in a major re-evaluation of pectinolytic bacterial taxonomy. Adeolu et al. (2016) reclassified the family *Enterobacteriaceae* as an order (*Enterobacterales*) that comprises the *Enterobacteriaceae* but also other families. One such family is *Pectobacteriaceae,* which contains the genera *Pectobacterium* and *Dickeya* together with the plant pathogen genera *Lonsdalea* and *Brenneria*. *Pectobacterium* and *Dickeya* spp., formerly termed 'Soft Rot erwiniae', and then 'Soft Rot *Enterobacteriaceae* in an attempt to use the same acronym (SRE), have more recently been termed 'Soft Rot *Pectobacteriaceae* (SRP)' as used throughout this book. Genomic analyses have more recently re-defined multiple SRP species leading to the current description of nineteen *Pectobacterium* and twelve *Dickeya* species as described in Chap. 2.

Taxonomy of the SRP has been and remains a complex field and so we have attempted to simplify it in the book by using, where possible, the most recent name for the genus or species. For example, a previous study that refers to *Erwinia chrysanthemi*, in the absence of further pathovar information has been referred to as '*Dickeya* spp.' but, where a pathovar is noted, e.g. *Erwinia chrysanthemi* pathovar *dianthicola*, we have referred to it by its current name '*Dickeya dianthicola*'.

1.2 Host Range and Environmental Sources

Collectively, SRP have a very broad host range. Indeed, Ma et al. (2007) recorded SRP hosts in 35 % of angiosperm plant orders, including both dicot and monocot plants (Ma et al. 2007) and this catalogue is still expanding to include woody plants (Charkowski 2018; Tian et al. 2016). While several *Pectobacterium* spp. have been reported on a specific host, e.g. *P. atrosepticum*, *P. parmentieri* and *P. polaris* on

potato and *P. betavascularum* on sugar beet, others have been isolated from a large variety of plants, often belonging to both dicots and monocots. Conversely, some plants may act as hosts for several SRP species. For example, potato is infected by half the SRP species currently described (5 *Dickeya* and 9 *Pectobacterium* spp.). This reflects, in part, the extensive research characterising potato blackleg causing agents over recent decades. The diversity of SRP plant hosts is described in Chap. 3 together with modes of infection and disease development. This chapter also explores the numerous environments outside plants that SRP inhabit and their role as possible sources of plant contamination.

1.3 The Nature of Disease

Because SRP can be found in association with asymptomatic plants and rely mainly on the production of plant cell wall degrading enzymes (PCWDE) for their pathogenicity, they have often been viewed as "brute force" opportunistic pathogens. However, characterisation of the virulence factors and the highly sophisticated regulatory networks that control their production, in addition to the intense cross-talk governing the interactions of these pathogens with their plant hosts, show that SRP are much more than producers of PCWDE but instead behave as true stealth force pathogens (De Boer 2003; Toth and Birch 2005).

SRP may survive in a latent state within the plant without producing symptoms. There is also now good evidence that SRP can live on plants [particularly roots] away from a susceptible host, suggestive of a more natural lifestyle in the wider environment as outlined in Chap. 3. Whether on such plants or in the apoplast of a susceptible host, they can multiply and persist using the nutrients present and have developed a large array of metabolic pathways to adapt to such environments. These metabolic pathways are tightly controlled by regulatory networks intertwined with those governing the production of PCWDE, and in some cases clearly act as virulence factors. The reason why these bacteria can grow on some plants in the absence of disease, while causing devastating diseases on others, remains unclear. However, when in a susceptible host, this biotrophic lifestyle may persist for months when the environmental conditions are not favourable to disease initiation (De Boer 2002), further suggesting the hemi-biotroph nature of these pathogens (Kraepiel and Barny 2016).

For decades, analyses of SRP-host plant interactions have led to the characterisation of a range of factors involved in SRP virulence, including the key virulence factors, PCWDE, and their secretion systems but also other protein secretion systems, the production of toxins and plant hormones, and motility, as well as plant responses to SRP infections. This has been extensively reviewed (Davisson et al. 2013; Reverchon et al. 2016; Toth et al. 2006; Charkowski et al. 2012). More recently, gene expression analyses, both in vitro, in conditions mimicking the plant environment, and directly *in planta*, have allowed the identification of complex regulatory networks that permit the sequential production of virulence factors in the different phases of

infection (Liu et al. 2008; Venkatesh et al. 2006; Jiang et al. 2016; Bellieny-Rabelo et al. 2019; Chapelle et al. 2015; Gorshkov et al. 2018; Pédron et al. 2018; Raoul des Essarts et al. 2019). The most recent advances in molecular interactions between SRP and plants are summarized in Chap. 4 with a special emphasis on the importance of metabolic activities in plant-bacteria interactions and a comparison of the strategies used by both *Pectobacterium* and *Dickeya* spp. for controlling the coordinated and "at the right time" expression of virulence factors during infection. Indeed, the control levers regulating virulence gene expression in both genera are quite similar, e.g. quorum sensing, metabolic status and environmental conditions but the genetic components governing the regulatory networks vary in both genera. While this cannot be all encompassing, Chap. 4 summarises the main areas and, like other chapters, helps to guide the reader to further detailed information.

Despite the high conservation of regulatory networks, the expression profiles of even closely related species during infection may vary for several genes, including those involved in virulence and regulation as exemplified in the comparison of the two closely related *D. solani* and *D. dianthicola* expression profiles in infected potato tubers (Raoul des Essarts et al. 2019). It is even more striking within the species *D. solani,* where several strains are clonal and differ at the DNA level in only a few SNP/InDels and genes, and yet exhibit widely contrasting aggressiveness and large variation in expression of virulence genes, including those encoding the PCWDE (Khayi et al. 2015; Golanowska et al. 2018). Genomic analyses have also revealed genes encoding virulence factors and metabolism associated with horizontally transferred genomic regions and prophages, indicating genome plasticity. Chapter 2 also explores the levers of evolution in SRP genomes and how this may pave the way to rapid evolution of SRP for easy adaptation to different environments and/or new hosts.

1.4 Managing Diseases

Diagnostics play an important part in disease management as they identify the presence and level of a pathogen even in the absence of symptoms. Indeed they are essential to identify and track disease outbreaks caused by SRP and play an important role in monitoring the presence of certifiable pathogens, e.g. as used in Scotland where there is legislation to prevent the import of *Dickeya* spp. (Toth et al. 2011). Detection and diagnostics are vital areas if we are to understand how these pathogens move between plants, through trade routes, contaminated crops and much more, and this area is covered in Chap. 5. Diagnostics have changed considerably and for the better in recent years thanks to advances in genomics and the characterisation of numerous new SRP species, linking closely information in Chap. 5 with that on taxonomy in Chap. 2.

Ultimately, research on the SRP has been undertaken for the purpose of improving existing or identifying new methods of disease control. Control of these pathogens has never been straightforward due mainly to the lack of chemical control options but

also, and at least currently, the lack of disease resistance (Czajkowski et al. 2011). With these two main control options unavailable, the industry has instead relied on a toolbox of different, less effective, but nevertheless useful options. Hygiene of machinery, equipment, glasshouses, and stores etc. also has a high priority. Simple disinfectants are highly effective against these pathogens but the logistics of undertaking hygiene measures in vast storage units or large constantly used machinery is very much more difficult and can often get neglected (Czajkowski et al. 2013). While not every region or crop production system undertakes control in the same way, control often begins with the use of microplants grown in the laboratory and free from the pathogen. In the case of potato, these are then grown as minitubers under covered conditions before going to the field. Certifying through inspection, removal of diseased plants (roguing) and, where necessary, rejecting or down-grading crops based on the presence of the pathogen or disease symptoms is also an essential part of any well-managed system. Chapter 6 looks at disease management and the different options available. This is perhaps the pinnacle of the book as all previous chapters are there exclusively with control in mind. This is a very difficult pathogen to control but with recent advances we are hoping that new possibilities may be just around the corner.

1.5 Population Dynamics

It is noteworthy that the dynamics of SRP populations responsible for potato diseases, and also perhaps less studied diseases, have been changing worldwide over the last decades. In Europe, for example, until the 1970s *P. atrosepticum* was the major SRP responsible for disease on potato. At this time, sporadic infections by *D. dianthicola* were recorded, which later increased across much of Europe. At the beginning of the twenty-first century, a new species emerged, *D. solani*, again spreading across potato-producing countries in Europe and, by around 2010, this species had become the most important blackleg agent in several of these countries. Over the past decade, other species of the so called "*P. carotovorum* complex", including *P. brasiliense* and *P. parmentieri,* have emerged and become predominant. Interestingly, several of these emerging SRP have recently been found in historic bacterial culture collections (classified under the generic term *Erwinia carotovora*), suggesting that they may have been previously 'unnoticed' rather than 'new-comers'. Nevertheless, the emergence of totally new SRP variants by genetic changes cannot be ruled out. Genomic analyses have revealed genes encoding virulence factors and metabolism associated with horizontally transferred genomic regions and prophages, indicating genome plasticity. As several SRP can occupy the same niche in infected plant, a rapid evolution of SRP can be expected to adapt to new environments and/or new hosts (Chap. 3). Chapter 7 describes the global reach of SRP, and the different genera and species responsible for diseases, by taking key examples from around the world of plants affected by SRP and the pathogens responsible. It has not been possible to include all

countries affected by these pathogens and we apologise in advance for those regions that have not been included.

1.6 Economic Impact

As recorded in Chap. 7, SRP cause severe losses in important economical crops in many countries around the world and, consequently, have been ranked in the top ten plant pathogenic bacteria in 2010 (Mansfield et al. 2012). They cause diseases in three of the four most important crops worldwide: rice, maize and potato (but not wheat). Over the last decades, the potato seed industry has faced several outbreaks corresponding to the emergence of SRP, including *D. solani* and *P. brasiliense* in Europe and *D. dianthicola* in the USA (Toth et al. 2011; Nunes Leite et al. 2014; Charkowski 2018). These outbreaks have led to the rejection or downgrading of substantial amounts of seed potato crops, e.g. leading to average annual losses of 12 million euros in the Netherlands (Prins and Breukers 2008). No recent reports are available on the global economic impacts of SRP on potato or on diseases of rice or maize, although Thind and Pavak (1985) reported bacterial stalk rot caused by *D. zeae* as one of the four major maize stalk diseases in India with an incidence of up to 80–85 %.

Soft rot is one of the most destructive diseases of vegetables during production and post-harvest with, for example, severe losses observed in the production of chicory in Europe since the 1990s. Under conditions favourable for the pathogens, crop losses of chicory roots due to soft rot infection by *P. carotovorum* can exceed 50 % and can rise to up to 90 % for *Dickeya* spp. (Le Hingrat et al. 2012). There are no accurate values for losses caused by SRP in fleshy fruits and vegetables post-harvest. However, estimates vary between 15 and 30 % of the harvested crop (Agrios 2006). A survey of the post-harvest fruit rot diseases of tomato in Nigeria revealed that soft rot can cause 25 % loss at harvest and 34 % loss of the remaining product in transit, storage and market stalls (Fajola 1978).

SRP also cause destruction of many flowers and ornamental crops both in the field and in glasshouses, including a recent finding of soft rot on orchid caused by *Dickeya fangzhongdai*—an ornamental that has an export market valued of 500 million euro in the Netherlands alone (Alic et al. 2017; Hinsley et al. 2018). In the 1950s bacterial stunt disease caused by *D. dianthicola* affected 26.5 % of carnation stocks in Denmark (Hellmers 1958). The disease was so destructive that the European and Mediterranean Plant Protection Organization (EPPO) classified *D. dianthicola* as a quarantine organism in carnation production. However, due to good phytosanitary measures, the absence of the bacterium and only negligible amounts of damage to carnation production in recent years, this regulation has recently been removed (EFSA 2013). Chapter 8 gives a specific example of the economic consequences of SRP diseases by taking an in depth look at the situation for potato in Switzerland. There is an expectation that the findings can be extended to losses that might occur in other countries and on other crops.

Finally, Chap. 9 gives a brief outlook moving ahead. It begins by reiterating the difficulties of finding solutions to SRP disease control but finishes with some tangible possibilities for the future. Options for control in the future may include improved diagnostics, novel resistances (with the use of biotechnology helping to achieve this) and biocontrol (Chap. 9; Czajkowski et al. 2011). The term integrated pest management (IPM) was first used in the 1970s mainly in response to the desire for reduced pesticide use and alternatives to disease control. In recent years, the term IPM is now widely used to reflect a desire to find new integrated solutions to replace or reduce the use of chemicals. It is interesting to reflect, therefore, that control of SRP has never been anything other than an IPM system and may be one of the original examples of its use.

We hope you enjoy!

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Chapter 2
Pectobacterium and *Dickeya*: Taxonomy and Evolution

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Abstract The taxonomy of soft rot *Pectobacteriaceae* (SRP) has been in a state of flux for the last century. With the advent of genomic technologies, there has been a flurry of new species just in the last 2 years and no doubt new ones will emerge in the future. Nevertheless, the use of these methods has greatly advanced our understanding of the relationships between these organisms and allowed ambiguities to be resolved. It is therefore hoped that the rate of change we have seen over the last century will begin to slow. This chapter provides an overview of the latest taxonomy of SRP, gives an overview of the recent genomic techniques being used and discusses how evolution, including through bacteriophage infection, has shaped the genome and ultimately the taxonomy of this group of organisms.

2.1 Introduction

Soft rot *Pectobacteriaceae* (SRP), belonging to the genera *Pectobacterium* and *Dickeya*, were recently reclassified from the *Enterobacteriaceae* to the *Pectobacteriaceae* Family of bacteria (Adeolu et al. 2016). Members of the *Pectobacterium* and *Dickeya* genera are Gram-negative rods that are usually motile by means of

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peritrichous flagella. They are facultative anaerobes that catabolize glucose by a fermentative pathway and reduce nitrate to nitrite (Hauben et al. 1998a). The SRP are the causal agents of blackleg and soft rot diseases of potato (the most affected crop economically) as well as soft rot and vascular wilts of other vegetables and ornamental plants (Ma et al. 2007; Pérombelon 2002; Toth et al. 2011). However, due to the general nature of disease symptoms, different SRP species may be involved. This non-specific nature means that these organisms are often reported generally as soft rot bacteria or as *Pectobacterium* spp. In such cases, taxonomy becomes a very important tool in allowing accurate identification of the organism(s) responsible, not least to enable epidemiological analyses of outbreaks and new pathogen incursions. A case in point is the spread of different genera and species across Europe over the last decades or the recent outbreak of *D. dianthicola* in the USA (Janse 2012; Patel et al. 2018; Toth et al. 2011; Ma et al. 2018). As taxonomic tools continue to improve, so too does the taxonomy of the SRP, splitting, renaming and refining the different groups, with many changes particularly over the last two decades. As we refine taxonomy, we also refine our understanding of the evolutionary differences between these bacteria in terms of their host ranges, ecological niches and geographic origins. In this chapter, we discuss the role of new genomic technologies in the study of both taxonomy and evolution of the SRP. We then describe in more detail the latest, and often changing, taxonomy of the *Pectobacterium* and *Dickeya* genera, before finishing with a look at the role of bacteriophages in the evolution of these pathogens.

2.2 Genomic Approaches to Evolution and Taxonomy

2.2.1 Genomics and Evolution

The first complete genome sequence of an SRP, *Pectobacterium atrosepticum* (then *Erwinia carotovora* subsp. *atroseptica*) strain SCRI1043, was published by Bell et al. (2004). The sequence revealed a genome of similar size to the well-studied human and animal pathogens *E. coli* and *Salmonella.* However, although much of the genome was shared between these two groups, a whole plethora of horizontally-acquired islands (HAIs) were present in *P. atrosepticum* that were absent in the animal pathogens, many of which were shown to play specific roles in plant colonisation and infection (Bell et al. 2004; Toth et al. 2006). This was the first indication that HAIs play such a key role in the evolution of *P. atrosepticum* and similar bacteria, with some evidence that different bacteria (plant and animal) share pathogenicity determinants through the transfer of common mobile elements. For example, in *P. atrosepticum* SCRI1043 a putative coronafacic acid-like phytotoxin (*cfa* gene cluster), similar to syringomycin produced by *Pseudomonas syringae,* is associated with a group of genes with high homology to the SPI-7 pathogenicity island in *Salmonella enterica* serovar Typhi, considered to be a mobile element (Bender et al. 1999; Pickard et al.

2003). Since then, genome comparisons have been used to examine and compare the structure of other genomes to better understand the evolutionary differences between the SRP, other bacterial plant pathogens, bacterial animal pathogens (e.g. *E. coli* and *Salmonella*), endosymbionts and eukaryotes, and their adaptation to specific ecological niches and modes of pathogenesis (Degnan et al. 2011; Husnik et al. 2011; Toth et al. 2006; Zhou et al. 2015). More specifically, genome studies have revealed the extent to which genomic variability occurs within and between SRP species, e.g. due to HAI transfer (including plasmids) but also through genetic changes such as single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) (Khayi et al. 2015). Recently, Golanowska et al. (2018) used comparative genomics to study the pan-genome of *D. solani,* identifying that 74.8 and 25.5 % of genes were grouped into the core and accessory genomes, respectively. A pan-regulon analysis then helped to predict the main regulons involved in the expression of virulence factors. While different strains of *D. solani* exhibited significant differences in their virulence phenotypes, relating to cell wall degrading enzymes and motility, only small differences were observed between their genomes, highlighting how relatively small changes in the genome can have a profound impact on phenotype.

While the above studies examined entire genomes, others have focussed on specific genes and operons. For example, the single housekeeping gene *fliC*, involved in motility, was used to delineate species of *Dickeya,* including the then unnamed *D. solani* (Van Vaerenbergh et al. 2012). Genes involved in housekeeping functions and in Type III secretion (*hrcC*, *hrcR*, *hrpJ* and *hrcV*) were used to study horizontal gene transfer in enterobacterial plant pathogens, indicating multiple gene transfer events amongst and between this group (Naum et al. 2009). The diversity and evolution of the secondary metabolite phenazine gene *phzF* was also investigated across a range of plant pathogenic bacterial species to show conservation of the gene in *Pseudomonas* spp. and horizontal gene transfer in *Burkholderia* spp. and *Pectobacterium* spp. (Mavrodi et al. 2010). Similarly, a genome-wide analysis across prokaryotes and eukaryotes was conducted to better understand the evolutionary history of chitin synthases which, as well as being found in probably all fungi and catalyse the formation of chitin, also showed evidence for horizontal gene transfer in certain classes of bacteria, including the SRP and other plant pathogens (Goncalves et al. 2016).

Some studies have used transcriptional profiling to study the regulation of genes both within and between SRP. For example, transcriptional profiling of *P. atrosepticum* strain SCRI1043 and *D. dadantii* 3937, under O_2-limited conditions, revealed that similar phenotypic outcomes are sometimes achieved using different genes and regulatory strategies that arise, at least in part, due to lateral gene transfer events (Babujee et al. 2012). One such regulator, *expI*, which controls quorum sensing (see Sect. 4.2.7), was shown by transcriptional profiling to control the expression of 26 % of genes within the genome of *P. atrosepticum* strain SCRI1043 (Liu et al. 2008). Duprey et al. (2016) studied the repositioning of transcriptional start sites during evolution, revealing that modifications in the transcriptional regulation of the pectinase genes *pelE-pelD* led to *pelE* becoming the initiator of pathogenesis, while *pelD* acted during the later stages of infection, thus providing a more in depth study of the evolution of these and other paralogous genes.

2.2.2 Genomics and Taxonomy

Non-genomic methods, including DNA-DNA hybridisation (DDH), 16S RNA analysis and phenotypic characterisation, have been the cornerstone of SRP taxonomy for many years, e.g. the seminal work by Samson et al. (2005), who re-classified *Pectobacterium chrysanthemi* into six species of a new genus, *Dickeya* (Samson et al. 2005). Nevertheless, whole-genome sequencing is having a major impact on traditional taxonomic studies, leading to the re-evaluation and naming of multiple SRP species and genera (Sects. 2.2 and 2.3). New genome-based approaches for improving taxonomic classification, including Average Nucleotide identity (ANI), in silico DNA-DNA hybridisation (*is*DDH) and the Microbial Species Identifier (MiSI) method, rather than disregarding traditional methods have instead complemented and helped to standardise them (Richter and Rosselló-Móra 2009; Pritchard et al. 2016; Varghese et al. 2015; Zhang et al. 2016).

ANI of whole genomes represents the degree of identity/similarity between homologous regions shared by two genomes and has emerged as a powerful genome-based criterion for establishing species identity amongst genetically related microorganisms (Konstantinidis and Tiedje 2005). The approach evaluates a large number of genes, including both slow and fast evolving ones, in the calculation and thus minimizes the effect of variable evolutionary rates or horizontal gene transfer events, as studied by Zhang et al. (2016) in a study to assign genomospecies to 83 *Pectobacterium* and *Dickeya* genomes. At the time of writing, ANI is calculated using the JSpecies software (Richter and Rosselló-Móra 2009) with the Nucleotide MUMmer algorithm (NUCmer) and default parameter settings. The degree of pairwise genome-based relatedness is calculated as an ANI value following the BLAST-based ANI calculation method described by Goris et al. (2007). An ANI threshold cut-off point of 96 % was determined to be a useful value for demarcation of bacterial species in a study comparing genomes from a massive genome sequence dataset (655 genera and 1738 species) of prokaryotes (Kim et al. 2014). Prichard et al. (2016) applied ANI to 257 genomes of the SRP and other genera to verify existing classifications for subsequent use in the development of diagnostics and, in doing so, also revealed inconsistencies in classification of strains from both culture collections and genome sequences in databases, together with new unclassified clades; some of which have since become new species, *e.g. P. parmentieri* (Khayi et al. 2016). Zoledowska et al. (2018) used comparative genomics, including ANI, to study the pan-genome of *P. parmentieri,* identifying that only 52.8 % of gene clusters comprised the core genome. The structure and gene content of the *P. parmentieri* pan-genome indicates its high genomic plasticity for easy adaptation to different environments.

While ANI represents core genome homology, genome-genome distance calculation (GGDC) or digital DNA-DNA hybridization (dDDH) measures the genome-to-genome distance between pairs of entirely or partially sequenced genomes. The digital pairwise estimator for the relatedness of genomes serves as an *in-silico* replacement for the wet-lab based DNA-DNA hybridization method (Auch et al. 2010; Meier-Kolthoff et al. 2013). A dDDH value of 70 % is the cut-off point

suggested as the gold standard threshold for bacterial species demarcation (Meier-Kolthoff et al. 2013). Similarly, this classification can be reliably predicted by Microbial species delineation (MiSI), which extends ANI to include the fraction of orthologous genes (alignment fraction), and identifies bacterial species based on > 96.5 % nucleotide identity over a common gene content of at least 60 % (Pritchard et al. 2016; Varghese et al. 2015).

Employing ANI and dDDH techniques, Zhang et al. (2016) analyzed 83 genome sequences from *Pectobacterium* and *Dickeya* spp. with other remotely related genera as out group references. The results indicated pectolytic soft rot strains analyzed with ANI values of ≥ 96 % and dDDH values of ≥ 70 % were consistently grouped together in the phylogenetic tree reflecting the whole-genome-based phylogeny from 895 single-copy orthologous genes encoded in these genome sequences. In other words, all the evidence from ANI, dDDH, and whole-genome-based phylogeny support the current species and subspecies taxonomy. Within *Pectobacterium*, the ANI, dDDH and whole-genome-based phylogeny not only support the establishment of *P. parmentieri* but also support the elevation of the four *P. carotovorum* subspecies (*actinidiae, odoriferum, carotovorum, and brasiliense*) to species level (Portier et al. 2019). More than 10 strains with genome sequences deposited at GenBank were mis-identified and some strains could not be classified to any of the existing species, which may suggest the existence of novel species (Zhang et al. 2016). In fact, ANI and MiSI, the latter in a study of 13,000 prokaryotic sequences, identified that ca 18 % of annotated genome sequences in public repositories are potentially mis-classified (Varghese et al. 2015). Genotyping of strains and isolates in historical collections of *Pectobacterium* and *Dickeya,* using whole genome sequence data (ANI, dDDH and phylogeny), has great potential for re-evaluating and clarifying remaining confusion surrounding species status (see Sects. 2.3 and 2.4).

Genome-wide microarrays, developed from even a relatively limited number of full or partial genome sequences, use large numbers of specific probes to distinguish between different strains/species both from the presence and relative abundance of hybridisation. This allows rapid classification of strains in the absence of a genome sequence (Aittamaa et al. 2008; Pritchard et al. 2009). Using Microarray Comparative Genomic Hybridisation (aCGH) analysis, Pritchard et al. (2009) incorporated spatial information on the location of genes relative to a reference genome, providing evolutionary information additional to a traditional array, e.g. operon structure and region of HGT. Similarly, Aittamaa et al. (2008) developed over 9,000 probes against the most harmful bacterial pathogens, including the SRP, to distinguish between them in the absence of further genome sequence data.

2.3 Taxonomy of *Pectobacterium*

In 1998 the genus *Erwinia* underwent a major revision resulting in the soft rot *Erwinia* spp. being reassigned to the genus *Pectobacterium* (Hauben et al. 1998a, b), a name originally proposed by Waldee (1945). Subsequent studies of these taxa

reassigned *Pectobacterium chrysanthemi* to the new genus *Dickeya* (Samson et al. 2005) and *Pectobacterium cypripedii* to the genus *Pantoea* (Brady et al. 2010). Over the past decade, advances in genomics have allowed the scientific community to clarify the taxonomy of species within the *Pectobacterium* genus, either by re-examining biological resources in international collections or sampling a wide spectrum of environments worldwide. The current species delineation within the genus *Pectobacterium* comprises 19 recognized species: *P. actinidiae*, *P. aquaticum*, *P. aroidearum, P. atrosepticum*, *P. betavasculorum P. brasiliense*, *P. cacticidum*, *P. carotovorum, P. fontis*, *P. oderiferum, P. parmentieri, P. parvum*, *P. peruviense, P. polaris, P. polonicum*, *P. punjabense, P. versatile, P. wasabiae and P. zantedeschiae* (Table 2.1; Fig. 2.1). Recent genomic analysis performed by Zhang et al. (2016) proposed that *P. carotovorum* be divided into genomospecies that largely follow the currently accepted species division.

Of the 19 species listed above, one (*P. cacticidum* – Alcorn et al. 1991) was already a species, three (*P. atrosepticum*, *P. betavasculorum, P. wasabiae)* were elevated from *P. carotovorum* subsp. by Gardan et al. (2003), while four others (*P. actinidiae, P. carotovorum, P. brasiliense,* and *P. oderiferum)* have been elevated only recently (Portier et al. 2019). The remaining 12 species are newly described.

Historically, *Pectobacterium* isolates from diseased potato that were not readily classified into a known species have often been identified as atypical *Pectobacterium* spp. or atypical *P. carotovorum* (Gallelli et al. 2009). Some of these strains, sometimes from culture collections over 40 years old, were recently classified as *P. wasabiae* (originally isolated from Japanese horseradish) (Waleron et al. 2013). However, a reanalysis of these strains, using genomic (including ANI and *is*DDH in silico) and phenotypic data, have identified that isolates on potato are taxonomically different from those on horseradish and therefore constitute a new species, which has been named *P. parmentieri* (Khayi et al. 2016). Isolates from potato in Pakistan and Peru, which are taxonomically similar to both *P. wasabiae* and *P. parmentieri*, again analysed by genomic methods, were described as new species and named *P. punjabense* (Pakistan isolates) and *P. peruviense* (Peru isolates) (Sarfraz et al. 2018; Waleron et al. 2018).

Isolates from potato in Norway and the Netherlands, previously described as atypical *P. carotovorum* when analysed using ANI, were found to be < 94 % ANI when compared to any other known *Pectobacterium* species. Additional investigations, including the use of phylogenetic data and genome-to-genome analyses, identified a new species named *P. polaris* (Dees et al. 2017). Finnish strains isolated from potato in 2004–2005 failed to amplify using diagnostic primers for *Pectobacterium* and *Dickeya* spp. and were later shown to be non-pathogenic when artificially inoculated into potato (Pasanen et al. 2013). ANI and *is*DDH of these and related isolates from potato in Finland, Poland and the Netherlands, and *Brassica rapa* in China showed these isolates to be highly similar to *P. polaris* but distinct enough to warrant a new species, named *P. parvum* (Pasanen et al. 2020).

Isolates of *Pectobacterium* from monocot plants, including Calla lily (*Zantedeschia* spp.) and previously classified as atypical *P. carotovorum* (or simply *P. carotovorum*), were found to be more pathogenic on monocots than dicots, suggesting a