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A review on *Dickeya solani,* a new pathogenic bacterium causing loss in potato yield in Europe

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Abstract

A pectinolytic bacteria of the genera *Dickeya* and a *Pectobacterium* (formerly, pectinolytic *Erwinia*) are the two causal agents of blackleg and soft rot diseases of potato and soft rot of other economically important vegetables and ornamental plants. *Dickeya* and *Pectobacterium* are in a group of top ten bacterial pathogens causing great damage and important economic losses of different crops. Potato yield reduction caused by *Dickeya* species has significantly increased in recent years. These bacteria have been frequently and increasingly isolated from diseased potato plants in Poland, Finland, France, the Netherlands, Switzerland, and other European countries, as well as Israel. Until the end of the last century, the only species of *Dickeya* genus that had caused disease symptoms on potato plants in Europe was *Dickeya dianthicola*. According to our current knowledge, since the beginning of the 21st century, bacteria showing characteristics typical of *Dickeya solani* have been isolated from potato in Europe. In Poland, they were isolated for the first time in 2005. Yet, it was only in 2014 that the *D. solani* species was established. Recent results indicate that *D. solani* strains can efficiently infect potato and cause disease symptoms in temperate climate. *D. solani* strains are considered to be more aggressive than other blackleg-causing bacteria. There is a need for intense research on the pathogenicity of *D. solani*, not only because it is fast spreading across Europe or because of its better adaptation to various climatic conditions, but also because of the lack of efficient means to control the pectinolytic bacteria during plant vegetation, transport of the potato tubers, and their storage.

Key words: blackleg, soft rot, pathogenicity, pectinolytic bacteria

Importance of the research on *Dickeya spp.*

Potato (*Solanum tuberosum* L.) is among the top ten commodities produced in the world. In 2013, it was sixth according to FAOSTAT, the statistics division of Food and Agriculture organization of United States (http://faostat3.fao.org/home/E). The first reported disease caused by *Erwinia chrysanthemi* (most probably belonging to the species *Dickeya dianthicola*) on potato in Europe occurred over 40 years ago (Toth et al., 2011).

In most European countries, losses attributable to the bacteria from the species *Dickeya* and *Pectobacterium* belonging to the Soft-rot Enterobacteriaceae (SRE) family have remained generally low. But in recent years, potato yield losses caused by *Dickeya* spp. have increased significantly in a number of European countries and in Israel, a major importer of European potato seed tubers (Fig. 1). Potato yield reduction caused by *Dickeya* spp. was evaluated as about 30% in the Netherlands and 20 to 25% in Israel. Up to 25% of the potato blackleg incidences in the Netherlands, Belgium, and France have been attributed to infections caused by *Dickeya* spp. (Toth et al., 2011). This may be associated with the emergence of a new *Dickeya* species – *D. solani* – that could have most likely spread by trade in seed tubers, and potentially in future years, could have a larger impact as a consequence of climate change (Toth et al., 2011; van der Wolf et al., 2014b). Until 2000, *Pectobacterium atrosepticum* and *D. dianthicola* were considered to be responsible for the majority of potato blackleg infections in Europe (Perombelon and Kelman, 1980; Perombelon, 2002). Recent results, obtained in different European countries, indicate that a new group of *Dic-*

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Fig. 1. The distribution of *Dickeya solani* on potato in Europe. On the basis of EUPHRESCO Report 2013 and Potrykus et al. 2016. The red color represents *Dickeya solani*, the yellow color represents *Dickeya dianthicola*

keya spp. strains can efficiently infect potato plants and cause disease symptoms in temperate climate. This new group of *Dickeya* spp. strains – *D. solani* was already described in 2009 (Sławiak et al., 2009b), but established as a new species only in 2014 (van der Wolf et al., 2014b). *D. solani* was first detected in Poland in 2005 (Sławiak et al., 2009a), but later on it was increasingly found on Polish potato seed plantations (Łojkowska et al., 2010; Potrykus et al., 2016).

All *D. solani* strains shared similar results in biochemical assays, identical molecular profiles based on rep-PCR and identical sequences of 16S rDNA and some of the housekeeping genes (e.g., *dnaX, recA, gyR rpoS*). Current studies indicate that all strains of *D. solani* studied so far show a high level of similarities in their characteristics, which may suggest a common origin, possibly a single introduction in the potato ecosystem (Sławiak et al., 2009b; Wolf et al., 2014b; Potrykus et al., 2016).

In general, *Dickeya* spp. are high-temperature pathogens, which are believed to play a major role in blackleg epidemiology in tropical and subtropical regions. In Europe, over the past 10 years, the incidence of infections of seed potato by the bacteria of genus *Dickeya* has increased relatively, compared to those from genus *Pectobacterium* (Laurila et al., 2008; Łojkowska et al., 2010; Toth et al., 2011; Potrykus et al., 2016).

D. solani strains are considered to be more aggressive than other blackleg-causing bacteria. The analysis presented here suggests that they need lower optimal temperatures for disease development as well as lower inoculum levels for infection threshold (Toth et al., 2011; Czajkowski et al., 2012). They seem to have a greater ability to colonize potato plant roots and to spread through the plants' vascular system (Czajkowski et al., 2010). In 3 years of field studies in the Netherlands with *D. dianthicola* and *D. solani*, disease severity varied annually and between species. Czajkowski and coworkers (2012) concluded that *D. solani* possesses features that allow more efficient plant colonization than *D. dianthicola*.

There is less information on the existence of *D. solani* outside European countries and Israel. There is no evidence of the presence of *D. solani* in the Americas or Australia. But *D. solani* on hyacinth bulbs has been reported to be found in China (Chen et al., 2015) and in

Malaysian waterfalls (GeneBank accession number JSXD 00000000.1, Win-Si Tan, University of Malaysia). *D. solani,* however, has not only been isolated from symptomatic potato plants, but also from the potato rhizosphere, as a potential biocontrol agent against *Rhizoctonia solani*, *Verticillium dahliae,* and *Phytophthora infestans* in the field study of genetically modified potatoes in Germany (Weinert et al., 2010). These strains possess identical rep-PCR and RFLP-PFGE profile as the type strain of *D. solani* IPO2222 and strains isolated in Poland in 2005 and 2009 (IFB0099 and IFB0158). However, they indicate a much lower ability to macerate potato tissue and a lower ability to produce plant cell wall degrading enzymes (PCWDE), (Potrykus et al., 2014a).

Apart from the availability of 7 *D. solani* genomes, the genetics of *D. solani* is poorly understood in comparison to what is available and known about *D. dadantii* (a species closely related to *D. solani*). The genomic analyzes on *D. solani* strains performed by Garlant and coworkers (2013) and Pedron and coworkers (2014) have revealed open reading frames (ORF) possibly involved in virulence and the production of toxic compounds. They were annotated as polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS), and amino acid adenylation domain proteins, suggesting that they encode proteins that may be involved in the production of such metabolites as antibiotics and toxins. Pedron and coworkers (2014) pointed out a higher variability in proteins transported *via* Type 5 and T6 secretion systems (5SS/T6SS) in *D. solani* 3337 than in *D. dadantii* 3937.

The main information about virulence and regulation is acquired from the knowledge about closely related and mostly studied species, *D. dadantii* 3937. This strain's metabolism and regulation of pectinolysis have been studied for about 40 years (Kotoujanski et al., 1982). Many tools have been developed for *D. dadantii* 3937, so that the vast features and pathogenicity strategy of *D. solani* can be better understood. The knowledge about regulation of the expression of genes encoding virulence factors like PCWDE in *D. solani* has been obtained in our laboratory (Potrykus et al., 2014a).

Nowadays, by means of comparative and functional genomics, researchers can improve the understanding of adaptation modes to different ecological niches and the genes involved in *D. solani* pathogenicity (Toth et al., 2006; Pritchard et al., 2016). It is worth insisting that there is a need for intense research on the molecular regulation of the *D. solani* pathogenicity not only because of its fast spreading nature across Europe or its better adaptation to various climatic conditions (not only temperate climates such as in Poland or cold climates in Finland, but also hot climates such as in Israel), but also because of the lack of efficient means to control the pectinolytic bacteria during the vegetation and storage (Toth et al., 2011; Czajkowski et al., 2011).

The enemy – Soft Rot Enterobacteriaceae – *D. solani*

Bacteria from genera *Dickeya* and *Pectobacterium* form a group of pectinolytic plant pathogenic bacteria. Based on their economic and scientific impact, these species have been included among the 10 most important bacterial plant pathogens (Mansfield et al., 2012). The most recent taxonomy of SRE states that these bacteria belong to the kingdom of Bacteria, phylum Proteobacteria, class Gammaproteobacteria, order Enterobacteriales, family Enterobacteriaceae. Since 1998, SRE have been divided in two genera: *Pectobacterium* and *Dickeya* (Hauben et al., 1998).

The taxonomy of SRE has been changing over the past decades. When the genus *Erwinia* was classified in 1917, for the first time, it comprised all members of the Enterobacteriaceae that were pathogenic to plants, including both pectinolytic (e.g., *Erwinia carotovora* and *Erwinia chrysanthemi*) and non-pectinolytic (*Erwinia amylovora*) species (Winslow et al., 1917). The name was chosen after one of the first phytopathologists, Erwin Smith. In 1953, Burkholder and coworkers assigned *E. chrysanthemi* to the genus *Erwinia* as a pathogen of chrysanthemum. Later studies proved that *E. chrysanthemi* cause disease on a wide variety of plant hosts (Samson et al., 2005; Ma et al., 2007). In 1984, Lelliott and Dickey, on the basis of host specificity, subdivided the species *E. chrysanthemi* into six pathovars (pv.), namely, *chrysanthemi, dianthicola, dieffenbachia, paradisiaca, parthenii,* and *zeae*. In 1987, Samson and coworkers developed a biovar system based on their biochemical characteristics (Samson et al., 1987).

Already in 1945, Waldee proposed to move the pectinolytic *Erwinia* into a new genus *Pectobacterium* based on their ability to produce pectinolytic enzymes. However, this was not done until 1998 when the 16S rDNA analysis provided more data for this proposal (Hauben et al., 1998). While the potato pathogens *P. c.* subsp. *ca-*

rotovorum (syn. *E c.* subsp. *carotovora*) and *P. atrosepticum* (syn. *E. c.* subsp. *atroseptica*) remain within this genus, a further analysis of *P. chrysanthemi* using 16S rDNA, DNA–DNA hybridization and biochemical characterization showed that it formed a distinct clade from *Pectobacterium* spp., so a new genus, *Dickeya,* was proposed (named after the microbiologist Robert S. Dickey) (Samson et al., 2005). Samson and coworkers (2005) proposed six species within the genus *Dickeya*, namely, *D. chrysanthemi, D. dadantii, D. dianthicola, D. dieffenbachia, D. paradisiaca,* and *D. zeae*. Then, Brady and coworkers (2012) reclassified *D. dieffenbachiae* into *D. dadantii* subsp. *dieffenbachiae*. In 2014, two new species of *Dickeya* were proposed: *D. aquatica* and *D. solani* (Parkinson et al., 2014; Van der Wolf et al., 2014b). All currently accepted species and subspecies of *Dickeya* and *Pectobacterium* are presented in Table 1.

The SRE are found worldwide and have been isolated from plants of more than half of angiosperm families as well as from soil, rivers, ground waters, insects, molluscs, and nematodes (Perombelon and Kelman, 1980; Perombelon, 2002; Ma et al., 2007; Laurila et al., 2008; Nykyri et al., 2014; Parkinson et al., 2014). They are Gram-stain-negative, rod-shaped, facultative anaerobes that produce a wide range of PCWDE and cause diseases such as blackleg and soft rot. They possess a wide range of hosts including 16 dicotyledonous families of plants in 11 orders and 10 monocotyledonous families in 5 orders (Samson et al., 2005; Ma et al., 2007). Soft rot losses may occur on plants growing in the field, garden, greenhouse, or after harvest during transit, storage, or marketing.

SRE have a climatic distribution that reflects their host diversity and increasing temperatures. *Pectobacterium atrosepticum* is mainly restricted to temperate climates and almost exclusively to potato. *Pectobacterium carotovorum* subsp. *carotovorum* infects a wide variety of plants, and is found in both temperate and tropical zones, causing soft rot to potatoes and many fruits and vegetables (Elphinstone, 1987; Toth, 2003). *Dickeya* spp. (formerly *Erwinia chrysanthemi*) affects a wide range of tropical and subtropical plants, including potatoes, many ornamental plants, maize, rice, and pineapple. *D. dianthicola* has not only causes disease of many ornamental plants worldwide, but also has played an important role in potato losses in Europe since the 1970s (Toth et al., 2011). Exemplary hosts of different species of SRE are presented in Table 1.

Soft rot and Blackleg symptoms – what are we dealing with?

SRE cause blackleg and soft rot diseases. They are often present in latent infections of many crops (Perombelon and Kelman, 1980). The symptoms of soft rot are similar in most plants. The disease symptoms appear first on leaves, stems, and/or underground parts as small, water-soaked, translucent lesions, which gradually enlarge in both diameter and depth. The host tissue softens and becomes squashy or watery. Slimy masses of bacteria and cellular debris frequently seep out from cracks in the tissues. In optimal conditions, within 20 to 72 hours, entire fleshy fruits, roots, tubers, stems and rhizomes, bulbs, corms, buds, leaf stalks, and leaves may rot and collapse, sometimes leaving only the outer skin intact. The decaying tissue may be opaque, white, cream-colored, gray, brown, or black and is always wet. Sometimes, characteristic putrid odor occurs caused by secondary invading bacteria that are growing in the decomposing tissues (Perombelon, 2002).

As the infected seed potato tubers rot in wet soil, new plants do not grow later. Shoots arising from infected tubers become watery, and then they wilt and finally collapse. This potato disease is commonly called blackleg. Blackleg is a soft rot that spreads from infected seed tubers into the stems of new potato plants, and hence, it is a seed-borne disease. If the soil moisture level is lowered, the base of the shoots may become soft, brown to inky black and shriveled. The leaves on such shoots are dwarfed, stiff, curled upward, and are yellowish, red, or bronzed. Affected shoots are also stunted, more upright, and pale in color. Such plants often die prematurely or their yield is reduced (RPD No. 943 July 1990). In Figure 2, symptoms of soft rot and blackleg on potato are presented.

Disease cycle

For the development of the disease, a compatible interaction between the bacterial strain and the host plant must be established. In addition, the environmental conditions have to be optimal for multiplication and spread of bacteria. Only under favorable conditions (for *Dickeya* spp., high humidity and favorable temperature), the disease can develop.

Bacteria from genus *Dickeya* can live as epiphytes or as saprotrophs in soil on the plant debris until they encounter a susceptible host. They are strong compe-

Table 1. Characteristics of the *Dickeya* spp. and *Pectobacterium* spp. and their host range

Species name	Host range	References
Dickeya aquatica	Isolated from water, host plant not determined	Parkinson et al. (2014)
Dickeya chrysanthemi bv. chrysanthemi	*Chrysanthemum spp.*, *Cynara scolymus*, *Cichorium intybus*, *Helianthus annuus*	Samson et al. (2005)
Dickeya chrysanthemi bv. parthenii	*Lycopersicon esculentum*, *Parthenium argentatum* *Philodendron spp.*	Samson et al. (2005)
Dickeya dianthicola	*Dianthus spp.*, *Cichorium intybus*, *Cynara scolymus*, *Dahlia variabilis*, *Kalanchoe blossfeldiana*, *Lycopersicon esculentum* *Solanum tuberosum.*	Samson et al. (2005)
Dickeya dadantii subsp. *dadantii*	*Pelargonium capitatum*, *Ananas comosus*, *Dianthus spp.*, *Euphorbia pulcherrima*, *Ipomoea batatas*, *Musa spp.*, *Philodendron spp.*, *Saintpaulia ionantha*, *Zea mays*	Samson et al. (2005)
Dickeya dadantii subsp. *dieffenbachiae*	*Dieffenbachia spp.*, *Lycopersicon esculentum*, *Musa spp.*	Brady et al. (2012)
Dickeya paradisiaca	*Musa spp.*, *Zea mays*	Samson et al. (2005)
Dickeya solani	*Solanum tuberosum*	Van der Wolf et al. (2014b)
Dickeya zeae	*Zea mays*, *Ananas comosus*, *Brachiaria ruziziensis*, *Chrysanthemum morifolium*, *Musa spp.*, *Nicotiana tabacum*, *Oryza sativa*, *Solanum tuberosum*,	Samson et al. (2005)
Pectobacterium atrosepticum	*Solanum tuberosum*, *Solanum lycopersicum*, *Cichorium intybus*	Gardan et al. (2003)
Pectobacterium betavasculatum	*Beta vulgaris*	Gardan et al. (2003)
Pectobacterium carotovorum subsp. *carotovorum*	*Solanum tuberosum*, *Beta vulgaris*	Hauben et al. (1998)
Pectobacterium carotovorum subsp. *brasiliense*	*Solanum tuberosum*	Hauben et al. (1998)
Pectobacterium carotovorum subsp. *odoriferum*	*Solanum tuberosum*, *Cichorium intybus*	Hauben et al. (1998)
Pectobacterium wasabiae	*Armoracia rusticana*, *Solanum tuberosum*	Gardan et al. (2003)

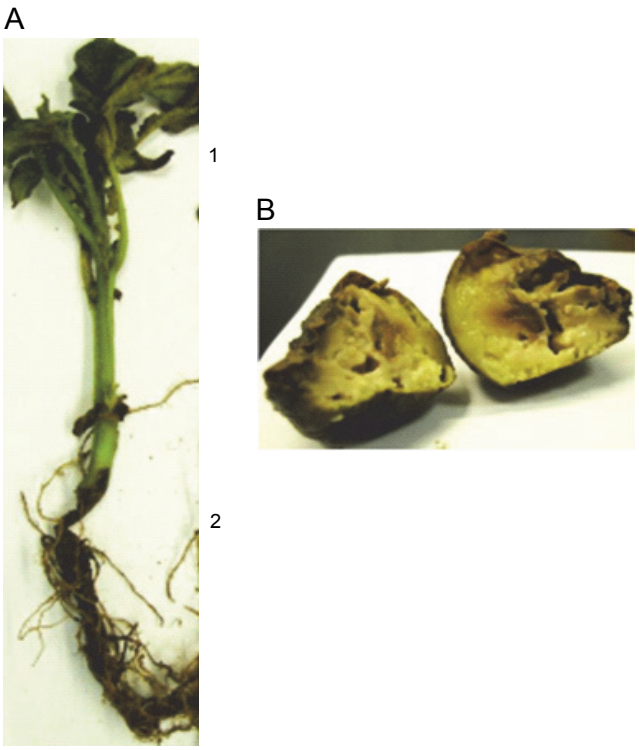

Fig. 2. Symptoms of soft rot and blackleg on potato caused by *D. solani*: A) potato plant: 1 – leaf wilting, 2 – blackleg; B) potato tuber with soft rot, cross section

titive bacteria that eliminate other microbes by contact-dependent growth inhibition mechanisms involving type 5 and type 6 secretion systems (T5SS, T6SS) (Charkowski et al., 2012; Pedron et al., 2014). SRE may also infect insects and these may then become dissemination vectors (Grenier et al., 2006). Nykyri and coworkers (2014) also showed that nematodes can act as vectors spreading *Dickeya* spp.

There are three main steps in the plant infection by bacteria from genera *Dickeya* and *Pectobacterium*: 1) adhesion to the plant surface and penetration into the plant tissues either through wounded sites or through natural openings such as stomata, 2) invasion of the apoplasts, and 3) plant cell wall degradation. SRE can reside in the vegetal intercellular spaces, causing a latent infection without any symptoms (Reverchon et al., 2013). If the bacteria encounter advantageous conditions such as favorable temperature, high humidity levels, and poor oxygen availability, the shift of disease symptoms occur (Pérombelon and Kelman, 1980; Pérombelon, 2002). Each of these disease stages requires the detection of information from the environment, and specific interaction between the host plant and the pathogen population. Reverchon and Nasser (2013) presented a list of genes involved in disease development that have been studied mostly in the *D. dadantii* 3937 strain. This strain has been isolated from *Saintpaulia ionantha* (Kotoujanski et al., 1982), and most of the genetic studies and virulence factors analyzes have been performed with the use of this strain; therefore, it has been accepted by a scientific community as a model for the molecular study of *Dickeya* spp.

In spring, the *Dickeya* species are commonly found on or in the seed tubers, which represent the primary inoculum. In late spring and in summer, bacteria from the infected seed spread to young stems and roots. They multiply in intercellular spaces, degrade the plant cell walls, and liquefy the mother tuber. Progeny tubers may become contaminated with *Dickeya* spp. as they develop in late summer and early fall. Emerging bacteria may migrate through the soil and infect the neighboring plants. Soft rot occurs when the bacteria gain access to the tuber through wounds and other entry points. Wounds caused during harvest and handling provide multiple points of entry into the tuber, so the infection may spread within the storage containers during storage. Discarded rotten tubers may allow the dispersal of *Dickeya* spp. in environment (in soil and water). Bacteria from different species of *Dickeya* and *Pectobacterium* are able to grow and produce virulence factors in different temperatures. In winter, *Dickeya* spp. can survive on plant residues in the soil. During the whole disease cycle, insects may be involved in disease spread either because they cause wounds on plant organs on which they feed or deposit their eggs and these allow bacteria to enter the plant, or because they transfer bacteria to other plants, where they might cause disease (Reverchon and Nasser, 2013).

Armory – virulence factors of *Dickeya* spp.

Bacteria from genus *Dickeya* use a combination of different compounds, such as pectin metabolites, acyl homoserine lactones, organic acids that regulate the expression of genes encoding pectate lyases, plant hormones (auxin) and other virulence factors at both transcriptional and post-transcriptional stages. It is thus possible to interfere with soft rot pathogenicity by disrupting these signaling cascades (Charkowski, 2009, 2012). The

main weapon in the SRE arsenal is the coordinated production of high levels of multiple exoenzymes, including pectinases, cellulases, and proteases, which break down plant cell walls and release nutrients for bacterial growth (Barras et al., 1994; Py et al., 1998; Thomson et al., 1999; Pérombelon, 2002; Hugouvieux-Cotte-Pattat et al., 1996, 2014).

Plant cell wall degrading enzymes (PCWDE)

Pectinases

The activity and regulation of pectinases produced by *D. dadantii* 3937 were described by Hugovieux-Cotte-Pattat and coworkers in 1996 and reviewed in 2014. The term "pectinases" describes different enzymes cleaving the glycosidic linkages or the methyl-ester bonds of the pectic polymers. Many of these pectinases, such as pectate lyase (Pel), pectin lyase (Pnl), pectin methyl esterase (Pme), and polygalacturonase (Peh) exist in multiple forms (isoenzymes) encoded by independent genes that, in some cases at least, are clustered and appear to be derived from successive rounds of gene duplication (Barras et al., 1987; McMillan et al., 1994). The *D. dadantii* 3937 strain produces two pectin methyl esterases, at least nine pectate lyases, a polygalacturonase, and a pectin lyase.

Historically, pectate lyases isolated from various organisms were often named as PelA, PelB, PelC, or Pel-1, Pel-2, Pel-3, and so on, depending on either their order of discovery or their characteristics (i.e., their isoelectric point in the case of the first enzymes described in *D. dadantii* 3937, (Bertheau et al., 1984)). With such a nomenclature, orthologous pectate lyases often have different names in various strains or *Dickeya* species. Pectate lyases (Pels) are the main pectinases in pathogenesis and, as with other exoenzymes, their number varies between species, subspecies, and strains. Pectate lyases generally indicate optimal activity at basic pH (from 7.3 to 9.5) and in the presence of a divalent cation (Ca^{2+} in most cases). They preferentially cleave polygalacturonate (PG) or partially methylated PG. However, the enzymatic properties can differ significantly among different pectate lyases. For instance, the optimal PG methylation level varies from 0 to 50% among the *D. dadantii* 3937 pectate lyases (Table 2). There are five major Pels that are grouped into two families (Pel A, D, E, and Pel B, C) and at least four secondary Pels (Pel I, L, Z, and X) in *D. dadantii* 3937. The secondary Pels have a lower enzymatic activity than the major ones but appear to play an important role in either the early phase of infection or host specificity (Łojkowska et al., 1995; Jafra et al., 1999; Hugouvieux-Cotte-Pattat et al., 2014). Additional isoenzymes of Pel, Pnl, Pme, and Peh are also induced in minimal medium in the presence of both pectate and pectin (McMillan et al., 1994). Pectinases are secreted through the commonly named Out secretion system (Type II Secretion System, T2SS).

All major and secondary pectate lyases present in *D. dadantii* are also present in the genome of *D. solani* and their sequences are homologous to those of *D. dadantii* 3937; they are also secreted by T2SS (MG, unpublished data).

Cellulases

The complete degradation of native cellulose to glucose requires three enzymes, namely, *endo-β-I*,4-glucanase, cellobiohydrolase, and β-glucosidase. Cellulases, which mainly exhibit endoglucanase (EG) activity, break down cellulose in the primary and secondary cell walls of the host plant. *D. dadantii* 3937 produces two EGs: EGZ, which comprises the major activity and is secreted and EGY, which is less abundant and is periplasmic (Barras et al., 1994). The genes encoding these enzymes are *celZ* and *celY*, respectively. They seem to be inessential for pathogenicity, but they do appear to act in synergy with other exoenzymes of various classes (i.e., pectinases) to attack the plant cell wall (Boccara et al., 1994). EGZ is secreted by so-called Out general secretion pathway (T2SS). EGZ contains two functionally independent domains: a catalytic domain (CD) and a cellulose-binding domain (CBD) joined by a Ser/Thr-rich linker region (LR) (Py et al., 1991). Both genes coding cellulases are present in the genome of *D. solani* and their sequences are identical to those of *D. dadantii* 3937 (MG, unpublished data).

Proteases

Many plant-pathogenic bacteria produce proteases. *Dickeya* strains produce metalloproteases A, B, C, and G (Delepelaire and Wandersman, 1990; Ghigo and Wandersman, 1992). The proteases are secreted into the external medium via Type I Secretion system, of which two are inactive precursors (zymogens B and C) where they are activated by divalent cations. Metalloproteases may

Table 2. Characteristics of *D. dadantii* 3937 pectate lyases (Modified after Hugouvieux-Cotte-Pattat et al. 2014)

Name	PL family (subfamily)	Substrate (optimal methylation)	Optimal pH	Cation(s)	Cell location (secretion system)
PelZ	1 (2)	PG (0 to 20%)	8.5-9.0	Mn (Ca)	external (Out)
PelB	1 (3)	PG (0 to 40%)	9.0-9.5	Ca	external (Out)
PelC	1 (3)	PG (0 to 20%)	9.0-9.5	Ca	external (Out)
PelA	1 (6)	PG (0%)	8.5	Ca	external (Out)
PelD	1 (6)	PG (0%)	8.5	Ca	external (Out)
PelE	1 (6)	PG (0%)	8.0	Ca	external (Out)
PnlH	1 (8)				outer membrane (Stt)
PelW	2 (2)	G3, G4, PG (0 to 60%)	8.5	Co, Mn, Ni	cytoplasm
PelI	3 (5)	PG (0 to 50%)	9.2	Ca	external (Out)
PelL	9 (1)	PG (0 to 30%)	8.0-9.0	Ca	external (Out)
PelN	9 (1)	PG (0 to 90 %)	7.4	Fe	external (Out)
PelX	9 (1)	G4 to G7, PG (0 to 20%)	8.0-8.5	Ca (Mn, Co, Ni)	periplasm

PG – polygalacturonate; G3, G4, G7 – saturated galacturonates; Out – type 2 secretion system

play a role in virulence by degrading plant cell wall proteins. They may either provide amino acids for biosynthesis of microbial proteins or lead to degradation of host proteins associated with resistance (Heilbronn and Lyon, 1990; Kyöstiö et al.,1991) or, according to the literature, have a minor role like cellulases, in pathogenesis (Marits et al., 2002).

Motility

Phytopathogenic bacteria can be motile by means of flagella, and genes coding for flagellar proteins contribute to virulence and to host-pathogen interactions, that is, for Dickeya – potato interaction (Van Vaerenbergh et al., 2012). Two different types of motility require the presence of bacterial flagella – swimming, which takes place in liquid media and swarming motility, on solid surfaces or in media of high viscosity. The ability to swarm can be dependent upon the growth media and temperature. The analysis of the virulence of the mutants in different host plants indicated that motility and chemotaxis play an important role in the pathogenicity of *D. dadantii* 3937 (Antunez-Lamas et al., 2009a,b). The results presented by Antunez-Lamas and coworkers (2009a) indicated that mutations affecting motility/chemotaxis (in genes *motA* and *cheY*) had an influence on the *D. dadantii* 3937 virulence toward several hosts, such as chicory, Saintpaulia, and potato. The mutants' *motA* and *cheY* showed a significant reduction of their virulence in those hosts. Motility/chemotaxis also plays a role in colonization of potato tubers (Antunez-Lamas et al., 2009b).

Iron uptake

Another process that is crucial for pathogenesis is iron uptake, which initially was linked to pathogenicity in *D. dadantii* 3937 through the analysis of bacteriocin-resistant mutants (Franza et al., 2005). Iron is a necessary cofactor for enzymes involved in important cellular functions. Bacteria from genus *Dickeya* regulate gene expression in response to iron. For example, low iron availability is a signal that triggers transcription of the genes encoding major pectate lyases PelD and PelE, as well as the genes that are involved in iron transport. This regulation is mediated by the transcriptional repressor Fur, Ferric Uptake Regulator (Franza et al., 2005). *D. dadantii* 3937 produces two siderophores: chrysobactin and achromobactin, in order to acquire iron from the iron-poor environment of the plant apoplast. Several studies demonstrated that the presence of chrysobactin and achromobactin highly contributes to the successful infection of the plant (Franza et al., 2005; Dellagi et al., 2005). Mutants defective in chrysobactin-mediated iron transport remain localized within Saintpaulia leaves, sug-

gesting a role in bacterial spread throughout the plant (Franza et al., 2005). The virulence of achromobactin-deficient mutants are also affected, but they are more aggressive than the chrysobactin non-producers; and double mutants, deficient in both achromobactin and chrysobactin production, are impaired in symptom initiation.

An analysis of *Dickeya* spp. and *Pectobacterium* spp. genomes revealed multiple TonB-dependent outer membrane receptors and TonB homologs (TonB-dependent transporters are bacterial outer membrane proteins that bind and transport ferric chelates), suggesting that the capacity of using diverse exogenous siderophores is common among SRE and may confer fitness in complex environments (Schauer et al., 2008). A study of *P. atrosepticum* SCRI1043 and *D. dadantii* 3937 revealed that besides the production and utilization of siderophores, they have the capacity to use other iron sources through siderophore-independent systems, such as Feo, Efe, UOB, or Hmu systems (reviewed by Franza and Expert, 2013). Both species are also able to uptake the haem-iron, while *P. atrosepticum* SCRI1043 can only transport the ferric citrate complex and only *D. dadantii* 3937 can acquire ferrous iron system (Franza and Expert, 2013).

Quorum sensing communications systems

Virulence genes are expressed in a concerted manner and they culminate when the bacterial quorum is reached. Numerous Gram-stain-negative species use acyl-homoserine lactone (AHL)-based quorum sensing (QS) system Exp to regulate the expression of genes involved in interactions with host cells. In *Pectobacterium* spp., which produces 3-oxo-C6-HSL and 3-oxo-C8-HSL, the AHL QS system is at the top of a regulatory cascade controlling over a quarter of the *Pectobacterium* spp. genes, including key virulence factors, such as pectinase, cellulase, and protease activities, and production of HrpN, a protein secreted via type III secretion system (T3SS) (Liu et al., 2008; Charkowski et al., 2012). Expression of the virulence genes encoding PCWDE is activated only when a sufficient amount of the AHL signal molecule has accumulated (Andersson et al., 2000). Expression of those genes is also induced by pectin metabolites. Pectin is both one of the main components of the plant cell wall and a carbon source that SRE are able to exploit. In contrast, in the closely related *D. dadantii* 3937, AHL appear to play a minor role in virulence gene regulation. Specific interactions have been observed *in vitro* between the AHL-related QS regulator ExpR and *pel* gene promoters, but no specific phenotype was associated to the *expI* or *expR* mutations (Nasser et al., 2013). In addition AHL-based QS system of *D. dadantii* 3937 does not play a pivotal role in the cell density-dependent control of virulence gene expression *in vitro* or *in planta* (Reverchon et al., 2002; Mhedbi-Hajri et al., 2011).

Besides AHL-based QS, a new communication system has been discovered in *D. dadantii* 3937 called Virulence Factor Modulating (Vfm) cluster (Nasser et al., 2013). The Vfm cluster does not seem to be widespread among bacterial species but is conserved in *Dickeya* species; the Vfm cluster is absent from the related pectinolytic enterobacteria of the *Pectobacterium* genus (Nasser et al., 2013). Studies on the mutants with decreased production of all PCWDE that could be complemented by cell-free culture supernatant have revealed that Vfm cluster controls the transcription of the PCWDE genes (Nasser et al., 2013).

Both QS systems, Exp and Vfm gene clusters, are present and they play an important role in the regulation of the biosynthesis of genes coding PCWDE in *D. solani* (Potrykus et al., 2014a; M. Potrykus, unpublished results). The work of Potrykus and coworkers (2014a) reveals the role of QS mediated by ExpI and ExpR in *D. solani* virulence on potato.

Not only the bullets, also the guns – secretion systems and their role in *Dickeya* spp. cells

Protein export is the key in the virulence of *Dickeya* spp. (Glasner et al., 2011; Garlant et al., 2013; Pedron et al., 2014). The rapid induction of expression of genes coding for exoenzymes and other pathogenicity factors within the bacterial cell is of little consequence, unless they can be efficiently targeted to the extracellular environment. To accomplish this, bacteria from *Dickeya* genus have all six secretion systems that are common for Gram-stain-negative bacteria, all of which exhibit very different mechanisms that appear to be conserved within SRE. These systems are involved in attacking host plants and competing bacteria (Charkowski et al., 2012).

Type I Secretion System (T1SS) secretes proteases from the cytoplasm to the extracellular space in a single step, but while this system has been studied in detail in

Dickeya spp., it appears to have a relatively minor role in pathogenicity (Dahler et al., 1990; Delepelaire and Wandersman, 1990; Létoffé et al., 1990). In *Pectobacterium* spp., the T1SS is upregulated by plant extracts and AHL-based QS, and controlled by GacAS regulator (Marits et al., 2002). In *Dickeya* spp., this system is controlled by PecS global negative regulator (Hommais et al., 2008; Mhedbi-Hajri et al., 2011) and GacAS (Reverchon et al., 2013). T1SS-associated ABC transporters with a noncatalytic C39 peptidase-like domain tend to secrete large, 50 to 1000 kDa, repeats-in-toxin (RTX) proteins with uncleaved C-terminal secretion signals (Linhartova et al., 2010). In *D. dadantii* 3937, RTX proteases (PrtG, PrtB, PrtC, and PrtA) are secreted by a T1SS (Letoffe et al., 1990; Delapelaire and Wandersman, 1990; Ghigo and Wandersman, 1992; Charkowski et al., 2012). Two of the proteases (PrtB, PrtC) are secreted as zymogens that are activated after secretion by divalent cations (Delapelaire and Wandersman, 1989). These characteristics are similar to other RTX metalloproteases, which belong to a subgroup of proteases with an extended zinc-binding motif (Linhartova et al., 2010). *D. dadantii* 3937 also exports a protease inhibitor to the periplasmic space in a Sec-dependent manner that is superficially analogous to the contact-dependent growth inhibition mechanism, but the inhibitor is nonspecific and can inhibit many proteases (Letoffe et al., 1989). Additionally, RTX protease mutants of *P. carotovorum* are modestly affected in virulence, suggesting a role in plant pathogenesis rather than toxicity against other bacteria (Marits et al., 2002).

Type II secretion system (T2SS), also called Out system, is essential for pathogenicity and it secretes pathogenicity determinants, such as pectinases and cellulases in a two-step mechanism. The first step is a Sec-dependent protein export system that exports proteins to the periplasm. The second step, controlled by a 15-gene Out cluster, includes the formation of a structure that spans the periplasmic compartment and outer membrane and channels proteins, recognized by a signal sequence, to the outside of the cell. Type II-secreted proteins have been identified during individual enzyme studies and enzyme or global secretome analyzes of *D. dadantii* or *P. atrosepticum* (Salmond et al., 1994; Charkowski et al., 2012). However, despite a high level of interspecies amino acid identity within the SRE, Out genes from *P. c.* subsp. *carotovorum* (strain SCRI193) do not complement mutations in equivalent genes in *D. dadantii* 3937 and *vice versa*, suggesting a degree of species-specificity (Py et al., 1991). Regulation of Type II system is, at least in part, under the control of KdgR global negative regulator and may also operate under a QS mechanism (Charkowski et al., 2012).

Type III secretion system (T3SS) has been more closely examined in hemibiotrophic phytopathogenic bacteria, such as *Pseudomonas syringae*, than in SRE, but is required for pathogenesis in both bacterial groups. Unlike *P. syringae*, which can have up to 30 potential type III – secreted effector proteins in individual strains, *Pectobacterium* spp. appear to have relatively few (Holeva et al., 2004; Kim et al., 2011), including a small number of harpins or helper proteins and the single known effector, DspA/E. Holeva and coworkers (2004) demonstrated that the T3SS helper HrpN and effector DspE/A are required for pathogenicity in *P. atrosepticum*.

In comparison to other secretion systems, type IV secretion system (T4SS) is unique in its ability to transport nucleic acids in addition to proteins into plant and animal cells, as well as into yeast and other bacteria (Christie et al., 2005). Genes that encode a similar system were discovered in the complete genome sequence of *P. atrosepticum* SCRI1043, and further investigation revealed that a mutation in the T4SS reduced its virulence on potato (Bell et al., 2004).

Type V secretion systems (T5SSs) have relatively simple structures and they are reviewed by Leo and coworkers (2012). T5SSs are subtyped into T5aSS-T5eSS on the basis of differences in the structure and mechanism of secretion. The functions of the T5SS secreted proteins are variable, but include serine proteases, lipases, cytotoxins, invasins, and adhesins that collectively generalize to influencing bacterial fitness, aggregation, biofilm formation, and virulence (Grijpstra et al., 2013). The T5SSs have also been implicated in a phenomenon referred to as contact-dependent growth inhibition, where, upon contact, toxic proteins are targeted to another bacterium (Ruhe et al., 2013). In *D. dadantii*, a >3,800 amino acid-long protein with homology to T5bSS adhesins was demonstrated to be necessary for bacterial adhesion to the surface of leaves, aggregation of bacteria, and incitation of host cell death (Rojas et al., 2002). On the basis of C-terminal extensions with homology to proteins toxic to bacteria, these putative T5SSs

likely function to suppress the growth of competing bacteria rather than suppressing their virulence toward plants (Ruhe et al., 2013)

Type VI secretion system (T6SS) is the most recently described of the Gram-stain-negative bacterial secretion systems and is widely distributed among diverse species. The T6SS has been shown to play an important role in pathogenicity toward eukaryotic host cells in a variety of important human pathogens, including *Pseudomonas aeruginosa, Burkholderia mallei, Vibrio cholerae,* and *Aeromonas hydrophila* (English et al., 2014). It is now clear that the T6SS can also be used to target other bacteria and is thus likely to play an important role in polymicrobial infections. Various Type VI-secreted antibacterial toxins have recently been identified. The biggest groups are peptidoglycan hydrolases including several families of peptidoglycan amidase and glycoside hydrolase enzymes, which attack the cell wall of target bacteria (Russell et al., 2012; Whitney et al., 2013). Additionally, a superfamily of phospholipase effectors, attacking the target cell membrane, were described recently and a small number of other, unrelated effector toxins have been experimentally identified, such as antimicrobial toxins and phopsholipases, but they are yet to be fully characterized (Russell et al., 2013). In *P. atrosepticum*, the T6SS-encoding genes are induced in response to potato extracts (Mattinen et al., 2008). *P. wasabiae* SCC3193 has two putative T6SSs and a large inventory of putative tail and spike homologs (Nykyri et al., 2012).

A mutant with deletions of unlinked genomic regions spanning 16 and 23 genes that included the two putative T6SS-encoding loci was modestly affected in its virulence on potato tuber slices (Nykyri et al., 2012). Pedron and coworkers (2014) revealed that *D. solani* genome encodes a distinctive arsenal of T5SS- and T6SS-related toxin-antitoxin systems.

Regulation – when and where to use armory?

Virulence determinants in the *Dickeya* are controlled by complex regulatory networks, which act either positively or negatively on one (targeted regulation) or several (global regulation) determinants. They are stimulated by factors such as oxygen and nitrogen availability, temperature, pH, osmolarity, iron deprivation, growth phase, catabolite repression, plant degradation intermediates, plant extracts, DNA-damaging agents, and other factors yet to be identified. Newly identified regulatory proteins, thanks to a better understanding of the existing ones, (Nasser et al., 2005; Nguyen et al., 2002) continue to be added to the list of known regulators that have been reviewed extensively by Hugouvieux-Cotte-Pattat et al. (1996) and Thomson et al. (1999).

Three regulatory loci, *kdgR, pecS*, and *pecT,* have been identified as the regulators that negatively control the expression of genes encoding pectinases in *D. dadantii* 3937 (Nasser et al., 2005; Praillet et al., 1997; Reverchon and Nasser, 2013; Surgey et al., 1996). Among these regulators, the KdgR protein is the main effector and it acts as a transcriptional repressor for all the genes coding for proteins involved in the pectin degradation pathway. In *D. dadantii* 3937, in the absence of pectic inducers, the KdgR protein binds to a specific 17-bp sequence (the KdgR box), which is conserved in the promoter region of the whole spectrum of genes encoding pectinases (Nasser et al., 2005). PecS negatively controls the production of pectinases and cellulases, as well as the production of the blue pigment indigoidine (antioxidant molecule), motility, and the harpin HrpN (Reverchon et al., 1994, 2002; Rouanet et al., 2004; Nasser et al., 2005). PecT represses the production of pectinases, EPS, motility, and the harpin HrpN (Surgey et al., 1996; Castillo and Reverchon, 1997; Condemine et al., 1999; Nasser et al., 2005).

Pectinase production is also positively regulated. The cAMP receptor protein (CRP) activates the transcription of the pectinolysis genes (Nasser et al., 2005). The histone-like nucleoid-structuring (H-NS) protein (a protein that influences nucleus structure and gene expression in response to numerous growth parameters) plays an important role within the *pel* gene regulatory network (Nasser et al., 2005). The studies have shown that H-NS is involved in the *D. dadantii* 3937 *pel* virulence pathway by exerting a negative effect on the expression of at least three regulatory loci, namely, *expI*, *expR* and *pecT*. For example, inactivation of H-NS results in the overproduction of PecT, which will in turn reduce the transcription of *pel* genes (Reverchon and Nasser, 2013).

The work of Potrykus and coworkers (2014a) revealed the role of KdgR, PecS, PecT, ExpI, and ExpR regulators in *D. solani* strains. Mutants of four *D. solani* strains were constructed by inactivating the genes coding either for one of the global negative regulators of

D. dadantii 3937 virulence (*kdgR, pecS*, and *pecT*) or for the synthesis and perception of signaling molecules (*expI* and *expR*). An analysis of these mutants indicated that PecS, PecT, and KdgR play a similar role in *D. dadantii* and *D. solani* cells, regulating the expression of genes coding pectinases, cellulases, and proteases. The thermoregulator PecT seems to be a major regulator of *D. solani* virulence (Potrykus et al., 2014a).

Identification and differentiation of the bacteria from genus *Dickeya*

The detection, identification, and differentiation of *Dickeya* spp. have been reviewed in an article recently published by Czajkowski and coworkers (2015). Here, we present only a short description of methods used for those purposes.

Phenotypic methods

The most used worldwide medium for isolation of *Dickeya* and *Pectobacterium* cells is CVP medium (Crystal Violet Pectate) (Helias et al., 2012). Detection of *Dickeya* spp. on CVP depends on the formation of characteristic deep cavities by the bacterial colonies. When the bacterial population are low, they need to be enriched above the detection level (Pérombelon, 2002). The tested material is incubated under anaerobic conditions in a liquid enrichment medium, PEB, containing sodium polypectate as the sole carbon source (Pérombelon and van der Wolf, 2002). The NGM medium was developed to differentiate *Dickeya* spp. from *Pectobacterium* spp. on the basis of the ability to produce the blue pigment, indigoidine (Lee and Yu, 2005).

In the past, biochemical tests were commonly used to differentiate strains of *Pectobacterium* spp. and *Dickeya* spp. from other bacteria. As the procedures are troublesome and time consuming, they were replaced with more rapid serological and molecular methods (Czajkowski et al., 2015). Palacio-Bielsa and coworkers (2006) developed a modern version of a biochemical test using a microtiter system for differentiation of *Dickeya* species. Sławiak and coworkers (2009a) modified the method and checked features such as, growth at 39°C, 41°C and 25°C on nutrient broth, anaerobic hydrolysis of arginine and polysaccharide inulin utilization in phenol red peptone water (inulin extracts from chicory and dahlia were used at 0.3% final concentration); eight carbon sources were tested by acidification/alkalization on liquid Ayers, Rupp and Johnson medium with bromothymol blue mixed with different 0.3% carbohydrates: (−)-D-arabinose, 5-keto-D-gluconate, mannitol, (+)-D-melibiose, (+)-D-raffinose and (−)-D-tartrate, β-gentiobiose and (+)-L-tartrate.

Other phenotypic methods used for distinguishing SRE are fatty acid methyl ester analysis, volatiles profiling, serological methods, immunofluorescence staining, and immunofluorescence colony staining (Czajkowski et al., 2015).

Genotyping methods

Molecular detection methods based on the analysis of bacterial genomic DNA have become the most frequently used methods to detect and differentiate tuber soft rot and blackleg pathogens in environmental samples. These are methods that can be applied to the mixture of bacterial strains and allow detection and identification of pathogens present in symptomatic or symptomless plant samples. Several single-PCR and multiplex-PCR assays with species-specific primers have been developed for the detection of soft rot and blackleg pathogens; multiplex-PCR assays can simultaneously detect a few kinds of pathogens in the tested sample. In our laboratory, a multiplex-PCR assay has been developed, which detects and distinguishes SRE from *P. c. subsp. carotovorum*, *P. wasabiae*, *P. atrosepticum,* and *Dickeya* spp. (Potrykus et al., 2014b). Real-time PCR procedures have also been developed for the detection of bacteria from the genus *Dickeya* and *Pectobacterium* (Brierley et al., 2008; Laurila et al., 2010; Kim et al., 2011, Pritchard et al., 2012; Van der Wolf et al., 2014a). Pritchard and coworkers (2012) presented a bioinformatics tool allowing an easy prediction of primer sets for specific detection of species and subspecies of *Dickeya* based on the raw genome sequence information.

On the other hand, there are more sophisticated methods for differentiating closely related strains, but they require isolation of pure bacterial culture. Restriction fragment length polymorphism of amplified PCR (PCR-RFLP) product (usually one of the housekeeping genes) may be applied specifically for the identification and differentiation of closely related isolates within species and subspecies. To differentiate between *Pectobacterium* and *Dickeya* species, two groups of genes were mainly used; those coding for virulence factors, such as *pel* genes (Darrasse et al., 1994) and the housekeeping genes,

such as 16S rDNA, 23S rDNA, *recA, gyrA, gyrB, rpoS,* and *dnaX*. Waleron and coworkers (2002ab, 2013) developed a PCR-RFLP assay to differentiate pectinolytic *Pectobacterium* and *Dickeya* species on the basis of the sequences of housekeeping genes *recA* and *rpoS*.

A multilocus sequence analysis (MLSA) using concatenated sequences of the intergenic spacer (IGS), as well as *dnaX, recA, dnaN, fusA, gapA, purA, rplB, rpoS,* and *gyrA* have been used in the study of *Dickeya* species regarding the establishment of *D. solani* genus (van der Wolf et al., 2014b). This study revealed that the group of *D. solani* is homogenous and distinct from other *Dickeya* species.

The methods mentioned above have been developed on the basis of single genes, but there are also methods applying a whole-genome approach such as repetitive sequence-based PCR (rep-PCR) or pulsed-field gel electrophoresis (PFGE) and, of course, genomes sequencing which are described below. Repetitive sequence-based PCR (rep-PCR) has been developed to target the repetitive sequences present in bacterial genomes, namely, Repetitive Extragenic Palindromic (REP) sequences, Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR), and BOX elements (BOX-PCR) (Versalovic et al., 1991 and 1994). The relative resolution of the generated patterns is high and allows phylogenetic classification of SRE from the genus down to the strain level. The rep-PCR analyzes were used, for example, in studies on the classification of a new clade of *Dickeya* spp. biovar 3 (Sławiak, 2009b) and *D. solani* (Degefu et al., 2013; Potrykus et al., 2016). The technique is easy to perform and its resolution is high. The macrorestriction analysis by PFGE described by Ribot et al. (2001) was successfully applied to confirm the identity and homogeneity of *D. solani* strains isolated in Finland, Israel, and Poland (Degefu et al., 2013; Tsror et al., 2009, 2013; Potrykus et al., 2016). Another method used for genotyping of D. *dianthicola* and *D. solani* on the basis of a whole genome is a variable number tandem repeat (VNTR) analysis; it allows to differentiate 19 profiles characteristic of *D. dianthicola* and only 3 profiles characteristic of *D. solani* (Parkinson et al., 2015).

The development of next-generation sequencing (NGS) techniques lets the scientists to delve into more detailed search for the differences among the strains of same or different species (Toth et al., 2003; Pritchard et al., 2012). Complete or draft genome sequences are now available for numerous strains from the genus *Dickeya* and *Pectobacterium*, with many more in draft formats. In the National Center for Biotechnology Information (NCBI) genomes database, already 53 genomes assemblies of *Pectobacterium* spp. and 41 genome assemblies of *Dickeya* spp. are available (http://www.ncbi.nlm.nih.gov/). A typical member of this group has a single circular chromosome of around 5 Mb and no large plasmids are present. Comparative genomics can reveal physiological and functional variation among bacteria that provide insight into their ability to exploit distinct ecological niches (Toth et al., 2006). Genome sequences provide a valuable resource for the discovery of molecular markers that can be used for reliable classification of prokaryotic taxa and for understanding evolutionary relationships among them (Lerat et al., 2005; Dutilh et al., 2008; Gupta, 2010; Bhandari et al., 2012; Gao and Gupta, 2012). The complete genome sequence of *D. dadantii* 3937 (Glasner et al., 2011) as well as genome sequences of seven *D. solani* strains at different stages of assembly have been published (Garlant et al., 2013; Pritchard et al., 2013a,b; Khayi et al., 2014; Golanowska et al., 2015).

Control of disease caused *Dickeya* spp.

Effective control of blackleg and soft rot diseases of potatoes caused by bacteria from the genus *Dickeya* has not yet been achieved although many different methods were employed. Disease control is based primarily on avoidance of bacterial contamination during the production of healthy certified seed (Czajkowski et al., 2011). This should be achieved by producing seed potato from bacteria-free minitubers, by the application of seed certification schemes and strict hygienic practices. Knowledge of the pathogen sources and contamination pathways and effective methods for detection of pectinolytic bacteria together with the application of hygienic measures during harvest and post-harvesting storage should allow the production of healthy plant material. The recent appearance of apparently more virulent strains of *Dickeya* spp. – *D. solani* – should also give a new impact on the development of new, efficient diagnostics tools for control of seed potato and other crop contamination and breeding of new cultivars with a higher level of resistance to pectinolytic bacteria than currently growing cultivars.

Progress related to enhanced resistance to pectinolytic bacteria in new cultivars is expected, since there

are new tools now available and breeders may be used for more efficient introduction and maintenance of desired traits in breeding pool. They include DNA markers providing tools for the analysis of QTL controlling genetic resistance and marker-assisted selection, better understanding of the host-pathogen interaction at the molecular level, new techniques for genetic improvement of potato by application of somatic hybridization, and transgenic approach besides sexual crosses conventionally in use (Zimnoch-Guzowska et al., 2005). Another possibility is control of SRE by newly isolated and fully characterized bacteriophages, specific for the broad range *Dickeya* spp. and *Pectobacterium* spp. strains (Czajkowski et al., 2015).

Conclusions

The review presented here sums up the available information about new, economically important plant pathogenic bacteria from the genus *Dickeya. D. solani* strains isolated in the past 10 years in different countries, including Poland, form a very homogenous group, whose genome contains all genes involved in the pathogenicity of SRE including a full set of plant cell wall degrading enzymes and at least two QS systems. The genome of *D. solani* contains two sets of virulence factors, such as polyketide synthases and non-ribosomal peptide synthetases as well as effective T6 secretion system effectors. There is a need for intense research on the factors involved in the pathogenicity of *D. solani*. This is especially important because of the lack of efficient chemical and biological means to fight these bacteria.

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