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Short survey

Molecular methods as tools to control plant diseases caused by *Dickeya* and *Pectobacterium* spp: A minireview

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ABSTRACT

Dickeya spp. and *Pectobacterium* spp. are etiological agents of soft rot on crops, vegetables, and ornamentals. They also cause blackleg on potato. These pectinolytic phytopathogens are responsible for significant economic losses, mostly within the potato production sector. Importantly, there are no methods to eradicate these microorganisms once they have infected plant material. Solely preventive measures remain, including early detection and identification of the pathogens, monitoring of their spread in addition to planting certified seed material tested for latent infections. As proper identification of the causative agent allows for efficient limitation of disease spread, numerous detection and differentiation methods have been developed. Most commonly followed procedures involve: isolation of viable bacterial cells (alternatively post-enrichment) on semi-selective media, identification to species level by PCR (single, multiplex, Real time), serology or fatty acids profiling. Differentiation of the isolates is often accomplished by sequencing the housekeeping genes or molecular fingerprinting. In view of lowering total costs of next-generation sequencing (NGS), a huge amount of generated data reveals subtle differences between strains that have proven to be potentially useful for the establishment of specific novel detection pipelines. Successful implementation of molecular diagnostic methods is exemplified by 20-year studies on the populations of pectinolytic bacteria on potatoes in Poland. The presented work aims to gather the characteristics of *Dickeya* spp. and *Pectobacterium* spp. important for the identification process in addition to providing an overview of modern and newly developed specific, rapid, high-throughput and cost-effective screening methods for the detection and identification of these phytopathogens.

Introduction

Pectinolytic bacteria belonging to *Dickeya* spp. and *Pectobacterium* spp. (currently soft rot *Pectobacteriaceae*, SRP; formerly soft rot *Enterobacteriaceae*, SRE) are causative agents of soft rot on economically important plants, both vegetables and ornamentals. They are also responsible for blackleg symptoms on potato plants [1]. SRP might be shifted over long distances by infected seed material. They also spread locally by plant remains, soil, waterways, air, aerosols, alternative hosts or agricultural machines [2]. Despite the fact that the blackleg and soft rot are considered seed-borne diseases, *Dickeya* spp. and *Pectobacterium* spp. may invade neighboring plants through natural openings or mechanical injuries. Under environmental conditions favorable for the pathogen, infection latency state is disrupted and SRP secrete plant cell wall-degrading enzymes (PCWDEs) breaking down host macromolecules e.g. cellulose, pectins and proteins, in order to exploit plant protoplasts as rich sources of nutrients [3]. Once the disease symptoms develop, there are no control methods available. To limit the spread of these phytopathogens, solely preventive measures apply i.e. avoiding contamination of plant material, screening seed potatoes for latent infections, providing appropriate storage conditions, performing field inspections and monitoring the spread of pathogens [2,4].

A strong demand exists for designing reliable and cost-effective methods intended for detection and identification of SRP in latently infected plant material. Until now, over thirty diverse procedures have been proposed [5] in order to identify distinct pectinolytic bacterial species, elucidate their origin and limit further spread. In this minireview we introduce crucial taxonomical and epidemiological aspects relevant to the proper understanding of considerable challenges related to an accurate identification of *Dickeya* spp. and *Pectobacterium* spp. Most of all, classical and molecular methods designated for the detection of pectinolytic bacteria are comprehensively summarized and discussed with particular focus on innovative and emerging techniques. Moreover, a case study that lasted over 20 years was included aiming to illustrate the successful utilization of molecular identification and differentiation methods to screen seed potato fields and waterways for

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Dickeya and *Pectobacterium* spp. under temperate climate in Poland.

The pathogens: *Dickeya* spp. and *Pectobacterium* spp.

Taxonomic classification

Dickeya spp. and *Pectobacterium* spp. used to be referred to as SRE or pectinolytic erwinias. Although these terms are easily recognizable by scientists and subsistence farmers, numerous classification reassignments involving these bacteria have followed as a result of continuous progress in phylogenetic and systematic analyses. Initially, soft rot phytopathogens were identified as *Bacillus carotovorus* [6]. Subsequently, they were included in the genus *Erwinia* as two separate species: *Erwinia carotovora* and *Erwinia chrysanthemi* [7]. In 1998, they were transferred to the genus *Pectobacterium* as *Pectobacterium carotovorum* and *Pectobacterium chrysanthemi*, respectively [8]. Currently, the genus *Pectobacterium* comprises seven species: *Pectobacterium aroidearum* [9], *Pectobacterium atrosepticum* [10], *Pectobacterium betavasculorum* [10], *Pectobacterium cacticida* [8], *Pectobacterium parmentieri* [11], *Pectobacterium wasabiae* [10], and *Pectobacterium carotovorum* (including four subspecies: *Pectobacterium carotovorum* subsp. *actinidiae*, *Pectobacterium carotovorum* subsp. *brasiliense*, *Pectobacterium carotovorum* subsp. *carotovorum*, and *Pectobacterium carotovorum* subsp. *odoriferum* [8,12–14]).

The current genus *Dickeya* was established in 2005 to comprise all *Pectobacterium chrysanthemi* strains [15]. According to the generally accepted classification, eight *Dickeya* species are distinguished nowadays, namely, *Dickeya aquatica* [16], *Dickeya chrysanthemi* [15], *Dickeya dadantii* (involving *D. dadantii* subsp. *dadantii* and *D. dadantii* subsp. *dieffenbachiae* [15,17]), *Dickeya dianthicola* [15], *Dickeya fangzhongdai* [18], *Dickeya paradisiaca* [15], *Dickeya solani* [19], and *Dickeya zeae* [15].

It is important to stress that a lot of new information on the relatedness between pectinolytic isolates arises every year and triggers further taxonomic changes. Adeolu et al. (2016) conducted one of the most spectacular rearrangements by creating a new order *Enterobacterales* including *Pectobacteriaceae* fam. nov. into which the *Pectobacterium* and *Dickeya* genera were transferred from the *Enterobacteriaceae* family [20].

Virulence factors

Dickeya spp. and *Pectobacterium* spp. are Gram-negative rods moving due to the presence of peritrichous flagella. In addition to lipopolysaccharides providing adherence to host tissues, ca. 7–10 nm diameter fimbriae were observed among certain strains [21,22]. The average cell size of e.g. *P. carotovorum* subsp. *carotovorum* was estimated to reach 0.5–0.7 × 1.2–2.2 μm [23]. As SRP are incapable of spore formation, their predicted survival in harsh environment is rather limited. For instance, scientists are doubtful about their ability to reside over winter in soil, however, access to plant remains and more humid environment significantly prolong the viability of cells [4]. As facultative anaerobes, *Dickeya* spp. and *Pectobacterium* spp. are survive both under aerobic and anaerobic conditions. The latter feature is notably crucial since anaerobiosis impairs host resistance systems such as production of phytoalexins, phenolics and free radicals, integrity of plant cellular membranes in addition to lignification and suberization of the cell wall. Further outcome involves breakdown of disease latency state and promotion of plant tissue rotting [1].

SRP are often described as pectinolytic, which results from their ability to produce a wide range of enzymes degrading primary cell wall components of terrestrial plants, namely pectins. Most crucial for developing disease symptoms are pectate and pectin lyases cleaving α-1,4 glycosidic linkages by the β-elimination mechanism. Polygalacturonases release oligogalacturonates from this acidic polysaccharide by classical hydrolysis. Pectin esterases such as methylesterases, acetylesterases or feruloyl esterases are frequently listed as important auxiliary pectinases [3]. It is worth noticing that the products of pectin degradation participate in a molecular dialogue between plants and phytopathogens *via* activation of the plant defense response [24].

Other PCWDEs include cellulases, proteases, phospholipases, and xylanases. For example, proteases were hypothesized to suppress plant defense response besides providing nutrients for the invading microorganism [25]. Siderophores were shown to be similarly important for the pathogenesis of *Dickeya* spp. and *Pectobacterium* spp. as they enable bacterial growth under iron-limited conditions [26]. Special emphasis has been attributed to population density-dependent regulatory mechanism i.e. quorum sensing that coordinates effective production of virulence factors in addition to their secretion [24].

The soft rot and blackleg diseases

Disease symptoms and spread

Dickeya spp. and *Pectobacterium* spp. are etiological agents of plant diseases on the species belonging to approx. 50% of angiosperm orders, both monocotyledons and dicotyledons [27]. In this minireview we want to focus on potato, an important crop listed among top 5 agricultural products worldwide (FAO, 2015). SRP cause in potato plants blackleg symptoms that are recognized by wilting, chlorosis of the leaves in addition to progressive browning and decay of the stem base (Fig. 1A). They are also responsible for the symptoms of soft rot i.e. water-soaked and macerated inner parenchymatous tissue (Fig. 1B) [2,28]. The above-mentioned diseases are considered seed-borne, however, their causative agents may infiltrate into neighboring plants or tubers through natural openings (stomata and lenticels) or wounds [29]. By now, the spread of SRP has been attributed to contaminated plant material, soil, waterways, aerosols, alternative hosts, insects, nematodes and agricultural machines (especially harvesters) [30,31] as shown in Fig. 2. Also, transfer by man and animals has been suggested [32].

It is worth mentioning that besides acting as phytopathogenic necrotrophs, *Dickeya* spp. and *Pectobacterium* spp. may play the roles of endophytes. The resulting symptomless infection period can be significantly prolonged due to host resistance or unfavorable environmental conditions encountered by the pathogen e.g. acidic shock, drought, nutrients depletion, osmotic and oxidative stresses [33], whereas low oxygen level and high humidity act in favor of SRP [34].

Economic impact and control

In view of a wide host range and common occurrence of *Dickeya* spp. and *Pectobacterium* spp. in different geographical zones, an assessment of total economic impact of these phytopathogens is quite challenging. Nonetheless, Perombelon and Kelman (1980) [2] estimated the losses as \$50 to \$100 × 10^6 annually on a worldwide basis. Almost 30 years later, Tsror et al. (2009) [35] reported 20–25% potato yield reductions caused by *Dickeya* infections with a disease incidence greater than 15%. It is important to underline that *Dickeya* spp. and *Pectobacterium* spp. were listed among top ten bacterial plant pathogens with regard to scientific/economic importance [36]. Direct losses in the potato production sector result mainly from downgrading and rejections during seed tuber certification. Because of no uniform international potato certification policy, total damages resulting from SRP infections vary significantly between individual countries [30]. In the Netherlands, for example, where distinctively strict potato certification policy is in force, direct losses reach 30 M € each year [30]. On the other hand, in Poland, where 90% of potato production is intended for storage lasting 6–7, 1–9, and up to 10 months for seed, table and industrial potatoes, respectively, on average 5–30% total loss is recorded during the above listed periods (Mazovian Center of Agricultural