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Review

# Host plant physiological transformation and microbial population heterogeneity as important determinants of the Soft Rot *Pectobacteriaceae*–plant interactions

Vladimir Gorshkov *, Olga Parfirova

*Kazan Institute of Biochemistry and Biophysics, Federal Research Center Kazan Scientific Center of Russian Academy of Sciences, Kazan, Russia*

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ABSTRACT

*Pectobacterium* and *Dickeya* species belonging to the Soft Rot *Pectobacteriaceae* (SRP) are one of the most devastating phytopathogens. They degrade plant tissues by producing an arsenal of plant cell wall degrading enzymes. However, SRP-plant interactions are not restricted to the production of these "brute force" weapons. Additionally, these bacteria apply stealth behavior related to (1) manipulation of the host plant via induction of susceptible responses and (2) formation of heterogeneous populations with functionally specialized cells. Our review aims to summarize current knowledge on SRP-induced plant susceptible responses and on the heterogeneity of SRP populations. The review shows that SRP are capable of adjusting the host's hormonal balance, inducing host-mediated plant cell wall modification, promoting iron assimilation by the host, stimulating the accumulation of reactive oxygen species and host cell death, and activating the synthesis of secondary metabolites that are ineffective in limiting disease progression. By this means, SRP facilitate host plant susceptibility. During host colonization, SRP populations produce various functionally specialized cells adapted for enhanced virulence, increased resistance, motility, vegetative growth, or colonization of the vascular system. This enables SRP to perform self-contradictory tasks, which benefits a population's overall fitness in various environments, including host plants. Such stealthy tactical actions facilitate plant-SRP interactions and disease progression.

## 1. Introduction

The bacteria of the *Pectobacterium* and *Dickeya* genera attributed to Soft Rot *Pectobacteriaceae* (SRP) belong to one of the most devastating and extensively studied phytopathogens [1]. Plant cell wall degrading enzymes (PCWDEs), which can be produced by SRP in high amounts, turning plant tissues into an amorphous rotting mass, are considered the major weapons of these bacteria [2]. Due to this, most studies on SRP have been oriented on the "brute force" side of their behavior as well as on searching for host plant defense reactions that can cause pathogen elimination or at least prevent or reduce its propagation. However, in addition to PCWDEs, virulence factors typical of stealth phytopathogens (e.g., type three secretion system, coronafacic acid) are required for full virulence of SRP, indicating that these bacteria rely on host plant manipulation to cause the disease [3–5]. In turn, the outcome of a plant-pathogen interaction largely depends on the induction of so-called plant susceptible responses [6] as well as on the morphophysiological structure of the microbial population, which, according to the contemporary view, consists of differentiated cell forms with different properties [7–9].

The activation of susceptible responses promotes pathogen fitness *in planta* and may also be the root cause of symptom formation [6]. Physiological transformation of the host plant due to the induction of susceptible responses may promote pathogen invasion and vascular colonization, provide nutrient and inorganic substance flow toward pathogens, facilitate plant cell wall decomposition, induce death or partial digestion of host cells, and reduce the levels of defense compounds, all in favor of the pathogen [6]. Pathogens induce susceptible responses via manipulating the expression of the host susceptibility (S) genes [10–12].

Undoubtedly, different plant reactions, including susceptible responses, are perceived by pathogens and drive their population behavior. Moreover, pathogens may induce susceptible responses precisely in order to facilitate the diversification of their population structure within the host [13]. The bacterial population structure is determined by a set of differentiated microbial cell forms that can be

* Corresponding author.
*E-mail address:* gvy84@mail.ru (V. Gorshkov).

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very different in morphological, physiological, and functional terms [7–9]. Such cell differentiation occurs because, under various environmental conditions, bacteria need to simultaneously perform several tasks that are usually self-conflicting at the single cell level since a limited amount of resources makes a cell focus on a particular task. To allow the implementation of a number of complementary tasks, within a bacterial population, "the division of labor" takes place to distribute energetically costly functions among discrete coexisting subpopulations, minimizing the burden placed on individuals and benefiting a population's overall fitness [8,14,15]. Here, some cells may be in charge of utilizing different substrates, others of intensive proliferation, others of the production of one or another virulence factor, others of spreading, others of resistance to different stressors, etc. Some morphological and physiological forms of bacterial cells with particular characteristics have been specifically named (e.g., swarmers, viable but non-culturable forms, L-forms [16–18]), while many other functionally specialized forms remain nameless to date.

The dissociation of microbial populations resulting in the formation of specialized cells is generally studied using in vitro cultures. Much less is known about the morphophysiological heterogeneity of bacterial populations within host plants, in which different cells and tissues with different local conditions within them are likely to promote the dissociation of microbial populations. Moreover, infection-related plant responses may exacerbate the dynamism and heterogeneity of the plant interior, thus making additional contributions to the manifestation of the heterogeneity of microbial populations.

During the past two decades, reactions that can be regarded as susceptible responses during plant-SRP interactions have been reported. Gene products necessary for manipulation of host plants typical of stealth phytopathogens were revealed in both *Pectobacterium* and *Dickeya* species. Various differentiated cell forms and multicellular biofilm-like structures have been described for SRP both in vitro and *in planta*. However, there is no comprehensive overview of the induced plant susceptibility to SRP or the population structure of these phytopathogens. This review aims to consider SRP-plant interactions from the perspectives of host physiological transformation and microbial

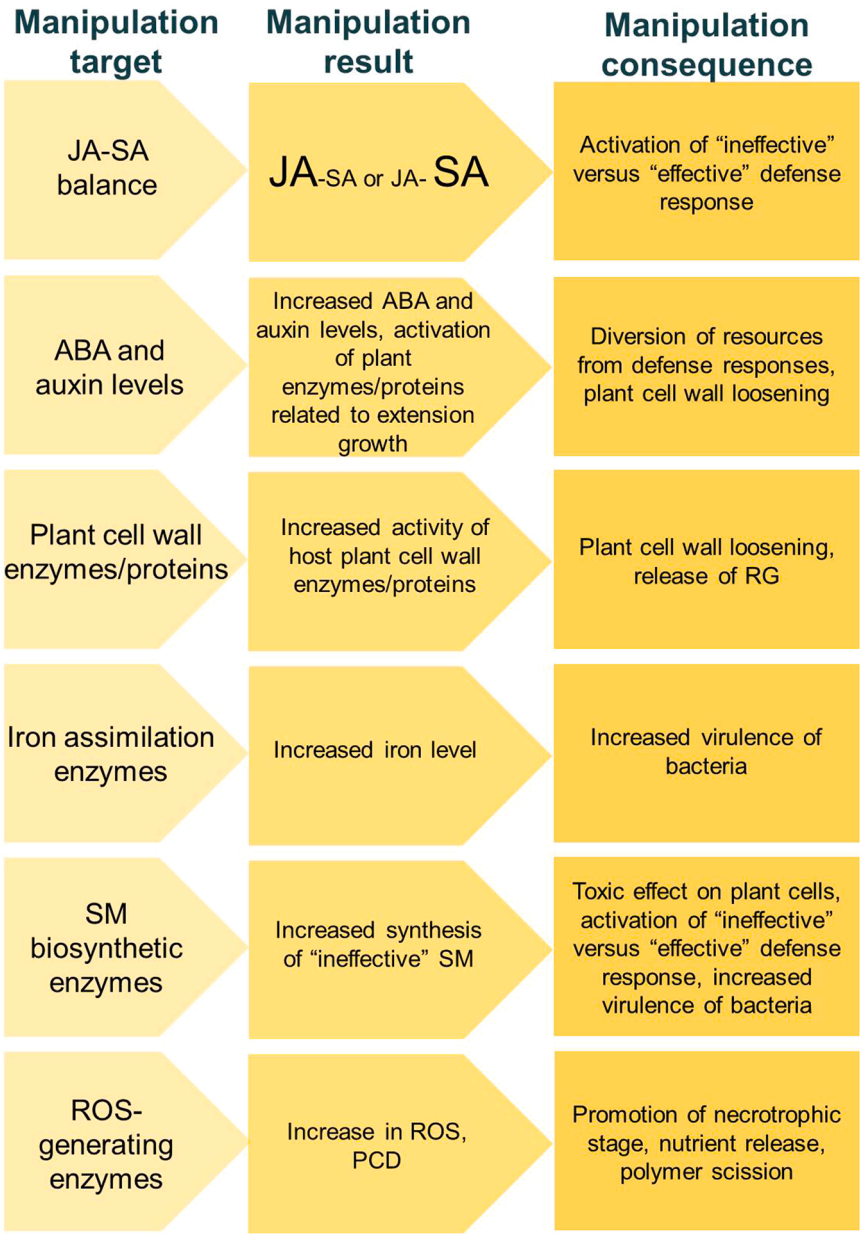


**Fig. 1.** The summary of host plant susceptible responses induced due to the manipulations by the Soft Rot *Pectobacteriaceae*. SA – salicylic acid; JA – jasmonic acid; ABA – abscisic acid; RG – rhamnogalacturonan; SM – secondary metabolite; ROS – reactive oxygen species; PCD – programmed cell death.

population heterogeneity.

## 2. Host plant reactions that may promote SRP fitness *in planta* and disease manifestation

The crosstalk between partners is the basis of plant-pathogen interactions and disease development. The pathogens cannot just consume plant-derived substances; they have to transform the interior of the host plant, creating a specific econiche from its body. In other words, a plant should be transformed from a relatively autonomous biological unit to a component of an integral pathosystem. The pathogen fails to systemically colonize the host plant and/or produce disease symptoms if the host plant does not undergo specific physiological changes throughout different interaction stages. Such host transformation is mediated by the susceptible responses—the reactions that promote pathogen fitness *in planta* and/or disease manifestation [6]. Confusingly, the susceptible and defense responses are often mediated by similar molecular actors, being the two sides of the same coin. Whether a particular reaction is a susceptible or defense response depends on the strength, timing, and dynamics of the response as well as on particular plant and pathogen species, environmental factors, infection stages, etc. All pathogen-induced plant responses are generally viewed through the lens of their involvement in defense reactions that reduce pathogen-caused damage. However, in fact, many of these responses encourage pathogen development and disease manifestation. Here, we consider how plant responses may determine their susceptibility to SRP (Fig. 1).

### 2.1. Hormonal perturbations

The most considered pathogen-induced plant responses are reactions related to salicylic (SA) and jasmonic (JA) acids. SA and JA, referred to as phytohormones of biotic stress, are often, but not always, mutually antagonistic, and thus a plant may usually give priority to only one type of response (either SA- or JA-mediated) [13,19]. SA is believed to mediate resistance to biotrophic pathogens, while JA – to necrotrophic ones [20]. However, since phytopathogenic bacteria (including SRP) are mostly hemibiotrophs [21], the role of SA and JA in bacteria-caused diseases is less univocal. Phytopathogens frequently exhibit a high susceptibility to one of these two phytohormones (or the result of its action) and tolerance to the other one. In the framework of a fraudulent tactic, some phytopathogens purposefully induce that host hormonal pathway, to which the microorganism displays tolerance or less susceptibility than to another one. The most explicit example of this is *Pseudomonas syringae*, which produces the phytotoxin coronatine, a functional analog of JA, in order to induce the JA pathway and thus repress the SA pathway that is lethal for this pathogen [22]. Both SA and JA have been shown to contribute to the resistant response to SRP [23–25]. However, there is strong evidence that these two phytohormones may also play important roles in mediating induced susceptibility to SRP.

*Pectobacterium* ssp.-caused diseases are associated with the down-regulation (or lack of regulation) of the SA pathway in susceptible plants [23–27], which is in accordance with the observation that these bacteria produce extracellular repressors of the SA pathway [23]. Presumably due to this, the SA-related mutant plants do not exhibit the increased susceptibility to *Pectobacterium* ssp. [23,24,28] since these bacteria themselves repress SA-regulated responses, and an additional exogenous repression of the SA pathway by genetic manipulations would hardly affect the host's susceptibility. Herewith, the SA treatment increases plant resistance [29,30] and represses the quorum sensing in *Pectobacterium* ssp. by binding the synthase and sensors of autoinducers [31,32].

Simultaneously, *Pectobacterium* ssp.-caused diseases are always associated with a strong upregulation of the JA pathway in susceptible plants, and this upregulation does not prevent the development of severe symptoms in *Pectobacterium*-infected plants of different species [5,25,33–35]. Furthermore, some *Pectobacterium* species produce coronafacic acid [36], a specific virulence factor that, similar to the coronatine of *Pseudomonas syringae*, induces the JA pathway of the host plant and thus promotes the transition from latent to symptomatic infection [5,34]. These facts indicate that the *Pectobacterium*-induced upregulation of the JA pathway represents a susceptible response, which likely prevents the activation of the SA-related responses that impede pathogen proliferation and/or reduce its aggressiveness. However, this scenario does not appear universal for all *Pectobacterium*-plant interactions, as the JA-related *Arabidopsis thaliana* mutants were more susceptible to *P. carotovorum* than wild type plants [24], and the treatment of calla lily plants with JA reduced *P. carotovorum*-caused symptoms more than treatment with the SA analog [33]. In Chinese cabbage, the JA also delayed disease development caused by *P. carotovorum*; however, the SA had a more pronounced effect on resistance, completely blocking symptom formation [37]. Whether JA can have a defensive effect against other *Pectobacterium* species besides *P. carotovorum* remains to be determined.

In the case of *Dickeya* ssp.-caused diseases, both the SA and JA pathways are upregulated, presumably resulting in a specific interaction of the two phytohormone pathways [38–40]. On the one hand, it has been demonstrated that the SA treatment confers resistance to *D. solani* [41], and the SA deficiency contributes to the formation of symptoms caused by this species [39]. On the other hand, SA has been shown to participate in the induced plant susceptibility to *D. dadantii*: *A. thaliana* mutants with elevated post-infection SA levels had repressed JA-related responses, resulting in increased susceptibility to *D. dadantii* compared to wild type plants with lower post-infection SA levels and enhanced JA responses [42]. Some studies also support the hypothesis that JA is a more effective phytohormone against *Dickeya* ssp. than SA [38,43]. However, JA was also shown to serve as a chemoattractant for both *Dickeya* and *Pectobacterium* species and an inducer of *D. dadantii* virulence gene expression [44,45]. Moreover, the treatment of *D. dadantii* cells by JA increased their virulence, and JA-deficient plants were more resistant to invasion by this species [44]. Such a dualistic role of JA can be explained by the fact that JA and JA-mediated responses can have opposite effects on plant-*D. dadantii* interactions at different infection stages, contributing to pathogen virulence at initial stages and improving plant tolerance at advanced stages.

Thus, the scenario of the SRP-plant interactions depends on the balance of the SA- and JA-related responses, and both *Pectobacterium* and *Dickeya* species interfere with these hormonal pathways by inducing plant responses that can shift the priority toward one or another phytohormone. In turn, pathogen-manipulated shifts in SA-JA balance can promote host plant susceptibility. Some SRP species even have specific virulence factors (e.g. coronafacic acid) whose action is required to alter the SA-JA balance. Since the abovementioned studies performed on different pathosystems yielded contradictory results on the roles of SA and JA in plant-SRP interactions, it can be assumed that representatives of the *Pectobacterium* and *Dickeya* genera (as well as species within a common genus) probably need genus- or species-specific exact parameters of SA-JA balance to thrive in the host plant. The exact roles of these two phytohormones in SRP-plant interactions may also depend on the amplitude, timing, and force of the SA and JA responses. The SRP-susceptibility-related parameters of SA-JA balance may also vary depending on the host plant species, its physiological status, and infection stages (including biotrophic and necrotrophic stages). For example, JA-deficient *A. thaliana* mutants were more tolerant to *D. dadantii* at the initial infection stages but more susceptible at the advanced interaction stages compared to wild type plants [44]. It is worth emphasizing that the increased resistance to *Pectobacterium/Dickeya* species induced by many different factors (e.g. iron-deficiency [46], treatment with hexadecane [47], overexpression of a negative regulator of ABA responses ERD15 [48] or WRKY70 transcription factor [49], down-regulation of chlorophyllase AtCLH1 [50]) is coupled with the upregulation of SA responses but not JA responses or even the suppression of the latter. This presumably suggests that SRP-induced activation of the JA pathways

more often reflects the susceptible response rather than the resistant one.

Along with SA and JA, SRP also affect the auxin- and abscisic acid (ABA)-mediated hormonal systems of the host plants. *Pectobacterium* and *Dickeya* species induce the accumulation of both auxin and ABA in infected plants [26,37,51], contributing to plant susceptibility. ABA-overproducing, ABA-hypersensitive, and auxin-hypersensitive mutant plants, and mutants deficient for the negative regulator of auxin transport were more susceptible to SRP, whereas ABA-deficiency resulted in increased plant resistance [48,51–56]. Exogenous spraying of plants with ABA or auxin makes them more susceptible to these pathogens [37,52,57]. Besides, *Dickeya* species produce auxin themselves, and auxin-deficient mutants of these bacteria have reduced virulence [58].

The role of ABA and auxin in plant susceptibility to SRP is likely related to the potential of these phytohormones to divert resources to abiotic stress response (ABA) and growth and development (auxin), reducing the power of an immune response [59]. Indeed, ABA has been demonstrated to repress the oxidative stress responsible for improved resistance to *D. dadantii* and *P. carotovorum* [51,57]. Auxin, in addition to its immunity-repressing potential [53,56], can also activate plant enzymes and proteins (expansins) involved in plant cell wall loosening [60], thus contributing to the manifestation of rotting symptoms following SRP infection. Thus, SRP may force host plants to accumulate those phytohormones that lower plant defense efficiency and promote other disease-related traits.

### 2.2. Plant cell wall modification

Many processes taking place *in muro* (i.e. in the plant cell wall) are widely known to contribute to pathogen resistance (e.g. lignification, polymer cross-linking, suberinization, callose deposition, etc.) [61]. Simultaneously, specific reactions implemented by host plant gene products *in muro* are required to make a plant susceptible to a pathogen. Phytopathogens may recruit plant cell wall enzymes and proteins to effectively interact with this complex compartment and induce "natural" host-mediated cell wall loosening that normally takes place during extension growth or fruit ripening [6]. Herewith, plant cell wall proteins of the host can presumably provide the breakdown of those polymer parts that are inaccessible to microbial PCWDEs or enable plant cell wall modification and polymer decomposition when bacterial density is insufficient to produce enough PCWDEs.

*P. atrosepticum* induces host-mediated modification of the plant cell wall [12]. During the initial stages of the colonization of the primary xylem vessels, when bacterial density is very low, *P. atrosepticum* stimulates considerable plant-mediated remodeling of the vessel cell wall structure, resulting in the release of pectic polysaccharide rhamnogalacturonan from cell walls into the vessel lumen. The released rhamnogalacturonan is used by bacteria as a component of the extracellular matrix to build up specific biofilm-like structures [12]. Based on the results of the transcriptomic analysis of infected tobacco and potato plants, xyloglucan endotransglycosylase/hydrolases, expansins, rhamnogalacturonan lyases, and polygalacturonases were hypothesized to provide the release of rhamnogalacturonan [35,62]. It is also possible that the products of infection-upregulated plant genes related to the plant cell wall may assist *P. atrosepticum* enzymes in maceration of the parenchymatous tissues in the advanced stages of infection.

### 2.3. Reactive oxygen species and programmed cell death

The oxidative burst due to the accumulation of reactive oxygen species (ROS) and various ROS-mediated forms of programmed cell death (PCD), including the hypersensitive response, are conventional attributes of plant defense reactions [63,64]. However, both PCD upregulation and ROS accumulation are often required for *in planta* pathogen accommodation and/or disease development [6]. Herewith, upregulation of PCD may enable pathogens to promote the selective elimination of defense compounds and digestion of host cells to release nutrients, whereas ROS can mediate PCD and cause polysaccharide scission.

ROS burst and “dead zones” in plant tissues formed due to PCD have been demonstrated to mediate increased resistance to both *Pectobacterium* and *Dickeya* species [38,40,65–67]. However, both ROS and PCD are also needed to promote plant susceptibility to SRP. Furthermore, the SRP, the bacteria that can pass through a relatively prolonged necrotrophic stage in plants, synthesize specific virulence factors (DspE/F type III effectors) whose function consists in inducing PCD in plants, which is necessary for further tissue maceration [68]. Additionally, the accumulation of ROS in the walls of the primary xylem vessels was hypothesized to promote polymer scission, thereby contributing to the release of rhamnogalacturonan into the lumen [12].

Since the successful infection cycle of SRP implies the accumulation of ROS in host plant tissues, SRP must cope with the increased ROS level. For this purpose, SRP synthesize various metabolites and enzymes with antioxidant properties: indigoidine, siderophores, exopolysaccharides, superoxide dismutase and other antioxidant enzymes [30,69,70]. Due to this, SRP can withstand host-produced ROS [71] even in mutant plants with an increased ROS level [72].

The particular role of ROS (resistance- or susceptibility-related) in plant-SRP interactions may be determined by the timing of ROS generation. It has been shown that in more resistant potato cultivar, ROS peaked as early as 6 h post infection with *P. brasilense* and then decreased, while in susceptible cultivar, the peak of ROS level was observed 24 h post inoculation [67]. Additionally, the outcome of the interaction may be determined by the type of generated ROS and the type of induced PCD.

### 2.4. Iron transport

Iron is essential for both plant defense responses and pathogen virulence [46,73,74]. Therefore, iron assimilation is an important part of plant-pathogen interactions. *D. dadantii* became a classical object for studying how pathogens steal host plant iron. For this purpose, *D. dadantii* produces the siderophores achromobactin and chrysobactin that sequester iron from host proteins and then transfer it into bacterial cells [75,76]. Importantly, chrysobactin also acts as a systemic iron-deficiency signal, causing plants to increase iron assimilation from the environment [77–79]. The assimilated iron can then be used for the pathogen’s needs. It cannot be ignored that assimilated iron can also mediate plant defense responses. However, since siderophore-deficient mutants of *D. dadantii* are avirulent [77,80], and plants grown under iron-deficient conditions have increased resistance to *D. dadantii* and *D. solani* [46,81], the pathogen-induced iron assimilation by plants during the infection is likely to have more beneficial traits for the pathogen and thus represents a susceptible response.

### 2.5. Secondary metabolite synthesis

The activation of the secondary metabolism in infected plants is routinely considered a defense response, although antiherbivorous properties are mostly described for the defense-related secondary metabolites and much less is known about the influence of these compounds on phytopathogenic bacteria [82]. During plant-SRP interactions, plant secondary metabolism is strongly induced [25,26,35,83,84]. However, although several plant secondary metabolites suppress the quorum sensing and PCWDEs in *Pectobacterium* species [31,32,85,86], no clear evidence has been obtained that plant secondary metabolites, even those produced by SRP-resistant plants, have apparent toxicity to SRP [85]. Particularly, since indole glucosinolate-deficient and wild-type *A. thaliana* plants exhibit similar levels of susceptibility to *P. carotovorum* or *D. dadantii*, it seems unlikely that indole glucosinolates prevent the spread of these pathogen species, although the

synthesis of these secondary metabolites is strongly activated following infection [43,87].

SRP are able to tolerate host plant secondary metabolites (at least some of them) due to the presence of genes whose products can provide their detoxication [88–90]. Furthermore, the spectrum of hosts that the pathogen can infect may depend on whether such genes are present or absent. For example, *P. odoriferum* and *P. versatile* have genes (*saxA*) encoding isothiocyanate hydrolase that catalyzes the cleavage of isothiocyanates that are accumulated in plants of the *Brassicaceae* family following infection [89]. The knockout of the *saxA* gene reduces the virulence of *P. odoriferum* and *P. versatile* on *A. thaliana* and *Brassica oleraceae*, whereas the heterologous expression of this gene makes the potato-limited species *P. parmentieri* (which normally lacks the *saxA* gene) able to cause disease in *A. thaliana* and *B. oleraceae* [90]. Taken together, the SRP have evidently adapted to the activated secondary metabolism of the host, and the synthesized defense metabolites (at least some of them) do not pose a serious threat to these pathogens. This casts doubt on the defensive nature of the activation of secondary metabolism during plant-SRP interactions.

In turn, the activation of secondary metabolism may contribute to the progression of diseases, including SRP-caused ones. Plant secondary metabolites can induce virulence in several phytopathogens, including *D. dadantii* [91]. Moreover, secondary metabolites can be toxic to plant cells and may thus impair defense reactions, exacerbate plant cell death, and promote increased susceptibility [92–94]. Furthermore, by forcing a host to synthesize "ineffective defense compounds", a pathogen can divert plant resources from effective defense reactions, making it more susceptible.

Taken together, as part of a manipulation tactic, SRP induce host plant reactions related to hormonal regulation, plant cell wall modification, PCD, ROS accumulation, iron transport, and secondary metabolite production in order to make the host more susceptible to pathogen propagation (Fig. 1). By now, it is difficult to conclude which of these reactions have a common susceptibility-related outcome for all (or most) plant-SRP interactions and which are specific for the susceptibility of particular plant species/genera to particular SRP species/genera. Nonetheless, it is clear that manipulating host plant reactions is a requirement for the progression of any SRP-caused disease. Deciphering the mechanisms of induced plant susceptibility to SRP will promote the improvement of disease management based on making plants less manipulable by the pathogen by using all classical and molecular breeding, genome editing, chemical, and biological approaches.

## 3. Morphological heterogeneity of SRP populations

Most studies on SRP consider microbial population as a summary of similar cells. The fact that a microbial population consists of heterogeneous differentiated cell forms is often neglected, except for some studies that are given below to create a contemporary picture of the SRP population heterogeneity (Fig. 2).

### 3.1. Biofilm-like structures

Biofilms have received special attention in studies of bacterial population heterogeneity and multicellular behavior. These structures are widely described for various bacteria, including phytopathogenic ones [95]. The major condition for biofilm formation is the synthesis of exopolysaccharides (EPS), which constitute an extracellular matrix that consolidates individual bacterial cells in a holistic structure. Within a biofilm, bacterial cells acquire “improved” properties, including enhanced stress resistance and virulence.

Biofilms formed by the *Dickeya* species were classified into two types: 1) surface-air-liquid interface biofilms (SAL-biofilms), which look like rings attached to the edge of the culture tube, and 2) air-liquid interface biofilms (or pellicles) – smooth and thick structures covering the air-liquid culture surface (without attachment to the solid surface of the

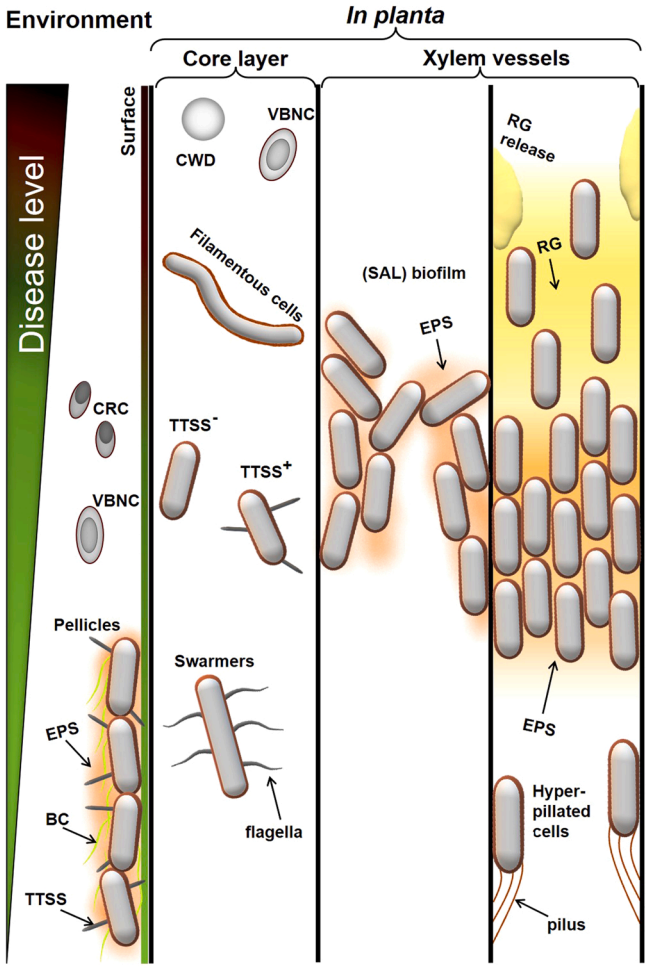


**Fig. 2.** Specialized cell phenotypes formed by the Soft Rot *Pectobacteriaceae* in host plants or environments. VBNC – viable but non-culturable; CWD – cell wall deficient; CRC – cells with reduced cytoplasm; TTSS – type three secretion system; EPS – exopolysaccharides; RG – rhamnogalacturonan; SAL – surface-air-liquid; BC – bacterial cellulose.

tube) [96–101]. SAL-biofilms and pellicles are genetically and chemically distinct. A type III secretion system is necessary for only pellicle but not SAL-biofilm formation. The assemblage of SAL-biofilms and pellicles is induced under different conditions [96]. Bacterial cellulose is the major structural component of pellicles but not SAL-biofilms. However, cellulose-deficient mutant can also form pellicles with more fragile cellulase-resistant matrix [96–98].

*Dickeya* species were demonstrated to form biofilm-like structures not only in vitro but also inside the host plant and on its surface [102, 103]. However, it is unclear, which type of biofilm *Dickeya* species form *in planta*. The fact that bacterial cellulose is required for *D. dadantii* to attach to the plant surface presumably indicates that pellicle-like structures are involved in plant surface colonization [103].

Within *Pectobacterium* genus, *P. brasilense* has been shown to form biofilms both in vitro and *in planta* [104,105]; however, the details of their structure and composition have not been elucidated. Moreover, the formation of biofilms in xylem vessels has been attributed to the aggressive behavior of *P. brasilense* since these structures were formed only in susceptible but not resistant plants. In contrast, *P. atrosepticum* has been shown to be cryptic in its biofilm-forming capacity under in vitro conditions. However, its di-GMP-overproducing mutant was able to form biofilms, and therefore, it was suggested that the formation of biofilms might be induced in *P. atrosepticum* under those conditions that promote the increase in the di-GMP level [106].

Under *in planta* conditions, *P. atrosepticum* has been shown to form specific biofilm-like structures called bacterial emboli [62,107]. Bacterial emboli are formed exclusively in the primary xylem vessels of the host plants and have distinctive features compared to typical biofilms in terms of morphological traits and the formation process. As a basis for the assemblage of bacterial emboli, a matrix not of bacterial EPS but of the plant pectic polysaccharide rhamnogalacturonan serves. Rhamnogalacturonan is released from the plant cell walls into the vessel lumen due to a specific plant susceptible response described above [12]. Thus, the "story" of bacterial emboli demonstrates that the differentiation of microbial cells may depend on the host plant's susceptible responses. EPS were also revealed in the composition of the extracellular matrix of bacterial emboli, but only at advanced stages of their development [108]. Importantly, unlike *P. brasilense* biofilms, the formation of bacterial emboli is unlikely to be coupled with the aggressive behavior of *P. atrosepticum*. The formation of bacterial emboli occurs during not only symptomatic but also asymptomatic colonization and affects only primary vessels, not secondary ones, and thus cannot significantly reduce water transport in the host plant as a whole [5].

The formation of different biofilm-like structures by SRP in the xylem vessels of the host plant likely promotes the downward xylem-mediated migration of bacteria toward subterranean host organs, which is widely described for SRP [102,104,105,107,109]. The entire or partial blockage of particular vessels by biofilm-like structures may locally reduce water transport, creating the conditions for the downward translocation of bacterial cells. Due to such a downward translocation, bacteria can invade subterranean host organs, including vegetative reproduction organs (tubers or bulbs), and thus transmit to the subsequent generation of the host plant. Therefore, the formation of biofilm-like structures, especially in xylem vessels, seems an important aspect of SRP-plant interaction.

### 3.2. Motility-related cells

Specific cell phenotypes are in charge of bacterial social motility (swarming and twitching). "Swarmers"—hyperflagellated and often elongated cells—implement flagella-mediated swarming motility [16]. SRP implement swarming [97,110]; however, whether this motility type is carried out by SRP during plant colonization remains contradictory. On the one hand, flagella genes of *P. brasiliense* were up-regulated during infection of potato tubers [111]. On the other hand, initially flagellated *P. brasiliense* cells lost flagella during plant colonization, and the mutant impaired in flagellar motility was still able to systemically colonize potato plants, albeit more slowly than the wild type [105]. Additionally, flagella-related genes in *P. atrosepticum* were down-regulated by plant extract and during the colonization of tobacco plants [5,112]. It is possible that flagellar-mediated motility and swarmers are involved in the colonization of tubers, but not shoots.

In turn, twitching motility, which is mediated by pili and implemented by hyperpiliated cells, seems important for SRP to systemically colonize host plant shoots. The *P. brasiliense* hyperpiliated mutant, in spite of lacking the flagella, was able to colonize potato plants systemically [105]. Besides, pili-related genes were upregulated in *P. atrosepticum* during the colonization of tobacco plants [5]. This agrees with the fact that bacterial downward migration via xylem vessels requires pili-mediated twitching motility [113,114].

### 3.3. Filamentous cells responsible for the increased metabolism

To establish a large population *in planta*, bacteria should manifest a high level of metabolic activity directed toward the consumption of a substrate and its expenditure for rapid reproduction. However, within the host plant, phytopathogens have to spend a lot of energy on the synthesis of virulence factors at the expense of resources that could be spent on proliferation. The simultaneous execution of both of these self-conflicting functions (vegetative growth and production of virulence factors) during host plant colonization in *D. dadantii* was shown to be provided by the specific dissociation of the bacterial population [115]. At late infection stages, the specific filamentous cells responsible for enhanced vegetative growth are formed in the *D. dadantii* population. These filamentous cells are non-motile and exhibit reduced production of virulence factors and enhanced metabolic activity compared to typical rod-shaped cells responsible for virulence properties [115]. Even though filamentous cells themselves produce few (if any) virulence factors, they are likely to play a significant role in the pathogen's aggressive behavior since their emergence results in an extensive increase in the bacterial population that rapidly depletes the resources of the host plant. Furthermore, by re-differentiation, filamentous cells may give rise to heaps of virulent "typical" rod cells.

### 3.4. Stress-related cell forms

Several stress-related cell forms have been described for the *Pectobacterium* genus: viable but non-culturable (VBNC) cells, cell wall-deficient (CWD)-forms or L-forms, and specific cells with reduced cytoplasm that are formed in starved cultures with low population density. VNBC cells characterized by the proliferative dormancy and specific morphology are formed by *P. atrosepticum* under starvation conditions in vitro as well as in host plant remnants [107,116]. *D. dianthicola* cells were also shown to transform to a VNBC state after treatment with copper sulfate [117].

CWD-forms can be formed by *P. atrosepticum* under starvation and in potato tubers [118,119]. The absence of a cell wall, which houses most elicitors, may make CWD-forms "invisible" to the plant immune system. Besides, it has been suggested that it is the L-form formation that enables *P. atrosepticum* to penetrate plant cell protoplasts and thus to behave as an intracellular pathogen [118].

Specific cells with reduced cytoplasm are formed by *P. atrosepticum* when the bacterial cell density is too small to implement the population behavior and the exogenous growth substrate is absent [120]. The reduction of the cytoplasm enables cells to support cell division until the population density reaches the quorum level, allowing effective adaptation and invasiveness [121]. Thus, various stress-related forms can promote SRP survival under unfavorable conditions, including nutrient deficiency, toxic compounds, and maybe plant defense compounds, since these forms possess increased stress tolerance [119,120,122]. Moreover, stress-related forms can serve as a reserve of the microbial population since they may revert to a "normal" state.

### 3.5. Cells with increased production of virulence factors

Cells within the SRP population were also shown to be differentiated in terms of virulence gene expression. Only a minor proportion of the *D. dadantii*'s population expressed genes related to the type three secretion system (TTSS) [123]. Herewith, the percentage of TTSS-expressing cells varied depending on the colonized host plant organ and infection stage. Such dissociation of the *D. dadantii* population was provided by epigenetic mechanisms rather than permanent genetic mutations since clonal populations derived from either TTSS+ or TTSS- cells became heterogeneous in terms of TTSS gene expression [123]. Differential production of particular virulence factors likely enables cells to share their responsibilities, leading to the formation of a consortium where particular cells are in charge of producing a particular virulence factor.

Thus, SRP cells within a population are multifaced and different cell types are specialized for implementing distinct tasks (Fig. 2). Within different populations of a species, different "sets" of specialized cell types are formed depending on environmental conditions. Due to heterogeneity, a population can perform self-contradictory tasks, which benefits a population's overall fitness in various environments, including host plants. The formation of a certain SRP cell variant *in planta* can be timed to a particular infection stage, colonized tissue type, and interaction

strategy (latent/moderate/acute infection). Different cell types are likely to contribute differentially to the devastating potential of SRP. Herewith, some cell types seem to determine a relatively commensalistic SRP behavior.

## 4. Concluding considerations

SRP are more than just PCWDE producers. To thrive *in planta*, SRP should be able to manipulate their host plants by inducing susceptible responses, thus transforming the plant's interior into a "proper" ecological niche. Within this niche, SRP create complex and heterogeneous populations, in which different cells acquire functional specialization and share different tasks. It is challenging to distinguish which susceptible responses or population structure parameters are genus- or species-specific and which are more or less universal for various SRP-plant interactions because our understanding of the global picture of SRP-induced susceptible responses and population heterogeneity is still in its infancy. A more comprehensive view on these issues will form the basis for the identification of plant S-genes targeted by SRP and for controlling SRP behavior during interactions with plants.

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## Conflict of Interest

The authors declare that they have no competing interests.

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