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Response of potato tubers to biocontrol agent and soft rot pathogen *Dickeya solani*: transcriptional regulation and hormone interactions

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DOCTORAL DISSERTATION

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ABSTRACT

The primary aim of this thesis was to develop biological control for potato soft rot disease caused by bacteria in the *Pectobacteriaceae* family. Especially the focus was on *Dickey solani*, an aggressive pathogen that has a potential to threaten potato production at all stages of cultivation, storage, and transit. The secondary aim was to discover molecular mechanisms underlying potato responses to *D. solani* and its biocontrol agent.

In the initial study, experiments focused on identifying the biocontrol potential of a collection of endophyte and rhizosphere bacteria in suppressing soft rot pathogens under storage conditions. Endophytic bacterium *Serratia plymuthica* strain A30 was identified as a superior biocontrol strain due to its effective colonization of potato tubers, cold tolerance, and direct inhibitory action against various soft rot pathogens, including *D. solani*. Two species-specific quantitative TaqMan PCR assays were employed to monitor the population dynamics of *D. solani* and S. *plymuthica* A30 during long-term storage and during the following year's cultivation in the field. Results indicated that *S. plymuthica* A30 could stably colonize potato tubers and survive for up to seven months, significantly reducing *D. solani* populations in cultivars with different susceptibility to soft rot disease. Bacterization of seed potatoes with *S. plymuthica* A30 after harvest effectively mitigated soft rot occurrence in stored seed material and reduced blackleg incidence in potato plants during field cultivation the following year. The second objective involved uncovering the potato tuber response to *D. solani* infection through RNA-Seq analysis. Gene expression patterns during the asymptomatic early phases of infection (1 and 24 hours after inoculation) displayed the induction of genes involved in the recognition of pathogen-associated molecular patterns (PAMPs), resistance proteins, reactive oxygen species, secondary metabolites, and hormonal pathways involving salicylic acid (SA) and jasmonic acid (JA). In symptomatic infections one week after inoculation, PAMP-triggered gene expression was downregulated, while genes linked to the production of free sugars and pectic enzymes influencing cell wall modeling were upregulated. Hormone production measurements indicated an increase in SA concentration in asymptomatic tubers and an increase in JA, coupled with a decrease in SA concentration in symptomatic tubers. The third objective aimed to identify mechanisms mediating plant defense in potato tubers treated with *S. plymuthica* A30 by RNA-Seq analysis in potato tubers exposed to *D. solani*, *S. plymuthica* A30 or both bacteria. Treatment with S. *plymuthica* A30 led to transcriptional reprogramming, enhancing host defense mechanisms through accumulation of structural barriers and the stimulation of many biochemical and molecular defense responses, effectively protecting tubers against *D. solani* infection. Early transcriptional responses were expressed at a much higher magnitude during combined inoculation with *S. plymuthica* A30 and *D. solani*, indicating the essential role of pathogen signals for

engaging plant priming and stimulation of A30-mediated host resistance. Changes in plant hormone biosynthesis and signaling played a prominent role in orchestrating interactions with this beneficial strain, as A30-induced systemic resistance dependent on the early activation of JA biosynthesis and signaling, while the SA-dependent defense response was impaired, possibly due to inhibitory crosstalk between JA and SA. Furthermore, A30 had a sustained effect on the expression of auxin-related genes, which are key regulators in promoting microbial colonization. In the late interaction phase, A30's endophytic colonization coincided with active interference in plant immunity by modulating the signal recognition system, reducing stress- and defense-related genes, and activating genes in cell wall remodeling, enhancing cell wall plasticity and symplastic trafficking for a successful symbiotic relationship with the host. Intriguingly, upregulation of genes in ethylene and abscisic acid biosynthesis and signaling was accompanied by downregulation of gibberellic acid- and cytokinin-related genes and alterations in several dormancy marker genes, suggested the maintenance of tuber dormancy and a delay in sprouting, ensuring potato tuber quality and durability during storage. This study contributes valuable insights into the tripartite interaction among *S. plymuthica* A30, *D. solani*, and potato, facilitating the development of biological control methods for soft rot pathogens under storage conditions.

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I dedicate this doctoral thesis to the cherished memory of my beloved mother, whose unlimited love, indomitable spirit, and boundless encouragement were the guiding forces behind my academic pursuits. Though she is no longer with us, her spirit lives on in the pages of this work, a testament to the values she instilled and the inspiration she continues to provide.

Helsinki, January 2024
Iman Hadizadeh

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications. The publications are referred to in the text by their roman numerals.

I **Iman Hadizadeh**, Bahram Peivastegan, Asko Hannukkala, Jean Martin van der Wolf, Riitta Nissinen and Minna Pirhonen. (2019), Biological control of potato soft rot caused by *Dickeya solani* and the survival of bacterial antagonists under cold storage conditions. Plant Pathology, 68: 297-311.

II **Iman Hadizadeh**, Bahram Peivastegan, Jinhui Wang, Nina Sipari, Kåre Lehmann Nielsen and Minna Pirhonen. 2022. Gene expression and phytohormone levels in the asymptomatic and symptomatic phases of infection in potato tubers inoculated with *Dickeya solani*. PloS one, 17(8), e0273481.

III **Iman Hadizadeh**, Bahram Peivastegan, Nina Sipari, Kåre Lehmann Nielsen and Minna Pirhonen. 2023. Transcriptome analysis unravels the biocontrol mechanism of *Serratia plymuthica* A30 against potato soft rot caused by *Dickeya solani*. Manuscript.

My contribution to the publications:

I **IH** collected the samples, screened antagonist activity against soft rot pathogens, calculated the population dynamics, and performed the field experiments. IH designed and conducted the TaqMan PCR and the RT-qPCR experiments. IH interpreted the results and wrote the manuscript.

II **IH** contributed to this publication in designing and preforming the experiments, conducting the bacterial population assay, constructing cDNA and RNA-Seq libraries, and carrying out qRT-PCR experiment of early time point samples. IH was responsible for designing the primers, statistically analysing the qRT-PCR data, performing functional analysis of the genes, and interpreting the transcriptome data. As shared first authors, IH and Bahram Peivastegan collaborated on writing the draft of the manuscript.

III **IH** planned and conducted the experiments, collected samples, executed bacterial population experiments, synthesized RNA-Seq libraries, and conducted qPCR. Additionally, IH undertook the statistical analysis of qPCR data, interpreted the transcriptome data, and wrote the manuscript.

ABBREVIATIONS

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylatedeaminase
AHSL	Acyl homoserine lactone
AM	Arbuscular mycorrhiza
BCA	Biological control agent
CFUs	Colony forming units
CK	Cytokinin
DAMPs	Damage-associated molecular patterns
DEG	Differentially expressed genes
ET	Ethylene
ETI	Effector-triggered immunity
GA	Gibberellin
GO	Gene ontology
HR	Hypersensitive response
IAA	Indole acetic acid (auxin)
ISR	Induced systemic resistance
JA	Jasmonic acid
MAMPs	Microbe-associated molecular patterns
M/ PTI	MAMPs/ PAMP-triggered immunity
OG	Oligogalacturonide
OPDA	12-oxophytodienoic acid
PALs	Phenylalanine ammonia lyases
PAMPs	Pathogen-associated molecular patterns
PCD	Programmed cell death
PCWDEs	Plant cell wall degrading enzymes
PGA	Polygalacturonan
PGPR	Plant growth-promoting rhizobacteria
PRs	Pathogenesis-related proteins
PGSC	Potato genome sequencing consortium
QS	Quorum sensing
RBOH	Respiratory burst oxidase homolog
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SRP	Soft rot *Pectobacteriaceae*

GENERAL ASPECTS

The world population is projected to reach about 9.7 billion people in the next three decades (Godfray *et al.*, 2014). Rapid world population growth, conflicts, migration, climate change, and most recently the Covid-19 pandemic and war in Ukraine, have engulfed the world in an ongoing crisis that increase the demand for food production (Goffart *et al.,* 2022). It has been estimated that by 2050, a global population will demand 70 % more food than is consumed today (Goffart *et al.,* 2022; Devaux *et al.,* 2021). To minimize this problem, it requires intensication of the quality and quantity of agricultural production that significantly contributes to the increase in chemical pollutants due to the exceeding use of synthetic pesticides and chemical fertilizers, which cause environmental pollution with possible risks to human health (de Janvry and Sadoulet, 2020). Therefore, the new Agri-food system approaches in sustainable agriculture concerned the side effects of chemical management, not only in the environmental dimension, but also in the socio-economic dimension (Zhang *et al*., 2018).

As a result of the integration of biotechnology with traditional agricultural practices, emerging developments on nontoxic microbial biofertilizers and biopesticides have shown some promises for application in plant protection, food safety, phytoremediation, and climate change (Pathma *et al*., 2021). Biological control or biologically based pest management includes technological, economical, and political approaches with the aim of developing sustainable agriculture with less ecological cost. This method utilizes the natural ability of microorganisms to limit the growth of each other to control crop diseases while causing no or minimal adverse effects on the environment to represent an ecologically friendly alternative to chemical control methods with the term of microbial biological control agents (MBCAs). MBCAs have been attracted considerable attention in agriculture because they function like vaccines without producing undesirable effects in plants and show an important role in improving of soil fertility and enhancing plant health due to their ability to promoting crop productivity and nutritional quality, as well as plants' resistance to pathogens and tolerance to abiotic stresses (Malgioglio *et al.,*

2022). MBCAs consist of a wide variety of microbes, such as plant growth-promoting rhizobacteria (PGPR), phosphate solubilizing microbes, nitrogen-fixing bacteria, actinomycetes, mycorrhizal fungi and endophytic bacteria and fungi. Bacteria belonging to different genera, such as *Bacillus*, *Pseudomonas, Burkholderia*, *Enterobacter*, *Azotobacter*, *Flavobacterium*, *Microbacterium*, *Rhizobium* and *Serratia*, are reported to be the most important inoculants used as MBCAs due to the wide range of their modes of action (Lahlali *et al.,* 2022).

A great diversity of potential mechanisms has been proposed to benefit plant hosts by MBCAs, such as directly improving plant nutrient uptake or modulating phytohormones to stimulate plant growth. Indirectly, they can improve plant health by inducing resistance or priming plant defense against a target pathogen or with other mechanisms modifying the growing conditions for suppression of pathogen development, leading to nutrient and niche competition. Such interactions are highly regulated by a sequence of events after the release of signaling compounds for establishment of the MBCA, and induction of a cascade of metabolic compounds with different modes of action are used to outcompete the pathogen or induce plant defense mechanisms (Afzal *et al*., 2019). Although the biocontrol method seems promising, its application faces several technical complications. Several traditional approaches have been developed for identification and isolation of potential MBACs but tend to be laborious and time-consuming and do not always translate to successful biocontrol outcomes. There has been limited effort to characterize the roles of beneficial microbes in natural ecosystems, particularly in assessing their diverse modes of action. Therefore, most research projects are finished before any natural product is developed and introduced to the market sector (Kurokawa *et al.,* 2022). Often, when researchers attempt to explain unpredicted results in biocontrol field experiments, they claim that any number of unknown and complex interactions between plants, microbes, and the environment may affect the outcome. Most of these researchers focus on single pathway of interest under defined growth conditions and fail to address how these mechanisms may be altered through tripartite interaction where both beneficial and pathogenic microbe exist together in the host (Bradáˇcová *et al*., 2019).

Understanding the mode of action of MBCAs with high potential in the induction of resistance is essential for optimal disease control and determination of possible danger for humans or the environment and risks of resistance development against the biocontrol agents. The final outcome of interactions of potential MBCAs with the plant and of the induced plant with the pathogen depends on the successful establishment of the mode of action of MBCA, the growing conditions for the MBCA for active colonization of plant, the physiology of the plant to develop a sufficiently high level of resistance, the cultivar genetics of the plant and the conditions for pathogen germination and infection. Although plant-microbe interaction is a complex process under the influence of many factors to study, identifying the molecular mechanisms responsible for biocontrol is a great challenge. The outlook for this field is bright, especially as omics techniques continue to develop, allowing us to gain insights into this incredible system and keep trying to exploit the plant's natural immune system during microbial interactions for the development of biocontrol techniques (Yang *et al*., 2022; Poluri and Czajkowski, 2022).

In this thesis, plant-microbe interactions were investigated in three cases. The first case focused on screening and selecting the best MBACs against bacterial soft rot disease caused by *Pectobacterium* and *Dickeya* in stored potato tubers. In addition, the survival of MBACs, their population dynamics in potato tubers, and their potential transmission to the next-generation seed tubers were investigated. The second case discusses a single potato-*D. solani* pathosystem to explore the molecular aspects of compatible interaction following the recognition of the soft rot bacteria *D. solani* by potato cells and how plants are able to defend themselves. The third case presented here concerns the early and late phase of interaction between plant and BCA in which plants recognize biocontrol endophytic bacteria *Serratia plymuthica* A30. This event leads to a symbiosis between the two organisms that have a dramatic effect on the plant’s ability to acquire resistance against pathogen. Understanding the molecular mechanisms of biocontrol responsible for the protective effect of *S. plymuthica* A30 will facilitate the optimization to fully utilize the biocontrol potential of this strain and to design of appropriate formulations and application methods in sustainable agriculture.

1. INTRODUCTION

1.1. Potato at the foundation of global food stability

Potato (*Solanum tuberosum*) is one of the important crops ensuring global food security due to the superior nutritive qualities of tubers, high potential yields in a short time, and diverse distribution patterns worldwide (Devaux *et al*., 2021). It's also the most important tuber crop for human consumption with over one billion consumers globally, grown in more than 150 countries worldwide with notable production volume (376 million from 19 million hectares with an average yield of 17 tonnes per hectare— FAO, 2021) through a wide range of environments as it exhibits remarkable phenotypic plasticity. Potato tubers are an excellent source of nutrients and vitamins, minerals, and high-quality protein that contribute to dietary supplementation. In Europe, 55 million tons of potatoes harvested across Germany, France, the Netherlands, the United Kingdom, and Belgium in 2020 continued to rebound away from the low in 2018. The success of the crop to a large extent depends on the quality and disease-free certification of seed tubers (Goffart *et al*., 2022). Improving the quality and storability of potatoes and conferring resistance to diseases are the key factors for successful economic and sustainable potato production (Forbes *et al.,* 2020).

1.2. Introduction to bacterial soft rot disease

1.2.1. Restrictive impact of soft rot disease on potato production

The harvested potato tubers are living and respiring stem-derived organs with perishable characteristics that risk a post-harvest loss of up to 40 % (Devaux *et al*., 2021). Production of seed potato tubers can be threatened by various environmental stresses, pathogenic organisms attack, and insects that lower yield and create a potential economic post-harvest loss. Potato is sensitive to various diseases caused by fungi, bacteria, and viruses at every stage of growth and storage. Among them, bacterial pathogens cause severe damage, especially on tubers, which has direct consequences such as yield loss and unmarketability of the produce or indirect long-term consequences such as economic, environmental, and social.

The bacterial soft rot and blackleg diseases after bacterial wilt disease caused by *Ralstonia solanacearum* are considered the most destructive bacterial diseases in seed potato production (Charkowski *et al*., 2020). Although potatoes are the most economically important crop affected by soft rot bacteria, 35 % of angiosperm plant orders were recorded as their host, including many economically important crops (e.g., maize, rice), fruit, vegetable, ornamental crops and weeds/ wild plant (Charkowski, 2018). As a result, soft rot bacteria have been ranked among the top ten plant pathogenic bacteria in 2010 based on their economic or scientific impact (Mansfield *et al*., 2012). Farmers lose millions annually to blackleg and tuber soft rot both in the field during the growing season and post-harvest during storage, transit, or during marketing, after the farmer has invested a full season of inputs into growing the crop (Toth *et al*., 2011). The main losses are linked to downgrading and rejection of substantial amounts of seed potato during certification, where the limit for rejection is from 0.0 to 0.5 % rotted tubers in storage (Directive 2014/20/UE and 2014/21/UE). Although it is difficult to determine the full extent of postharvest losses caused by soft rot bacteria, conservative estimates indicate that the overall loss for the European potato sector is estimated at 46 million euros per year, with 32 % of losses for the seed potato sector. The accounts exhibited high variability of economic losses over the years, depending upon the climate, the value of the crop, conditions of potato growth and storage, soft rot bacterial species, and environmental conditions for pathogen growth (Dupuis *et al*., 2021).

1.2.2. A brief overview of soft rot bacteria

Dickeya (formerly *Erwinia chrysanthemi*) and *Pectobacterium*, key genera belong to pectinolytic soft rot *Pectobacteriaceae* (SRP; formerly known as pectinolytic *Erwinia* spp. in the *Enterobacteriaceae*) are main causal agents of blackleg and soft rot on potato, leading to economic losses in seed tuber production worldwide (Adeolu *et al*., 2016). These genera are genetically highly diverse and composed of various species with different host preferences and wide geographic distribution (Charkowski *et al*., 2020; van der Wolf *et al*., 2021). However, multiple pathogens of both genera are frequently isolated from

the same soft rot sample due to overlapping host ranges and, in many cases, are distributed in the same geographical areas (Charkowski, 2018). The prevailing SRP pathogens can also vary over time, probably as a result of the changes in host plant, environmental factors, and the evolution of pathogens themselves (Ge *et al.,* 2021). The SRP possesses several levels of evolution in genomes that may lead to rapid adaptation to different environments and new hosts, raising serious concerns about potential damage to new crops (Klair *et al*., 2021; Boluk *et al.,* 2020).

The taxonomy of SRP species was recently revisited following genomic studies of isolates present in international culture collections and originating from diverse ecosystems around the world (Zhang *et al.,* 2016). Recently, several new *Pectobacterium* and *Dickeya* species were reported. By now, the genus *Pectobacterium* is divided into 19 recognized species, and the genus *Dickeya* encompasses 12 species, including *D. solani, D. undicola, D. zeae*, *D. dianthicola*, *D. fangzhongdai*, *D. lacustris*, *D. paradisiaca*, *D. dadantii* (including *D. dadantii subsp. dadantiiand* and *D. dadantii subsp. dieffenbachiae*), *D. aquatica*, *D. chrysanthemi, D. poaceiphila (*Adeolu *et al.,* 2016) and *D. oryza* (Wang *et al*., 2020). Some *Dickeya* species were reported to be associated with devastating outbreaks of blackleg or soft rot (Curland *et al.,* 2021; van der Wolf *et al.,* 2021; Charkowski, 2015). At the beginning of the twenty-first century, an outbreak of blackleg and soft rot in the European potato ecosystem has been attributed to the rapid establishment of *D. solani* due to the importation of infested seed tubers and climate change, and, by around 2010, this species caused an increasingly serious problem that severely affects potato production in field and storage in many countries (Toth *et al*., 2011; Tsror *et al*., 2013).

D. solani was introduced in 2014 as a new *Dickeya* pathogen on potato, as a gram-negative, rod-shaped, facultative anaerobes bacteria with peritrichous flagella, which was considered to be more aggressive than the closely related bacterial species with lower infection threshold (van der Wolf *et al*., 2014). Outbreaks of *Dickeya* in potato highlighted the failure of soft rot management for decades. *D. solani* is able to cause disease symptoms under a variety of temperatures, while it has a higher optimal growth temperature in when

compared to other closely related *Dickeya* (e.g., *D. dadantii; D. dianthicola*) and consequently can be more virulent under hot climatic conditions. Climate change and global trade provide favorable conditions for their proliferation and global dispersal (Potrykus *et al.,* 2014).

All *Dickeya* species, except *D. paradisiaca,* have been isolated from a wide range of ornamentals (Golanowska *et al.,* 2018). In Europe, crop rotation most likely promoted the spread of *D. solani* from monocot ornamental bulb crops to potatoes. *D. solani* strains isolated from a different sources and geographically distant locations were shown to be genetically closely related, indicating very limited genetic diversity between isolates on potato and ornamental hosts and fueling speculation that these pathogens have independently spread from an ornamental plant such as hyacinth onto potato. Unlike potato, the import of ornamentals is not controlled in Europe or other parts of the world, this can facilitate transfer to or from potatoes and possibly other crops (Parkinson *et al.*, 2014a).

Dickeya species inhabit numerous environments as possible sources of plant contamination. This pathogen can be found in water (Toth *et al.*, 2011), weeds/wild plants (Tsror *et al.*, 2011), volunteer potatoes and spreads by insects (Rossmann *et al.*, 2018), as well as can be present on the surface of processing machinery, stores and storage boxes and grading tools, etc. (Fehres and Linkies, 2018). The presence of a great diversity of SRP has been identified in water samples from different sources (such as irrigation, surface water, aerosols, waterways, water reservoirs, rain, snow, sea, and ground water), which indicate the role of water in the dissemination of these bacteria (Laurila *et al.*, 2010; Pédron *et al.*, 2014; 2017). The presence of natural populations of water-borne *Dickeya* spp. have recently been described following identification of several new species including *D. aquatica, D. lacustris* and *D. undicola*. In addition, *D. aquatica* was found in Finland, Scotland and France, and characterized having low pathogenicity in potato field trials (Parkinson *et al.*, 2014b; Laurila *et al.*, 2010; Hugouvieux Cotte–Pattat *et al.*, 2019). Numerous findings have indicated the association of various bacteria in *Pectobacterium* and *Dickeya* genera with high proportion, variety, and ubiquity with insect species, suggesting a contribution of insects in the transmission of SRP, which is especially relevant

in the initial infection of clean seed material such as potato mini tubers (Rossmann *et al.*, 2018). There are also examples that show nematodes may be a vector for these bacteria (Nykyri *et al.*, 2013). Since *Pectobacterium and Dickey* are common in the environment and appear to spread mainly at harvest, it is not practicable to produce potatoes free of these pathogens (Charkowski, 2015).

1.2.3. Nature of disease and ecology of *Dickeya*

Although *Pectobacterium* and *Dickeya* are in different genera with some important differences in gene regulation that may explain some of the differences in disease symptoms, they are often discussed together because of similarity in source and method of spread; and process of infection. They are often found together, leading to disease development more frequently than when only one of them is present. SRP species can enter and colonize plant host through natural openings (e.g., lenticel, hydathodes, stomata, and tuber stolon), and wounds that often incurred by insect feeding or bruising during harvest and handling (Toth *et al.*, 2011). These niches play a significant role in the long-term survival of soft rot bacteria and also support the spreading of these pathogens from a few rotting tubers to numerous nearby healthy ones (Charkowski, 2015). The contamination may be internal, in the plant vascular system and the bacteria can also spread down to reach the stolon of developing progeny tubers. Study on *D. solani* and *P. parmentieri* showed the translocation of pathogens infected haulms through the soil into progeny tubers tissues (Kastelein *et al.*, 2020).

In potato, soft rot bacteria can cause a variety of disease symptoms at every stage of the potato production cycle. Early growth of the bacteria in contaminated seed tubers after planting results in pre-emergence decay of seed tubers below ground (blanking). Under disease-inducing conditions, the bacteria colonize parenchyma and then xylem tissues leading to browning of the plant vascular system, necrosis of the stem, leaf chlorosis, and wilting, known as blackleg (Figure 1). Contamination of the progeny tubers via the vascular system causes stolon-end rot, which under extreme conditions can lead to the complete decay of progeny tubers (Charkowski, 2018). Bacteria can be

transmitted to progeny tubers via soil water during rainy weather or irrigation that causes lenticel rot/pit rot. Incidence and development of visible symptoms during the growing season are mainly dependent on environmental conditions (Toth *et al.*, 2011). For example, blackleg symptoms without clear wilting can develop for *Dickeya* similar to *Pectobacterium* species under wet conditions regardless of the temperature (Tsror *et al.*, 2013; Czajkowski *et al.*, 2011a). In contrast, under a hot-dry climate (> 27 °C), *D. solani* initiate slow wilting of the plant accompanied by hollow stems and desiccation of the entire plant, often without any visible blackleg symptom (Tsror *et al.*, 2008). Temperatures above 30 °C appear to be particularly favorable to disease development caused by *D. solani* with severe blackleg outbreaks (Adeolu *et al.,* 2016), while most *Pectobacterium* species are the main cause of blackleg at cooler temperatures, lower than 25 °C (Dees *et al.*, 2017). However, there is an overlap in the core temperature optimum and growing range of blackleg SRPs, making it difficult to argue that the growing season temperature is the sole mechanism for species selection and pathogenicity differentiation (du Raan *et al.*, 2016). Most likely, other factors such as the unique ability of *D. solani* to produce bacteriocins and bacteriocin-like substances can be responsible for its ability to outcompete or overtake *Pectobacterium* species, and relatively higher aggressiveness of *D. solani* in causing disease symptoms from lower inoculum levels compared to other species (Degefu, 2021). However, investigation of the effect of temperature on the pathogenicity-related gene expression in *D. solani*, revealed that only a low percentage of the bacterial genes are thermo-regulated (Czajkowski *et al.*, 2017).

Figure 1. Symptoms caused by *Dickeya solani* in potato stems and tubers.

Upon infection, bacteria first colonize the cortex and are rapidly transported to apoplastic spaces and then to the vascular system. Inside the plant, bacteria can remain in a latent state at an undetectable level ($<10^1$ cells/g of tissue) when the environmental conditions are not favorable to disease initiation. These latently infected potato tubers are the major source of SRP in the environment (Pérombelon, 2002). The bacteria can survive up to several months to bridge between two growing seasons. After the storage period, SRP inoculum in latently infected tubers can reach to relatively high population (10^2–10^4 viable cells/g of tuber tissue), which is enough to initiate soft rot symptoms in the next season. However, latent infections may help pathogens to spread unnoticed through several generations of tubers before the occurrence of disease symptoms (Czajkowski *et al.,* 2009).

The majority of seed lots are usually contaminated at least one generation in the field during plant growth, at harvest, or seed grading (Frost *et al*., 2013). However, asymptomatic infections are common during storage, as the bacteria remain dormant unless conditions are favorable for bacterial multiplication. Free water and high-water status of tubers, the optimal temperature for bacterial growth, which differs between species, storage with poor ventilation and high humidity together lead to local oxygen depletion (hypoxia/anoxia), are the main post-harvest environmental factors that affect pathogen colonization and increase the sensitivity of tubers to soft rot, causing greater disease incidence and symptom progression (Lisicka *et al*., 2018). The onset of soft rot diseases is associated with bacterial population in critical density of about 10^7–10^8 bacterial cells/g of potato tissue in order to secrete enough pectinolytic enzymes to start tissue maceration. The periderm of the tuber is resistant to decay (Figure 1) and can generate an anaerobic environment suitable for wet and foul-smelling rot inside the tuber. (Toth *et al*., 2003).

Under favorable conditions for pathogen growth, even low initial inoculum levels can cause tuber maceration. The tuber soft rotting occurs very slowly in the storage with a temperature less than 10 °C. It may take 3-5 days for a whole tuber to rot at a combination of poor oxygen availability with high humidity level, which is accompanied by a production of volatile metabolites and a foul odor (Kushalappa and Zulfiquar, 2001). Poor ventilation causes an increase in

the temperature due to high respiration rate of the tubers, especially in freshly harvested tubers. These conditions can lead to rising of the warm air that consequently condensates on the tuber surface and creates a layer of water, which is an early sign of rotting and is known as wet conditions (Pérombelon, 2002). Under wet conditions, a layer of water film around tubers can restrict the oxygen-dependent respiration and create an anaerobic environment, which causes the opening of the lenticels, swelling of the cortical cells, and increased cell membrane permeability resulting in solute leakage of cell content and increased nutrient availability (Lisicka *et al.*, 2018). Accessibility of nutrition and entry site during anaerobiosis may increase the relative fitness of the pathogen and allow SRP to microaerophilic grow. Therefore, soft rot pathogens have a competitive advantage over other co-occurring aerobic (antagonistic) bacteria at low oxygen concentrations (Charkowski, 2006). On the other hand, wet conditions impair the effectiveness of oxygen-dependent plant defense mechanisms via the decline of cell wall lignification and suberization (inhibition of wound healing) and lack of antibacterial protein synthesis resulting in a decrease of host resistance (Peivastegan *et al.*, 2019).

1.2.4. Pathogenicity and virulence strategies of soft rot bacteria

In a susceptible host, soft rot bacteria can multiply and develop a large array of metabolites to adapt to the host environment. They rely mainly on synthesizing and secretion of plant cell wall degrading enzymes (PCWDEs) for their pathogenicity to disintegrate plant cell wall and provoke maceration symptoms and have therefore long been considered as “brute force” necrotrophic and opportunistic phytopathogens (Yang *et al.,* 2008; Pérombelon, 2002). This symptomatic phase is associated with the liberation of the cell content and the release of carbon and energy sources in the form of oligosaccharides that can be assimilated by bacteria for a new cycle of multiplication. Further characterization of soft rot pathogens suggests the existence of some biotrophic features for these pathogens in their interactions with plant hosts as “stealth force” pathogens that evade or suppress the host defenses. During the asymptomatic phase, SRP may survive latently at a low population level in the intercellular space, where they use simple sugars or small oligosaccharides for

growth and also induce defense responses in the host (Effantin *et al.,* 2011; Davidsson *et al*., 2013). This biotrophic lifestyle may persist for a short or long time until environmental conditions are conducive to bacterial multiplication. The clear boundaries between these two phases are not clear for bacterial pathogens, and it has been proposed that SRPs like most Gram-negative phytopathogens can be described as hemibiotrophs regardless of their ability to produce necrotrophic symptoms (Kraepiel and Barny, 2016).

The transition from the latent (asymptomatic) to the soft-rotting necrotrophic phase, requires an intensive multiplication and production of a wide range of pathogenicity determinants, including PCWDEs (Davidsson *et al*., 2013). PCWDEs consist of pectinases, cellulases, xylanases, and proteases, which by their properties, presence, regulation, and secretion differ between *Pectobacterium* and *Dickeya* species (Hugouvieux-Cotte-Pattat *et al.,* 2014). Primary cell wall is a complex of cellulose/hemicellulose and structural glycoproteins network embedded in pectin. Pectin is a jelly-like matrix of acidic polysaccharide composed of both long-chain polygalacturonan (PGA) and ramified regions [rhamnogalacturonan I and II (RG-I and RG-II)] (Caffall and Mohnen, 2009). *Dickeya* spp. has adapted to the complexity of pectin by synthesis and secretion of vast array of PGA- and RG-degrading enzymes, including polygalacturonase (PGs), pectate lyase (Pel), pectin lyase, and rhamnogalacturonate lyase. The bacteria also produce one pectin acetyl esterase and two pectin methyl esterases, which are removing methyl or acetyl groups from pectic polymers making them amenable to the action of pectate lyases (Hugouvieux-Cotte-Pattat*,* 2016; Golanowska *et al.,* 2016). *Dickeya spp*. also secret cellulase and proteases to complement the pectinase activity to facilitate the breakdown of the cell wall components (potrykus *et al*., 2014). However, in *Dickeya* spp. most enzymes and genes involved in pectin degradation are not activated at the earlier (8 hpi) colonization step of infection (Chapelle *et al.,* 2015). Despite the high conservation of regulatory networks and similarities in degrading enzyme arsenal, the expression profiles of even closely related species (such as *D. solani* and *D. dianthicola* in infected potato tubers) or even more striking difference between more distant species*,* showes variation in the expression of virulence genes, including those encoding the PCWDE and

secretion systems, which results in widely contrasting aggressiveness (Khayi *et al*., 2015; Golanowska *et al*., 2018).

Bacteria belonging to to SRP secret and transport several virulence determinants from the bacterial cytosol into environment or directly into intercellular spaces of the plant tissue through one or a combination of type I-VI secretion systems (T1SS-T6SS) (Hugouvieux-Cotte-Pattat *et al.,* 2014). All *Dickeya* and *Pectobacterium* spp. employ the ABC-type PrtDEF type I secretion system (T1SS) to secrete proteases, whereas the Type II secretion system (T2SS), known as the Out system (T2SS Out system) is devoted to the delivered of most PCWDEs to the host cells (Charkowski *et al*., 2012). The T2SS Out system is also responsible for the transit of necrosis-inducing protein NipE and the two paralogues AvrL-AvrM proteins in interaction with plants. The type III secretion system (T3SS) has significant roles in hemibiotrophic plant pathogenic bacteria by contributing to secret effectors and transport virulence factors into the plant cell to induce the hypersensitive reaction (HR) and programmed cell death (PCD). Except for *DspE*, no additional T3SS effectors have been identified in SRPs. DspE has been shown to elicit plant cell death in leaf tissue at the beginning of the infection (Hogan *et al*., 2013). DspE and necrosis inducing protein Nip are responsible for full virulence (rather than pathogenicity) but there is no evidence that they function in the suppression of basal defense responses. In SRPs, T3SS secretion and induction of necrosis promote plant tissue maceration, providing nutrients for the multiplication and colonization of these necrotrophic pathogens (Hogan *et al*., 2013; Kim *et al*. 2011; Fan *et al.,* 2020). Therefore, the pathogenicity of SRPs does not rely on T3SS to supress defence mechanisms of the host plants. More diversity was observed for T4SS as a conjugation system able to transport nucleic acids in addition to effector proteins into host cells during infection via a cell contact-dependent way. It has been suggested to act as a virulence factor in *P. atrosepticum* (Bell *et al*., 2004). Both T5SS and T6SS participate in contact-dependent competition systems and secrete plenty of proteins to induce stress responses for host adaptation and bacterial survival. The role of T6SS effectors in bacterial pathogenicity has been associated with biofilm formation and antibacterial activity in several *Pectobacterium* and *Dickeya* spp. (Bellieny-

Rabelo *et al.,* 2019; Yu *et al*., 2021).

In planta, the virulence-related process involved in metabolism and cell wall composition is controlled by highly sophisticated regulatory networks, especially for PCWDEs-encoding genes. Quorum sensing (QS) as a crucial key in this network allows the expression of genes only at high population density (Charkowski *et al.,* 2012; Reverchon and Nasser, 2013). QS control system is mediated by the perception of signaling molecules *N*-acyl homoserine lactone (NAHSL) via the ExpI/ExpR proteins or achieved via the VFM system that leads to a coordinated gene expression in a bacterial population. The conserved role of VFM-QS in modulating the production of PCWDEs has been demonstrated in several species in the *Dickeya* genus (Lv *et al*., 2019; Potrykus *et al*., 2014). AHL-dependent QS system is placed at the top regulatory cascade of the virulence factor production of all *Pectobacterium* and some *Dickeya* spp., mainly during the late phase of infection when the population density reaches a certain threshold level (Hussain *et al*., 2008). In *Dickeya*, the global regulator PecS negatively controls the induction of genes encoding many identified virulence factors dedicated to primary metabolism, transport, and chemotaxis mainly during the early stage of infection, and plays an active role in the adaptation of bacterium to the epiphytic environment (Pédron *et al*., 2017).

For successful colonization, pathogens require many additional virulence elements, such as exopolysaccharides and biosurfactants that allow the adhesion of bacteria to the plant surface, and motility and chemotaxis that are essential for the bacteria when searching for favorable sites to enter into the plant apoplast, and also production of siderophores that are critical for successful infection due to their ability to acquire iron (des Essarts *et al*., 2019). To attach on the plant surface, *D. dadantii* produces cellulose fibrils that are embedded within exopolysaccharides to develop aggregates on the plant surface and cover the bacterial cell to maintain the hydrated surface around the bacteria, thus helping them to survive under desiccation conditions (Prigent-Combaret *et al.,* 2012). During early in the virulence stage, biosurfactants and the type V secretion system are activated in *D. dadantii* to promote wettability and surface colonization. Subsequently, flagella-mediated adhesion, and motility and chemotaxis play a pronounced role in searching out nutrients and

identifying sites for entry into the plant apoplast (Rio-Alvarez *et al.,* 2015). *D. dadantii* exhibits a strong chemotactic response to wound-induced jasmonic acid, favoring bacterial access to the wound and facilitating the systemic invasion of the plant (Antunez-Lamas *et al.,* 2009). Inside the xylem tissues, these exopolysaccharide fibers together with other bacterial structures (e.g., adhesin) and plant cell wall RG-I (a bacteria-induced release of pectic polysaccharides) lead to bacterial cell aggregation and biofilm formation to withstand water flow within the xylem and support bacteria against plant defense mechanisms, which is thought to be necessary for successful xylem colonization that is eventually followed by wilting and rotting. At some point while in the xylem, probably following the production of biofilm structures, exopolysaccharides-based multi-cellular structures called bacterial 'emboli' may be present, where bacteria begin to multiply and reach cell densities sufficiently high to trigger quorum sensing (Gorshkov *et al.,* 2016).

1.3. Interaction between plant and soft rot bacteria

1.3.1. General molecular basis of plant defense responses

In natural environments, plants are confronted with diverse microorganisms that interact within complex relationships from symbiosis to parasitism. Plants perceive the invading microbe by interactions between pattern recognition receptors (PRRs) on the cell surface and conserved molecular signature molecules known as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) such as flagella, lipopolysaccharides, and peptidoglycans or plant-derived signals arising from injured tissues called damage-associated molecular patterns (DAMPs). Plants organize a large number of receptor-like kinases (RLKs) and receptor-like proteins (RLPs) as PRRs to detect microbe- and host- derived molecular stimuli. Subsequently, recognition of endogenous or microbial elicitors triggers a genetically imprinted innate immune system, known as MAMPs/PAMP-triggered immunity (M/PTI) with an important role in preventing non-adapted microbes from infecting the host and in restricting infection of adapted pathogens in susceptible hosts (Pritchard and Birch, 2014). Over time, specific pathogens target immune receptors to prevent the perception of signaling components or secrete a broad arsenal of virulence

factors that aim to deactivate PTI and promote their virulence. In turn, plants use a repertoire of intracellular receptors belonging to the nucleotide-binding site and/or leucine-rich repeat domain (also identified as NB-LRRs), to recognize effectors and initiate a second line of defense known as effector-triggered immunity (ETI), which involves stronger and longer-lasting responses than the first line of defense, PTI. During ETI, induction of genes encoding specific disease resistance (R) proteins triggers HR that leads to localized PCD to restrict the growth and colonization of biotrophic and hemibiotrophic pathogens (Dodds and Rathjen, 2010). This dead plant tissue could instead be beneficial for necrotrophic pathogens by preparing more nutrients for pathogen multiplication.

In general, PTI and ETI commonly induce a similar downstream immune response but vary in magnitude. Thus, many cellular events are attributed to both PTI and ETI, including the production of ROS, rapid ion flux across the plasma membrane and increasing cytosolic Ca^{2+}, activation of the mitogen-activated protein kinases (MAPKs) cascades, induction of phytohormone-mediated defense networking, and the synthesis of phenolic compounds (Lu and Tsuda, 2021; Mengiste, 2012). Perception of M/DAMP stimulates an influx of Ca^{2+} ion as a second messenger that participates in signaling pathways, which in turn activate downstream protein kinases, promote immediate gene expression and protein synthesis to constitute one of the earliest immune responses to enable plants to adapt to environmental changes in their surroundings. These processes are implemented via several Ca^{2+} sensing proteins such as calmodulins, calmodulin-like proteins, calcineurin B-like proteins, calcium-dependent proteins kinases, and calcium channels to elicit appropriate defense responses (Aldon *et al.,* 2018). Plant immunity activates a signal transduction pathway mediated by cytoplasmic MAPK cascade with a critical role in the PTI pathway by transcriptional regulation of downstream basal defenses leading to stimulation of WRKY-type transcription factors, generation of chloroplast-derived ROS; and modulation of synthesis and signaling of defense-related hormones (Zhang and Zhang, 2022).

1.3.2. Context of plant defense responses against bacterial soft rot

Due to the limited data available, interpretation of plant response to *D. solani* has been described using *Pectobacterium* and other *Dickeya* species such as *D. dadantii* on different hosts. During the biotrophic latent phase of SRP infection, bacteria invade the host plant without causing any damage and the bacterial population has not reached a density that can be recognized by the host plant. No resistance genes (R genes) were identified for *Pectobacterium and Dickeya* spp. that could recognize type III-effectors and mediate ETI, unlike observed in other bacteria with T3SS-dependent systems such as *Pseudomonas* or *Xanthomonas* spp. (Davidsson *et al.,* 2013; 2017). By switching in the mode of action of bacteria as a brute force pathogen, putative signals trigger host recognition leading to activation of the plant defense responses. Besides the production of necrosis-inducing toxins, bacterial structures such as conserved fig22 domain of flagellin and elongation factor Tu (EF-Tu), which are recognized by the plant receptors flagellin sensing 2 (FLS2) and EF-TU receptors, respectively; are active PAMPs during plant-SRP interactions. Specifically, plant cell wall fragments released by the action of PCWDEs secreted by SRPs are the major elicitors, because they are recognized as DAMPs to activate PTI immunity toward these pathogens (Mengiste, 2012; Davidsson *et al.,* 2017; Expert *et al.,* 2018).

The most obvious DAMP signals are the oligogalacturonides (OGs) produced by the degradation of the pectic polysaccharide homogalacturonan and methanol released from the pectin demethylation by bacterial pectin methylesterase, as well as molecules arising from necrotic cells such as cutin monomers and cellulose oligomers, that were shown to be potent elicitors of plant defense in monocots and dicots (Hou *et al.,* 2019). Likewise, exposure to different abiotic stresses or mechanical wounding may change the integrity, fluidity, and membrane functions that lead to generation of DAMPs. In Arabidopsis, wall-associated kinases 1 (WAK1) and WAK 2 as receptors perceive the OGs of several necrotrophs and monitor pectin degradation by PCWDE activity. Fragments of OGs vary in degree of polymerization and unsaturation depending on the pectinolytic enzyme activity. Long OGs are predominantly released during the early stages of infection and perceived by WAK receptors.

In contrast, short OGs exhibit less efficacy as elicitors of plant defense which do not trigger high oxidative stress and probably improve resistance with different mechanisms in signaling and perception than long OG (Davidsson *et al.,* 2017). For example, potato receptor-like protein kinases (PRK1-4) has been identified as a likely receptor for short OG perception in response to *P. carotovorum* (Montesano *et al.,* 2001). Induction of genes encoding WAK2 and WAK4 in response to OG molecules were involved in PTI immunity as a central plant defense process of Chinese cabbage against *P. carotovorum* spp. *carotovorum* (*Pcc*). The other genes involved in PTI response were MAPK signaling cascade (MPK3, and MPK4), calcium-binding protein, calcium-dependent protein kinase, respiratory burst oxidase homolog (RBOH), and defense signaling transcription factor WRKY33 (Liu *et al.,* 2019). The defense response was investigated by comparison of transcriptomes between susceptible cultivar and tolerant cultivar of potato plant varieties, during the early stages of infection with *P. brasiliense* (Kwenda *et al.,* 2016). Several differentially expressed genes associated with PRRs in pathogen recognition, particularly those encoding FLS2 and EF-Tu proteins, WAK1, and receptor-like kinases BAK1 were shared between tolerant and susceptible cultivars, emphasizing that both cultivars employ similar sets of genes in pathogen recognition and wounding response. However, some upregulated genes were observed only in the tolerant cultivar, possibly contributing to strong PTI defense signaling pathways. The results also revealed that induction of the MPK3/MPK6 cascade in the early stage of infection in tolerant cultivar was accompanied by enrichment of the WRKY33 transcription factors with function in the activation of downstream defense genes, which may be key components of the potato immune response. Plant immune responses during the *D. dadantii*-Arabidopsis interaction appear to be a complex process including multiple lines of defense, such as competition for iron acquisition in Arabidopsis (Segond *et al.,* 2009), early production of extracellular ROS associated with plant cell wall reinforcement, modulation of plant cell death for limitation of disease progression and activation of plant defense marker genes associated with hormonal signaling pathways (Fagard *et al.,* 2007; Asselbergh *et al.,* 2008; Kraepiel *et al.,* 2011; Van Gijsegem *et al.,* 2017; 2018). Although *Pectobacterium* and *Dickeya* spp. are considered as

brute force pathogens, whose virulence characteristics are often sufficient to overcome plant immune responses, the outcome of their interaction with the host depends on the physiological status of the plant and on the external environmental conditions that determine whether the host reaction is a component of resistance, tolerance, or susceptibility.

1.3.2.1. Oxidative burst: Early plant response to soft rot pathogen

In plant, the intracellular ROS is typically generated via photorespiration and various metabolic processes and are utilized in the regulation of several physiological and developmental processes, as well as response to various abiotic and biotic stresses. As a downstream marker of PTI, low level of ROS functions as signaling molecule for initiation of plant resistance associated with various processes, such as cell wall protein cross-linking, defense gene activation, SA-related responses, and synthesis of phytoalexins, while a high level of ROS leads to cell death via oxidative damage (van Gijsegem *et al.*, 2017). The most well-known enzymes in control of apoplastic ROS production are the plasma membrane-localized NADPH oxidases RBOHs and the cell wall localized peroxidase III.

Oxidative burst is a common and powerful response that plays multifunctional role in defense mechanisms, mainly at early infection phase between 9 and 24 hpi (Fagard *et al.,* 2007; Van Gijsegem *et al.,* 2017). Production of ROS is initiated through the activation of RBOHD and peroxidases III in Arabidopsis (Survila *et al.,* 2016) and tomato (Asselbergh *et al.,* 2008) upon inoculation with *P. parmentieri* or *D. dadanti*, respectively. Mutants lacking these components show heightened susceptibility to the bacteria, providing confirmation of the defensive role played by ROS. Following infection by *P. carotovorum*, the plant recognition of cell-wall derived DAMPs, such as OGs, induced expression of several class III peroxidases. Increased peroxidase activity leads to the downregulation of genes involved in cuticle biosynthesis and subsequent increased cuticle permeability and resistance to the necrotrophic bacteria, which is at least partially independent of SA and JA signaling pathways. However, abscisic acid (ABA) appears to function as a major regulator in this mechanism by removal of the ROS via increased

peroxidase activity leading to cuticle biosynthesis (Survila *et al.,* 2016). Another signaling role of ROS in plant defense responses has been highlighted by Arabidopsis *bos1* mutant, where the *D. dadantii*-induced systemic necrosis at late time points around the maceration zone is associated with a strong extracellular oxidative burst, which appears to be an efficient defense mechanism against pathogen spread and survival. This necrosis is modulated by the earlier produced ROS in infected tissues which is associated with strengthening of the plant cell wall by protein cross-linking (Kraepiel *et al.,* 2011). In addition, the production of hydrogen peroxide as a substrate of peroxidases in the plant cell wall generates covalent links of cell wall proteins and lignin resulting in the strengthening of physical barrier to prevent bacterial invasion during infection by *D. dadantii* in tomato and Arabidopsis (Asselbergh *et al.,* 2008; Kraepiel *et al.,* 2011). However, ROS accumulation in xylem vessels upon *P. atrosepticum* infection does not hamper the bacterial cell and may be regarded as part of the susceptible response of the plant due to the promotion of ROS-mediated plant cell wall loosening (Gorshkov *et al.,* 2016). Although high ROS concentration exerts antimicrobial activity against bacteria in vitro, a direct antimicrobial effect on *D. dadantii* during infection has not been observed (Miguel *et al.,* 2000). This may be related to the antioxidant machinery that has been developed in SRP, which in turn acutely modifies the redox state of the host at infection sites to promote pathogenesis through the production of the extracellular ROS scavenging, such as the production of superoxide dismutase, or antioxidant molecules, such as indigoidine in planta by *Dickeya* spp (Hugouvieux-Cotte-Pattat, 2016).

1.3.2.2. Hormonal regulation in host response to soft rot bacteria

Following MAMPs and DAMPs perception, the plant immune system orchestrates a network of intricate and interconnected hormonal signaling pathways. Plant hormones SA, JA, and ET are the primary signaling molecules that activate immune responses in plants and determine the outcome of plant-pathogen interaction. Beyond these, other hormones, such as auxins, ABA, gibberellins and cytokinins, are considered as modulators in plant–pathogen interactions, acting as either positive or negative regulators of disease resistance

by regulating key physiological processes, responding to abiotic stresses, and influencing growth and development (Yang *et al.,* 2015).

1.3.2.2.1. Role of salicylate, jasmonates, and ethylene in plant defense response

SA signaling typically governs plant defense against hemibiotrophic pathogens, while JA- and ET-dependent signaling pathways are activated in response to wounding and required for resistance to necrotrophic pathogens (Pieterse *et al.,* 2012). In Arabidopsis, JA signaling can be divided into two distinct branches known as MYC and ERF, exhibiting antagonistic crosstalk within the JA defense pathway (Glazebrook*,* 2005). The ERF-branch predominantly responds to necrotrophs, whereas the MYC-branch is primarily activated in response to wounding and herbivorous insects (Pieterse *et al.,* 2012). Antagonistic crosstalk between the SA and JA pathways has been observed in various pathosystems. SA-mediated suppression of the JA pathway relies on regulatory proteins like pathogenesis-related protein 1 (PR1), NPR1, nuclear TGA, WRKY, and MAPKs. WRKY70 plays a pivotal role in balancing SA-dependent and JA-dependent signaling for defense against *Pcc*, while WRKY75 positively regulates JA- or SA-dependent defense responses to *Pcc* (Choi *et al.,* 2014).

The comparison and characterization of the immune response to SRP in most studied pathosystems revealed an increase in endogenous level of both SA and JA/ET. However, the activation of genes in the SA-mediated pathway is primarily detected in response to *Dickeya* infection rather than *Pectobacterium*. The predominance of each hormone in different pathosystems depends on factors such as the host's susceptibility, the pathogen species and its virulence, incubation conditions, and the timing of sampling (Alvarez *et al*., 2016; Burra *et al.,* 2015; Fagard *et al.,* 2007; Liu *et al.,* 2019). In the model plant *A. thaliana,* in response to *D. dadantii*, JA induces an effective plant defense response that does not involve SA (Fagard *et al.,* 2007; Van Gijsegem *et al.,* 2018). The JA biosynthesis mutant *jar1* demonstrated increased susceptibility to *D. dadantii* infection, while the SA-deficient sid2 mutant showed susceptibility similar to the wild type (Fagard *et al.,* 2007). Both SA and JA pathways played a significant role in the induction of plant immunity in

Nicotiana benthamiana against *D. dadantii* (Pérez- Bueno *et al.,* 2015). A study on potato transgenic lines conducted by Burra et al. (2015) revealed that SA-deficient mutants (NahG) and JA signaling-deficient coi1 mutants were both more sensitive to *D. solani* infection, indicating the importance of SA and JA pathways in resistance against this aggressive bacterium. In addition, treating potato plants with SA was found to enhance their resistance to *D. solani* (Czajkowski *et al.,* 2015b). The role of SA-mediated defenses in resistance against *Pectobacterium* varies depending on studied pathosystem. In some Arabidopsis studies, SA demonstrated efficacy as a defense response against *P. carotovorum* (Kariola *et al.,* 2003; Liu *et al.,* 2019). Analysis of Arabidopsis responses to *P. parmentieri* revealed two distinct signaling pathways. The first route requires activation of ERF branch of JA pathway that is enhanced by SA and leads to induction of JA and ET marker genes, whereas in second route triggers JA responses that is repressed by SA (Norman-Setterblad *et al.,* 2000). In Arabidopsis, systemic resistance and cell death were induced after *P. parmentieri* infection, accompanied by the expression of marker genes PR1 and PDF1.2 involved in SA- and JA-dependent defense signaling, respectively (Kariola *et al.,* 2003). In other plants like tobacco, the application of SA was demonstrated to enhance resistance to *P. parmentieri* (Vidal *et al.,* 1997). Subsequent studies suggested a more direct role for the SA hormone in reducing *Pectobacterium* virulence, potentially by inhibiting the production of CWDEs through direct interference with the QS machinery (Joshi *et al.,* 2015; 2016a; 2016b).

Several studies have provided substantial support for the role of JA/ET signaling in resistance to SRP. Measurement of host tissue hormonal content after inoculation with soft rot bacteria consistently revealed an increase in JA concentration (Montesano *et al.,* 2005; Kariola *et al.,* 2005; Norman-Setterblad *et al.,* 2000; Chen *et al.,* 2020), and transcriptome profiling demonstrated the upregulation of JA biosynthesis or signaling genes in infected plants (Gorshkov *et al.,* 2022; Tsers *et al.,* 2020; Gorshkov *et al.,* 2018; Chen *et al.,* 2020; Liu *et al.,* 2019; Kwenda *et al.,* 2016). Studies using JA-insensitive and JA-deficient mutants of Arabidopsis, as well as ET-insensitive mutants of Arabidopsis and tobacco, consistently indicated increased susceptibility to SRP infection,

emphasizing the efficacy of JA-dependent defense against SRP (Catinot *et al.,* 2015; Geraats *et al.,* 2003; Fagard *et al.,* 2007; Norman-Setterblad *et al.,* 2000).

Additionally, exogenously applied ET and JA were shown to enhance the accumulation of plant defense-related transcripts, indicating the role of these two signaling molecules in potato defense against *P. parmentieri* (Montesano *et al.,* 2005). In calla lily (*Zantedeschia aethiopica*) leaves, MeJA application significantly inhibited *P. carotovorum* disease development compared to other chemicals related to the SA pathway (Bion and β-aminobutyric acid, BABA). Furthermore, MeJA was able to reduce disease symptoms for a longer period, suggesting that JA/ET-signaling pathway is required for long-lasting protection (Luzzatto *et al.,* 2007a; 2007b). Priming of plant defence response of *Z. aethiopica* to *P. carotovorum* was investigated by application of either MeJA or SA analog benzothiadiazole (BTH) before pathogen infection. The results clearly indicated the involvement of JA in priming the defence response, leading to the upregulation of several proteins, including MAPK, methyltransferases, S-adenosyl methionine synthase, thioredoxin and oxidoreductases (Luzzatto-Knaan *et al.,* 2014). Moreover, JA is responsible for inducing synthesis of secondary metabolites, which reduce fitness of soft rot-causing bacteria (joshi *et al.,* 2015; Luzzato *et al.,* 2007b).

In Chinese cabbage, resistance against *Pcc* was enhanced by the induction of JA- and ET-mediated genes, while no gene was triggered in SA signaling. Treatment with a single application or combinations of JA and ET induced resistance in *Pcc*-infected leaves, and the assessment of phytohormone production revealed increased levels of JA and ET, while SA production was inhibited. The hypothesis put forward suggested that the SA-dependent pathway might not play a role in resistance to *Pcc* infection, whereas JA and ET could act synergistically to defend against infection (Chen *et al.,* 2020). These findings contrasted with the results of Liu et al. (2019), who proposed that hormonal signals required both SA- and JA/ET-dependent pathways to induce resistance response against *Pcc*. Moreover, a study on the molecular basis of resistance in potato stems infected by *P. brasiliense* revealed enrichment of ethylene biosynthesis and signaling pathways in the tolerance-specific gene category with upregulation of EBF1, EIL3, and ACC synthase and oxidases. The

expression of downstream defense-related genes, specifically ERF1 and ERF2, was induced to a higher level in tolerant potato cultivars compared to susceptible cultivars. ERFs served as a control point for integrating signals from both JA and ET pathways to regulate defense response genes. In susceptible cultivars at the late stage of infection, induction of MYC2 occurred along with downregulation of genes involved in secondary cell wall synthesis, such as cellulose and lignin biosynthesis (Kwenda *et al.,* 2016).

In general, the production of PCWDEs by *Pectobacterium* and the release of OGs activate genes associated with JA and ET pathways, as well as responses to wounding and innate immunity (Davidsson *et al.,* 2013). This perspective aligns with studies demonstrating the central role of JA and other oxylipins in defense responses following tissue damage caused by pathogens or wounding (Davidsson *et al.,* 2017). The accumulation of JA and its precursors OPDA occurs upon wounding and activates signaling toward undamaged roots (distal site). Adding complexity to the issue, Antunez-Lamas et al. (2009) reported a "dual role" for JA—while acting as a strong chemoattractant guiding *D. dadantii* to entry the apoplast through finding wounded tissues. However, other studies stated that JA does not confer true plant resistance to SRP but reflects the plant susceptibility response to facilitate brute force behavior of pathogen (Gorshkov *et al.*, 2018; Tsers *et al.*, 2020). According to this theory, JA acts as a switch for the transition from a latent asymptomatic phase to a typical symptomatic soft rot infection (Gorshkov *et al.*, 2018; Tsers *et al.*, 2020).

1.3.2.2.2. Role of abscisic acid and auxin in the hormonal network

The phytohormone ABA, known as a global regulator of abiotic stress adaptation, orchestrates various physiological processes to ensure plant fitness and survival under adverse growth conditions. Additionally, ABA plays a multifaceted role in plant immunity (Vishwakarma *et al.,* 2017). Numerous pathosystems have confirmed the role of ABA in susceptibility to bacterial diseases. In the post-invasive defense phase, ABA negatively affects the plant's response by inhibiting the accumulation of ROS, callose deposition, and the expression of PAMP-induced genes stimulated by bacterial pathogens (Mohr and Cahill, 2007; de Torres Zabala *et al.,* 2009). Nevertheless, ABA can act as a

signal for plant resistance to necrotrophic pathogens. During the early stages of defense, ABA promotes physical defense barriers, such as stomatal pre-invasive resistance to bacteria and possibly callose deposition in papillae in response to fungal pathogens (Ton *et al.,* 2009; Lievens *et al.,* 2017; Asselbergh *et al.,* 2008). Therefore, the contrasting effects of ABA in plant basal defense depend on the infection phase and the lifestyle of the pathogen (Cao *et al.,* 2011). There is substantial evidence confirming the effect of ABA on the outcome of host-SRP interactions. Exogenous ABA application rendered the host susceptible to these pathogens (Asselbergh *et al.,* 2008). In this context, resistance to *Pectobacterium* and *Dickeya* spp. is conferred by the disruption of ABA biosynthesis in ABA-deficient mutants, while ABA-overproducing plants exhibit an activated ABA response and increased susceptibility to soft rot pathogens (Van Gijsegem *et al.,* 2017). The direct effect of ABA on plant susceptibility in tomato and Arabidopsis appears to result from its negative control over H2O2-producing peroxidases (Asselbergh *et al.,* 2008). ABA inhibits the oxidative stress triggered by *D. dadantii* and reduces resistance to infection without involving JA signaling (Van Gijsegem *et al.,* 2017). Alteration in cuticle integrity leads to ROS production and allows a faster defense reaction to pathogen elicitors (L'Haridon *et al.,* 2011). Furthermore, ABA facilitates the multiplication and spread of *D. dadantii* within its hosts (Asselbergh *et al.,* 2008; Pérez-Bueno *et al.,* 2015). *D. dadantii* infection increases transcript abundance in the last step of ABA-biosynthesis and triggers ABA accumulation in Arabidopsis leaves, suggesting that ABA is a susceptibility factor for bacteria. The bacteria hijack the ABA biosynthesis to inflict disease as part of its virulence strategy. *D. dadantii*, like some other bacteria, has developed virulence strategies by manipulating ABA homeostasis in the plant through the secretion of type III virulence factors in the early stages of infection (Van Gijsegem *et al.,* 2017; 2018). As reported for *P. syringae*, ABA promotes stomatal closure as its defensive role against *D. dadantii* by inhibiting pathogen entry following early MAMPs recognition in *N. benthamiana* leaf (Pérez-Bueno *et al.,* 2015). This ABA-activated resistance to SRP is controlled by SA signaling. In this regard, Po-Wen et al. (2013) reported that non-protein amino acid BABA may also directly boost Arabidopsis stomatal immunity to *Pcc* by activation of ABA and

SA-mediated defense response. BABA-induced resistance against both hemibiotrophic and necrotrophic pathogens is correlated with a primed PTI response and activation of both JA and SA pathways to achieve a full stomatal closure response to infection (Ren *et al.,* 2022).

In many pathosystems, ABA regulates plant defense against pathogens through the modulation of plant hormone signaling pathways in a complex manner. Therefore, synergetic crosstalk of ABA with JA exhibits a complex antagonistic relationship with SA disease development (García-Andrade *et al.,* 2020; Ton *et al.,* 2009; Mohr and Cahill, 2007). In Arabidopsis, the drought-responsive gene early responsive to dehydration 15 (ERD 15) is one of the key negative regulators of ABA responses. Transgenic plants with overexpression of ERD15 reduced responsiveness to ABA and led to enhanced SA-dependent resistance to *Pectobacterium*, while *ERD15* silencing resulted in hypersensitivity to ABA and reduced stomatal conductance. These results describe a strong antagonistic effect of ABA on SA signaling, but it does not appear to interfere with JA signaling (Kariola *et al.,* 2006). A recent study highlighted the involvement of auxin and ABA in the regulation network of plant defense response towards *Pectobacterium* infection in Chinese cabbage (Chen *et al.,* 2020).

Auxin could be considered a virulence factor in SRP and potentially establishes favorable physiological conditions for disease progression, as bacterial mutants lacking auxin production genes exhibit reduced aggressiveness (Yang *et al.,* 2007). Some SRP harbor *iaaH* and *iaaM* genes in their genome, allowing them to produce auxin in planta after bacterial invasion. This auxin production is thought to enhance bacterial fitness by reducing plant defense efficiency. The interaction between Arabidopsis and SRP highlights the role of auxin status, with auxin-hypersensitive mutants and mutants deficient in a negative regulator of auxin transport being more susceptible to *P. carotovorum* (Piisilä *et al.,* 2015). In addition, SA, JA, and ET induce expression of the GLIP2 gene, which is a negative regulator of auxin responses and actively participates in the defense mechanisms against *P. carotovorum* (Lee *et al.,* 2009). Furthermore, auxin has been shown to stimulate cell wall loosening by inducing the expression of plant pectate lyases in the context of cell elongation,

facilitating penetration and nutrient availability for SRP (Sun *et al.,* 2018).

1.3.2.3. Metabolic regulation in host defense response to soft rot pathogen

Primary metabolites play a pivotal role in the plant's adaptive defense response against SRP. The competition for iron uptake between the plant and bacterial pathogens is a component of nutritional immunity, a mechanism employed by the host to inhibit bacterial growth by restricting nutrient availability post-infection (Herlihy *et al.,* 2020). Regulating iron homeostasis is a critical factor in establishing plant innate immunity against SRP. Accordingly, infection of Arabidopsis leaves with *D. dadantii* triggered an iron-deficiency signal and iron starvation that led to the accumulation of transcripts related to iron-acquisition genes in the roots (Segond *et al.,* 2009). These findings indicate that Arabidopsis competes with *D. dadantii* for iron acquisition during infection. A similar immune-stimulatory effect was linked to the iron-sequestering activity of the Arabidopsis PDF1.2 protein in response to *P. carotovorum* invasion. Arabidopsis lines with overexpressing PDF1.2 exhibited more resistance to the pathogen (Hsiao *et al.,* 2017). The host can also use iron as a modulator of oxidoreductase activities in ROS production and photosynthetic metabolism.

The host's defense response is a a high energy demand process that appears to impose a fitness cost. It has been suggested that infected plants save energy by downregulating processes, such as photosynthesis, normally vital for plant growth and fertility. The diverted energy is then used for defense responses (Rojas *et al.,* 2014). In *D. dadantii-Nicotiana benthamiana* pathosystem, the reduction of oxygen-evolving complex components disrupts photosystem II efficiency, leading to an increase in the capacity of energy dissipation or reversible non-photochemical quenching. These responses are likely part of the plant's defense mechanism to limit carbon source availability for pathogens, redirect resources to secondary metabolite production, and potentially contribute to the accumulation of key hormones regulating biotic stress responses, including ABA, JA, and SA (Pérez-Bueno *et al.,* 2015). Moreover, primary metaboliets as a source of signaling molecules can directly or indirectly trigger defense responses. In the *A. thaliana-P. syringae* pathosystem,

numerous genes involved in glycolysis, tricarboxylic acid cycle, and the oxidative pentose pathway are upregulated during plant defense responses. Notably, only specific genes, such as cell wall invertase and hexokinase 1, are induced in defense responses, leading to the initiation of HR and the activation of PR genes. Interestingly, the upregulation of genes encoding PR proteins has been observed in tobacco and Arabidopsis even in the absence of a pathogen and in response to the presence of glucose, fructose, and sucrose (Rojas *et al.,* 2014).

Modifications in plant metabolism during infection by *Pectobacterium* and *Dickeya* spp. are frequently documented as part of the host's defense response. Several secondary metabolites have great potential to suppress specific virulence determinants and act as quorum-quenching compounds that disturb cell-to-cell communication (Alvarez *et al.,* 2016; Joshi *et al.,* 2015; 2016a). Conversely, some plant secondary metabolites can act as virulence inducers for SRP such as inducers of the virulence-related genes *hrpL* and *rsmB* for *D. dadantii* (Yang *et al.,* 2008). Therefore, the synthesis and composition of secondary metabolites are the determining factors in the overall dynamics of plant-microbe interactions. In another study, a genome-scale transcriptome analysis of Arabidopsis during the early stages of *D. dadantii* infection revealed the induction of genes involved in the biosynthesis of tryptophan, indole glucosinolates, and the JA biosynthesis and signaling pathway (Van Gijsegem *et al.,* 2018). Another aspect of host defense is the activation of the phenylpropanoid pathway involved in the generation of various chlorogenic, flavonoid, and phenolamide compounds. These compounds exhibit antimicrobial properties and serve as signaling molecules in response to cell wall breakdown by pathogens. The quantitative defense response is closely linked to the production levels of these phenolic compounds among different host genotypes (Yadav *et al.,* 2020). Some key players like phenylalanine ammonia lyases (PALs) in this pathway are proposed to act in cell wall mediated immunity and in broad-spectrum disease resistance. The total content of chlorogenic acids, flavonoids, and phenolamides increases in response to infection by Pcc and enhances resistance levels in potatoes (Kröner *et al.,* 2011). Additionally, two naturally occurring antimicrobial components, α-solanine

and α-chaconine, constitute 95 % of the glycoalkaloid content in potato tubers, with the highest concentrations in the periderm and buds. Exposure of the plant to abiotic and biotic stresses can elevate glycoalkaloid content and improve its resistance response (Okamoto *et al.*, 2020). Furthermore, the glycoalkaloid produced by potato tubers during greening has been tested as a method of blackleg control. The effect of glycoalkaloids extracted from four different potato cultivars were examined for bacteriostatic and bactericidal effects on Dickeya and Pectobacterium strains in growth media. Glycoalkaloids of different cultivars had different inhibitory effects, but almost all plants grown from green seed tubers exhibited a lower incidence of blackleg without changes in yield (Soltys-Kalina *et al.*, 2023).

The wild potato, *Solanum chacoense*, is known for its partial resistance to soft rot pathogens. Stems and tubers of this wild potato exhibit unique chemical properties with bactericidal, bacteriostatic, or antivirulence activities that confer resistance to SRP. Interestingly, extracts from the stems and tubers contain phenolic amines, phenols, peptides, and alkaloids, demonstrating a significant reduction in pectinase, cellulase, and protease activities in *P. brasiliense* (Joshi *et al.,* 2021). In another study, the antimicrobial properties of compounds such as cinnamic, coumaric, and syringic acids, SA, and catechol—derived from the plant's phenylpropanoid pathway—were screened for their effect on virulence determinants such as motility, biofilm formation, and extracellular enzyme activity in various *Pectobacteria*. The results indicated that these small plant molecules can markedly reduce disease severity and exert a direct reducing effect on *Pectobacterium's* virulence such as pectolytic and proteolytic exoenzyme activities (Joshi *et al.,* 2015).

1.3.2.4. Plant cell wall modification in response to soft rot pathogen

In the course of infection, plant cell wall modification represents a "double-edged sword" directing both the increase and reduction of barrier properties of this cellular compartment. On one hand, the plant host undergoes various modifications to the primary cell wall, such as the deposition of callose, insolubilization of hydroxy-proline-rich glycoproteins, and the accumulation of cross-linking proteins. These changes result in a strengthened cell wall,

rendering the polymers unavailable to microbial PCWDEs, contributing to plant defense mechanisms (Fagard *et al.,* 2007; Asselbergh *et al.,* 2008; Kraepiel *et al.,* 2011). Similarly, the secondary cell wall, a complex and dynamic barrier during pathogen interaction, plays a crucial role in host resistance. The accumulation of components like hemicellulose, xylan, lignin, and suberin in the secondary cell wall architecture is required for maintaining cell wall integrity and supporting the defense mechanism. On the other hand, some *Pectobacterium* and *Dickeya* spp. manipulate specific plant enzymes and proteins involved in "natural" cell wall loosening processes that typically occur during growth extension or fruit ripening, such as pectin methylesterases (Raiola *et al.,* 2011) to facilitate bacterial invasion. Therefore, SRP induce infection-promoting alterations in plant cell walls, using not only their own enzymes and proteins but also exploiting those of the host. These CWDEs contribute to susceptibility response to pathogens. The overexpression of the gene encoding polygalacturonase-inhibiting protein 2 (PGIP2), which inhibits the degradation of the plant cell wall by binding to polygalacturonase, has been shown to enhance resistance to *Pcc* infection in resistant cultivar of Chinese cabbage (Liu *et al.,* 2019; Hwang *et al.,* 2010). Moreover, a decrease in the expression of genes related to secondary cell wall biosynthesis, including cellulose synthase and lignin biosynthesis, was predominantly observed in susceptible potato cultivars in response to the necrotrophic pathogen. Transcription factors like NAC domain proteins and MYB83 in secondary cell wall were downregulated in response to *P. brasiliense* in susceptible potato tubers but induced in tolerant cultivars (Kwenda *et al.,* 2016).

Lignin functions in maintaining the plant cell wall integrity and preventing the loss of water and nutrients. As the final product of the phenylpropanoid pathway, lignin is closely correlated with host resistance to pathogen invasion. The process of lignification and the formation of lignols in the middle lamella are activated 4-8 hours after infection by soft rot pathogens, leading to wound healing. The expression of genes encoding PALs and other phenylpropanoid-related genes involved in lignin biosynthesis, such as cinnamoyl CoA reductase (CCR), caffeoyl-CoA O-methyltransferase (CCoAOMT), and cinnamyl alcohol dehydrogenase (CAD) were increased in host resistance response against *Pcc*

(Liu *et al.,* 2019). Additionally, the cuticle and wax play a critical role in establishing the first barrier to restrict pathogen entry and provide mechanical support to protect plants against injuries and abiotic stresses. Underground plant tissues, including roots and tubers, as well as damaged areas and secondary growth of stems, are covered by the periderm. The cuticle and periderm share similar main compositions, including polysaccharides, embedded waxes (soluble lipids), and insoluble polyester as cutin in the cuticle or as suberin in the periderm (Li and Chen, 2009).

1.4. Control strategies against soft rot pathogens in potato

1.4.1. Prophylactic strategies and genetics selection for resistance

The control of plant diseases caused by SRP is highly challenging, primarily due to the widespread contamination, the absence of commercially effective control agents, and the lack of resistant cultivars in agriculturally important crops (Charkowski, 2015). Until now, there are no control methods available for eradicating SRP post-infection. Contact with infected plant material and contaminated machinery during harvest and post-harvest activities has been identified as a factor that increases infection rates and reduces yield (Pérombelon, 2002). As a result, current integrated management strategies largely rely on preventive and curative measures across multiple layers to reduce the incidence of SRP-induced diseases.

Application of good hygiene practices prevents the entry and spread of the bacteria, such as washing and disinfecting machines used during planting, spraying, haulm killing, harvesting, and grading, as well as avoiding of tuber wounding (Czajkowski *et al.,* 2011a; Gill *et al.,* 2014). Given that SRP can be seed-borne pathogens, the use of pathogen-free propagative material produced from axenic planting culture is a key strategy to limit cross-contamination within and between seed lots (Czajkowski *et al.,* 2011a). While the initial use of clean, SRP-free seed tubers in the early stages of potato multiplication has led to a reduction in blackleg and soft rot incidences below the threshold, challenges persist. Pathogen-free mini tubers, despite being initially clean, are often infected through unknown sources, leading to contamination in subsequent field generations. The effectiveness of this method is influenced significantly by

prevailing environmental conditions (van der Wolf *et al.,* 2017; Charkowski, 2015). Within the framework of the seed tuber certification scheme, the examination of potato seed quality is inspected to limit the risks associated with the use of infected planting material. Typically, screening relies on a visual assessment of the crop (Frost *et al.,* 2013). However, the efficiency of this control measure varies due to latent infections occurring at different stages of potato seed reproduction. Most protocols are unable to detect latent infections in progeny tubers from asymptomatic plants. Consequently, some countries employ laboratory tests, including ELISA, PCR, qPCR, and Next Generation Sequencing (NGS), to detect and manage latent or late infections. In addition to laboratory testing, application of adequate agricultural practices proves to be a highly effective strategy in reducing contamination and preventing disease outbreaks. These practices include crop rotation, proper field drainage to avoid waterlogging, provision of proper plant nutrition, and regular field inspections during vegetation to monitor pathogen spread (Ansermet *et al.,* 2016; Czajkowski *et al.,* 2015a).

Despite these measures, SRP can persist in the soil even in the absence of a host or plant debris for a few months. Survival is prolonged when connected to plant material, with soil characteristics such as moisture, temperature, nutrient status, organic matter, and microbial composition influencing pathogen persistence (Czajkowski *et al.,* 2011a; Moh *et al.,* 2011). Maintaining balanced nutrition is crucial as it impacts plant defense status; for instance, high levels of nitrogen or magnesium correlate with lower disease incidence, while high calcium enhances resistance/tolerance response against SRP (Gill *et al.,* 2014; Ngadze, 2018). Volunteer plants during rotation or certain weeds can also infect potato crops, emphasizing the importance of annual and perennial weed control to prevent pathogen prevalence (Charkowski, 2018).

Various physical and chemical treatments have been applied to improve the management and selection of healthy potato tubers prior to culture or storage, aiming to reduce the population of SRP in latently infected tubers. Physical treatments, such as hot water, steam-dried hot air, UV radiation, and solar radiation, provide some level of control, however, they are expensive, time-consuming, and unable to kill soft rot bacteria living deep in the vascular system

or lenticels (Czajkowski *et al.,* 2011a). These methods are also impractical for large-scale potato tuber disinfection (Charkowski, 2015). Improved storage management practices, such as drying harvested tubers and storing them in well-ventilated atmospheres at low temperatures (below 10 °C), can prevent SRP multiplication and the spread of soft rot diseases during storage (Elphinstone *et al.,* 2018). Chemical compounds with bactericidal or antiseptic properties, including antibiotics, inorganic or organic salts, or their combinations, have presented effectiveness in controlling SRP infection (Czajkowski *et al.,* 2013). However, they are no longer recommended because of their high impact on environment and human health as well as the risk of selecting multidrug-resistant bacterial strains. Essential oils extracted from plants, such as those containing aromatic volatiles like carvacrol and eugenol, have shown promise in reducing the incidence and severity of post-harvest soft rot (Schollenberger *et al.,* 2021; Hajian-Maleki *et al.,* 2019). Some aromatic volatiles, including carvacrol and eugenol act through the QS machinery to inhibit specific virulence factors in SRP and have great potential for soft rot control (Joshi *et al.,* 2016a).

Modern potato varieties have a narrow genetic base (originate from a limited number of potato clones) may lack immunity against SRP, although tolerance to soft rot pathogens is widely different among potato cultivars (Lebecka, 2017; Czajkowski *et al*., 2011a). Chung et al. (2013) examined the resistance of 65 cultivars and 13 breeding lines of potato under storage conditions, which indicated a partial resistance of three cultivars, Freedom Russet, Anett and Alaska Red Eye to soft rot disease. Developing cost-effective, high-throughput bioassays is crucial for predicting resistance in the field and testing multiple traits of potato plants, including resistance against tuber soft rot, transmission from seed to stolon and progeny tubers, pathogen population growth, and symptom development (Marquez-Villavicencio *et al.,* 2011). Factors affecting tuber resistance include tuber age, changes in respiration rates, membrane integrity, physiological changes due to abiotic stresses, and loss of dormancy during storage (Chung *et al*., 2013). The identification of numerous new SRP species with potentially high virulence revealed the pathogen's diversity, making the development of tolerant potato varieties

challenging. The influence of environmental factors on disease development of blackleg further complicates the correlation between laboratory assays for tolerance and field observations. Importantly, resistance tests are often not conducted under anaerobic conditions, potentially impairing the plant's resistance mechanism (Gill *et al.,* 2014).

Resistance to blackleg and soft rot diseases has been reported in some wild diploid species of S*olanum* spp. (e.g., *S. microdontum S. chacoense, S. commersonii*), which are exceptionally high in level of toxins (e.g., glycoalkaloids) and other undesirable traits. Studies on *S. chacoense* indicate a quantitative genetic base of resistance to *Pectobacterium*, which is distributed across multiple chromosomes, suggesting a multi-genic nature of resistance (Chung *et al.,* 2017; Lebecka *et al.,* 2021). Previous research has identified active resistance genes against *Pectobacterium* in potato and other host species (Zimnoch Guzowska *et al.,* 2000; Lebecka *et al.,* 2021). Resistance to SRP has been associated with the activation of genes encoding proteinase inhibitors, antimicrobial peptides, and cell wall-related enzymes, serving as a primary source of pre-formed resistance in potato plants (Joshi *et al.,* 2021). Other traits, such as total soluble phenols, polyphenol oxidase, peroxidase, PAL, and chlorogenic acid, have been correlated with tuber soft rot resistance in 18 tested potato cultivars (Ngadze *et al.,* 2012). JA marker (lipoxygenase) and SA marker (PR1) have also been found to be associated with resistance to blackleg in potato plants infected with *D. solani* (Burra *et al*., 2015). (Burra *et al.,* 2015). In addition, a high level of methyl esterification of pectin present in the cell wall, which is present even under anaerobic conditions, can be effectively applied in resistance base in practice (Goulao, 2010).

Breeding selection in potato cultivars has its own complexities and difficulties due to the polygenic nature of resistance against SRP, a lack of suitable techniques for resistance testing, and limited genetic diversity (Zimnoch Guzowska *et al.,* 2000; Charkowski, 2018). Despite these challenges, a limited number of transgenic lines have been released for commercialization (Hameed *et al.,* 2018). Although the generation of SRP-resistant crops using biotechnology holds promise, the use of them is not widely accepted by concerns about human health and environmental risks. Recently, new breeding

technologies, such as CRISPR/Cas9, offer the potential for specific editing of targeted genome sequences and new perspectives on generation of resistant cultivars.

1.4.2. Biological control of *Pectobacterium* and *Dickeya* spp.

In addition to the prophylactic approach, the application of biocontrol agents, such as natural compounds and microorganisms, provide a promising approach within an integrated management strategy for strategy in potato protection towards soft rot pathogens (Lahlali *et al*., 2022). Intensive research has been conducted to discover a wide variety of microbial biocontrol agents, including antagonistic bacteria (Table 1), bacteriophages (Poluri and Czajkowski, 2022), and natural predators (Youdkes *et al*., 2020), aiming for effective control of *Dickeya* and *Pectobacterium* pathogens. The most studies are restricted to *in vitro* laboratory assays, small-scale pathogenicity tests on tuber slices, or using culture tube-raised potato plants (Osei *et al.,* 2022; Diallo *et al*., 2011; Czajkowski *et al*., 2011a). So far, few experiments have been conducted for the evaluation of putative biocontrol agents for SRP, especially *D. solani*, under greenhouse, field, or storage conditions for consistency of results (Czajkowski *et al.,* 2012a; Des Essarts *et al.,* 2016).

Different strategies have been developed for the biological control of potato soft rot. Several bacterial competitors or plant growth-promoting rhizobacteria from the potato rhizosphere, endorhiza or tuber periderm were isolated and described with encouraging results in biological control of bacterial soft rot in potato that some of them is listed in Table 1. They are mainly classified into the genera *Pseudomonas*, *Bacillus*, *Serratia*, *Lactobacillus*, *Actinobacteria,* and *Lactococcus* directed at the control of the *P. atrosepticum*, *Pcc*, and, to a lesser extent, *Dickeya* sp. The mechanisms behind their ability as a BCA are strain-specific and depend on the host plant physiology, the pathogen species, and several environmental factors (Diallo *et al*., 2011; Czajkowski *et al.,* 2011b; Des Essarts *et al.,* 2016).

Bacterial biocontrol agents may use one or a combination of different mechanisms that directly or indirectly interact with the pathogen (Zamioudis and Pieterse, 2012; Köhl *et al.,* 2019). The biocontrol activity of antagonistic

bacteria against SRP can be related to (i) antibiosis, achieved through the secretion of secondary metabolites with broad-spectrum antimicrobial properties; (ii) interference with pathogen virulence through quorum quenching; (iii) utilization of motility, and competition for nutrients and niches suitable for SRP colonization; and (iv) indirectly, by triggering defense response or promoting plant growth, as outlined in Table 1. Many introduced biocontrol isolates produce diverse allelochemicals, including antibiotics, bacteriocins, lipopeptides, biosurfactants, hydrogen peroxide, enzyme inhibitors, cell-wall-degrading enzymes, and microbial volatile organic compounds (VOCs), which interfere with the metabolism of the pathogen and thereby inhibit the pathogen development (Pandit *et al.,* 2022; Köhl *et al.,* 2019). In addition, bacterial biocontrol agents may colonize infection sites and exhibiting a more efficient nutrient uptake system and survival ability than the pathogen. This colonization strategy can reduce infection pressure through competitive elimination of pathogens, limiting their growth without killing them, for example, by production of siderophores with an affinity for ferric iron. Low-molecular-weight siderophores have a significant effect on the uptake of iron by plants when other metals such as cadmium and nickel are present and play an important role in the competitive fitness of bacteria within the rhizosphere microbial community (Lahlali *et al.,* 2022; Gerayeli *et al.,* 2018). The formation of an iron-siderophore complex enhances iron solubilization and extraction from natural complexes or minerals (Zhou *et al.,* 2016; Hibbing *et al.,* 2009), which ultimately excludes the pathogen from its niche by depriving it of essential iron uptake.

The initial screening for potential biocontrol candidates against blackleg and soft rot focused on their ability to primarily produce siderophores, antibiotics, and surfactants (Loper and Henkels, 1999; Compant *et al.,* 2005; Sharga and Lyon, 1998; Gross, 1988). It was confirmed that various antagonistic bacteria, including members of the *Bacillus* and *Pseudomonas* genera, could generate and release antibiotics (e.g., 2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid, macrolactin A, pyrrolnitrin) or biosurfactants (e.g., lipopeptides and glycolipids). These compounds serve to minimize or prevent the growth and metabolic activities of SRP pathogens (Kastelein *et al.,*

1999; Jafra *et al.,* 2006; Ongena *et al.,* 2007; Bakker *et al.,* 2002). Early studies indicated that direct bacterization of tuber periderm with both fluorescent and non-fluorescent *Pseudomonas* strains could effectively manage soft rot in potatoes and enhance yields in field sites (Colyer and Mount, 1984; Geels and Schippers, 1983; Cronin *et al.,* 1997), but failed in the large-scale application.

Bacterial BCA can exchange signals and interfere with pathogens' cell-to-cell communication through QS mechanism. They achieve this by enzymatically degrading or inhibiting the synthesis of the signal molecules essential for initiating infections. This antivirulence ability, targeting QS regulation, has been observed in the biocontrol activities of bacteria belonging to *Bacillus, Pseudomonas, Delftia, Ochrobactrum, Chromobacterium,* and *Rhodococcus* genera against *Dickeya* and *Pectobacterium* pathogens (Jafra *et al.,* 2006; Cirou *et al.,* 2007; Krzyzanowska *et al.,* 2012a; 2012b; Ha *et al.,* 2018). In quorum-quenching strategies, bacterial agents producing QS-degrading enzymes, such as lactonases, oxidoreductases, and acylases that suppress QS signal molecules, impair pathogen infection, and reduce diseases symptoms (Kalia *et al.,* 2019). Additionally, some natural and synthetic low-molecular-weight compounds with a range of QS-inhibitor activity are shown to inactivate quorum sensing signals and diminish the severity of the *Pectobacterium*-induced disease (Des Essarts *et al.*, 2013; Palmer *et al.,* 2011; Pun *et al.,* 2021). The two growth-stimulating agents, gamma-caprolactone and gamma-heptalactone, efficiently enhance the colonization of the plant by the quorum-quenching bacterium *Rhodococcus* in the potato rhizosphere (Cirou *et al.,* 2012).

The beneficial impacts of PGPRs on promotion of plant growth and health are directly linked to their ability to produce compounds that stimulate plant growth, such as hormones (phytostimulators), synthesize of compounds that provide the plant with a rich source of energy and nutrients, or facilitate the acquisition of minerals and water from the environment (bio-fertilizers). Indirectly, PGPRs contribute to plant well-being by inhibiting the harmful effects of pathogens (bio-antagonists). Mechanisms like nitrogen fixation associated with roots, the production of the auxin phytohormone, and the reduction of plant ethylene levels through bacterial production of the 1-

aminocyclopropane-1-carboxylate (ACC) deaminase enzyme in response to stress and physiological damage are examples of both direct growth promotion and indirect plant protection (Beneduzi *et al.,* 2012; Zhou *et al.,* 2016; Osei *et al.,* 2021). Some PGPRs function as endophytes, residing systemically in plants within the same ecological niche as their pathogenic counterparts without causing harm to plant tissues. This competition for internal colonization aims to combat pectinolytic SRP in locations (such as vascular tissue) that cannot be reached by chemical and physical control treatments (Czajkowski *et al.,* 2012b). Additionally, endophytic bacteria with biocontrol potential can directly reduce the population of soft rot bacteria and/or suppress their virulence, while also inducing changes in plant physiology and activating the plant immune response. Numerous studies have sought to identify endophytic bacterial antagonists against SRP on potato tubers (Krzyzanowska *et al.,* 2012b; Zhang *et al.,* 2020).

Table 1. List of selected publications focused on biological control of soft rot bacteria within the *Dickeya* and *Pectobacterium* genera, and reported bacterial antagonistic isolates used in these studies as potential biocontrol agents.

References	Biocontrol agents	Target pathogens	Mechanisms	Biocontrol assays
Kloepper,1983 Gross,1988 Rhodes and Logan,1987 Axelrood *et al.,*1988 Kastelein *et al.,*1999	Fluorescent *Pseudomonads*	*Pectobacterium* spp. and *Dickeya* spp.	Antibiosis, iron competition, production of siderophores, antibiotics and surfactants	*In vitro* screening, tuber assay and field trial
Colyer and Mount, 1984	Fluorescent *Pseudomonads*	*Pectobacterium* spp.	Antibiosis	Tuber bacterization and field trial
Jafra *et al.,* 2006	*Ochrobactrum* sp. *Delftia acidovorans* *Rhodococcus* spp.	*P. parmentieri* *P. carotovorum subsp. carotovorum*	AHL inactivation, Antibiosis	*In vitro* screening, Tuber assay
Cladera-Olivera *et al.,* 2006	*Bacillus licheniformis* P40	*P. carotovorum subsp. carotovorum*	Production of bacteriocin-like substance	Bacteriocin activity, whole tuber assay

Cirou *et al.,* 2007	*Rhodococcus erythropolis* *Delftia acidovorans*	*P. atrosepticum*	AHL inactivation	Tuber assay
Trias *et al.,* 2008	*Lactobacillus plantarum* *Lactobacillus acidophilus* *Lactobacillus buchneri* *Leuconostoc* spp. *Weissella cibaria*	*P. carotovorum* subsp. *carotovorum*	Antibiosis, production of organic acids, hydrogen peroxidase and siderophores	*In vitro* screening
Jafra *et al.,* 2009	*Rahnella aquatilis* *Erwinia persicinus*	*Dickeya zeae*	AHL inactivation, siderophore production	*In vitro* screening and tissue maceration assay on hyacinth bulb
Crépin *et al.,* 2012	*Rhodococcus erythropolis* R138	*P. atrosepticum*	AHL inactivation	Whole tuber assay
Baz *et al.,* 2012	*Streptomyces* sp. OE7	*P. carotovorum* *P. atrosepticum*	Antibiosis	Tuber assay
Krzyzanowska *et al.,* 2012a	*Bacillus subtilis* *Pseudomonas* sp. *Ochrobactrum* sp.	*Pectobacterium* spp. and *Dickeya* spp.	AHL inactivation, potato rhizosphere colonization	Seed tuber bacterization, fluorescence and confocal laser scanning microscopy, REP-PCR
Krzyzanowska *et al.,* 2012b	*P. donghuensis* *Bacillus weihenstephanensis* *Delftia acidovorans*	*D. solani* D. dadantii *P. carotovorum* subsp. *carotovorum* *P. atrosepticum*	AHL inactivation, antibiosis, motility, siderophore and biosurfactant production	*In vitro* screening, tuber assay, AHL inactivation assay
Czajkowski *et al.,* 2012b	*Serratia plymuthica* A30	*D. solani*	Antibiosis, AHL inactivation, production of siderophore, biosurfactant and auxin.	Tuber assay, greenhouse and field trial
Khayi *et al.,* 2015	*P. brassicacearum* *Bacillus simplex*	*Pectobacterium* spp.	Antibiosis	*In vitro* screening
Krzyzanowska *et al.,* 2016	*P. donghuensis* P482	*D. solani* *P. brasiliense*	Antibiosis, iron competition, production of antibacterial metabolites and siderophore	Antibacterial activity assay, genome mining
Des Essarts *et al.,* 2016	*P. putida* *P. fluorescens* *Bacillus simplex* *P. brassicacearum*	*D. dianthicola* *P. atrosepticum*	Antibiosis, production of siderophore and antibiotics	*In vitro* screening, tuber assay, greenhouse assays

Garge and Nerurkar, 2017	*Bacillus firmus* *Bacillus subtilis* *Bacillus thuringiensis*	*P. carotovorum* subsp. *carotovorum* BR1	AHL inactivation, antibiotic production, inhibition of pathogen virulence enzyme production	*In vitro* biocontrol assay on potato, carrot and cucumber, *in planta* assay on mung beans
Li *et al.,* 2018	*Myxococcus* sp.	*P. carotovorum*	Multicellular swarming capacity "wolf-pack" for predatory feeding	Predation assay, and pot experiments
Azaiez *et al.,* 2018	*Bacillus amyloliquefaciens*	*P. carotovorum*	Production of glycolipid-like antibacterial compound	In vitro screening, tuber assay, thin layer chromatography
Gerayeli *et al.,* 2018	*Bacillus pumilus* *Bacillus myloliquefaciens*	*P. carotovorum* subsp. *carotovorum*	Producing PAL, polyphenol oxidase, peroxidase and total phenols	Tuber assay, genome mining
Tsuda *et al.,* 2016	*Lactobacillus plantarum*	*P. carotovorum* subsp. *carotovorum*	Antibiosis, long survival assay and inhibition of pathogen invasion	*In vitro* antibiosis assay, population dynamics, field trial on Chinese cabbage
Krzyzanowska *et al.,* 2019a; b	*Enterobacter amnigenus* A167 *Rahnella aquatilis* *Serratia rubidaea* *Serratia rubidaea Serratia plymuthica* A294	*P. atrosepticum* *P. carotovorum subsp. carotovorum* *P. parmentieri* *D. solani* *D. dianthicola*	Antibiosis, biosurfactant production, siderophore production, AHL inactivation	Potato slices and whole tuber assays, tuber injection assay
Zhou *et al.,* 2022	*Rhodococcus pyridinivorans*	*P. carotovorum*	AHL inactivation	Antagonistic activity assay on Chinese cabbage, carrot and potato
Cigna *et al.,* 2023	*Pseudomonas* sp. *P. brassicacearum*	*D. solani* *D. dianthicola* *P. atrosepticum* *P. parmentieri*	Antibiosis	*In vitro* antibiosis assay
Hossain *et al.,* 2023	*Paenibacillus polymyxa shx301*	*Dickeya* spp. *Pectobacterium* spp.	Production of polymyxin and multiple lipopeptides	*In Vitro* and *in vivo* antagonistic screening on sweet potato

An alternative biological approach for controlling soft rot is the use of bacteriophages with the ability to infect and destroy bacterial cells (Poluri and Czajkowski, 2022). Bacteriophages are host specific, self-replicating, consistently present in the environment, and safe to use without any hazardous

effect in the environment. Several experiments have assessed the biocontrol efficacy of lytic phages against SRP in various scenarios (Buttimer *et al.,* 2018; Youdkes *et al.,* 2020; Czajkowski *et al.,* 2015c). Although their applications are primarily limited to laboratory conditions, they can control pathogens rapidly, and have a long-lasting effect. The use of phage cocktails with diverse receptor specificities has been proposed to mitigate the development of resistance to phage treatment.

Contradictions in the performance of BCAs have limited their adoption in commercial agriculture. Several factors such as intrinsic pathosystem characteristics (pathogen sensitivity, host cultivar, and physical properties), environmental conditions (biotic and abiotic stresses, chemical residues, nutrient availability, temperature, and moisture), as well as delivery methods and formulation, can influence BCA effectiveness by affecting their mechanisms of action or multitrophic interactions within the plant-pathogen-microbial community (Bonaterra *et al.,* 2022). Strategies involving nutritional enhancement, physiological adaptation to stress, and the application of mixtures with improved formulation, termed "synthetic communities" or "microbial consortia," can enhance the efficacy and viability of biocontrol across diverse environmental conditions (Czajkowski *et al*., 2020). Using a mixture of antagonists often provides a broader spectrum of activity for plant protection due to the synergistic effect of different mechanisms of action. Beneficial microorganisms may increase in the synthesis of compounds involved in the control of the pathogens. As well as distinct strains exhibiting variations in environmental preferences, that are expected to ensure the survival of at least a subset of strains under variable conditions. The complex mode of action exhibited by BCAs in consortia diminishes the probability of pathogens developing resistance to them. However, the registration and marketing of consortia face challenges, including difficulties in understanding the specific roles of each component and their biological activity (Czajkowski *et al.,* 2020; Maciąg *et al.,* 2020). Limited studies have been conducted to develop compatible mixtures of bacterial antagonists or phages for protecting potato tubers from soft rot caused by *Pectobacterium* spp. and *Dickeya* spp. Recently, an artificial bacterial consortium known as "The Great Five" was developed and

tested against a combination of SRP pathogens in seed potato tubers, showing promise under high disease pressure conditions (Krzyzanowska *et al.,* 2019a; b). Newly developed formulations of this micro-consortium are being investigated for potential commercial use, providing good shelf life during long-term storage at 8°C, mimicking conditions in commercial potato storage facilities (Maciąg *et al.,* 2020). In a separate effort, Carstens et al. (2018) designed and applied a complex phage cocktail comprising six diverse phages to treat soft rot in potatoes under simulated storage conditions, resulting in a significant reduction in the incidence and disease severity of *D. solani*, offering a promising phage-based biocontrol against SRP infection in potatoes. Despite numerous attempts to identify efficient BCAs and use them for SRP control on potatoes, little progress has been made in elucidating the molecular mechanisms of antagonism in interactions with pathogen-host combinations.

1.4.3. Endophytic bacterium *Serratia plymuthica* A30

The endophytic bacterium *S. plymuthica*, a Gram-negative member of the *Enterobacteriaceae* family, is widely distributed globally and emerges as a highly promising BCA against various fungal and bacterial plant pathogens (de Vleesschauwer *et al.,* 2009; Pang *et al.,* 2009). Well-known for its production of secondary metabolites with impressive biocontrol properties (Koo and Cho, 2009). Several strains of this genus also exhibit plant growth-promoting and phytoremediation traits by effectively accumulating various toxic heavy metals from the soil (Khan *et al.,* 2017). Notably, the *S. plymuthica* strain A30 stands out for its significant antagonistic activity against *D. solani*. It possesses features such as the production of biosurfactants, motility, and the ability to thrive under both aerobic and anaerobic conditions, even at relatively low temperatures (10 °C) (Czajkowski *et al.,* 2012a). The study on the effectiveness of the *S. plymuthica* A30 against *D. solani* in potato plants under greenhouse conditions confirmed that A30 is able to stably grow inside potato roots and stems and colonize xylem vessels and apoplast between parenchyma cells, leading to the reduction of pathogen populations (Czajkowski *et al.*, 2012b). These characteristics identify *S. plymuthica* A30 as a valuable endophyte with biocontrol properties.

1.5. Interaction between beneficial bacterial endophytes and host

1.5.1. Perception of bacterial endophytes and induction of plant resistance

Endophytic bacteria enter plant cells by secreting biofilm, establishing close contact with the plant host to provide direct benefits in exchange for a continuous supply of nutrients and shelter. Interactions between endophytic bacteria and plants occur at different levels from pathogenic to beneficial (Brader *et al.,* 2017). The symbiotic relationship between them improves the physiology, fitness, and metabolite profile of plants through the synthesis of secondary metabolites or signaling molecules, the modulation of phytohormones, and the improvement of abiotic and biotic stress tolerances. Endophytes, characterized by a short life cycle and rapid evolution within the host, undergo a greater selection of antagonistic forms against pathogens (Hardoim *et al.,* 2015). These microbes can induce or enhance resistance against pathogen infections in plant tissues without being hindered by the local host immune response (Pieterse *et al.,* 2014).

In the initial stages, the specific interaction between beneficial microbes and plants assists hosts to trigger an immune system, supporting an innate response similar to that activated against pathogens. This collaboration enhances the overall defensive capacity of the plant against various invaders. The immune system depends on a complex sequence of events of events where the beneficial microbe initially releases specific MAMPs that are recognized by specific PRRs, allowing the microbe to localize itself on the host. Subsequently, signals are transmitted within the host plant, initiating signaling pathways that result in the high expression of plant defense genes and the activation of antimicrobial responses. This, in turn, suppresses the growth of microbial pathogen populations (Hacquard *et al.,* 2017). A diverse array of potential bacterial elicitors acts as resistance-inducing stimuli belonging to very different groups of compounds, including flagella, antibiotics, quorum-sensing molecules, biosurfactants, siderophores, lipopolysaccharides, superoxide dismutase and volatiles compounds, which are recognized by their corresponding extracellular

PRRs (Pieterse *et al*., 2014).

The colonization of beneficial microbes elicits a wide array of plant responses, potentially enhancing the plant's defensive capabilities through Induced Systemic Resistance (ISR). Specifically, for endophytic biopriming resistance, it is termed endophyte-induced resistance (E-IR) (De Kesel *et al.,* 2021). Endophyte-mediated ISR resembles pathogen-induced systemic acquired resistance (SAR) since both of them are mediated by the plant's innate immune system against a variety of pathogens. However, ISR-mediated resistance is significantly less effective than that achieved through SAR. SAR is mainly restricted to local infections, can lead to tissue necrosis, and is associated with the activation of pathogen response genes (PRs) and various endogenous signals like the accumulation of SA, N-hydroxy pipecolic acid, and azelaic acid/glycerol 3-phosphate in plants during pathogen attacks (Klessig *et al.,* 2018). The nature of endophytic ISR depends on the presence or absence of the pathogen (type of elicitor) and plant hormones. The ISR was established by an endophyte in the absence of a pathogen is termed ISR-Prime, preparing the plant for future challenges by pathogens. On the other hand, induced resistance in conjunction with pathogen infection is called IR-Boost (De Kesel *et al.,* 2021; Oukala *et al.,* 2021; Pieterse *et al.,* 2014). In contrast to directly induced resistance (ISR-Boost), defense priming (ISR-Prime) prompts plants to respond to stimuli in future pathogenic attacks through a long-lasting system of faster or stronger defense mechanisms with lower energy costs (Mauch-Mani *et al.,* 2017). There have been reports of the potential for transgenerational defense priming (Conrath *et al.,* 2015).

Endophytic bacterial strains belonging to the genera *Pseudomonas*, *Bacillus*, and *Serratia* have been studied as groups that employ defense priming through ISR to protect plant hosts (Pieterse *et al.,* 2012). Extensive studies revealed that ISR operates through various phytohormone pathways to regulate the defense signaling network. Among them, JA and ET are central players in priming plant resistance by beneficial microbes (Van der Ent *et al.,* 2009; Pieterse *et al.,* 2009). However, with the characterization of more resistance-inducing agents, several endophytic bacteria and PGPRs have demonstrated the involvement of both SA and JA/ET signaling pathways in resistance induction,

forming a complex network of interconnected hormone pathways (Niu *et al.,* 2011; Yuan *et al.,* 2019; Ilham *et al.,* 2019). The crosstalk among multiple hormonal signaling in shaping ISR provides a powerful capacity for fine regulation of immune response, however, the predominance of each signaling pathway strongly depends on the players that are interacting. For instance, endophytic *Actinobacteria* exhibited the ability to prime different pathways, conferring resistance against two distinct pathogens. Resistance to *Erwinia carotovora* was mediated through the JA/ET pathway, while resistance against *Fusarium oxysporum* involved the SA pathway (Conn *et al.,* 2008). In Arabidopsis, activated ISR by *Bacillus cereus* AR156 triggered both SA and JA/ET signaling pathways against *P. syringae* pv. *tomato*, while requiring only the JA/ET to induce resistance against *B. cinerea* (Niu *et al*., 2011). The endophytic bacterium *Paraburkholderia phytofirmans* PsJN induced ISR through both SA- and JA/ET pathways when challenged with *Pst* DC3000 (Timmermann *et al.,* 2019). In line with these findings, ISR triggered the upregulation of SA- and JA/ET-related gene expressions in *Arabidopsis thaliana* during infection with *Pst* DC3000 by *Pseudomonas* sp. SCA7 (Kuhl-Nagel *et al.,* 2022). Conversely, *Pseudomonas simiae* WCS417-induced ISR functioned independently of SA and did not involve the accumulation of PR proteins (Pieterse *et al.,* 2021).

In the early events of ISR, endophytic bacteria exert a broad-spectrum response in priming the plant defensive capacity. This response includes ion fluxes, activation of MAPK cascade, extracellular alkalization, and production of ROS. Subsequent changes involve alterations in basal levels of defense-related hormones and activation of various molecular and cellular host defense responses. These responses encompass increased expression of PR genes, reinforcement of the cell wall (such as deposition of pectin and callose at pathogen infection sites), and the accumulation of antimicrobial compounds (e.g., phenylalanine ammonia lyase, polyphenol oxidase, peroxidase, β-1,3 glucanase, and chitinase) (Yu *et al.,* 2022). Upon a pathogen attack, more robust defense mechanisms are activated, potentially linked to changes in tissue sensitivity, indicating the fitness costs associated with plant priming during direct defense against pathogens (Conrath *et al.,* 2006). After successfully

limiting pathogen growth, the expression of genes related to defense and antimicrobial activity returns to pre-infection levels (Hacquard *et al.,* 2017). Endophytic bacteria employ adaptive strategies to circumvent plant defense signaling, enabling successful colonization as avirulent symbionts. This symbiotic relationship allows endophytes to tolerate the plant's immune system and resist competition with pathogens. These adaptations enable them to maintain homeostasis within plants, fine-tuning the interaction to benefit both partners. In this mutually beneficial dynamic, the plant is protected and receives increased nutrients, while the bacteria obtain organic compounds and a growth niche (Lu *et al.,* 2021).

1.5.2. Modulation of plant immunity by endophyte for successful colonization

Beneficial microbes produce a large number of MAMPs that can initiate early host immunity, Known as MAMP-Triggered Immunity (MTI). Activation of MTI efficiently hinders penetration and colonization of the mutualists. Plants seem incapable of distinguishing between pathogenic and beneficial microbes due to significant overlap in symbiotic and immune signaling, along with cross-regulations within host Pattern Recognition Receptor (PRR) pathways. However, when endophytes engage in apoplastic communication with the plant, these signals may differ, and microbial receptors function as an “Identification Code”, playing a crucial role in mediating microbial accommodation to defend plant immunity. Furthermore, beneficial microbes associated with plants have evolved various strategies to temporarily suppress MAMP-triggered responses, allowing them to escape the early host defense system and establish a mutual interaction. These bacterial strategies involve evading PRR recognition through the divergence of MAMPs, degradation or masking of excessive MAMPs, developing variants from the same MAMP, or secreting compounds to neutralize their MAMPs. Additionally, bacteria release effectors to suppress the host immune system or induce changes in the gene expression reprogramming of plant hormone defense signals (Yu *et al*., 2019; Trda *et al*., 2014; Lopez-Gomez *et al*., 2012).

Endophytes also produce proteins serving as molecular mimics to restrict

plant immune functions by disrupting key plant protein interactions, representing a strategy to prevent MTI (Stringlis *et al.*, 2018a). Notably, different recognition patterns of the bacterial MAMP flagellin (flg22) by plants have been observed between pathogenic (*Bacillus phytofirmans*) and non-pathogenic (*Xanthomonas campestris*) bacteria (Trada *et al.*, 2014). For instance, *Bacillus subtilis* produces the antibiotic subtilomycin, which binds to its flagellin, preventing an overresponse of plant defenses, probably because of a non-full perception of the bacteria (Deng *et al.*, 2019). In general, pathogenic bacterial strains use T3SS and T4SS for effector transmission in plant cells to induce ETI. In contrast, endophytic bacteria either do not use this system, or if so, in a very low abundance (Liu *et al.*, 2017). Beneficial microbes, unlike pathogen-caused PTI with severe cellular damage, induce a transient and relatively mild local ISR due to their manipulation of the host immune mechanisms. This is a common feature of ISR-eliciting beneficial microbes, leading to symbiotic colonization with the host (Pieterse *et al.,* 2014). For example, the colonization of *Pseudomonas fluorescens* WCS417 on Arabidopsis roots requires local suppression of flagellin-triggered PTI response through the secretion of low molecular weight compounds and the inhibition of callose deposition (Millet *et al.*, 2010). Similarly, the flg22 peptide from the beneficial bacterium *Burkholderia phytofirmans* induces only a limitted oxidative burst, sufficient to trigger the transient induction of defense genes without negatively affecting plant growth (Felix *et al.*, 1999). In cases where oxidative burst is a plant defense mechanism, some bacterial endophytes protect themselves by secreting antioxidant enzymes such as catalases, superoxide dismutase, peroxidases, alkyl hydroperoxide reductases, and glutathione-S-transferases (Alquéres *et al.*, 2013).

The alteration in Arabidopsis defense-related gene transcription occurs through the downregulation of both JA and SA pathways following a 24-hour incubation with the endophytic fungus *Trichoderma harzianum* (Moran-Diez *et al.,* 2012). In the context of mutual interactions, the root colonization by the beneficial rhizobacteria *Pseudomonas simiae* WCS417 actively inhibited more than half of the MAMP-triggered transcriptional responses. The remaining responses exhibit a robust auxin signature, confirming the dual role of auxin

signaling in finely balancing growth-promoting and defense-eliciting activities in plant roots (Stringlis *et al.,* 2018a).

It has been reported that some PGPRs and ISR-inducing endophytes appeared to be highly tolerant to antimicrobial agents such as coumarin scopoletin, which is exerted through plant's immune response against soil-borne fungal pathogens, resulting in plants selectivity to shape the root-associated microbial community (Stringlis *et al*., 2018b). Most endophytes do not break down lignin and carbohydrates, leading to nonpathogenic and asymptomatic responses, but damage-gating of immune responses on the plant side avoids constitutive activation of defenses against nonpathogenic root colonizers with a tolerant attitude. In contrast, when plants are infected by a pathogen, plants will immediately recognize damaged cells and trigger the immune response with a "zero tolerance attitude" to prevent further invasion of local pathogens (Zhou *et al*., 2020).

1.5.3. Transcriptional responses of plant to bacterial biocontrol agents

Knowledge about mechanisms of action for most biocontrol agents is relatively superficial due to the lack of an understanding of the key biochemical and molecular processes within host-pathogen-biocontrol agent interaction that determine the effectiveness of a biocontrol system. Dissecting the nature of these interactions goes beyond merely isolation and characterization of individual genes or proteins. Advanced molecular techniques, such as NGS technologies, play a pivotal role in making significant advancements in this field (Pieterse *et al.,* 2014). Transcriptome profiling, particularly by RNA-Seq, has developed the identification of a wide range of genes involved in tritrophic interactions.

For example, the beneficial bacterium *Paraburkholderia phytofirmans* PsJN induced resistance through ISR in *Arabidopsis thaliana* against *Pseudomonas syringae* pv. tomato. Transcriptome analysis of PsJN-inoculated Arabidopsis, both before and after pathogenic infection, revealed distinct responses compared to the plant's response to the pathogen or PsJN alone. Less than 2 % of transcripts were exclusively expressed in PsJN-inoculated Arabidopsis. In the absence of the pathogen, PsJN-inoculated plants exhibited

a rapid response, with more upregulated genes at 1 hpi compared to the 24 hpi sample. Genes related to auxin, ET, brassinosteroid, and JA signaling were significantly induced in PsJN-inoculated plants, along with the upregulation of genes associated with the cell wall, peroxidases, PR proteins, and transcription factors (e.g., ERF, bZIP, WRKY, and MYB). In Arabidopsis inoculated by both PsJN and pathogen, induction of transcription factors belonging to LHY, WRKY28, MYB31, and RRTF1 groups may act as hub regulators in JA, ET, SA, and ROS (Timmerman *et al.,* 2019).

In a comparative analysis, the transcriptional response of PsJN-inoculated grapevine (*Vitis vinifera*), with or without the challenge of the bacterial pathogen *Pseudomonas syringae pv. Pisi*, revealed that PsJN perception triggers a low-level defense, facilitating its colonization throughout the plant. Full defense gene induction occurred in the presence of the pathogen, leading to sustained extracellular alkalinization, ROS-related expression, and a HR-like reaction, characteristic of non-host and incompatible interactions. PsJN-inoculated grapevine exhibited a compatible interaction profile, with transient extracellular alkalinization, no ROS production, no disease symptoms, and concurrent plant growth promotion and pathogen resistance. Results suggested the involvement of both SA and JA in the defense response, with significant induction of marker genes in the grapevine for the SA and JA pathways. The accumulation of SA in PsJN-inoculated cells may activate defense responses through ISR (Bordiec *et al.,* 2011).

In another study, the PGPR bacterium *Bacillus velezensis* F21 was introduced as a BCA against *Fusarium oxysporum f. sp. niveum* (Fon), the causal agent of *Fusarium* wilt in watermelon. Transcriptome analysis elucidated the response of watermelon roots treated with F21 alone and in combination with Fon inoculation. The results indicated that F21 suppressed the growth and spore germination of Fon by secreting secondary metabolites with antimicrobial activity. Additionally, F21 enhanced the plant's basal immunity to Fon by inducing genes related to defense and detoxification. F21 inoculation led to the significant production of hydrolases with strong and broad-spectrum bacteriostatic activity against Fon. The findings revealed the upregulation of ripening-related genes and transcription factors from WRKY, MYB, bZIP, AP2,

and NAC families. The findings indicated the contribution of both JA and SA signaling in ISR triggered by F21 in watermelon (Jiang *et al.,* 2019).

The BCA candidate *Pseudomonas chlororaphis* PA23 demonstrated its ability to protect *Brassica napus* against the necrotrophic fungus *Sclerotinia sclerotiorum* (Ss) (Duke *et al.,* 2017). Several defense mechanisms were activated in S. *sclerotiorum*-canola pathosystem. Canola infected by Ss exhibited a substantial number of shared genes with PA23 + Ss treatment. Inoculation with PA23 reduced the number of genes upregulated in response to Ss, possibly linked to the suppression of the host defense response through the detection of biocontrol bacterial effectors. Gene expression patterns after inoculation of PA23 alone revealed that the recognition of non-pathogenic bacteria led to systemic defense against SAR. Genes encoding receptors involved in the detection of flagellin and peptidoglycan in MAMP-triggered immunity were downregulated in plants treated with PA23 alone, consistent with post-stimulus desensitization. Subsequently, the reduction in ROS production was accompanied by the overexpression of genes related to glycerol-3-phosphate (G3P)-mediated SAR, a known defense priming mechanism linked with pathogen infection. Enrichment of genes associated with cuticle or wax deposition was observed in PA23 + Ss treated samples, potentially enhancing tolerance to the pathogen. In another survey, the native endophyte *Pseudomonas fluorescens* PICF7, known as an effective biocontrol agent against *Verticillium* wilt during colonization of olive, was employed to study induced defense responses in olive trees (Schiliro *et al*., 2012). The persistence of PICF7 in olive root tissues suggested that the plant did not recognize it as a threat, highlighting its ability to overcome the host's defense response. PICF7 colonization significantly induced transcripts related to plant hormone production, such as lipoxygenase, phenylpropanoids biosynthesis and genes encoding PR proteins (e.g., β-1,3-glucanase [PR-2], class IV endochitinases [PR-3, 4], lignin-forming peroxidases [PR-9], and Bet v1-like proteins [PR-10]), which are part of the plant ISR response (Schiliro *et al.,* 2012).

The beneficial rhizobacterium *Pseudomonas simiae* WCS417 as a PGPR model can both promote plant growth and induce broad-spectrum disease resistance in plants. A time-course RNA-Seq was conducted to investigate the

early transcriptional responses of Arabidopsis roots to free-living mutualist's colonization of WCS417 and compared to MAMPs flg22^{417} (from WCS417), flg22^{Pa} (from pathogenic *Pseudomonas aeruginosa*) and fungal chitin. While the root response to MAMPs flg22^{417}, flg22^{Pa}, and chitin exhibited temporal differences, a substantial overlap displayed in the genes affected. Both flg22^{Pa} and flg22^{417} strongly upregulated genes involved in immunity, while downregulating those linked to growth and development—a characteristic feature of the growth-defense tradeoff typical for MTI responses. Although the host's transcriptional response to living WCS417 largely overlaped with those mediated by the MAMPs, about half of them were suppressed by WCS417 inoculation, which may facilitate establishment of a mutually beneficial interaction with the host root. The root response to tested MAMPs included significant activation of both MTI immunity marker genes MYB51 and CYP71A12, while live cells of WCS417 activated only MYB51. Roots colonization by WCS417 induced the expression of MYB72, IRT1 and GH3.3 as marker genes of ISR, iron deficiency response and auxin response, respectively. The transcriptome results indicated a dual role of auxin signaling in finely balancing growth-promoting and defense-eliciting activities (Stringlis *et al.,* 2018b). WCS417 also induced alterations in the expression of several SWEET and sugar transporter families, highlighting their involvement in the plant growth-promoting (Desrut *et al.,* 2020).

2. AIMS OF THE STUDY

The first phase of this study focused on evaluating the efficacy of biocontrol agents in preventing soft rot pathogens during the cold storage of potato tubers. Subsequently, the effectiveness of the selected BCAs was investigated in a field setting. The survival and population dynamics of the soft rot bacterium *D. solani* and the promising antagonist *S. plymuthica* strain A30 were evaluated to advance our understanding of latent pathogen life and the antagonist's colonization capabilities under storage conditions. In the second part of the study, the objective was to unravel the global defense response of potatoes to the soft rot pathogen *D. solani* by identifying genes with differential expression

during both early and late phases of infection. The third and final part aimed to understand the comprehensive transcriptional response of potato tubers to *S. plymuthica* A30 colonization, both individually and in conjunction with *D. solani* under conditions mimicking storage. The study was strategically divided into these three segments to address the outlined objectives.

(1) The screening process for antagonist ability and biocontrol efficiency involved multiple steps and assays conducted to identify the most effective antagonist under cold storage conditions. Two species-specific TaqMan real-time qPCR assays were developed to examine the population dynamics of both the pathogen and selected biocontrol agents in storage and the field.

(2) The study employed a combination of time-course RNA-seq analysis, quantitative real-time PCR, and UPLC-MS analysis of hormonal content in potato tubers inoculated with *D. solani* to distinguish the tuber's response to *D. solani* during both the early (non-symptomatic) and late (symptomatic) phases of infection.

(3) Time-course RNA-Seq analysis, bacterial population measurement by TaqMan qPCR, quantitative real-time PCR, and hormonal content measurement by UPLC-MS analysis were employed to understand the transcriptional crosstalk triggered in tri–dimensional interactions of potato tuber–*D. solani*–*S. plymuthica* A30. The goal was to understand how potato tuber defense and adaptation mechanisms are influenced during both the early and late stages of infection or beneficial interaction.

3. MATERIALS AND METHODS

The materials and methods used in this study are summarized in Table 2. A detailed description has been provided in the original publications from I to III.

Table 2. Materials and methods used in this study.

Category	Method/model	Publication
Organisms	Potato (*Solanum tuberosum)*	I; II; III
	Dickeya solani	I; II; III
	Serratia plymuthica A30	I; III
	Pectobacterium carotovorum subsp. carotovorum SCC1	I
	Pectobacterium parmentieri strain SCC3193	I
	Pectobacterium atrosepticum strain SCRI1043	I
Laboratory task	DNA extraction	I
	RNA extraction and purification	II; III
	Ribosomal RNA depletion (Ribo-zero)	II; III
	cDNA synthesis	II; III
	Primer design	II; III
	qRT-PCR	II; III
	High-throughput (HT) Tru-Seq RNA sample preparation	II; III
	Illumina HiSeq2000	II; III
	NextSeq500	III
	Quantitative TaqMan real-time PCR	I; III
	Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS)	II; III
	Antagonist activity screening	I
	Assessment of temperature on antagonist colonization	I
	Survival and population dynamics of bacteria	I
	Tuber soft rot assay	II; III
	Bacterial growth quantification	II; III
	Growth assessment in the greenhouse	I
Computational works	RNA-Seq data analysis	II; III
	qRT-PCR analysis	II; III
	Statistical analysis of TaqMan assays	I; III
	UPLC-MS analysis	II; III

4. SUMMARY OF RESULTS AND DISCUSSION

4.1. Application of bacterial antagonists in control of potato soft rot and their survival in storage and field

Controlling potato soft rot presents a significant challenge, and the current trend is moving towards a safer approach for postharvest disease management. Specifically, the use of antagonistic microorganisms appears to be a successful strategy for minimizing the occurrence of potato soft rot in storage (Des Essarts *et al*., 2016). The possibilities of success for postharvest biological control can be numerous with more advantages compared to the field because the storage conditions such as temperature and humidity are partially controlled, and therefore, the host-pathogen-antagonist equilibrium can more easily switched toward the antagonist (Dukare *et al*., 2019). Once tubers are placed in cold storage, the BCAs need to be cold-tolerant and able to survive and could also be metabolically active during long-term storage. Finding and selecting such antagonistic strains can be challenging and require selective quantitative monitoring methods to investigate the survival and colonization ability of BCAs in different biological materials. This study aimed to develop methods for biocontrol of soft rot in low-temperature storage, which makes it important to select cold-tolerant BCAs. For this purpose, the survival and population dynamics of the latent soft rot bacterial pathogen and the best-selected antagonist were investigated during prolonged storage and in the field, which investigates the possibility of adaptation and transfer of biological control to the following growing seasons and progeny tubers.

4.1.1. Screening for antagonist activity against soft rot pathogens

Bacterial antagonist candidates were obtained from different sources including potato rhizosphere and rotting potato tubers, and endophytic bacteria isolated from arctic plant species. Initially, *in vitro* inhibition growth ability of 150 bacterial isolates was tested against the four soft rot species (*D. solani, P. carotovorum, P. atrosepticum,* and *P. parmentieri*) in an overlay plate assay, consequently, 21 strains were chosen and added to a collection of 53 bacterial strains isolated from various host plants and obtained from other laboratories. In the next step, 31 strains out of 74 isolates were selected based on growth inhibition of at least one soft rot pathogen and production of siderophores. The screening process was followed by performing a whole potato tuber assay to identify strains that could significantly suppress tuber tissue maceration caused

by tested soft rot bacteria. Although *in vitro* antagonism of the examined isolates may not consistently align with biocontrol effectiveness in a plant assay, a good correlation was observed in seven bacteria belonging to the genera *Serratia*, *Pseudomonas*, and *Bacillus*. These bacteria demonstrated significant concordance between Petri dish growth inhibition assay results and their efficacy in reducing all four tested soft rot bacteria, especially on *D. solani* and *P. carotovorum*.

4.1.2. Colonization ability of antagonists under low temperature storage

The selected isolates showed sufficient colonization on potato peel with density in a range of 4.1-5.8 log CFU mL^{-1} at 15 °C after 30dpi. At 4 °C, a significant reduction in cell density was evident for all strains except *Serratia plymuthica* strain A30 (isolated from rotten potato tubers) and *Pseudomonas* sp. S2H1 (isolated from arctic plants). Both strains are mesophilic and psychrotolerant as they survived between 4 °C and 20 °C with almost constant CFU numbers during a 4-week storage period.

4.1.3. Biocontrol efficiency of selected strains on wounded tubers under storage conditions

The efficacy of tuber bacterization with antagonists was expressed as a reduction of soft rot maceration and visual evaluation of disease severity. *S. plymuthica* A30 was the most effective BCA with a 93.2 % reduction of soft rot disease. In a biocontrol assay with *S. plymuthica* A30 against six different *D. solani* strains, a significant reduction in soft rot maceration was observed in infected tubers up to 15 days after inoculation. Biocontrol strain *S. plymuthica* A30 was characterized as the most promising BCA under cold storage conditions due to its superior adaptability to low temperatures.

4.1.4. Survival and population dynamic of bacterial pathogen and antagonist during storage

The population dynamics of *S. plymuthica* A30 and *D. solani* were evaluated over time on stored potato with two separate TaqMan real-time PCR assays (Figure 2). For detection of *D. solani*, species-specific TaqMan real-time PCR

using the primer pair *SOL-Cf/SOL-Cr* was used (Pritchard *et al.*, 2013). To quantify the *S. plymuthica* A30 population in potato samples, a TaqMan PCR based on the single-copy *luxS* gene was used (Czajkowski and van der Wolf, 2012). The population dynamics of *D. solani* and *S. plymuthica* A30 were investigated in stored tubers of three potato cultivars with different susceptibility during seven months from October to April of the following year.

In tubers infected with *D. solani* alone, the pathogen population decreased continuously until the end of November, then was not detected in December and January, and again became detectable in February and March depending on cultivars. Subsequently, the pathogen density remained constant until the end of the experiment (270 days after inoculation), and this increase coincided with the onset of soft rot incidence on the inoculated tubers. In contrast, in tubers pretreated with the A30 antagonist, the cell density of *D. solani* decreased rapidly within 14 or 21 days, depending on the cultivar, and did not increase later in the storage period, indicating that the antagonist could still be effective several months after the treatment. On the antagonist side, the population density of strain A30 decreased slightly in 30 days and remained at this level up to 270 days. In tubers co-inoculated with both *D. solani* and A30, the population of A30 followed a similar pattern as tubers treated with A30 alone but remained at higher cell density. A larger antagonist population in the presence of the pathogen could be a result of better epiphytic colonization and survival or due to nutrients released by the pathogen. In summary, the TaqMan assay results showed that pathogen cell density decreased faster in stored potatoes pretreated with the antagonist, and also, soft rot symptoms were reduced in treated tubers.

4.1.5. Population dynamic changes in the field

The effect of seed tuber bacterization using *S. plymuthica* A30 on disease development was monitored throughout the subsequent summer in the field. The severity and incidence of blackleg and soft rot were significantly lower in potato plants grown from A30-treated tubers. There was no significant difference among cultivars in the average percentage of surviving plants without symptoms. The soft rot incidence on the harvested daughter tubers notably decreased. TaqMan PCR detection of *D. solani* was conducted on asymptomatic

tubers harvested from plants exhibiting no visible symptoms. The treated tubers with A30 resulted in a notable reduction in the pathogen population within the tuber progeny. Consequently, a decline in latent infections in harvested tubers was observed across all cultivars, with a more pronounced reduction in the partially resistant cultivar compared to the fully susceptible one. Conversely, biocontrol A30 was not detected by TaqMan PCR in harvested daughter tubers, suggesting a likely decrease in its population due to microbial competition and unfavorable environmental conditions.

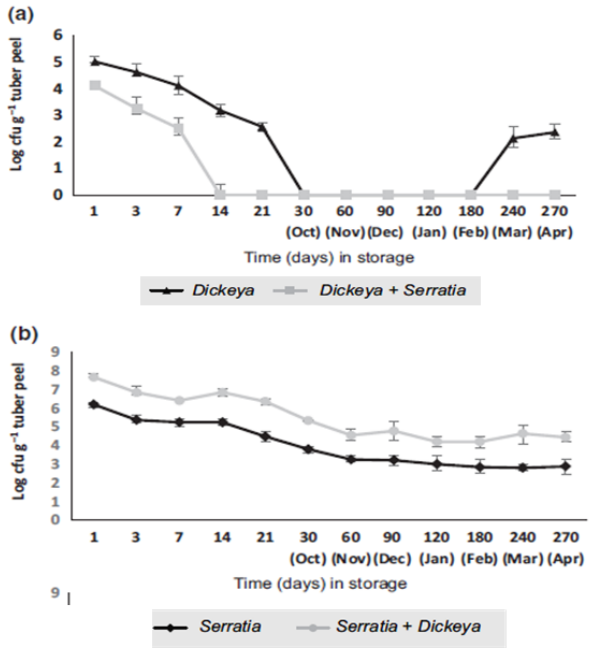

Figure 2. Population dynamics and survival of soft rot pathogen *Dickeya solani* (a) and biocontrol agent *Serratia plymuthica* A30 (b) during storage.

The findings demonstrated that treatment of potato tubers with the antagonist in the autumn contributed to their health during storage and offering protection of potato plants in the following summer. By increasing the population of antagonistic microbes on seed tubers, there is a potential to inhibit pathogen proliferation and disease transmission, thereby reducing the incidence of soft

rot and the risk of infection in the progeny tubers, which are used as seeds for the next generation.

4.2. Molecular mechanisms of defense responses in potato tubers infected by *D. solani*

D. solani, recognized as a highly virulent soft rot pathogen, caused tuber soft rot and stem blackleg in potato plants worldwide (Charkowski, 2018; Toth *et al.,* 2011). Soft rot bacteria are traditionally associated with a typical necrotrophic lifestyle; however, emerging evidence suggests suggests the potential for a hemibiotrophic existence. This involves an initially asymptomatic biotrophic phase within plant tissues, followed by a symptomatic necrotrophic phase when environmental conditions favor bacterial proliferation, leading to high cell density in potato tissues and caused rotting (Davidsson *et al.,* 2013). The ability of these pathogens to reside deeply within plant hosts and persist across multiple generations without inducing symptoms, known as latent infection, poses a significant challenge for soft rot control. Notably, there is almost zero evidence supporting the existence of qualitative disease resistance controlled by the existence of a single gene that complicates the efforts of plant breeders to establish effective resistance against members of the SRP. This study aims to unravel the molecular basis of activated defense responses during both asymptomatic and symptomatic phases of potato tuber infection with *D. solani*. Understanding the molecular mechanisms in the potato tuber-*D. solani* pathosystem holds the potential for identifying genetic markers utilized in potato breeding programs (Charkowski, 2015).

4.2.1. Potato tuber assay and soft rot development

A tuber sot rot assay was performed to investigate the soft rot development and disease severity at 24 and 168 hpi. Two distinct phases were clearly observed in tubers infected by *D. solani*. The brown tissue coloring with no significant soft rot symptom was observed at the early time point, while significant soft rotting was detected in infected tubers at 168 hpi. Accordingly, a time-course RNA-seq analysis was implemented to explore the transcriptional response of potato to *D. solani* infection at early and late time-points.

4.2.2. Distinct transcriptional changes in the early and late responses to *D. solani*

The RNA-seq analysis allowed us to identify 241, 268, and 413 differential expressed genes (DEGs) at 1, 24, and 168 hpi samples, respectively. The overlap in the clustering analysis of 1 and 24 hpi indicated the similarity in the potato tuber response at both of the early time points. Transcriptional dynamics highlight an exponential increase of DEGs in the late time point, which reflects a higher pathogen population during this phase resulting in increased responses in the host. However, a marked increase in the number of upregulatated DEGs was observed at 24 hpi, indicating differences in defense responses in these two stages of the infection process.

4.2.2.1. Expression profiling of potato defense genes during the early phase of *D. solani* infection

Transcriptome profiling of the early response of potato tubers to *D. solani* revealed the induction of PTI defense signaling pathway. This category encompassed the induction of defense-related signal transduction of genes associated with activation of Ca^{2+} internal flow, MAPKKK cascade, and NADPH oxidase RBOHB involved in ROS generation. Downstream defense responses were activated by the induction of genes encoding PR1 homologs, WRKY70 and WRKY3 in the SA signaling pathway. Downstream defense responses were stimulated through the induction of genes encoding PR1 homologs, WRKY70, and WRKY3 in SA signaling. Interestingly, several DEGs encoding resistance (R) proteins were mostly upregulated. Generally, R-gene-mediated ETI responses are not directly effective against necrotrophic pathogens like *Pectobacterium* (Davidsson *et al.,* 2013). Previous studies suggested no correlation between the upregulation of R genes and susceptibility of potato infected with *P. brasiliense*, indicating no direct link to defense induction in this pathosystem (Kewenda *et al.,* 2016). PTI and ETI regulate similar downstream responsive genes involved in plant resistance to the pathogen but differ in magnitude. It is plausible that PTI activation induces R genes in potatoes due to crosstalk between PTI and ETI responses, as observed in other plants (Yuan *et al.,* 2021). The upregulation of antimicrobial PR genes, including PR1, PR2,

PR3, PR5, and pathogenesis-related protein P69G, suggests the activation of the PR protein-mediated immune system, particularly SAR, enhancing resistance against *D. solani* infection in potatoes.

The findings also indicated the upregulation of genes associated with different classes of proteinase inhibitors (PIs), a significant category of PR proteins. Immune response activation leads to the accumulation of ROS and the induction of ROS-mediated oxidative bursts, acting as pathogen executioners and potentially causing damage in the form of HR in host cells. The high level of ROS in the cell was regulated by homeostasis in the ROS production-scavenging cycle, with the expression of genes associated with enzymatic or non-enzymatic scavenging systems mostly induced during the early response to *D. solani* inoculation. The onset of *D. solani* infection triggered the overrepresentation of genes involved in wound and cell wall-associated defense, including the upregulation of xyloglucan endotransglucosylase/hydrolase and expansins, alongside the downregulation of cellulose synthase. The primary defense response was associated with the induction of genes related to secondary cell wall biosynthesis in the phenylpropanoid pathway, particularly in lignin synthesis. Alteration of other genes in the phenylpropanoid and flavonoid pathways at the beginning of tuber infection could functionally contribute to bacterial resistance, acting as potato phytoanticipins either as phytotoxins or by disrupting virulence pathways.

4.2.2.2. Transcriptional reprogramming of potato during the late (symptomatic) phase of infection with *D. solani*

During the late response, potatoes employed the same set of genes in pathogen recognition and PTI immunity as observed in the early response, but with an opposite expression direction, where a majority of receptor-like kinases and WRKY70 were downregulated. The GO term “cell wall organization” was significantly enriched at the late time point 168 hpi. Within this category, the upregulation of genes encoding pectinesterase, pectate lyases, and polygalacturonase, involved in cell wall degradation, is likely linked to the promotion of tuber rotting during the symptomatic phase of infection. During the necrotrophic phase of *D. solani* infection, the induction of

polygalacturonase and pectinases is considered a susceptibility factor, potentially promoted by virulent pathogens (Gorshkov and Tsers, 2022), but it also suggests that an increase in pectin fragments may activate DAMP-mediated resistance response in the host.

RNA-Seq results indicated alterations in the expression pattern of genes related to primary metabolism during the late symptomatic phase of infection. Genes associated with sugar metabolism were significantly induced in infected tubers, potentially elevating the sugar content of the infected tissue. This could be related to the modification of host sugar efflux by bacteria, exploiting the host for their advantage. The transport of sugar, water, and other nutrients can either play a role in plant defense against pathogens or facilitate bacterial growth by providing a suitable environment for pathogenesis, thereby accelerating the host's susceptibility response (Bezrutczyk *et al.*, 2018). In this category, genes involved in polyamine biosynthesis, particularly spermidine biosynthesis, were upregulated. In potatoes, polyamine content has been positively correlated with wound healing defense response (Lulai *et al.,* 2015). Regarding lipid metabolism, genes related to phospholipid or sphingolipid metabolism, crucial for the plasma membrane or the cell wall, were mostly downregulated. However, two genes encoding lipoxygenases were upregulated, suggesting changes in oxylipin biosynthesis that may lead to JA production.

4.2.3. Potato responses to infection involve changes in defense hormones

During the early asymptomatic phase of *D. solani* infection, genes associated with SA biosynthesis, including PAL, were upregulated. No isochorismate synthase was expressed, suggesting SA production via the phenylalanine pathways in potato. Moreover, genes involved in SA signaling were upregulated early after inoculation, with WRKY70 upregulated at the early time points and downregulated later, indicating early stimulation of SA signaling. The overall data concluded that SA plays a crucial role in mediating the full defense response of potato tubers after *D. solani* inoculation. Transcriptome outcomes, verified by hormonal measurements, revealed a significant increase in SA content at the early, followed by a decrease at the later stage of infection.

In plant-pathogen interaction, the JA/ET signaling pathway serves as the main defense response to necrotrophic pathogens, showing an antagonistic relationship with SA. ET signaling components were highly expressed at early stage, indicating induction of ET-mediated signal transduction in infected potato tubers. Although the JA biosynthesis and downstream genes were induced at early stages, JA signaling was obviously activated at the late stage of infection, consistent with hormonal measurement results showing increased JA levels in symptomatic potato tubers. Two DEGs encoding JA negative regulator jasmonate ZIM-domain containing protein (JAZ) and JA receptor coronatine insensitive protein 1 (COI1) were enriched and upregulated at the early and late time points of interaction, respectively. Other genes involved in wound-induced JA signaling, such as MYC2 and MYC2-like genes, and ERF2-like gene as a positive regulator of JA-mediated defense signaling, were upregulated at the late time point, suggesting their potential role in JA production in potato tubers. The increase in endogenous JA levels is a typical response during the symptomatic phase of infection caused by soft rot bacteria (Gorshkov *et al.,* 2022; Van Gijsegem *et al.,* 2021). Accordingly, a high population density of *D. solani* was detected at the late phase of potato tuber infection (section 4.1.4) and this bacterial concentration can activate quorum sensing (Charkowski *et al.,* 2012). Similar to Arabidopsis, quorum sensing in the transition from asymptomatic infection to active rotting in potato tubers can lead to the activation of polygalacturonase (PCWDE) biosynthesis, producing oligogalacturonides that induce wound response, JA biosynthesis, and signaling (Davidsson *et al.,* 2013). This, in turn, triggers host cell wall modification and potentially activates wall-associated defenses (Mielke and Gasperini, 2019). In the *D. solani*-potato pathosystem, the early induction of PTI- and SA-mediated defenses during the asymptomatic phase is followed by the activation of JA signaling pathways after the switch to the necrotrophic phase in the later stages of infection, suggesting that *D. solani* acts as a hemibiotrophic pathogen.

4.3. Transcriptome profiling of defense response induced by *S. plymuthica* A30 against *D. solani*

The endophytic bacterium *S. plymuthica* A30, originally isolated from rotting

potato tuber tissue, exhibits considerable antagonistic activity toward blackleg- and soft rot-causing *D. solani*. This antagonistic activity has been observed in planta under greenhouse conditions (Czajkowski *et al.*, 2012b), as well as in seed material during storage and potato plants in the next growing season at the field (Hadizadeh *et al.*, 2019). Our objective is to unravel the intricate interaction between the potato host, the beneficial S. plymuthica A30, and the pathogenic *D. solani*. This investigation seeks to clarify how the antagonist influences plant fitness and defense mechanisms. Specifically, we aim to understand the role of plant immune responses and signal molecules in the efficiency of biocontrol. Additionally, we aimed to decipher the interaction of the potato host with the beneficial and pathogenic bacteria to clarify how the antagonist alters plant fitness and defense systems, where plant immune responses and derived signal molecules are important in biocontrol efficiency, and how plant regulate their gene expression patterns to adapt with beneficial microbe during symbiosis and successful microbial colonization.

4.3.1. Distinct plant response during the early and late phases of interaction with *S. plymuthica* A30

TaqMan-based qPCR assays of treated tubers with biocontrol strain A30 and *D. solani* demonestrated that the population density of A30 was boosted rapidly during first hours after inoculation and consistently remained at a high level up to 7 days after inoculation, however, population density of A30 was higher in tubers treated with combination of both pathogenic and antagonist bacterial strains compared to treatment with A30 alone. The biocontrol bacterium A30 effectively reduce *D. solani* population, and its antagonism to *D. solani* was confirmed by a significant reduction of pathogen DNA level in co-inoculated tubers. Tubers inoculated with *D. solani* almost rotted in 7 days following inoculation, while co-inoculated tubers with A30 and *D. solani* showed less than 2 % of soft rot. The brown phenolic protection layers, a typical feature of tuber defense against pathogen invasion was increased in co-inoculated tubers, while not observed in absence of the pathogen (Figure 3).

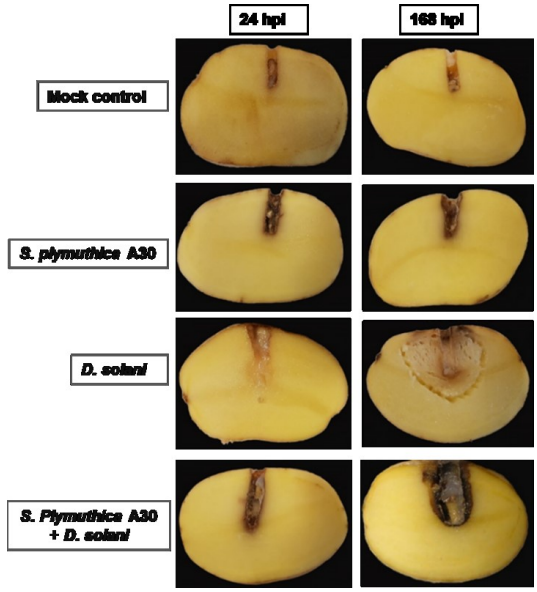

Figure 3. Effect of biocontrol *Serratia plymuthica* A30 on suppression of soft rot caused by *Dickeya solani*. Image of potato tubers with disease symptoms at 24 and 168 hpi with water, *S. plymuthica* A30, *D. solani* or their combination.

In this study, gene expression profiling of A30-treated tubers was characterized both before and after infection with *D. solani* during the early (1 and 24 hpi) and late (168 hpi) sampling time points. More specifically, transcriptome profiling of *D. solani*-infected plants with or without pretreatment by A30 was also investigated at 24 hpi, to allow a further comparison between endophyte-induced systemic resistance and pathogen-activated systemic acquired resistance. In the early response, A30 alone has only subtle effects on the host transcript levels in the absence of the pathogen, while more intense transcriptional reprogramming was observed upon *D. solani* infection, indicating a dual effect of A30. This is in line with other endophytic bacteria, which typically do not require additional host plant resources in the absence of the pathogen. Application of *S. plymuthica* A30 triggered a clear and pronounced expression of potato genes detected 24 hours after inoculation with *D. solani*, highlighting the complex mechanism of A30-induced resistance. RNA-Seq analyses of treated tuber samples collected at the late time point

revealed a considerable overlap, both in numbers and in gene identity between individual strain A30 and combined strains (pathogenic and biocontrol) treatments. GO term enrichment analyses of highly expressed genes revealed of host responses belonging to functional categories response to stimulus and signal transduction, transport, primary metabolism processes (carbohydrate, protein, and lipid metabolic process), and macromolecule metabolic processes (hormonal and cell-wall metabolism) that were stimulated by the introduction of strain A30 alone or in combination with *D. solani*.

4.3.2. Transcriptional changes in response to A30 perception share overlap with, but appear to be distinct in, tubers expressing systemic acquired resistance to *D. solani*

Transcriptional profiling revealed both analogy and convergence among genes associated with the signal transduction category, particularly those altered during ISR or SAR. These alterations signify the shared events within the plant's recognition system, emphasizing the detection of common structural features present in both beneficial and pathogenic bacterial strains. Indeed, ISR induced by beneficial microbes like strain A30 includes increase of the plant's basic immunity or MAMP-triggered defense reaction upon detection of a variety of MAMPs. The observed upregulation of genes annotated as FLS2 receptor-like kinase, brassinosteroid insensitive 1 (BRI1), and somatic embryogenesis receptor-like kinase (SERK1 and SERK4) suggests the potential recognition of flagellin, a MAMP, during the initial phase of interaction. However, the combination of bacterial strains resulted in a higher number and expression level of identified genes. The identification of diverse microbial ligands may facilitate reaching the signaling threshold required for the activation of innate immunity. Additionally, we identified genes associated with L-type and G-type lectin receptor-like kinases (LecRLKs) and receptor WAKs, specifically responding to A30 at early time points. These findings suggest their potential role as host symbiotic receptors contributing to the plant-microbe symbiotic relationship (Cope *et al.*, 2019). The data provide evidence for stimulation for the initiation of downstream defense signaling cascades, including oxidative burst, Ca^{2+} influx, and activation of protein kinase cascades as an early reaction

to A30, a response that becomes more pronounced when combined with the pathogen.

Treatment with strain A30 led to the upregulation of genes encoding MAPKKKs and EDR1, which play crucial roles in regulating the MAP kinase cascade and negatively regulates SA-dependent defense responses, ABA signaling, and ET-mediated pathway. The recognition of strain A30 by the tubers triggered the activation of genes associated with ROS generation, as evidenced by the swift and transient induction of RBOH and other regulatory genes in ROS signaling during the early phases of interaction. This suggests that the accumulation of apoplastic ROS is a component of the A30-induced systemic disease resistance. This event seems mild in response to individual A30 strain treatment due to less abundant transcripts, likely results in a low level of ROS production, serving as both a local and long-distance signal for host defense priming. Additionally, the study revealed the involvement of a substantial group of R genes activated in a typical ETI response in A30-treated tubers, demonstrated by the induction of genes encoding disease resistance proteins closely homologous to Arabidopsis RPM1 and RPS2.

In contrast to pathogen-induced PTI, which often results in significant cellular damage, the early transcriptional reprogramming of tubers in response to individual A30 was transient and relatively moderate. This mild response may be attributed to A30's capacity to manipulate plant immune mechanisms, facilitating the establishment of a mutually beneficial relationship with the host. The colonization by beneficial microbes activates host priming, enhancing the ability to mount more robust and rapid defense responses against future pathogenic attacks—a characteristic commonly observed in induced systemic resistance by beneficial bacteria. Consequently, immune responses induced by A30 exhibit heightened sensitivity and speed upon subsequent pathogen inoculation, prompting speculation that signals from the pathogen are essential to accelerate endophyte performance and evoke a fully-fledged ISR response.

4.3.3. Early A30-induced resistance triggers transcriptional regulation of stress signaling, cell wall modification, and defense metabolism

The expression of genes associated with a general response to stimulus or stress

was partially activated as an immediate response to A30, but this activation was significantly more pronounced when tubers were treated with combination of A30 and *D. solani*. Notably, there was a transient upregulation of defense-related genes, including β-1,3-glucanases (PR2), endochitinase (PR3), thaumatin-like proteins (PR5), the JA signaling marker defensin 1.2, potato antibiotic peptide snakin-1, and heat shock proteins, observed at 24 hpi. This heightened gene activity plays a crucial role in inducing systemic resistance and reducing soft rot in tubers colonized by A30. The beneficial A30 strain also exerted regulatory control over the expression of transcription factors associated with plant stress tolerance, particularly within TF families such as WRKY, MYB, bHLH (e.g., MYC2), ERF/AP2, and HSF, which play roles in both compatibility and resistance processes. WRKY genes exhibited time-dependent differential expression; positive regulators of the JA-mediated pathways, such as WRKY33, were more expressed at early time points, while those regulated by the SA pathway were predominantly downregulated during the later stages of the interaction. Furthermore, a range of ROS scavengers were activated upon A30-induced ISR. However, a more robust antioxidant defense was triggered after the pathogen challenge, which probably reduced ROS concentration and initiating retrograde ROS signaling to counter oxidative stress. Additionaly, genes encoding numerous proteolytic enzymes and protease inhibitors, crucial for priming the plant immune response, were induced in A30-treated tubers, particularly after *D. solani* inoculation.

The RNA-Seq data uncovered that strain A30 has the capability to boost the plant's basal immunity by influencing the expression of genes involved in structural modifications of the plant cell wall. Alterations in cell wall dynamics can impact intracellular signal transduction and the defense response at the site of pathogen invasion. This event is linked to the activation of genes related to crucial components of cell wall proteins, including extensins, arabinogalactan proteins, and hydroxyproline and proline-rich proteins. These proteins play critical roles in cell wall signal transduction cascades, plant growth, and stress tolerance (Kavi Kishor *et al.*, 2015). In addition, A30-mediated cell wall defense involves the upregulation of genes associated with cell wall deposition, including callose synthases, cellulose synthases, and wound-induced defense

proteins WIN1-like and WIN2. Interestingly, in contrast to *D. solani* infection, the beneficial strain A30 suppresses host pectin- and RG-degrading enzymes (polygalacturonase, pectate lyase, pectinesterase, and β-galactosidases). Simultaneously, A30 upregulates pectin synthases (glucuronosyltransferase) and pectin methyl esterase/pectinesterase inhibitors, contributing to the rigidification of the cell wall by increasing the degree of methyl esterification of pectin or potentially inhibiting pathogen pectin methyl esterases (Wormit and Usadel, 2018). A30 manipulated the host's physical barrier to upgrade cell wall strengthening to restrict pathogen spread. This enhancement included lignification, as evidenced by the upregulation of key enzymes in monolignol biosynthesis and oxidative enzymes involved in their polymerization to form lignin, such as laccases and peroxidases. Beyond lignin metabolism, upregulated genes within the core phenylpropanoid pathway were associated with the biosynthesis of suberin and wax, primarily induced in the early response to pathogen infection in A30-bacterized tubers.

The results indicated that A30 colonization may elevate the production of defensive secondary metabolites derived from phenylpropanoids, terpenes, and sesquiterpenes, commonly used as phytoalexins. The upregulation of genes involved in flavonoid antimicrobial compounds (e.g., quercetin), as well as anthocyanin and aromatic compounds were observed. Other upregulated secondary metabolite genes are associated with sesquiterpenoid phytoalexin vetispiradiene, solavetivone, rishitin (Takahashi *et al.*, 2007), stilbenoid phytoalexin pterostilbene, antimicrobial compound neomenthol, allelopathic benzoxazinoids (BXs) and their derivatives such as DIBOA, DIMBOA, and BX6 with antimicrobial activity contributing to cell wall defenses (Cotton *et al.*, 2019).

Endophyte inoculation altered genes related to primary metabolism. Despite a general downregulation of photosynthesis, upregulation of mitochondrial membrane metabolite transporters and components of the respiratory chain complex during the early response indicated the activation of the mitochondrial electron transport chain (ETC), supporting cellular energy supply for metabolic changes during the initial priming of host defense against *D. solani*. Early upregulation of genes involved in carbohydrate metabolism

suggested that endophyte A30 modified plant carbon partitioning, promoting the accumulation of precursors for metabolites fueling plant defense or providing nutrients for endophyte growth. Genes encoding SWEET bidirectional sugar transporter, invertase, phloem protein, and nitrate transporter were detected upon A30 treatment, contributing to the balance of carbon and nitrogen metabolism. The beneficial association with endophyte A30 activated genes related to fatty acid metabolism, supplying precursors for oxylipin or wax/suberin biosynthesis. Among the upregulated genes were those involved in fatty acid β-oxidation pathways, supporting JA production in tubers co-inoculated with A30 and *D. solani*. The A30 influenced the expression of genes related to phenylalanine, tryptophan, and methionine biosynthesis, potentially preparing precursors for hormones, cell wall compounds, and antimicrobial proteins to prioritize ISR response after pathogen invasion. Moreover, the induction of genes involved in cysteine and methionine biosynthesis accompanied the activation of polyamine biosynthesis and the methionine salvage pathway, linking to ethylene biosynthesis. Genes related to polyamine histamine and spermidine biosynthesis, crucial for plant stress response, were among the upregulated genes.

4.3.4. *Serratia plymuthica* A30 modulates the host immune system for successful colonization

The beneficial endophyte A30 must engage in intricate communication with its host, allowing for colonization while evading the host's MAMP-triggered immunity response. At 168 hpi, the suppression of genes related to plant immunity and signaling suggests the inhibition of active defenses initiated during early stages. Many signaling components, plant receptors, disease resistance proteins, and defense genes, including PR1b1, MYBs, and WRKYs (such as WRKY33 and WRKY70), were repressed, representing a weakened recognition of M/PAMPs, which is required to establish the intimate associations with plants. Concurrently, the upregulation of three members of the plant U-box family of ubiquitin E3 ligases (PUB22, PUB23, and PUB24), involved in the proteasomal degradation of PRR complexes for the negative regulation of MTI (Trujillo *et al.*, 2008), introduces an additional layer of

complexity.

Due to a weakened signaling network in the late phase of interaction, genes encoding RBOHs and Ca^{2+} signaling tended to be downregulated. Thus, the suppression of the respiratory burst, downregulation of genes associated with heat shock proteins (e.g., HSP90 and HSP70), and components of endoplasmic reticulum stress (e.g., syntaxin 121) suggest A30's ability to prevent continuous stimulation or better tolerate stresses without inducing oxidative stress in the plant. Despite this negative impact on general plant defense responses, there is no heightened susceptibility to host-pathogen interactions or increased symptom development after *D. solani* infection. Thus, it is reasonable to conclude that A30's biocontrol activity may primarily rely on direct antibiosis against pathogens or stimulation of the plant in the production of antimicrobial metabolites, such as proteinase inhibitors and phenolic compounds. Nonetheless, some ISR-inducing endophytes have evolved robust tolerance to antimicrobial compounds generated by the plant immune response (Lu *et al.*, 2021). The absence of constitutively elevated defense-related genes can be seen as a factor contributing to the low metabolic cost of A30-induced resistance, allowing for better energy allocation to primary or secondary plant metabolism. Moreover, endophyte A30 does not degrade lignin and carbohydrates at the late response, resulting in nonpathogenic and asymptomatic responses of treated host. The genes encoding the sucrose transporter SWEET and invertases were notably downregulated at the late response, while patatin, contributing to starch accumulation, showed upregulation.

A30 has other mechanisms to suppress the activation of host defense. Similar to observations with other biocontrol bacteria (Liu *et al.,* 2020; Lastochkina *et al.,* 2020), A30 can reduce pathogen-induced accumulation of glycoalkaloids α-solanine and α-chaconine. This is achieved by downregulating marker genes in steroid glycoalkaloid biosynthesis (hydroxymethylglutaryl-CoA reductase 1, Squalene monooxygenase, cycloartenol synthase, chalcone synthase, and solanidine glucosyltransferase) at a late time point of interaction. Consequently, the downregulation of genes related to light-regulated chlorophyll synthesis and involved in tuber greening (e.g., glutamyl-tRNA reductase and magnesium chelatase subunit I) (Okamoto *et al.,* 2020) may also

lead to reduced greening. Nevertheless, the early upregulation of genes in glycoalkaloid biosynthesis in A30-treated tubers is part of the defense response to *D. solani*. It has been recently established that the increased levels of glycoalkaloids in tuber peels after greening can serve as a source of soft rot control (Sołtys-Kalina *et al.,* 2023). Notably, during adaptation to the plant environment, endophytes can modulate plant cell wall-associated genes to establish a molecular invisibility cloak for successful colonization. In the late phase of interaction with A30, there was a transcriptional alteration of genes in cell wall remodeling, potentially increasing cell wall plasticity and facilitating A30 colonization. This includes genes correlated to synthesis and degradation of cross-linking glycans such as xyloglucan endotransglycosylases/hydrolases (XTHs), disrupting and reassembling the cellulose-xyglucan complex structure. The late upregulation of genes encoding expansin-like protein may lead to the accumulation of an auxin-inducible expansin, mediate long-term cell wall enlargement which can provide an opportunity for endophyte colonization, a phenomenon well-established in plant-symbiotic interactions (Mohanty *et al.*, 2018; Li *et al.*, 2014).

4.3.5. Hormonal changes in the early and late potato responses to A30 pre- and post-infection with *D. solani*

Recent investigations have demonstrated that both SA and JA pathways play a role in the induction of plant immunity in potatoes against *D. solani* (Burra *et al.,* 2015; Hadizadeh *et al.*, 2022). In our results, the early induction of the lipoxygenase (LOX) pathway for the production of the JA precursor cis-12-OPDA and other JA-responsive genes was observed in tubers treated with A30 across both individual and combined treatments with the pathogen. Whereas genes related to both LOX and fatty acid β-oxidation pathways, involved in the conversion of OPDA to JA, were induced only in combined treatment at 24 hpi. OPDA levels were significantly elevated in A30-treated tubers before and after the challenge with *D. solani* across both early and late time points, while JA content increased only at 24 hpi under the combined strains treatment. The A30-induced JA pathway aligns with the well-known JA accumulation in ISR response. OPDA signaling in the plant has been proposed to have intrinsic roles

in activating and fine-tuning defense responses following interactions with plant growth-promoting microbes (Wang *et al.,* 2020). Our data also indicated a reduction in both JA biosynthesis and signaling at the late time point in combined treatment (A30 and *D. solani*). Conversely, the induction of the JA-mediated defense pathway is a crucial feature of the late (necrotrophic) phase of *D. solani* infection in the tuber. The presence of a sufficient pathogen population activates the QS system, leading to the production of a high level of CWDEs, resulting in cell wall degradation and the production of OGs, which is known to trigger JA production and signaling. The reduction in the pathogen (*D. solani*) population level in treated tubers by A30 at the late phase of interaction likely caused the inactivation of the QS system, preventing the conversion of OPDA into JA in the plant when there is no challenge or infection to defend against. SA synthesis and SA-mediated defense in potato tubers were suppressed by strain A30 at both early and late responses. The decline in SA levels was accompanied by an increase in the inactive form of salicylic acid glucoside (SAG), particularly evident at 168 hpi. This reduction in SA supports the potential inhibitory crosstalk between JA and SA signaling, highlighting the role of JA in preventing excessive SA-mediated defense signaling during the early stages of interaction. This regulatory mechanism helps prevent the abortion of the symbiotic colonization event, a recognized phenomenon in plant-symbiotic microbe interactions.

Following A30 treatment, genes involved in ET biosynthesis were induced at both early and late time points. This activation of ET metabolism aligns with prior findings indicating the involvement of JA/ET signals in beneficial microbe-induced resistance. However, the endophytic bacterium A30 may directly reduce ethylene level in the host during the early phase of interaction by inducing ACC deaminase and its homolog D-cysteine desulfiderase (Nascimento *et al.*, 2014) in treated tubers. This enzyme participates in cleaving ethylene precursor ACC into ammonia and α-ketobutyrate which can be used by endophytic microbes as carbon and nitrogen source. This promotes microbial growth and enhances symbiosis by suppressing the host's defense system. In contrast, the lack of ACC deaminase expression in A30-treated tubers at 168 hpi may indicate a return to normal endogenous ethylene levels. This can regulate

seed tuber dormancy by increasing ABA levels and inhibiting potato tuber sprouting (Tosetti *et al.,* 2021; Sonnewald and Sonnewald, 2014).

Although ABA controls the proliferation and spreading of the pathogen through the suppression of plant innate immunity (Van Gijsegem *et al.*, 2017), it plays a crucial role in establishing the plant-microbe symbiotic relationship (Peskan-Berghofer *et al.*, 2015). ABA is actively involved in wound-induced suberization of potato tubers through callose deposition, required for JA-dependent defense response (Ton and Mauch-Mani, 2009; García-Andrade *et al.,* 2011). The activation of ABA biosynthesis confirms its role in both the early and later responses of tubers to strain A30, both before and after the challenge with the pathogen. A significant increase in ABA levels in A30-treated tubers was observed in all treatments, with an almost three-fold accumulation at 168 hpi compared to the respective samples at 24 hpi. It can be hypothesized that ABA promotes the colonization of potato tuber tissue by strain A30, similar to other mutualistic relationships, or induces bacterial adaptation to existing abiotic stresses such as wounding and cold storage conditions. ABA biosynthesis and signaling also play an important role in dormancy induction and maintenance in potatoes (Alamar *et al.*, 2017). During the late response to A30, the high level of ABA, coupled with noticeable induction of core components of ABA signaling, invites speculation on the role of ABA in maintaining dormancy and delaying the germination of tubers treated with strain A30. These events were accompanied by changes in the expression of genes involved in ABA-mediated dormancy or ABA-negative regulation of seed germination (e.g., Mother of FT and TFL 1, protein FRIGIDA, dormancy-associated MADS-box factors, and HVA22).

Both transcriptome analyses and hormonal measurements revealed an increase in IAA content throughout the experiment. The upregulation of genes involved in IAA biosynthesis indicate the activation of auxin production in tubers under A30 colonization. Numerous genes associated with auxin-mediated responses and auxin transport were identified, with most induction occurring at early time points in the presence of both the pathogen and A30. The alteration in IAA levels can be attributed to either beneficial or pathogenic microbes, either through modulation of the host auxin biosynthesis pathway or

direct production by A30 for its own purposes. In the case of IAA-producing endophytic bacteria like *S. plymuthica* A30, IAA can stimulate cell wall loosening to facilitate bacterial invasion and nutrient uptake while suppressing the SA pathway to evade the plant's defense strategy (Wang and Fu, 2011; Ma and Ma, 2016). High auxin content in potato tubers inhibits sprout growth, leads to increased ethylene biosynthesis, and prolongs dormancy. IAA also requires ABA for the maintenance of tuber dormancy (Liu *et al.,* 2012; Kolachevskaya *et al.,* 2019). The biosynthesis and signaling of two growth-related hormones GA and CK were suppressed in late samples, which potentially inhibit the sprouting, germination, and growth of tubers inoculated with A30. It is well known that initiation of tuber sprouting, and dormancy termination are under the control of ABA, GA, and CK (Gong *et al*., 2021).

5. CONCLUDING REMARKS

Despite extensive research on soft rot management, the development of biocontrol tools remains an important issue in controlling potato soft rot. This study study was designed with the goal to develop a better biocontrol system against *D. solani*, using environmentally safe bacterial strains capable of surviving post-harvest conditions and expressing antagonism during low-temperature storage. *S. plymuthica* A30 demonstrated effectiveness in reducing pathogen populations in potato tubers, showing great potential for controlling soft rot in wounded tubers with high pathogen inoculum levels. The endophytic strain A30 exhibited excellent colonization and survival capabilities on the tuber surface and wound sites during prolonged cold storage. The study also applied beneficial strain A30 in post-harvest treatment of seed potatoes, confirming its efficacy in protecting seed material during storage and subsequent field cultivation, reducing soft rot occurrence during storage, blackleg incidence, and pathogen transmission from mother to progeny tubers during field cultivation.

A comprehensive time-course RNA-Seq analysis was conducted to study the tritrophic interactions between potato tubers (host), *D. solani* (pathogen), S. *plymuthica* A30 (biocontrol agent), and their combination (*D. solani* and *S. plymuthica* A30) at early (1 and 24 hpi) and late (168 hpi) phases, to describe

how microbe alter plant fitness and defense signaling pathways. The RNA-Seq results revealed complex transcriptional responses, indicating significant alterations in gene expression in potato tubers, leading to diverse defense strategies at different infection stages with *D. solani*. During the early infection phase, genes involved in bacterial recognition, PTI response, SA production, ROS generation, and biosynthesis of secondary metabolite were induced. In the late infection phase, the induction of susceptibility factors like pectic enzymes coupled with the development of soft rot symptoms, leading to the activation of the pathogen-induced wound response, JA biosynthesis, and primary metabolism facilitate the availability of metabolites such as free sugar to serve the pathogen's needs.

In the early response (recognition phase), A30 directly modulates the microbial recognition system, showing higher gene expression when co-inoculated with *D. solani*. Combinations of pathogenic and beneficial strains activated numerous genes in defense signaling, including detoxification system, cell wall strengthening by callose, lignin, suberin, and antimicrobial production. A30-induced systemic resistance was observed via the induction of OPDA and JA-related genes and enhancement of their content in the tuber. The early induction of ACC deaminase enzyme suppressed ET production, affecting ethylene-induced stress. In the late response (colonization phase), A30 actively suppressed plant immunity, downregulating early-acting defense genes, facilitating the establishment of microbial biofilm and symbiotic associations. Concurrently, genes in cell wall remodeling and IAA were activated to increase cell wall plasticity, controlling symplastic trafficking to facilitate A30 colonization. A30 treatment provided fitness benefits to potato tubers, including preventing soft rot disease, inhibiting tuber greening, stress suppression, and prolonged dormancy, mediated by changes in dormancy-related hormones (ABA and ET) and growth-related hormones (GA and CK).

These findings offer valuable biomarkers for selecting novel resistance inducers and analyzing factors affecting induced resistance mechanisms, enhancing biological control systems for optimal efficacy in harvested potato tubers and improved tuber quality under storage conditions.

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