Chapter 1

General Introduction
POTATO PRODUCTION

Potato plants originated from the Andes mountains in South America where potato tubers were consumed as early as 8000 years ago. In the late XVI century (around 1570) potato was introduced into Europe by Spanish explorers initially as a decorative plant and then, later also for consumption (Hawkes & Francisco-Ortega, 1993). From there, the potato quickly spread to Italy, England and The Low Countries. By 1650, potatoes were cultivated as a field crop in Flanders, and had spread to the north of The Netherlands; to Zeeland by 1697, to Utrecht by 1731 to Overijssel by 1746 and to Friesland by 1765 (Messer, 2000). By 1800, the potato tubers were accepted as part of Dutch national diet (Davidson, 1992).

Potato is currently produced in 126 countries and the area of potato cultivation is rapidly increasing especially in developing regions (Leff et al., 2004). Today more than 325 million tonnes of potato are produced all over the world but the majority of potato production still occurs in Europe and Asia (approximately 80% of world potato production) (van der Zaag & Horton, 1983). In Europe ware potatoes are mainly cultivated in Russia, Ukraine, Poland, Germany, Belarus, France and in the United Kingdom. Simultaneously, The Netherlands with an export of ca. 700 000 tons per year for more than 40 years is the world leading producer of certified seed potatoes (NAO - Nederlandse Aardappel Organisatie; International Potato Center, Lima, Peru).

Today, in Europe, The Netherlands is among the top 10 potato producers with a harvest of approximately 7.2 million tonnes a year. Almost 25% of the Dutch arable land (around 160 000 ha) is used for potato production with an average yield of 45 tonnes per hectare. Around 50% of the potato crop is grown for food, 30% for starch production and the last 20% are seed tubers. The annual production of seed potatoes in The Netherlands is estimated to be about 1.2 million tonnes, which is 37% of the total seed potato production in the European Union. In 2009, the total area in which seed potatoes were cultivated in The Netherlands was 37.000 hectares. In the season 2008/2009 the export of seed potatoes to the European Union, Asia and America (altogether approximately export to 80 countries) reached 662.000 tonnes, which is 70% of export worldwide (J Gottschall, NAO, personal communication).

The intensive production of (seed) potato tubers increases the risks for spread of potato diseases. Potato plants and tubers are affected by approximately one hundred sixty diseases from which fifty are caused by fungi, ten by bacteria,
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Forty diseases are caused by viruses and the rest are based on the unknown-yet origin (Arora & Khurana, 2004). Diseases may influence potato production in any stage of crop growth and in storage, and can effect foliage, tubers or both. Potato diseases may reduce heavily crop yield and quality (Hooker, 1981).

From all pathogens infecting potato seed production, bacteria are recognized as the most serious problem (Van der Wolf & De Boer, 2007). Bacteria are able to infect potato plants and tubers from wounds and natural openings like stomata and lenticels and openings that occur during lateral root formation. They are often introduced via infected seed potatoes and are spread easily within a seed lot via the soil, water or/and contaminated machines, in particular during harvesting. If the environmental conditions favor their multiplication, bacteria will easily establish infections in potato tissues and spread through the entire plant. The most serious aspect of bacterial pathogenesis is that there are hardly any possibility to control bacterial pathogens on potato. Frequently, symptomless infections are present, not found during field inspections. Resistance in commercial cultivars is largely absent and chemicals to cure tubers and plants during cultivation are not available, moreover hygienic measures are insufficient to prevent seed infections (Van der Wolf & De Boer, 2007).

BLACKLEG AND SOFT ROT OF POTATO

Most harmful and damaging bacterial diseases of seed potato production in Europe are blackleg and soft rot caused by *Pectobacterium* and *Dickeya* species. The economic losses in seed potato production in The Netherlands due to the blackleg and soft rot diseases are estimated between 15 and 30 million euro annually (Prins & Breukers, 2008).

Blackleg and soft rot are seed-borne diseases. Production of pathogen-free seed tubers is therefore of great economic importance and of major growers’ interest (Perombelon, 2002).

Bacterial species belonging to different genera like *Pectobacterium*, *Dickeya*, *Pseudomonas*, *Bacillus*, *Clostridium*, *Aerobacter*, *Flavobacterium* and *Rhodococcus* are able to cause tuber rot. All these bacteria possess the ability to produce plant tissue macerating enzymes. Of these, soft rot and blackleg causing *Pectobacterium* and *Dickeya* spp. are regarded as the most important. *Pectobacterium* and *Dickeya* spp. are primary pathogens whereas species belonging to the other genera are in general only able to enhance decay after rot.
has been initiated by *Dickeya* and/or *Pectobacterium* spp. (Perombelon & Lowe, 1975).

Blackleg and soft rot causing *Pectobacterium* and *Dickeya* species are responsible also for diseases in other than potato crops like carrots, onion, cucumber, cabbage and in ornamentals such as hyacinth and cyclamen (Kado, 2006).

Potato blackleg can be caused by *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliensis*, *P. wasabiae* and *Dickeya* spp. wherever potato is cultivated (Pitman et al., 2010, Duarte et al., 2004, Perombelon, 2002, Samson et al., 2005). Disease symptoms caused by these different pathogens are indistinguishable.

All species easily cause soft rot of tubers during storage if environmental conditions favor disease progression, but the bacteria differ in their relative contribution to blackleg incidences (Perombelon, 2002). For a long time, *P. carotovorum* subsp. *carotovorum* was considered to play a minor role in potato blackleg, but recently it has been proven that *P. carotovorum* infections can result in typical blackleg in Europe (De Haan et al., 2008).

In 2004, a first report on a *P. carotovorum* subsp. *brasiliensis* causing severe blackleg symptoms in potato in Brazil was published (Duarte et al., 2004). This pathogen is more virulent than *P. atrosepticum* under warm climate conditions. So far *P. c. subsp. brasiliensis* was only isolated in South America and Africa (van der Merwe et al., 2010).

In the past, in temperate climate zones, particularly in Europe and in North America, *P. atrosepticum* was regarded as the dominant causative agent of blackleg. Molina and Harrison (1977) reported that the high blackleg incidences in potato crop in Colorado were dominantly caused by *P. atrosepticum* (Molina & Harrison, 1977). Perombelon (1972) in Scotland showed that up to 80% of progeny tubers were contaminated with *P. atrosepticum* although that plants often did not show any symptoms (Perombelon, 1972).

*Dickeya* spp. were recognized as pathogens of tropical and subtropical regions, being associated mainly with ornamentals (Perombelon & Salmond, 1995). Nevertheless, strains of *D. dianthicola* were frequently isolated from the blackleg-diseased plants in Northern and Western Europe. These “cold tolerant” *D. dianthicola* strains possessed a lower growth temperature optimum than other *Dickeya* species (Janse & Ruissen, 1988). In the last five years an increase in the blackleg incidences caused by *Dickeya* spp. bacteria in Europe was observed. Since 2005, *Dickeya* spp. was responsible for 50 to 100% of field infections in The Netherlands and France (Van der Wolf et al., 2008). A similar increase in *Dickeya*
spp. caused blackleg incidences were reported in other countries like Finland (Laurila et al., 2010) and in Israel (Tsror et al., 2008).

PRESENCE OF A NEW GENETIC CLADE OF BIOVAR 3 Dickeya SPP. – “D. Solani”

The increase in blackleg incidences caused by Dickeya spp. is related to the occurrence of a new, highly virulent genetic clade of Dickeya spp. Since 2005, this new genetic clade, representing probably a new Dickeya species has spread all over Europe (Tsror et al., 2008, Slawiak et al., 2009). Strains belonging to this clade were isolated from potatoes in France, Finland, Poland, England, the Netherlands, Germany, Sweden and in Israel (van der Wolf, personal communication). In the Netherlands, in last five years this clade was almost exclusively isolated from seed potatoes.

The strains cannot be classified in any of the six, known Dickeya species described till now (Samson et al., 2005). Results from dnaX and 16S rDNA sequence analyses, Rep-PCR and biochemical assays showed that all new Dickeya isolates from Europe and Israel that belonged to this new genetic clade were clonal. This points to their common origin and possibly a single introduction event. All isolates belong to biovar 3, a group of biochemically distinct strains which were isolated from crops grown in a warm climate or cultivated in greenhouses. The new unclassified biovar 3 strain is provisionally called “D. solani”. D. solani possesses a higher growth temperature optimum than the European D. dianthicola strains previously isolated from potato (Janse & Ruissen, 1988).

OBJECTIVES

The main objectives of this study were to gain knowledge on the ecology of the new genetic clade of biovar 3 Dickeya spp. “D. solani” currently found in seed potatoes in Europe and to find an effective biocontrol strategy to cure infected potato tubers from blackleg caused by D. solani.
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APPROACHES

Direct isolation of viable bacteria cells using (selective) plating techniques combined with molecular (16S rDNA and Rep-PCR), serological (DAS-ELISA) and biochemical (biovar determination) characterization of the isolates were used to evaluate the presence of Dickeya spp. and Pectobacterium spp. in different plant parts and tissues.

Bacterial population dynamics in planta was studied in greenhouse experiments. For the studies on colonization of potato tissues by biovar 3 Dickeya spp. (“D. solani”), and Serratia plymuthica A30, bacterial strains were transformed with plasmid-based genes coding for red or green fluorescent proteins (DsRed and GFP respectively) that were constitutively expressed in bacterial cells. Epifluorescence stereo microscopy and confocal laser scanning microscopy were used to visualize bacterial presence in different tissues of potato tubers or plants.

For isolation and characterization of bacterial isolates able to decrease and/or eradicate biovar 3 Dickeya sp. populations in infected plants, bacterial strains were isolated from rotten potato tissue. Selected isolates were assessed for features that are important in antagonism (i.e. antibiosis, siderophore, auxin and biosurfactant production, motility, quorum quenching) in plate assays, semi in planta potato slice assays and under greenhouse conditions favorable for blackleg disease development. Strains were characterized by partial sequencing of 16S rDNA and classified into risk categories.

Interactions of biocontrol Serratia plymuthica A30 and blackleg causing biovar 3 Dickeya sp. type strain (IPO2222) were evaluated in potato slice assay and in planta under greenhouse conditions. Population dynamics was assessed by direct bacterial isolation on selective media and by studies with epifluorescence stereomicroscopy and confocal laser scanning microscopy.

OUTLINE OF THE THESIS

Chapter 2 provides a literature review on the possible methods to control blackleg and soft rot causing bacteria. The paper summarizes major characteristics of the methods used in practice and evaluates the development and application of cultivars resistant to blackleg causing pathogens, the use of genetically modified potatoes and chemical, physical and biological treatments. This review provides
also basic information on the major (bacterial) pathogens and on the ecology of *Pectobacterium* and *Dickeya* species in relation to control strategies.

**Chapter 3** presents a study on the distribution of blackleg causing bacteria and their population structure in naturally, latently infected tubers of two potato seed lots. Knowledge on the distribution in (seed) tuber tissue, which is largely missing, is required for sampling of tuber tissue in seed testing programmes and for the development of efficient tuber sanitation procedures.

**Chapter 4** describes the ability of a strain from the new genetic clade of biovar 3 *Dickeya* sp. to infect roots from infested soil and to further colonize potato plants systemically after root infection. Potato tuber decay during growth results in the release of a large bacterial inoculum and infestation of soil. During wet weather conditions bacteria will migrate with free soil water and may infect neighboring plants and machines used for tubers collecting, which will increase the chances of infection or latent contamination of progeny tubers. These soil-borne bacteria may either directly infect tuber lenticels or, after root infection, may infect progeny tubers systemically.

**Chapter 5** describes the ability of a new *Dickeya* sp. strain to latently infect progeny tubers after haulm infection. Pathogen-free potato plants may become contaminated during cultivation and the infection may originate from a variety of sources such as contaminated machines contaminated insects, irrigation water, rain water, aerosols, human activity during field inspections or via animals entering potato fields. For all these routes, contamination will result in infection of haulms rather than of underground plant parts. Aerial stem rot, which is frequently found in the field under wet conditions, may be the result of these introductions but knowledge on the contribution to the contamination and infection of progeny tubers is lacking.

**Chapter 6** provides information on the possibilities for the (bio)control of a new clade of biovar 3 *Dickeya* sp. in potato by using antagonistic bacteria isolated from rotten potato tissue. Selection procedures are described to obtain bacterial antagonists able to survive and multiply in (rotting) potato tubers. The chapter also provides information on the characterization of the obtained antagonists for features potentially important in antagonism and for their ability to survive in different environments and different growth conditions. Finally, an antagonist is described that showed a high level of protection against infections caused by biovar 3 *Dickeya* sp. under greenhouse conditions.

**Chapter 7** describes the study on the control of the biovar 3 *Dickeya* sp. ("*D. solani*") by *Serratia plymuthica* A30 in planta under greenhouse conditions. The *S. plymuthica* A30 was isolated and characterized in the study presented in
Chapter 6. The strain showed good potential in control of Dickeya sp. in in vitro and in planta tests. This chapter also provides information of Dickeya sp. and S. plymuthica A30 interactions inside roots and shoots of potato plants and survival of biocontrol agent in this environment. Information is also provided on the ability of S. plymuthica A30 to colonize potato roots and stems from soil-borne inoculum and how this will contribute to the control of Dickeya sp. in potato.

Chapter 8, gives a summarizing discussion on the implications of the results for the control of biovar 3 Dickeya sp. (“D. solani”)