Incidence of Pectolytic Erwinias Associated with Blackleg of Potato in Rio Grande do Sul[1]

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ABSTRACT

Erwinia carotovora subsp. *atroseptica* (Eca), *E. carotovora* subsp. *carotovora* (Ecc) and *E. chrysanthemi* (Ech) may cause potato (*Solanum tuberosum*) blackleg. To determine the occurrence of these pathogens in the conditions found in the State of Rio Grande do Sul (RS), potato plants showing blackleg symptoms were harvested from 22 fields in nine counties in Serra do Nordeste, Planalto, Depressão Central, and Grandes Lagoas, from September to December of 1999 (Spring-Summer season). Green pepper (*Capsicum annuum*) fruits were used as a host to enrich for pectolytic erwinia from potato stems with blackleg symptoms. Bacteria were subsequently isolated on non-selective medium. Isolates that were Gram-negative, facultatively anaerobic, and pitted crystal-violet-pectate medium were tested for biochemical traits to identify the species and subspecies. Four hundred strains were identified as either Eca, Ecc or Ech. Although the three erwinias were found in RS potato fields, only three strains of Ech were found in one field. Frequencies of Eca and Ecc were 55 and 42%, respectively. Eight strains could not be assigned based on the biochemical characterization.

Additional key words: *Erwinia carotovora* subsp. *atroseptica, E. carotovora* subsp. *carotovora, Erwinia chrysanthemi,* epidemiology, *Solanum tuberosum*, soft rot.

RESUMO

Incidência de erwinias pectolíticas associadas com a canela preta em batata no Rio Grande do Sul

Erwinia carotovora subsp. *atroseptica* (Eca), *E. carotovora* subsp. *carotovora* (Ecc) e *E. chrysanthemi* (Ech) podem causar canela preta em batata (S*olanum tuberosum*). Com o objetivo de verificar a ocorrência destes patógenos em lavouras do Rio Grande do Sul, plantas com sintomas de canela preta foram coletadas em 22 lavouras em nove municípios de quatro regiões produtoras: Serra do Nordeste, Planalto, Depressão Central e Grandes Lagoas, nos meses de setembro a dezembro de 1999 (safra primavera-verão). Frutos de pimentão verde foram inoculados com palito de dente previamente espetados em tecido doente na haste de batata. Isolados com células Gram negativas, anaeróbios facultativos, formando cavidade em meio cristal-violeta pectato, foram identificados ao nível de espécie e subspécie através das características bioquímicas. Quatrocentos isolados foram identificados como Eca, Ecc ou Ech. Embora as três erwinias estejam presentes no RS, apenas três estirpes de Ech foram encontradas numa lavoura; as frequências de Eca e Ecc foram 55 e 42%, respectivamente. Oito isolados não se ajustaram na classificação baseada nas características bioquímicas.

INTRODUCTION

Blackleg is endemic wherever potato (*Solanum tuberosum* L.) is grown in Brazil, but there are few reports (Jabuonski *et al*., 1986b) about the causal agent and its incidence. *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye (Eca) has been cited as the exclusive causal agent in cool and temperate regions. However, *E. carotovora* subsp. *carotovora* (Jones) Bergey *et al*. (Ecc) and *E. chrysanthemi* Burkholder *et al*. (Ech) can also cause similar symptoms (Gumestad & Secor, 1993; Duarte, 1999).

Identification of Eca, Ecc and Ech is based on biochemical and phenotypic characteristics (Pérombelon & Hyman, 1986; Dickey & Kelman, 1988; Hyman *et al*., 1998). Serology (De Boer & McNaughton, 1987; Singh *et al.,* 2000) and, more recently, molecular techniques (De Boer & Ward, 1995; Helias *et al*., 1998; Fréchon *et al*., 1998) have also been applied.

Ecological and epidemiological studies of these pathogens have shown Eca, Ecc and Ech to have a wide geographical distribution, correlated with temperature, and host range (Pérombelon & Kelman, 1980). Diversity within Ecc and Ech is far greater than that within Eca, making it possible to relate diversity within Ech and, to a lesser degree, Ecc with host range and geographical location. Ecc occurs in regions with different temperatures, in soil and in surface water of lakes, rivers and oceans, and in the rhizosphere of host and nonhost plants (Pérombelon & Kelman, 1980). Ech is pathogenic to many important crop and ornamental plants

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in tropical and subtropical climates, but is composed of strains that show some host specificity (Jabuonski *et al*., 1986a).

Few epidemiological studies involving species and subspecies identification of *Erwinia* affecting potatoes have been done in subtropical and tropical climates, such as Brazil. Most publications are reports of occurrence of Ecc (Takatsu, 1983). Jabuonski *et al*. (1986a) assessed species of pectolytic erwinias in several hosts, including potato, and found that Ecc was predominant in nine out of ten states of Brazil. Only Eca was isolated from potato in Rio Grande do Sul. Other references to the occurrence of Eca were made by Robbs (1981) and Silveira (1992).

Control of blackleg is based on crop management practices such as crop rotation, elimination of plant debris, and use of pathogen-free seed tubers (Gumestad & Secor, 1993). As Eca, Ecc and Ech have different survival mechanisms and host ranges, knowledge of the etiology of blackleg is necessary to establish suitable control measures. The objective of this study was to identify the species and subspecies of *Erwinia* associated with blackleg in potato fields of Rio Grande do Sul in order to evaluate local disease management strategies.

MATERIALs AND METHODS

Sampling and isolation of pectolytic erwinias

Plants showing blackleg symptoms were collected during September through December of 1999. Twenty samples were collected per field from 22 potato fields in nine counties, in four regions of RS: Serra do Nordeste, Planalto Superior, Depressão Central and Grandes Lagoas (Figure 1).

In the lab, toothpicks were stabbed into the margin of blackleg lesions in potato stems and then placed into surface-disinfested (alcohol 70%, 30 s; NaOCl 1%, 30 s; and washed with sterile distilled water) green pepper (*Capsicum annuum* L.) fruits (Takatsu *et al*., 1981). The inoculated fruits were kept in a humid chamber at 28 °C for 24-48 h. Decayed tissue was peeled off with a scalpel. A loopful was used to touch the tissue and to streak the surface of K & H 523 culture medium (Kado & Heskett, 1970). Single colonies were harvested and tested for identity. Gram negative, facultatively anaerobic (Oxidation/Fermentation positive), straight rod-shaped bacteria, forming cream opaque colonies with irregular borders were considered to belong to the soft rot group of bacteria of the genus *Erwinia* (Dickey & Kelman, 1988; Hyman *et al.*, 1998). One bacterial isolate per plant was maintained in filter paper at 4 °C (Takatsu, 1970) and glycerol-water (15:85) at -80 °C.

Biochemical tests

Bacterial isolates were tested for production of reducing substances from sucrose and acid production from a-methyl-glucoside, maltose D(+), and lactose, growth at 37 °C, and sensitivity to erythromycin (Dickey & Kelman, 1988). Reference strains of *E. carotovora* subsp. *atroseptica* (Eca IBSBF 839), *E. carotovora* subsp. *carotovora* (Ecc IBSBF 1023) and *E. chrysanthemi* (Ech IBSBF 231), from the culture collection of Instituto Biológico, São Paulo, SP, Brazil, were used in each test.

Culture medium (peptone, 10 g; bromocresol purple 1.5 %, 0.7 ml; agar, 0.3 g; sugar 10%, 100 ml/l, pH 6.8) (Dickey & Kelman, 1988) with each sugar was loaded in 96-well microtiter plates. Bacteria from 24-48 h-old colonies were transferred to test media with a sterile toothpick, and incubated at 28 °C. Results were recorded for two-seven days after inoculation. A color change from purple to yellow was considered positive. The tests were conducted twice.

RESULTS AND DISCUSSION

Identification of pectolytic erwinias

Four hundred and eight isolates of pectolytic erwinias were obtained from plants showing blackleg symptoms in potato fields in the four main production areas of RS. Among these, 224 isolates were determined to be Eca, 173 to be Ecc and three to be Ech (Table 1). Eight isolates presented traits that did not correspond to those of any of the three species and subspecies and thus were not identified. Ninety and 79% of the Eca and Ecc strains, respectively, did not produce acid from maltose. According to Lelliot & Dick (1984), 21-79% of *E. carotovora* strains should be positive. This behavior may indicate a striking difference between the Brazilian and the other strains. Ninety-two percent of Eca and 75% of Ecc produced acid from lactose, which is consistent with the

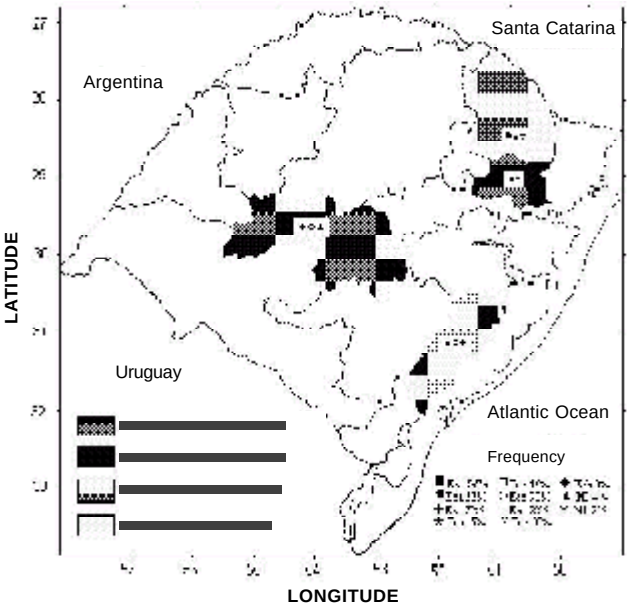

FIG. 1 - Agroecological regions of the State of Rio Grande do Sul where plants showing blackleg symptoms were collected from potato (*Solanum tuberosum*) fields. Frequency of *Erwinia carotovora* subsp. *atroseptica* (Eca), *E. carotovora* subsp. *carotovora* (Ecc) and *E. chrysanthemi* (Ech) in each region is indicated

TABLE 1 - Physiological and biochemical tests of 400 pectolytic erwinias isolated from plants showing blackleg symptoms sampled from 22 potato (*Solanum tuberosum*) fields in the State of Rio Grande do Sul

Species and subspecies	Number of strains	Strains (%)							
		Pectolytic activity	O/F*	Growth at 37 ºC	Resistance to erythromycin	Produced reducing substances from sucrose	Acid from		
							α-methyl glucoside	Lactose	Maltose
Eca**	224	100	100	10	0	100	100	92	10
Ecc	173	100	100	100	0	0	0	75	21
Ech	3	100	100	100	100	0	0	0	0

* O/F = Oxidation/Fermentation

** Eca = *Erwinia carotovora* subsp. *atroseptica*; Ecc = *E. carotovora* subsp. *carotovora*; Ech = *E. chrysanthemi*

subspecies descriptions (Lelliot & Dickey, 1984).

Acid production from α-methyl-glucoside and growth at 37 °C are among the most important biochemical and physiological characteristics that differentiate Eca from Ecc (Pérombelon & Kelman, 1980; Jabuonski *et al*., 1986a). However, there are conflicting data in the literature regarding the ability of Ecc to grow at 37 °C (Lelliot & Dickey, 1984; Jabuonski *et al*., 1986a; Pérombelon & Kelman, 1980; Hyman *et al*., 1998).

In the current study, none of the strains identified as Ecc produced acid from α-methyl-glucoside but all grew at 37 °C, consistent with current description (Dickey & Kelman, 1988). Among the strains collected from the different regions of Brazil by Jabuonski *et al*. (1986a), 44 were considered Ecc and did not grow at 37 °C. This was not confirmed here (Table 1). Phenotypic variability was observed among Ecc strains occurring in Brazil.

Among Eca strains, 10% grew at 37 °C in 24 h (Table 1), which is characteristic of Ecc and Ech. These strains might be atypical Eca, similar to those reported by other authors (Thomson *et al*., 1981; Helias *et al*., 1998).

Eight isolates did not fit into any identification profile. The occurrence of intermediate forms of Eca, Ecc and Ech, sharing common characteristics, has been reported (Thomson *et al*., 1981; Jabuonski *et al*., 1986a, 1986b). Thomson *et al*. (1981) described *E. carotovora* subsp. *betavasculorum* (Ecb), which occurs in sugar beet (*Beta vulgaris* L.), sunflower (*Helianthus annuus* L.), artichoke (*Cynara cardunculus* L.) and potato (Samson *et al*., 1998, cited by Helias *et al*., 1998), showing common characteristics with Eca and Ech. Therefore, the identity of these isolates should be investigated further, to see if they correspond to Ecb.

Polymerase Chain Reaction (PCR), using the primers ECA 1f and ECA 2r (De Boer & Ward, 1995) and Y45 and Y46 (Fréchon *et al*., 1998), specific to Eca, amplified DNA from a control Eca strain (Eca 31 provided by S. H. De Boer, Center for Animal and Plant Health, Charlottetown, Canada), but did not amplify DNA from any of the strains identified as Eca in the current study (data not shown).

Incidence and distribution of pectolytic erwinias

Eca was prevalent in all geographic regions of RS, except Grandes Lagoas, where 90% of the strains were identified as Ecc and 5% as Ech (Table 2). Since temperatures in RS are lower than most of the other states, the frequent occurrence of Ecc (42% of strains) may be considered unexpectedly high.

The association of Eca, Ecc or Ech with blackleg of potato is related to the environmental conditions, particularly temperature (Pérombelon & Kelman, 1980; Pérombelon *et al*., 1979; Powelson, 1980). The distribution of Eca, Ecc and Ech in RS has no apparent relation to geographic region or temperature. A much higher incidence of Eca than Ecc would be expected in Serra do Nordeste, where temperatures are lower than in other regions (Table 2).

Rio Grande do Sul is characterized by a humid subtropical climate. The four potato production areas (Figure 1) are located in very distinct regions based on type of soil, temperature, and amount of rain. The results showed that these conditions favored a balanced incidence of Eca (55%) and Ecc (42 %), but very low incidence of Ech (3%) (Table 2). Based on studies of population dynamics, Pérombelon *et al*. (1979) suggested that, when both Eca and Ecc are present, Eca predominates at low temperatures (16 °C), and Ecc at high temperatures (26 °C). The populations are similar at 22 °C. The study of seasonal incidence of Eca and Ecc in potato plants with blackleg symptoms in Oregon, U.S.A., at temperatures of 16 to 22 °C, showed that both subspecies were associated with the disease and Eca and Ecc predominated in cooler and warmer seasons, respectively (Powelson, 1980).

Temperature was not associated with the predominance of Ecc (90%) in the region of Grandes Lagoas (Table 2). This suggests that other epidemiological factors, like contaminated soil or seed potato, are involved. Powelson & Apple (1984) studied the importance of soil as an inoculum source through the identification of serogroups of strains found in the soil and in blackleg infected tissues. They concluded that the possibility of contamination through the soil was low. Besides contaminated soil, such factors as potato seed, other host plants, insects, weed rhizosphere, and irrigation water must be considered to explain the survival and dissemination of Ecc (Armond *et al*., 1995). Therefore, the source of the inoculum for contamination of potatoes in Grande Lagoas may be primarily the seed tuber. The seed produced outside this region and the erwinia contamination may reflect the

TABLE 2 - Incidence of pectolytic erwinias associated with blackleg in 22 potato (*Solanum tuberosum*) fields in four production areas in the State of Rio Grande do Sul. 1999

Region	County	Number of isolates						
		Potato field			Identification			
		1	2	3	Eca*	Ecc	Ech	NI
Serra do Nordeste	Caxias do Sul	20^{Ba***}	0	0	15	5	0	0
(17.1 °C)**	Farroupilha	20^{Ma}	12^{Ma}	0	12	20	0	0
	Garibaldi	20^{Ma}	0	0	12	8	0	0
	(%)				**54**	**46**	**0**	**0**
Planalto Superior	Ibiraiaras	20^{Ba}	16^{Ma}	20^{El}	30	26	0	0
(19.0 °C)	Nova Prata	20^{Ag}	20^{Ma}	17^{As}	49	5	0	3
	São Jorge	20^{Ma}	18^{El}	20^{Ma}	30	25	0	3
	(%)				**63**	**33**	**0**	**4**
Depressão Central	Santa Maria	20^{Ba}	20^{Ma}	20^{Ba}	38	20	0	2
(20.4 °C)	Silveira Martins	15^{Ma}	20^{Ma}	10^{Ba}	35	10	0	0
	(%)				**70**	**28**	**0**	**2**
Grandes Lagoas	São Lourenço do Sul	20^{Ma}	20^{Ma}	20^{Sa}	3	54	3	0
(18.8 °C)	**(%)**				**5**	**90**	**5**	**0**
Total			408		224	173	3	8
(%)					55	42	1	2

*Eca = *Erwinia carotovora* subsp. *atroseptica*; Ecc = *E. carotovora* subsp. *carotovora* ; Ech = *E. chrysanthemi*; NI = Not identified.
**Mean temperature from September to December of 1999 (Spring-Summer Season). Source: Fundação Estadual de Pesquisa Agropecuária do Estado do Rio Grande do Sul – FEPAGRO.
***Cultivar: Ag = Ágria, As = Asterix, Ba = Baronesa, El = Elvira, Ma = Macaca, Sa = Santo Amor

area where the seed was produced more than where the crop was sampled.

Considering that Ech is better adapted to warm regions than Eca or Ecc (Pérombelon & Kelman, 1980), the low incidence (1%) was expected. However, the occurrence of Ech associated with blackleg at 20 °C in Brazil was previously reported (Jabuonski *et al*., 1986b).

Eight strains out of 408 could not be identified in this study by the biochemical and physiological tests used. Jabuonski *et al*. (1986b) reported that four out of 22 isolates from potato blackleg in RS could not be identified. Also, Silveira (1992) reported on 23 isolates that did not fit in any of the *Erwinia* species or subspecies. The consistent presence of strains with traits that do not fit in the identification profile may show the variability of Brazilian strains. The identification and epidemiological importance of such strains need to be studied.

Besides the previous reports (Jabuonski *et al*., 1986b; Silveira, 1992), this is the first broad survey of pectolytic erwinias associated with potato blackleg in RS. It was shown that Ecc was associated with potato blackleg as frequently as Eca. Therefore, the management of this disease in RS must take into consideration the wider host range and the different ability of Ecc to survive in the environment. Epidemiological studies are crucial to establish the incidence of these subspecies in or on seed potatoes under local conditions.

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