Lemessa, F., Zeller, W.
Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany; E-mail:lemessaf@yahoo.com

Biological control of potato bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: Determination of biovars of *Ralstonia solanacearum*

Abstract

Ralstonia solanacearum is a very destructive pathogen that causes wilt in potato and many other solanacean crops in Ethiopia. In order to select effective antagonistic biocontrol agents for *R. solanacearum* strains, it is necessary to characterize the population of pathogenic strains. Therefore, 62 strains collected from wilted potato, tomato and pepper plants and potato tubers from the major potato producing regions of Ethiopia were characterized culturally and classified physiologically, based on their capacity to oxidize 3 disaccharides (lactose, maltose and cellobiose) and 3 hexose alcohols (mannitol, sorbitol and dulcitol). The results of this study indicated that all virulent strains from Ethiopia produce fluidal and irregular colonies with red centre and whitish periphery on triphenyl tetrazolium chloride (TZC) medium and irregular, fluidal, and creamy white colonies on casamino acids-pepton-glucose (CPG) medium. Physiologically, 19 strains were grouped to biovar I and 43 strains to biovar II. Previous studies from Ethiopia reported the availability of only biovar II of *R. solanacearum.* Thus for biovar I this is the first report concerning the Ethiopian *R. solanacearum* population.

Introduction

R. solanacearum E.F. Smith (Yabucchi et al. 1995), the causal agent of bacterial wilt, produces a severe and devastating disease affecting many crops in tropical and temperate regions (Hayward 1991). In Ethiopia it is a very destructive pathogen that causes wilt on potato and many other solanacean crops with incidence on potato as high as 63% in major potato growing areas (Bekele 1996).

To date, no effective control methods exist for potato bacterial wilt disease. Plant breeding, field sanitation, crop rotation and use of bactericides had only limited success (Ciampi-Panno et al. 1989). An increasing number of reports have indicated that biological control of potato bacterial wilt could be achieved using antagonistic micro-organisms. In order to select effective antagonistic biocontrol agents for the *R. solanacearum* strains, it is necessary first to characterize the variability of the strains. Therefore, the objective of this study was to culturally and physiologically characterize Ethiopian *R. solanacearum* populations.

Materials and methods

Bacterial strains and growth conditions: 62 strains collected from potato producing regions of Ethiopia from infected potato, tomato, and pepper plants and potato tubers were cultured on tetrazolium chloride (TZC) medium (Kelman 1954) and on casamino acids-pepton-glucose (CPG) medium. Cultures were maintained in sterile water at room temperature and revived by plating a loopful on TZC agar medium and CPG agar (0.1% peptone, 0.01% casamino acids (Difco), 0.05% glucose, and 1.5% (wt/volume) agar at 30°C.

Cultural and physiological tests: Isolates were culturally characterized by growing them on TZC and CPG medium at 30°C and recording the colony characters. The oxidation of sugars and sugar alcohols was tested on the basal medium according to Hayward (1964). Lactose, maltose, and cellobiose solutions were filter sterilized, while mannitol, sorbitol and dulcitol were autoclaved for 20 min as 10% (w/v) solutions. Five ml of each sugar and sugar alcohol solution were added to 45 ml of Hayward's medium and 10 ml of the resulting amended medium was dispensed into test tubes (Hayward 1964).

A suspension of each strain was prepared by inoculating 300 µl of sterile water with a loopful of cells (Williamson et al. 2002) from each strain grown on CPG for 48 h at 30°C. The test tubes with Hayward's

medium were inoculated with 30 µl of the suspension, incubated at 30°C and checked for acid production (yellow colour) (Hayward 1964) at various intervals for up to 5 weeks.

Results and discussion

All virulent strains of *R. solanacearum* from Ethiopia produce fluidal and irregular colonies with a red centre and whitish periphery on triphenyl tetrazolium chloride (TZC) medium after 48 h of incubation. However, when the strains lost their virulence upon storage, the colony becomes smaller and round with deep colour. On CPG medium Ethiopian isolates produce larger and whitish fluidal colonies which turn brown after 48 h of incubation. The colony appearance on TZC is typical to *R. solanacearum* (Kelman 1954), in agreement with a report from Ethiopia by Yaynu (1989) 17 years ago.

Marked differences were observed in the ability of strains from Ethiopia to oxidise disaccharides and sugar alcohols. Based on Hayward's classification scheme (Hayward 1964), 43 of 62 strains were classified as biovar II and 19 as biovar I (Table). Biovar II strains produced acid from lactose, maltose and cellobiose but failed to oxidise mannitol, sorbitol and dulcitol, while biovar I strains oxidized none of the disaccharides and sugar alcohols even after 5 weeks of incubation.

Table Oxidation of carbohydrates by strains of *Ralstonia solanacearum* from Ethiopia

Number of strains	Carbohydrate[a]						Biovar
	Lactose	Maltose	Cellobiose	Dulcitol	Mannitol	Sorbitol	
19	-	-	-	-	-	-	I
43	+	+	+	-	-	-	II

[a] + = reaction; - = negative reaction

Previous studies from Ethiopia reported the availability of only biovar II of *R. solanacearum.* Thus biovar I is observation the first time in the Ethiopian *R. solanacearum* population. Since this biotype is present in most parts of the world (He et al. 1983) this result is not surprising because Ethiopia has been introducing several thousands of potato genotypes (Berga et al. 1994) from the International Potato Center (CIP) and other parts of the world to develop high yields and adaptable cultivars with resistance to major stresses. Hence biovar I strains might have been introduced to Ethiopia from other parts of the world with latently infected tubers.

References

Bekele, K. 1996. Incidence and distribution of major potato diseases in 1993 and 1994 off-seasons in central Ethiopia (Abstract). The 4th annual conference of Crop Protection Society of Ethiopia, May 1996, Addis Ababa, Ethiopia.

Berga, L., Gebremedhin, W., Teressa, J., Bereke-Tsehai, T., Yaynu, H. 1994. Potato improvement research. In: Edward, H., Lemma, D. (eds.). Horticulture research and development in Ethiopia, Proceedings of the 2nd National Horticultural Workshop of Ethiopia, December 1992, Addis Ababa, Ethiopia.

Ciampi-Panno, L., Fernandez, C., Bustamante, P., Andrade, N., Ojeda, S., Contreras, A. 1989. Biological control of bacterial wilt of potatoes caused by *Pseudomonas solanacearum.* Am. Potato J. 66: 315-332.

Hayward, A.C. 1964. Characteristics of *Pseudomonas solanacearum.* J. App. Bacteriol. 27: 265-277.

Hayward, A.C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearm.* Annu. Rev. Phytopathol. 29: 65-87.

He, L.Y., Sequeira, L., Kelman, A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis. 67: 1357-1361.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathol.44: 693-695.

Williamson, L., Nakaho, K., Hudelson, B., Allen, C. 2002. *Ralstonia solanacearum* race 3, biovar 2 strains isolated from *Geranium* are pathogenic to potato. Plant Dis. 86: 987-991.

Yabuchi, E., Kosako, Y., Yano, I., Hota, H., Nishiuchi, Y. 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov., *Ralstonia solanacearum* (Smith, 1986) comb. nov. Microbiol. Immunol. 39: 897-904.

Yaynu, H. 1989. Characteristics of isolates of *Pseudomonas solanacearum* in Ethiopia. Ethiop. J. Agric. Sci. 11: 7-13.

Lebecka, R., Flis, B., Zimnoch-Guzowska, E.
Plant Breeding Acclimatization Institute, Młochów Research Center, 19 Platanowa str, 05-831-Młochów, Poland

Resistance to E*rwinia carotovora* introduced to *Solanum tuberosum* from wild species

Abstract

Resistance of potato tubers to soft rot, caused by the bacterium *Erwinia carotovora* subsp. *atroseptica* (Eca), was introduced to tetraploid potatoes from diploid hybrids of *Solanum tuberosum*, *S. chacoense*, *S. yungasense* and *S. phureja*.

Introduction

Potato blackleg and soft rot are caused by bacteria: *E. carotovora* subsp. *atroseptica,* van Hall, 1902 (Dye 1969), *E. carotovora* subsp. *Carotovora*, Jones, 1901 (Bergey et al. 1923) and *E. chrysanthemi* (Burkholder et al. 1953); synonyms are respectively: *Pectobacterium carotovorum* subsp. *atrosepticum*, *P. carotovorum* subsp. *carotovorum* and *P. chrysanthemi* (Hauben et al. 1998). Distribution of different *Erwinia* species depends on the temperature, but in general in temperate climate *Erwinia carotovora* is common and the global losses due to *Erwinia* spp. were estimated to 50 to 100 million USD (Pérombelon and Kelman 1980). Chemical control is not applied in practice and control of this disease is mainly based on prevention: planting of healthy seeds and avoiding the risks of damage and wetting of tubers during harvest as well as preventing anaerobic conditions in storage and transport (Pérombelon 2000). As another possibility to control soft rot of potatoes by a biological method we tested the level of resistance to *Erwinia* species in a tetraploid potato cultivar, because its resistance is relatively low (Krauze et al. 1982) and breeding of resistant cultivars would be a good solution to minimise the losses caused by these bacteria.

Results and Discussion

Resistance of potato tubers to soft rot, caused by the bacterium *Erwinia carotovora* subsp. *atroseptica* (Eca), was introduced to tetraploid potatoes from diploid hybrids of *Solanum tuberosum*, *S. chacoense*, *S. yungasense* and *S. phureja* (Lebecka et al. 2004).

Six clones were selected (out of 1353 individuals) in the course of breeding programs, based on their good agronomical characteristics (yield, phenotypic appearance, starch content, chipping quality) and presence of additional resistance to *Synchytrium endobioticum*, PV.Y, PLRV, PV.M and resistance to *Phytophthora infestans*. The high level of resistance to *Eca* found in these clones was confirmed in two consecutive years of evaluation using the point inoculation method described by Lebecka et al. (2004). The five-year-mean values of rotten tissue diameter ranged from 5.8 to 6.8 mm in resistant clones (Table), while the average value for susceptible cultivar Irys was 16.2 mm. Screening for resistance to *Ecc* indicated a high resistance of four clones (mean diameter of rotten tissue from 5.3 to 7.9 mm) and medium resistance of two other clones (9.9 and 10.5 mm) as compared with 14.0, 17.4 and 16.9 mm measured for the susceptible cv. Irys and the susceptible tetraploid parental clones PS 646 and PW 378, respectively.

Table Soft rot symptoms of six potato clones, their parents and standards, expressed as diameter of rotten tissue of inoculated tubers with *Erwinia carotovora* subsp. *atroseptica* and *Erwinia carotovora* subsp. *carotovora*

Clone/cultivar	Pedigree or type of tested material	Diameter of rotten tissue (mm) after inoculation with	
		Eca **(five-year-mean ± SD)**	***Ecc*** **(one-year-mean)**
E97-908	PS646 x DG88-9	5.8 ± 0.3	7.9
E97-678	PW378 x DG88-9	5.9 ± 0.9	5.7
E97-1954	M62564 x DG88-9	6.4 ± 1.7	6.5
E97-572	PW378 x HT/HZ84PH151	6.7 ± 1.7	5.3
E97-2075	M62564 x DG 94-112	6.8 ± 0.7	10.6
E97-1112	Cv. Glada x HT/HZ84PH151	6.8 ± 1.4	9.9
DG 88-9	Resistant parent	6.1 ± 0.9	6.7
HT/HZ84PH151	Resistant parent	6.4 ± 0.5	8.4
DG 94-112	Resistant parent	8.3 ± 1.7	10.5
Cv. Glada	Resistant parent	8.5 ± 2.2	10.9
M62654	Medium-resistant parent	9.2 ± 1.5	12.8
PS646	Susceptible parent	11.5 ± 3.5	17.4
PW378	Susceptible parent	11.7 ± 3.8	16.9
USA 249	Resistant standard	8.6 ± 2.3	16.3
Cv. Irys	Susceptible standard	16.2 ± 4.0	14.0

The source of resistance to potato soft rot demonstrated its value in a breeding program and some of these clones will be used as parental lines in breeding new cultivars.

References

Bergey, D.H., Harrison, F.C., Breed, R.S., Hammer, B.W., Huntoon, F.M. 1923. In: Bergey's Manual of Determinative Bacteriology, 1st ed., The Williams and Wilkins Co, Baltimore, pp. 1-442.

Burkholder, W.H., McFadden, L.A., Dimock, A.V. 1953. A bacterial blight of chrysanthemums. Phytopathology 43: 522-526.

Dye, D.W. 1969. A taxonomic study of the genus *Erwinia*. II. The "*carotovora*" group. New Zealand J. Sci. 12: 81-97.

Hauben, L., Moore, E.R.B., Vauterin, L., Steenackers, M., Verdonck, L. Swings, J. 1998. Phylogenetic position of phytopathogens within the *Enterobacteriacae*. Syst. Appl. Microbiol. 21: 384-397.

Krauze, B., Koczy, T., Komorowska-Jedrys, J., Ratuszniak, E. 1982. Laboratory assessment of tuber resistance of world potato cultivar collection to main causes of storage rots. Biuletyn Instytutu Ziemniaka 27: 111-134.

Lebecka, R., Zimnoch-Guzowska, E., Kaczmarek, Z. 2004. Resistance to soft rot (*Erwinia carotovora* subsp. *atroseptica*) in tetraploid potato families obtained from 4*x*-2*x* crosses. Am. J. Potato Res. 82: 107-114.

Pérombelon, M.C.M. 2000. Blackleg risk potential of seed potatoes determined by quantification of tuber contamination by the causal agent and *Erwinia carotovora* subsp. *atroseptica*: a critical review. EPPO/OEPP Bulletin 30: 413-420.

Pérombelon, M.C.M., Kelman, A. 1980. Ecology of the soft rot erwinias. Annu. Rev. Phytopathol. 18: 361-387.