Pseudomonas marginalis Associated with Soft Rot of *Zantedeschia* spp.

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Abstract

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For the first time in the Czech Republic, bacteria identified as *Pseudomonas marginalis, Pectobacterium carotovorum* subsp. *carotovorum* and *Pseudomonas putida* were isolated from tubers of *Zantedeschia* spp. with symptoms of tuber soft rot. The symptoms occurred on mother tubers as well as on new daughter tubers of different calla lily hybrids with yellow spathe, calla lily cv. Mango with bright orange spathe and *Zantedeschia rehmanii* with pink spathe. The percentage of diseased plants of the total plants in the plot was around 10%. When inoculated into potato tuber slices, strains of *P. marginalis* and *P. c.* subsp. *carotovorum* produced soft rot. Pectolytic activity of *P. marginalis* strains was less intensive than that of the *P. c.* subsp. *carotovorum* strain. The results confirm that bacterial soft rot of *Zantedeschia* spp. may have several causes.

Keywords: bacterial soft rot; *Zantedeschia* spp.; *Pseudomonas marginalis*; *Pseudomonas putida*; *Pectobacterium carotovorum* subsp. *carotovorum*; Czech Republic

Zantedeschia spp., members of the *Araceae* family, are herbaceous perennial plants which are appreciated world-wide as ornamental crops. They are mainly exploit for their striking, large flower spathes (outer "petal" shaped like a funnel) and decorative leaves and are grown both as ornamental plants and for cut flowers.

Aracea family consists of two sections, namely the *Zantedeschia* section (comprising two species with white flowers and rhizome storage organs) and *Aestivea* section (comprising six species with coloured flower and tuberous storage organ) (Singh *et al*. 1996; Snijder 2004). Cultivars have been bred for ornamental value of either flowers or entire plants. The types with coloured flowers are more sensitive to the environment and are therefore more susceptible to disease. Most cultivars are propagated vegetatively (Snijder 2004).

The list of the main pathogens that attack *Zantedeschia* spp., and require control, include two viruses (*Dasheen mosaic virus*, *Tomato spotted wilt virus*), two oomycetes (*Pythium ultimum*, *Phytophthora cryptogea*), one fungus (*Rhizoctonia solani*), and one bacterium (*Erwinia carotovora*)

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(Raabe *et al.* 2004). Until now, *Cucumber mosaic virus, Dasheen mosaic virus* (Mokrá & Gotzová 1994), *Tomato spotted wilt virus* and *Impatiens necrotic spot virus* (Mertelík *et al.* 2002) has been detected in *Zantedeschia* spp. plants in the Czech Republic.

According to Buonaurio *et al.* (2002), in Italy, bacterial soft rot of *Zantedeschia* spp. has been recorded by Petri in 1934 and subsequently by Mazzetti in 1951, who identified *Bacterium aroidea* (currently named as *Erwinia carotovora* subsp. *carotovora*) as the causal agent. Beside Italy, bacterial soft rot has also been reported in *Zantedeschia* spp. in the USA (Pirone 1978), Japan (Horita 1994), New Zealand (Wright 1998), Lithuania (Snieskiene 1995), the Netherlands (Snijder 2004) and Korea Republic (Cho *et al.* 2005). In some countries, e.g. in New Zealand, bacterial soft rot causes substantial losses to *Zantedeschia* plants in the field and to tubers in storage (Wright 1998). It is a major factor affecting the viability of this crop (Wright *et al.* 2005).

In past years, symptoms of bacterial soft rot were sporadically observed on *Zantedeschia* spp. in the Czech Republic, but, to the best of our knowledge, no records are available regarding of pathogen isolation and determination.

The bacterium *Erwinia carotovora* subsp. *carotovora* is regarded as the cause of bacterial soft rot of *Zantedeschia* species (*Z. aethiopica, Z. aethiopica* var. *minor, Z. childsiana, Z. elliottiana*) (Pirone 1978; Bradbury 1986; Cho *et al.* 2005) particularly in cultivars from the section *Aestivae* (Snijder 2004). In 1998, *Erwinia carotovora* subsp. *carotovora* was reclassified as *Pectobacterium carotovorum* subsp. *carotovorum* (Hauben *et al.* 1998). However, at present, *Pectobacterium* has not been widely adopted by the *Erwinia* research community (Toth *et al.* 2003).

Although pectolytic *Pectobacterium* (formerly *Erwinia*) species are commonly assumed to be the principal cause of the bacterial soft rot of *Zantedeschia* spp., the question can arise if pectolytic bacteria of other genera, i.e. *Pseudomonas*, *Dickeya, Bacillus*, *Flavobacterium*, *Clostridium* etc., are associated with soft rot of *Zantedeschia* spp.

The objectives of this study were: (*i*) to isolate and determine species and subspecies spectrum of bacteria associated with soft rot of *Zantedeschia* spp. in the Czech Republic; (*ii*) to verify the presumption that bacterial soft rot of *Zantedeschia* spp. is caused by more than one pathogen.

MATERIALS AND METHODS

Plant and localities. Tuber rot symptoms were observed on different *Zantedeschia* spp. plants at Děčín in the North Bohemia. The calla lily plants were imported to the Czech Republic in 1990 from the Netherlands.

Isolations. *Pythium* spp. and *Phytophthora* spp. were excluded as causal agents. Therefore, the isolation of suspected bacteria was carried out on King's medium B and nutrient agar (NA), using standard isolation techniques (Schaad *et al.* 2001). Pieces of wet and totally disintegrated tissue of calla tubers were placed in a droplet of sterilised water in a flamed watch glass and mechanically crushed. A loopful of macerate was streaked onto nutrient agar in Petri dishes and incubated at 26°C. Single bacterial colonies with cultural characteristics resembling that of pectobacteria or pseudomonads were re-streaked to obtain pure cultures of representative strains.

Pectolytic ability. Potato tubers were surface sterilised with alcohol, peeled aseptically and sliced into disks about 7 mm dick. These were placed in sterile Petri dishes with a sterile moistened filter paper. A very heavy cell suspension was applied into shallow pits made in the centre of potato disks, and the dishes were incubated at 28°C. The inoculated disks were examined at 24 and 48 h for soft rot probing the tissue surrounding the inoculum site with a loop to assess ability to macerate potato tuber tissue at 27°C (Bradbury 1970; Schaad *et al.* 2001).

Identification of bacterial isolates. Bacterial isolates from calla tubers were identified using the Biolog Identification System GN MicroPlate system (developed by Biolog, Inc., Hayward, USA). The test yields a characteristic metabolic fingerprint. The microplates were incubated for 4 and 24 hours. Biolog's MicroLog 2 4.2 software was used to identify the bacterium from its metabolic pattern. The calculations for the identification of bacteria as to genus, species and other taxonomic units are based on similarity indices (Schaad *et al.* 2001).

RESULTS

Symptoms of soft rot after natural infection

In September 2007, tuber soft rot symptoms of calla lilies (*Zantedeschia* spp.) were observed in the collection of calla lily plants in the private

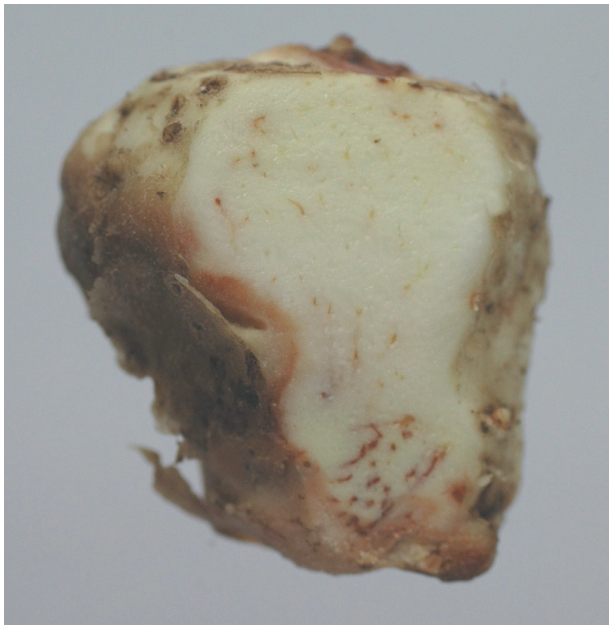

Figure 1. Cross-section through cala tuber showing soft rot symptom (Photo V. Krejzar)

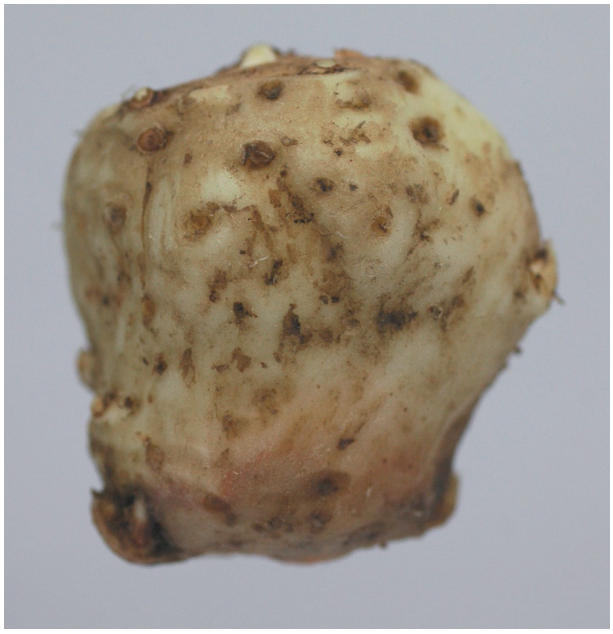

Figure 2. Soft rot lesions located in the place of attachment of rhizomes to calla tuber (Photo V. Krejzar)

garden in the North Bohemia. The percentage of affected plants in the plot was around 10%. The foliage and flower stems attached to rotted parts of tubers quickly became yellowed and wilted. In some cases, when the greater part of tubers were decayed, the entire plant completely collapsed and rotted. At an advanced stage of disease, rotted tubers were reduced to a mushy, whitish, foul-smelling pulp (Figure 1). The rot began sometimes as small, water-soaked soft lesions at the place of an attachment of roots to a tuber (Figure 2). The symptoms occurred on mother tubers as well as on new daughter tubers of different calla lily hybrids with a yellow spathe, calla lily cv. Mango with a bright orange spathe and *Zantedeschia rehmanii* with a pink spathe.

Characterisation of bacteria

A total of more than 30 bacterial isolates were obtained from tubers of *Zantedeschia* spp. showing symptoms of soft rot. According to the appearance of the colonies on nutrient media, the isolates belong to six colony types. Seventeen isolates were tested using the Biolog system and rate of potato tuber maceration was determined using tuber disks.

The most frequent species isolated from soft rot lesions was *Pseudomonas marginalis* (Brown 1918); Stevens 1925. Among other species, *Pectobacterium carotovorum* subsp. *carotovorum* (Jones) Hauben et al. 1999 and *Pseudomonas putida* (Trevisan) Migula 1895 were identified. Moreover, with *P. c.* subsp. *carotovorum*, and *P. putida*, undetermined Gram-negative, oxidase-positive, enteric and non-enteric, non-fluorescent bacteria were isolated (Table 1).

When inoculated into potato tuber slices, strains of *P. marginalis* and *P. c*. subsp. *carotovorum* produced soft rot. Pectolytic activity of *P. marginalis* strains was less severe than that of *P. c*. subsp. *carotovorum* strain (Table 1).

DISCUSSION

As expected, *P. c.* subsp. *carotovorum* was isolated from tubers of *Zantedeschia* spp. with symptoms of soft rot. However, *P. marginalis* was isolated more often than *P. c.* subsp. *carotovorum* (Table 1). In Central European climatic conditions, *P. c.* subsp. *carotovorum* is thought to be the most important soft-rot bacterium affecting wide range of host plants including *Zantedeschia* spp. Host specificity is not recognised among strains of *P. c.* subsp. *carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*) (Pérombelon & Kelman 1980).

There have been three levels of aggressiveness (Smith & Bartz 1990) (quantity of host tissue rot-

Table 1. Identification of bacterial isolates from tubers of *Zantedeschia* spp. using Biolog system and evaluation of pectolytic activity using potato tuber disks

Isolate or strain number	Names of bacterium	Type of bacterium	Similarity index	Probability (%)	Pectolytic activity
347	*Pseudomonas marginalis*	GN-NENT OXI+	0.90	100	+
422	*Pseudomonas marginalis*	GN-NENT OXI+	0.82	100	+
511	*Pseudomonas marginalis*	GN-NENT OXI+	0.79	100	+
429	*Pseudomonas marginalis*	GN-NENT OXI+	0.83	99	+
4410	*Pseudomonas marginalis*	GN-NENT OXI+	0.83	99	+
341	*Pseudomonas marginalis*	GN-NENT OXI+	0.65	93	+
521	*Pseudomonas putida*	GN-NENT OXI+	0.59	100	–
132	*Pectobacterium carotovorum* subsp. *carotovorum*	GN-ENT	0.56	94	+++

GN-ENT = Gram-negative ENTeric; GN-NENT = Gram-negative Non-ENTeric; OXI+ = oxidase-positive
At 16–24 hours of incubation, the similarity index must be at least 0.50 to be considered an acceptable species identification
% probability allows to compare identifications to other systems that use this type of calculation
+++ high pectolytic activity (tissue maceration has extended from the site of inoculation trough all potato disk; + weak pectolytic activity (tissue maceration has extended 5 to 20 mm from the site of inoculation); – no pectolytic activity

ted) among 45 strains of soft-rot bacteria reported. A strain *P. c.* subsp. *carotovorum* obtained from calla lily was in the most aggressive group, whereas the type strain of the *P. c.* subsp. *carotovorum* isolated from carrot was typical of strains in the least aggressive group. According to Smith and Bartz (1990), strains isolated from a particular host were not always more aggressive than those recovered from other plants when inoculated to that host. Thus, certain strains of *P. c.* subsp. *carotovorum* may exhibit a host specificity that is not related to their original host or to their relative aggressiveness in common hosts (such as potato tuber or tomato fruit). Smith and Bartz (1990) suggested that plant reaction groups can be set up for strains of *P. c.* subsp. *carotovorum*, just as it has been done for those of *Pectobacterium chrysanthemi*.

A prominent feature of bacteria soft rot is due to bacterial extracellular pectolytic enzymes such as pectin methylesterase, pectin lyase, polygalacturonase, and pectate lyase. Pectolytic enzymes breakdown pectic substances which are effective as intercellular cement in plant tissues. Development of soft rot usually involves conditions (such as high temperature and humidity, free water, low oxygen concentration, wounding of the host protective tissue, etc.) which are favourable for these pathogen(s) and unfavourable to the host plant (Pérombelon 1982; Liao 1991). The soft rot pectobacteria (erwiniae) are now entering the genomics era, and we will soon have catalogued the genes that these organisms possess (Toth *et al*. 2003).

Our results confirm the suggestion that bacterial soft rot of *Zantedeschia* spp. could be polyaetiological in nature. A number of species can cause the same symptoms and may be present in diseased tissues at the same time (Pérombelon 1982; Kennedy & Lacy 1982). Therefore, our finding of joint occurrence of *P. c.* subsp. *carotovorum* in soft-rotted *Zantedeschia* tuber tissues with *P. marginalis* is not totally unexpected. However, we can regard our finding as unusual. In temperate climates, symptom development following infection by soft rot bacteria is allegedly associated with erwinias (pectobacteria) and rarely with species of other genera (Pérombelon 1982).

Temperature is the main factor affecting the relative virulence of soft rot bacteria and its level may determine which organism predominates in a lesion. Optimal growth temperature for *P. c.* subsp. *carotovorum* is ca. 28°C and for *P. marginalis* 25–26°C. However, the differential effect of temperature on the relative virulence of soft rot bacteria is influenced not only by the effect of temperature on the *in vitro* growth rate of the specific bacterium but also on the level of production of various pectic

enzymes (Pérombelon 1982). *P. marginalis* and *P. c.* subsp. *carotovorum* exhibit differences in their relation to oxygen tension. *P. marginalis* can grow aerobically while *P. carotovorum* subsp. *carotovorum* is a facultative anaerobic organism also capable of growing anaerobically.

Pectolytic fluorescent pseudomonads have been isolated from soil, the rhizospheres of several plant species, and decaying plant material. Several soft rot diseases of vegetables have been attributed to strains of these bacteria, which frequently were classified as *Pseudomonas marginalis* or *P. fluorescens. P. marginalis* has not been thoroughly studied and its ability to macerate potato tissue, at present, serves as the principal character separating it from other groups in *P. fluorescens* and *P. putida* tribes (Sands & Hankin 1975; Cuppels & Kelman 1980). However, the studies of Liao (1991) suggest that some strains of fluorescent pseudomonads which exhibit a nonpectolytic phenotype under one set of conditions may become pectolytic under others.

The taxonomic and phytopathogenic status of *Pseudomonas marginalis* is not well known. Originally this species was restricted to one distinct pathogen, the causal agent of marginal necrosis of leaves of lettuce (Brown 1918) and a few other plants. A list of pathovar names of phytopathogenic bacteria from 1980 (Dye *et al.* 1980) encompass three pathovars of *P. marginalis* species including: pv. *alfalfae* that causes browning of roots and stunting of lucerne; pv. *marginalis* that has been reported to occur naturally on a wide range of host plans (but *Zantendeschia* spp. in not among them); pv. *pastinacae* that attacks *Pastinaca sativa* in natural conditions (however, this pathovar is probably synonymous with pv. *marginalis*) (Bradbury 1986).

The very complex group of fluorescent, oxidase positive soft rot *Pseudomonas* bacteria are opportunistic plant pathogens. They have been found to be biochemically indistinguishable from saprophytic pseudomonads such as *P. fluorescent* biovars, *P. putida* and *P. chlororaphis* (now includes *P. aureofaciens*). On the basis of their ability to hydrolyze pectin and to cause soft rot, they have been named *P. marginalis*. Recently, based on 16S rRNA analysis, *P. marginalis* has been placed in the *P. fluorescens* group (Anzai *et al.* 2000).

According to Snijder and van Tuyl (2002), the disease can be partly controlled by cultural measures. A combination of cultural methods with resistant plant material is regarded as a promising strategy for control of soft rot. Nevertheless, in the Czech Republic, the economic importance of bacterial soft rot of *Zantedeschia* spp. is probably minimal, because this ornamental plant is grown on a small scale and the occurrence of the disease is only sporadic.

References

Anzai Y.A., Kim H., Park J.U., Wakabayashi H., Oyaitu H. (2000): Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. International Journal of Systematic and Evolutionary Microbiology, **50**: 1563–1589.

Bradbury J.F. (1970): Isolation and preliminary study of bacteria from plants. Review of Plant Pathology, **49**: 213–218.

Bradbury J.F. (1986): Guide to Plant Pathogenic Bacteria. Kew, Surrey, CAB International, Mycological Institute.

Brown N.A. (1918): Some bacterial diseases of lettuce. Journal of Agricultural Research, **13**: 367–388.

Buonaurio R., Caglioti C., Pires M.M., Moretti Ch., Innocenti M. (2002): Occurrence of a soft rot of calla (*Zantedeschia aethiopica*) caused by *Pectobacterium carotovorum* subsp. *carotovorum* in central Italy. Phytopathologia Mediterranea, **41**: 152–156.

Cho H.R., Lim J.H., Yun K.J., Snijder R.C., Goo D.H., Rhee H.K., Kim K.S., Joung H.Y., Kirn Y.J. (2005): Virulence variation in 20 isolates of *Erwinia carotovora* subsp. *carotovora* on *Zantedeschia* cultivars in Korea. Acta Horticulturae, **673**: 653–659.

Cuppels D.A., Kelman A. (1980): Isolation of pectolytic fluorescent pseudomonads from soil and potatoes. Phytopathology, **70**: 1110–1115.

Dye D.W., Bradbury J.F., Goto M., Hayward A.C., Lelliot R.A., Schroth M.N. (1980): International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Review of Plant Pathology, **59**: 153–168.

Hauben L., Moore E.R.B., Vauterin L., Steenackers M., Mergaert J., Verdonck L., Swings J. (1998): Phylogenetic position of phytopathogens within the Enterobacteriaceae. Systematic and Applied Microbiology, **21**: 384–397.

Horita H. (1994): Occurrence of bacterial soft rot of calla incited by *Erwinia carotovora* subsp. *carotovora* in Hokkaido Prefecture. Annual Report of the Society of Plant Protection of North Japan, **45**: 100–103.

Kennedy R.W., Lacy G.H. (1982): Phytopathogenic prokaryotes: an overview. In: Mount M.S., Lacy G.H.: Phytopathogenic Prokaryotes. Vol. 1. Academic Press, New York: 3–17.

Liao C.-H. (1991): Cloning of pectate lyase gene *pel* from *Pseudomonas fluorescens* and detection of sequences homologous to *pel* in *Pseudomonas viridiflava* and *Pseudomonas putida*. Journal of Bacteriology, **173**: 4386–4393.

Mertelík J., Kloudová K., Mokrá V., Götzová B., Gabrielová Š., Nerušilová H. (2002): Occurrence of a two tospoviruses in the Czech Republic in the period 1992–2002. In: Proceedings of the Conference Plant Protection in the III. Millennium. Ivanka pri Dunaji, 1–2 October 2002: 109–112.

Mokrá V., Gotzová B. (1994): Identification of virus infections in *Diffenbachia* and *Zantedeschia* in Czechoslovakia. Acta Horticulturae, **377**: 361–362.

Pérombelon M.C.M (1982): The impaired host and soft rot bacteria. In: Mount M.S., Lacy G.H.: Phytopathogenic Prokaryotes. Vol. 2. Academic Press, New York: 55–69.

Pérombelon M.C.M., Kelman A. (1980): Ecology of the soft rot erwinias. Annual Review of Phytopathology, **18**: 361–387.

Pirone P.P. (1978): Diseases and pests of ornamental plants. 5th Ed. John Wiley & Sons, New York: 535–536.

Raabe R.D., Grebus M.E., Wilen C.A., McCain A.H. (2004): UC IPM Pest Management Guidelines: Floriculture and Ornamental Nurseries. Publication 3392. University of California Agriculture and Natural Resources, Oakland.

Sands D.C., Hankin L. (1975): Ecology and physiology of fluorescent pectolytic pseudomonads. Phytopathology, **65**: 921–924.

Schaad N.W., Jones J.B., Chun W. (eds) (2001): Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd Ed. APS Press, St. Paul.

Singh Y., Baijnath H., van Wyk A.E. (1996): Taxonomic notes on genus *Zantedeschia* Spreng. (*Araceae*) in southern Africa. South African Journal of Botany, **62**: 321–324. Cit. Snijder R.C. (2004).

Smith C., Bartz J.A. (1990): Variation in the pathogenicity and aggressiveness of strains of *Erwinia carotovora* subsp. *carotovora* isolated from different hosts. Plant Disease, **74**: 505–509.

Snieskiene V. (1995): Bacterial diseases found in Lithuania in 1983–1994 in flowers grown on closed ground. Biologija, **3/4**: 148–149.

Snijder R.C. (2004): Genetics of *Erwinia* resistance in *Zantedeschia*: impact of plastome-genome incompatibility. [PhD Thesis.] Wageningen University.

Snijder R.C., van Tuyl J.M. (2002): Evaluation of tests to determine resistance in *Zantedeschia* spp. (*Araceae*) to soft rot caused by *Erwinia carotovora* subsp. *carotovora.* European Journal of Plant Pathology, **108**: 565–571.

Toth I., Bell K.S., Holeva M.C., Birch R.J. (2003): Soft rot erwiniae: from genes to genomes. Molecular Plant Pathology, **4**: 17–30.

Wright P.J. (1998): A soft rot of calla (*Zantedeschia* spp.) caused by *Erwinia carotovora* subspecies *carotovora*. New Zealand Journal of Crop and Horticultural Science, **26**: 331–334.

Wright P.J., Triggs C.M., Burge G.K. (2005): Control of bacterial soft rot of calla (*Zantedeschia* spp.) by pathogen exclusion, elimination and removal. New Zealand Journal of Crop and Horticultural Science, **33**: 117–123.

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