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Pectobacterium carotovorum subsp. *brasiliensis* causing a soft rot on Styrian oil pumpkin in Austria

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Styrian oil pumpkin (*Cucurbita pepo* subsp. *pepo* var. *styriaca*) is an important crop in the eastern part of Austria, with a cultivated area in 2010 of about 26,000 ha yielding 4 million litres of edible oil worth \in 60 million per annum. In September 2014, fruits with soft rot symptoms, i.e. collapsing of the inner pumpkin pulp, resembling that caused by pectolytic Enterobacteriaceae, were sampled in East Styria. The impact of rot on the oil pumpkin fruits in the affected area was more obvious than in preceding years, affecting over 50% of the crop. Some symptoms differed from the black rot-diseased pumpkins observed previously on the same site attributed to *Didymella bryoniae*, and associated with *Pectobacterium atrosepticum*, *Pseudomonas viridiflava*, *Pseudomonas syringae* and *Xanthomonas cucurbitae* (Grube *et al.*, 2011).

Bacteria morphologically similar to *Pectobacterium* spp. were isolated from the symptomatic pumpkin pulp. DNA from five pure cultures was amplified by PCR using primers targeting the 16S rDNA according to Weisburg *et al.* (1991) and the amplicon sequenced (GenBank Accession No. KU899096). The sequences matched 100% with *P. carotovorum* subsp. *brasiliensis* (*Pcb*) isolated in China from *Brassica rapa* (KP187492). The identity of the pumpkin isolates was confirmed by a subspecies-specific PCR targeting the 16S-23S intergenic spacer (Duarte *et al.*, 2004).

Healthy potato plants (from tubers) and oil pumpkin plants cv. Gleisdorfer Ölkürbis (from seeds) were grown in a greenhouse until the four-leaf stage. Suspensions (10⁸ cfu/ml) of a single isolate of *Pcb* (deposited as DSM 102074 in the German Collection of Microorganisms and Cell Cultures, DSMZ) were injected into the stalk of the seedlings using a hypodermic needle, in parallel with a potato strain (DSM 30170) of P. c. subsp. carotovorum (Pcc) and sterile water as controls. Ten plants were inoculated per isolate. After two weeks, 60% of the Pcb-inoculated potato plants showed blackleg symptoms compared to 30% of the Pcc-inoculated plants. All the pumpkin plants inoculated with Pcb showed pronounced symptoms (collapse of entire vines) after three days (Fig. 1), dying shortly afterwards, whereas after two weeks only 10% of the Pcc-inoculated plants were symptomatic. Water controls did not show any symptoms. Pcb was reisolated from all inoculated symptomatic plants and the subspecies confirmed by PCR. The enhanced aggressiveness of the strain isolated from oil pumpkin was demonstrated by artificial inoculation of both potato

and pumpkin plants compared to the *Pcc* DSMZ strain. However, it should be noted that inoculations were done on young stems rather than fruits.

Although a number of pectolytic bacteria have been described to be responsible for rot diseases in Cucurbitaceae (Balestra, 1999; Sanogo *et al.*, 2011; Yi & Kim, 1996), to our knowledge, this is the first time *Pectobacterium carotovorum* subsp. *brasiliensis* has been isolated from and identified as causing bacterial soft rot in this plant family. This is also the first report of *Pcb* on any crop in Austria; the route of introduction is unknown.

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Figure 1

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