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FIRST OR UNUSUAL DISEASE REPORT

First report of collar rot caused by *Pseudomonas aeruginosa* on calla lily (*Zantedeschia elliottiana*)

Veerubommu SHANMUGAM[1,2*], Himankshi THAKUR[2,3], Surender PAUL[2], Priyanka BHADWAL[2], Surbhi MAHAJAN[2] and Kalyan KUMAR MONDAL[1]

[1] ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India
[2] CSIR-Institute of Himalayan Bioresource Technology, Palampur-176061, India
[3] Academy of Scientific and Innovative Research, New Delhi, India

Summary. Collar rot of calla lily cultivated in Himachal Pradesh, India, was recorded during 2011–2013, and the pathogen causing the disease was identified as *Pseudomonas aeruginosa*. The disease occurred on roots, rhizomes and stems of host plants, causing severe rotting and breakage, and was predominant during the rainy season (June-August) each year. . This is the first record of *Pseudomonas aeruginosa* affecting calla lily worldwide.

Key words: Himchal Pradesh, India.

Introduction

Calla lily (*Zantedeschia elliottiana* (H. Knight) Engl.) of the family Araceae is an exotic, rhizomatous and herbaceous perennial flowering crop plant native to southern Africa. Calla lilly plants are an evergreen or deciduous perennials (Letty, 1973), whose leaves are entire and borne on long petioles. Inflorescences consist of spadices each carrying the male and female flowers enclosed by a spathe. In India, the calla lilies are grown as cut flower crops in polyhouses or greenhouses, and Himachal Pradesh (H.P.) is one of the leading calla lilly producers of the country. In June-August, 2011 through 2013, crops of *Z. elliottiana* 'Black Magic' cultivated in polyhouses at different locations of Palampur (H.P) (≈1300 m above mean sea level; 32°06'32"N; 76°33'43"E) exhibited suspected bacterial symptoms consisting of extensive rotting, yellowing and wilting that caused severe yield losses. Hence, studies were initiated to establish the causal agent of the observed symptoms.

Corresponding author: Veerubommu Shanmugam
E-mail: shanpatho@yahoo.com

Isolation and pathogenicity tests of bacteria associated with collar rot

Six symptomatic plants were collected from six polyhouses (one each) of different locations and diseased collar tissues, which were subjected to the ooze test following standard protocols (Johnston and Booth, 1983). Isolations of the bacteria were made from the collar tissues by the direct plating method. Briefly, the tissues were surface-sterilized in 2% sodium hypochlorite, rinsed repeatedly in distilled water, dried by blotting, and then plated directly onto nutrient agar (NA) medium (Hi-media, India) in triplicate. After incubating the plates at 28 ± 2ºC for 24–48 h, colonies around the tissues were selected from each plate and further purified by sub-culturing onto NA medium. Three purified isolates were then subjected to pathogenicity tests. The pathogenicity of the isolated bacterium was established by inoculating 1 mL of a bacterial suspension (1×10^8 cfu mL^{-1}) through injection into stem vascular tissues of calla lily plants (cv. Black Magic) at the five- to six-leaf stage. Four plants were inoculated with each of the six isolates, and four plants were inoculated with sterilized water or nutrient broth as experimental

controls. The isolates were also subjected to a hypersensitivity reaction test by detached leaf bioassay. Bacterial suspensions (1×10^8 cfu mL^{-1}) were infiltrated on three *Nicotiana benthamiana* leaves of 4-week-old plants as described by Nemchinov *et al.* (2008). Leaves infiltrated with sterilized water or nutrient broth were also similarly treated as controls.

Characterization of the pathogen

Purified bacterial isolates were characterized by biochemical (Whitman *et al*., 2012) and physiological tests (Table 1), and compared to a reference *P. aeruginosa* strain (MTCC 647) from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, IMTECH (Chandigarh, India). The identity of the bacteria was further confirmed by cloning and partial sequencing of 16S rRNA (831 bp) and *gyrB* (1,102 bp) genes from the genomic DNA (Shanmugam *et al.*, 2011). Sequences were submitted to GenBank with accession numbers KT962175 and KX239873.

Results and discussion

Collar rot was observed affecting up to 10% of the plants in the polyhouses, so the bacterial pathogen inciting the disease was isolated, characterized and identified. The infected plants each initially exhibited browning followed by development of sunken water-soaked lesions at the collar region. The symptoms rapidly developed into a soft, watery, decayed mass within 3–5 d (Figure 1). In advance stages, the foliage appeared water soaked, soft, turning yellow, and later the entire plant wilted and died (Figure 1). In general, the symptoms were common in moist conditions and were consistent with that of bacterial rots.

Isolations of microorganisms from the infected vascular tissues yielded white fluidal bacterial colonies with small, round, convex elevation and entire margins on NA (Figure 2) at 28 ± 2°C. The bacterium cells were rod-shaped and Gram negative. In pathogenicity tests, the inoculated plants upon incubation at 16 to 21°C and 65 to 85% humidity for 7 d, exhibited the same symptoms as the polyhouse-grown diseased calla lily plants (Figure 3A–C). The bacterium re-isolated from the diseased tissues of the inoculated plants displayed characteristic colony morphology of the inoculated bacterium. No bacteria were isolated from the asymptomatic control plants. In the hypersensitivity test, the *N. benthamiana* leaves inoculated with the bacterial suspension of Call-IHBT-4 exhibited necrotic symptoms (Figure 4A, B) similar to those of the reference strain within 12–24 h. No

Table 1. Biochemical and physiological characteristics of calla lily bacterial isolates

Biochemical test[a]	Reference *Pseudomonas aeruginosa* (MTCC 647)	Isolates from calla lily*
Gram staining	Negative	Negative
KOH string test	Positive	Positive
Catalase	Positive	Positive
Oxidase	Positive	Positive
Citrate	Positive	Positive
Nitrate reduction	Positive	Positive
Starch hydrolysis	Negative	Negative
Methyl Red (MR)	Negative	Negative
Voges Proskauer (VP)	Negative	Negative
Urease	Negative	Negative
Lipase	Positive	Positive
Gelatin hydrolysis	Positive	Positive
Cellobiose	Negative	Negative
Dextrose	Positive	Positive
Maltose	Positive	Positive
Mannitol	Positive	Positive
Mannose	Positive	Positive
Sucrose	Negative	Negative
Trehalose	Negative	Negative
Glucose	Positive	Positive
Fructose	Negative	Negative
Ribose	Negative	Negative
24–41°C	Positive	Positive
pH 4.5–11	Positive	Positive
NaCl 0–7.5%	Positive	Positive

[a] Six isolates were tested; each of the tests was replicated three times, and were repeated twice.

Figure 1. Collar rot of calla lily caused by *Pseudomonas aeruginosa* (strain Calla-IHBT-4).

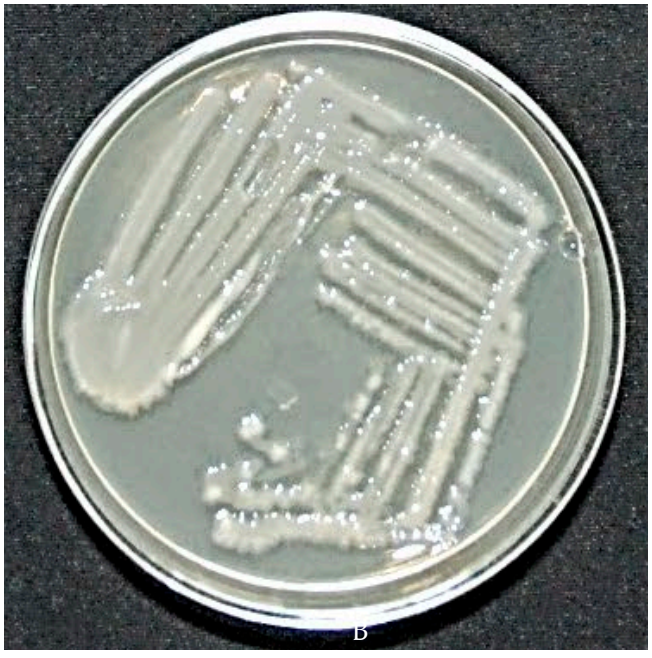

Figure 2. *Pseudomonas aeruginosa* strain Calla-IHBT-4 on NA.

Figure 3. Symptoms induced by *Pseudomonas aeruginosa* strain Calla-IHBT-4 on *Zantedeschia elliottiana* cv. Black Magic plants. Uninoculated control (A); Inoculated plants ($1x10^8$ cfu mL^{-1}) showing rotting 7 d after inoculation (B, C).

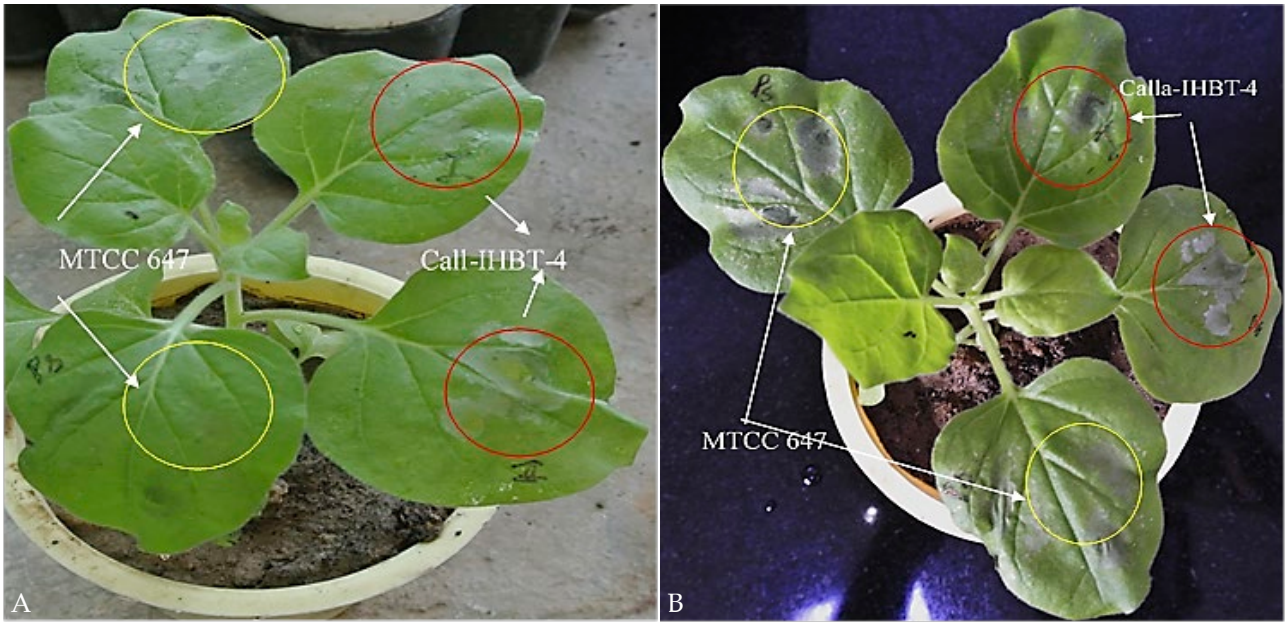

Figure 4. Hypersensitive reaction induced by *Pseudomonas aeruginosa* strain Calla-IHBT-4 ($1x10^8$ cfu mL^{-1}) on *Nicotiana benthamiana* leaves. A) 12 h post-infiltration (B) 24 h post-infiltration.

symptoms developed on control plants. The bacterium was re-isolated from inoculated leaves.

The isolates were subjected to biochemical and physiological characterization followed by molecular assays to establish identity. The cells were Gram-negative, short-straight rods (Figure 2B), aerobic, motile and non-endospore forming. The strain tested positive for the KOH string test, catalase, oxidase, citrate, nitrate reduction, lipase, gelatin hydrolysis, dextrose, maltose, mannitol, mannose, and glucose. Growth occurred at temperatures of 24–41°C, pH levels of 4.5–11 and NaCl levels of 0–7.5% (Table 1). Based on these characteristics, the strains were putatively identified as *Pseudomonas aeruginosa* (Garrity

et al., 2006). Results of 16S rRNA and *gyrB* sequencing of a calla lily strain designated as Calla-IHBT-4 (GenBank Accession No. KT962175 and KX239873) displayed, respectively) 100 and 98% sequence identity to that of the strain VA-134 of *P. aeruginosa* from the database. Thus, based on colony morphology, physiological and biochemical tests, sequence analysis of the 16S rRNA gene, and by fulfilling Koch's postulates, the strain was identified as *P. aeruginosa.* A culture of this bacterium has been deposited in the MTCC (Chandigarh, India).

Soft rot of calla lily caused by *Pectobacterium carotovorum* subsp. c*arotovorum* (Ni *et al*., 2010) and *Erwinia chrysanthemi* (*Dickeya* spp.) (Lee *et al*., 2002) have been reported elsewhere. *Pseudomonas aeruginosa* incites rotting of ornamental plants under favourable environmental conditions (Cho *et al*., 1975). Although *P. aeruginosa* causing brown rot of onion (Cother *et al*., 1976; Hao and Xie, 2006), root rot of ginseng (Gao *et al*., 2014), fruit rot of tinda (*Praecitrullus fistulosus*) (Mondal *et al*., 2012), and leaf spot of tobacco (Yu *et al*., 2008) have been previously reported, the present record of this pathogen causing collar rot in calla lily appears to be the first report worldwide.

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