

First Report of *Dickeya chrysanthemi* Causing Bacterial Soft Rot of *Aloe vera* in Sri Lanka

W.A.P.G. Weeraratne* and D.M.K.K. Dissanayake

Received: 29th June 2020 / Accepted: 23rd March 2021

ABSTRACT

Purpose: In September 2019, a rapidly spreading soft rot disease was observed in an *Aloe vera* cultivation in Puttalam District, Sri Lanka causing rotting of leaves and whole plant death, within three to five days. Identification of causal pathogen of soft rot was the main objective for effective management of the disease. The aim of this study was to isolate and molecular identification of causal pathogen of soft rot disease of *A. vera* and to confirm its pathogenicity.

Research Method: The causal bacterium was isolated on Nutrient Agar medium and initially identified by colony characters. Pathogenicity was assessed by inoculation of leaves of potted *A. vera* plants with bacterial suspension of each isolates ($\times 10^8$ cfu ml⁻¹). Bacterial DNA was extracted and subjected to PCR, using universal primers 27F and 1492R to amplify 16S rRNA region of the bacterium. The PCR products were sequenced and homology search was performed with GenBank database.

Findings: Typical field symptoms began appearing after 24 hrs on inoculated plants, with the death of whole plant within two-three days after inoculation. Based on PCR analysis, sequencing and homology search results, the causal pathogen of the bacterial soft rot disease of *A. vera* was identified as *Dickeya chrysanthemi* (former *E. chrysanthemi*). Sequences were deposited in GenBank for two bacterial isolates, kb (Accession No.MT028490.1) and kd (Accession No.MT028539.1). A BLAST search revealed 99 % identity with *D. chrysanthemi* from China (JF779681.1).

Originality/Value: To our knowledge, this is the first report of *D. chrysanthemi* causing bacterial soft rot of *A. vera* in Sri Lanka. This finding will be very useful to formulating disease management strategies for bacterial soft rot of *A. vera* and to prevent outbreak of the disease.

Keywords: *Aloe vera*, *Dickeya chrysanthemi*, Soft rot, 16S rRNA


INTRODUCTION

Aloe vera (*Aloe barbadensis* Miller) belongs to family Liliaceae, a perennial, succulent plant adapted widely through the world. The gel of the leaf of *A. vera* contains 96 percent of water and the remainder is a rich source of minerals, amino acids, enzymes and phytochemicals; hence it is extensively used in the cosmetic industry, in ayurvedic medicine and also as a food supplement such as drinks or mixed with yoghurt (Rajeswari *et al.*, 2012). There is a growing demand for such products among European consumers because of rising health consciousness; the European market offers good opportunities to *A. vera* suppliers in

developing countries. In Sri Lanka, it has become a popular large scale cultivation in recent times with the increased demand for *A. vera* leaves and the potential of export of *A. vera* based products at local and international levels.

Diseases are a major threat to *A. vera* cultivation worldwide, accounting for huge losses ranging from 25-75 percent. Among these, bacterial soft

* Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka
gethweeraratne@gmail.com

 ORCID <https://orcid.org/0000-0002-9600-8300>

rot of *A. vera* caused by *Erwinia chrysanthemi* is economically important as it causes yield losses up to 80 percent (Syamala and Ciba, 2017). In Sri Lanka, a bacterial soft rot was observed for the first time in September, 2019 in commercial *A. vera* cultivation in Puttlam District of Sri Lanka. About 40-60 % of plants were affected with the rotting of leaves, collapse and whole plant death within three to five days. Later it was reported from all the *A. vera* cultivation areas of Jaffna, Kaluthara, Hambanthota and Anuradhapura Districts in the country.

Since there are no previous records of *A. vera* diseases in Sri Lanka, a rapid and accurate identification of causal pathogen of soft rot disease of *A. vera* is needed to prevent further spread and any subsequent disease outbreaks. Analysis of the 16S rRNA gene is a suitable tool for bacterial species identification and taxonomic characterization by amplifying this gene using PCR and resultant PCR product sequenced (Sun *et al.*, 2008).

Soft rot is a rapidly-spreading disease of *A. vera* in Sri Lanka and pathogenicity of the disease has not been confirmed yet. Therefore, the objectives of the present study were to isolate the pathogen, prove its pathogenicity and the molecular identification of the causal pathogen associated with symptomatic *A. vera* plants.

MATERIALS AND METHODS

Collection of Plant Samples

A. vera plants showing soft rot symptoms and potted suckers (offsets) of infected mother plants were collected from two *A. vera* cultivations in Puttlam area, in the North Western Province of Sri Lanka in October, 2019. Disease incidence and field symptoms of affected plants from two locations were recorded.

Isolation of Causal Pathogen

Stems of affected *A. vera* plants and suckers were washed under tap water to remove adhering soil

particles and subsequently rinsed with sterilized distilled water. A small infected portion of the stem was cut with a sterile blade, then surface sterilized by immersing in 70 % ethanol for 1-2 min, followed by three washes in sterilized distilled water. After blot drying, each part was placed in 10 ml of sterile distilled water and allowed to ooze. A loop-full of this suspension was streaked on nutrient agar (NA) and plates were incubated at room temperature (28 ± 2 °C) for 3-4 days. Pure cultures of three bacterial isolates were kept at 4 °C by culturing them on NA slants for subsequent studies.

Potassium Hydroxide solubility test was performed for all the isolates to determine their Gram positive and Gram negative status. A loop-full of each bacterium was placed on a glass slide with a drop of 3 % (v/v) KOH solution, stirred for 10 seconds and observed for the formation of slime threads (Punawati *et al.*, 2014)

Confirmation of Pathogenicity

The pathogenicity of three selected bacterial isolates namely kb, kd and ke from the suckers of mother plant (kd) was confirmed by following the steps of Koch's postulates. The inoculation technique was carried out according to the method explained by Mandal and Maiti (2005). Pathogenicity assays were performed on healthy *A. vera* plants raised in plastic pots containing autoclaved soil, in the greenhouse.

The inoculum of each isolate prepared in nutrient broth for 2 days at room temperature (28 ± 2 °C), centrifuged and suspended in sterile water (adjusted to $\times 10^8$ cfu/ml) using spectrophotometer at 620 nm. The plants at 4-5 leaf stage of growth were injected at the base of the leaves of *A. vera* plants using a disposable syringe. Control plants were inoculated with sterile water. Plants were maintained at room temperature and soft rot symptoms were examined after 1-3 days. The pathogen was re-isolated from inoculated plants and plated on NA plates and the morphology of each isolate was compared with that of original cultures.

Molecular Identification of two Bacterial Isolates

Genomic DNA was extracted from 24 hrs old pure broth cultures of five bacterial isolates (kb, kd, ke and the re-isolates kbr and kdr) according to the cell lysis DNA extraction method, described by Weller *et al.* (2000). The quality of the DNA of bacterial isolates was verified using 0.8 % agarose gel electrophoresis.

The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGACMTGGCTCAG-3') and 1492R (5'-CGGYTACCTTGTTACGACTT-3'), which will amplify an approximately 1500 bp product (Sun *et al.*, 2008). PCR amplifications were performed in a total volume of 10 µl by mixing 5 µl of Taq PCR master mixture (25 units Taq DNA polymerase, 200 µM of each dNTP and 1X PCR buffer and 1.5 mM MgCl₂), 0.8 µl of each primer (10 mM), 0.5 µl of diluted (1:10) DNA template and 2.9 µl of sterilized distilled water. PCR amplification was done with an initial denaturation at 94 °C for 4 min followed by 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 58 °C, 1 min extension at 72 °C and a final extension step of 10 min at 72 °C. The PCR products obtained by amplification were analyzed on a 1.4 % (w/v) agarose gel.

Further confirmation was done by DNA sequencing for selected PCR products (kb and kd) and BLAST searches were performed for sequences obtained to find the similarity with sequence data from the GenBank (<http://www.ncbi.nlm.gov/BLAST/>).

ncbi.nlm.gov/BLAST/).

RESULTS AND DISCUSSION

Disease Symptoms

Soft rot symptoms of *A. vera* plant started as small, water soaked lesions at the base of the leaves and progressed very fast. Leaves became soft, collapsed and whole plant died within two to five days (Figure 01A, B) and the disease incidence ranged from 40-60 %. Mandal and Maiti (2005) reported that the leaf epidermis bulges out due to gas formation and the leaf content is converted into a slimy mass while gas is eventually released and that the disease is serious when abundant moisture was available either through irrigation or rains. It is observed that suckers of infected mother plants used for propagation also found to be infected with soft rot disease (Figure 01C).

Isolation of Causal Pathogen

Isolation of the pathogen on NA medium yielded similar colonies of three isolates (kb, kd and ke) which were creamy white colonies, convex and round with entire margins, after 48 hrs of incubation at 28±2 °C (Figure 02 A, B and C). All these bacterial isolates formed a slimy thread. 3% KOH test confirmed that isolates are Gram negative bacteria and were selected for further confirmation of their pathogenicity.



Figure 01: Bacterial soft rot symptoms in the field. (A- Initial stage, B- Later stage, C- Infected suckers)

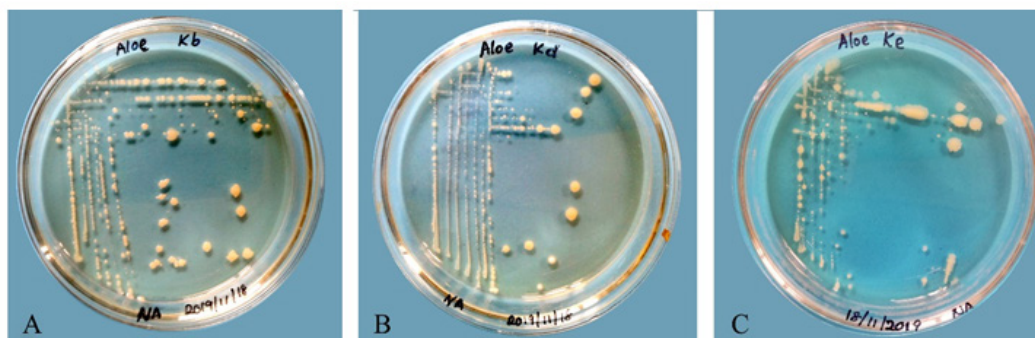


Figure 02: Three day old pure bacterial isolates (A-kb, B-kd and C-ke isolates) on NA medium

Confirmation of Pathogenicity

Typical soft rot symptoms of those observed in the field began to appear after 24 hrs on inoculated plants and the whole plant died within two-three days after inoculation with bacterial isolates (Figure 03A).

Plants inoculated with ke isolate (from infected suckers) also showed similar soft rot symptoms within two days, which indicated that bacterial pathogen of soft rot disease can be spread through infected suckers when they are used as planting materials.

All the isolates were successfully re-isolated from symptomatic plants, fulfilling Koch's postulates and identity of the re-isolates was further confirmed through PCR. No disease symptoms were observed on the plants that were artificially inoculated with sterile distilled water (Figure 03B).

Molecular Identification of two Bacterial Isolates

An amplicon of approximately 1500 bp was obtained from each of the three pure bacterial isolates (kb, kd and ke from suckers) and two re-isolates (kbr and kdr) and no bands were produced with water control (Figure 04).

BLAST analysis of 16S rRNA gene sequences revealed that the two sequences of kb and kd isolates were identical and showed 99 % identity with *Dickeya Chrysanthemi* (syn.: *Erwinia chrysanthemi*) from China (GeneBank Accession No. JF779681.1). Sequences were deposited in GenBank for two bacterial isolates, kb (Accession No. MT028490.1) and kd (Accession No. MT028539.1).

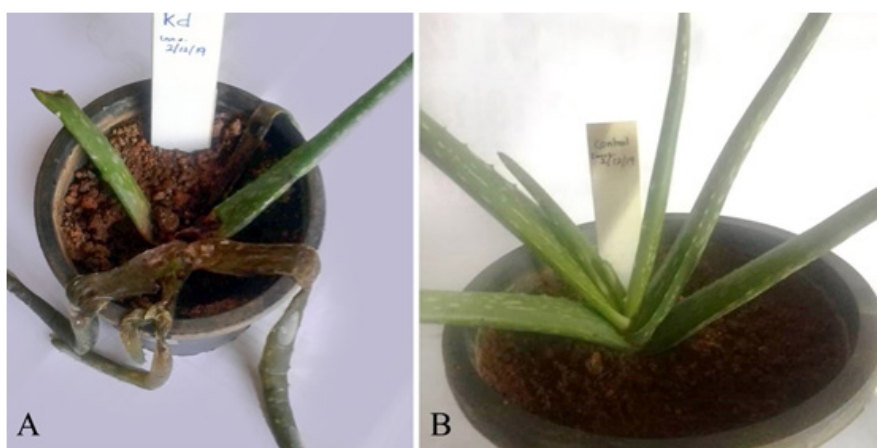


Figure 03: Soft rot symptoms in Aloe vera plants after 3 days of inoculation with kd bacterial isolate (A- leaves become soft rot, collapse, B- Control plant inoculated with sterile distilled water)

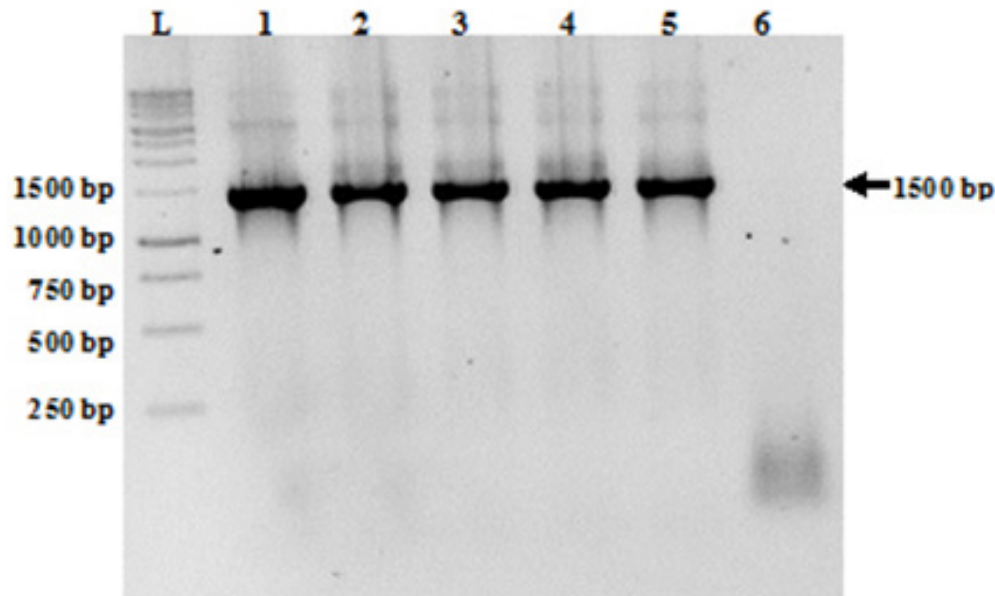


Figure 04: PCR products resulted by universal primers (*Uni-27F* and *Uni-1492R*) of five selected bacterial isolates, Lanes L: DNA Ladder, Isolates 1: kb, 2: kd, 3: kbr, 4: kdr, 5: ke, 6: negative control

Soft rot of *A. vera* caused by *E. chrysanthemi* (syn. *Pectobacterium chrysanthemi*) has been reported from different parts of the world. This disease was first reported from Caribbean island of Aruba in 1994 (De Laat *et al.*, 1994) and Korea (Jin *et al.*, 1994), later from India in 2005 (Mandal and Maiti, 2005) and Bangladesh in 2016 (Pervez *et al.*, 2016).

E. chrysanthemi that was recently reclassified as a new genus *Dickeya* based on genetic differences and divided into six new species; *D. chrysanthemi*, *D. dadantii*, *D. zea*, *D. dianthicola*, *D. dieffenbachia* and *D. paradisiaca* (Samson *et al.*, 2005). *D. chrysanthemi* causes disease in a broad host range of crops and ornamental plants in Tropical and subtropical regions (Toth *et al.*, 2011).

Lack of Pathogen free planting material and knowledge on disease management practices were identified as the key constraints to extensive cultivation of *A. vera* in Sri Lanka. This is the first report of *D. chrysanthemi* causing soft rot of *A. vera* in Sri Lanka.

CONCLUSION

The causal pathogen of the bacterial soft rot disease of *A. vera* was identified as *D. chrysanthemi*. A BLAST search revealed that two sequences of kb and kd bacterial isolates were identical and 99 % identity with *D. chrysanthemi* from China (GenBank Accession No. JF779681.1). Sequences were deposited in GenBank for two bacterial isolates, kb (MT028490.1) and kd (MT028539.1). The pathogen can be transmitted through suckers of infected mother plants.

ACKNOWLEDGEMENT

The authors wish to express their appreciation to the Department of Agriculture, Sri Lanka for funding this study.

Declaration of conflict of interest

The authors declare no conflict of interests.

REFERENCES

- De Laat, P.C.A., Verhoeven, J.T.W. and Janse, J.D. (1994). Bacterial leaf rot of *Aloe vera* L., caused by *Erwinia chrysanthemi* biovar 3, *European Journal of Plant Pathology*.100: 81-84.
- Jin, K.S., Lee, S.W., Kim, J.J. and Ryu, H.Y. (1994). Identification of bacterial isolates obtained from diseased orchids and Aloe plants caused by *Erwinia chrysanthemi*, *RDA Journal of Agriculture Science and Crop Protection*.36: 301-306.
- Mandal, K. and Maiti, S. (2005). Bacterial soft rot of aloe caused by *Pectobacterium chrysanthemi*, New Disease Reports, National Research Center for Medicinal and Aromatic Plants, Gujarat, India. 11, 1. doi:10.1111/j.1365-3059.2005.01216.x
- Pervez, Z., Alam, M.S. and Islam, M.S. (2016). First Report of Bacterial Soft Rot of Aloe vera caused by *Pectobacterium chrysanthemi* in Bangladesh, *Journal of Plant Pathology Microbiology*. 7, 1000e110. doi:10.4172/2157-7471.1000e110.
- Purnawati, A., Sastrahidayat, I. R., Abdul, L.A. and Hadiastono, T. (2014). Endophytic Bacteria as a Bio control agents of Tomato Bacterial Wilt Disease. *Journal of Tropical Life Science*, 4(1). 33-36. Doi: 10.11594/jtls.04.01.06
- Rajeswari, R., Umadevi, M., Rahale, S.C., Pushpa, R., Selvavenkadesh, S., Kumar, S.K.P. and Bhowmik, D. (2012). Aloe vera: Themiracle plant its medicinal and traditional uses in India, *Journal of Pharmacognosy and Phytochemistry*. 1: 118–124.
- Samson, R., Legendre, J.B., Christen, R., Fischer-Le Saux, M., Achouak, W. and Gardan, L. (2005). Transfer of *Pectobacterium chrysanthemi* (Burkholder *et al.*, 1953) Bernner *et al.*, 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi chrysanthemi* comb.nov. *Dickeya paradisiaca* comb.nov. and delineation of four novel species, *D. dadantii* sp.nov., *D. dianthicola* sp.nov., *D. dieffenbachiae* sp.nov., *D. zaeae* and. *International Journal of Systematic and Evaluationary Microbiology*, 55, 1415-1427. doi: 10.1099/ijms.0.02791-0
- Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X. and Song, W. (2008). Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis, *Journal Microbial Ecology*. 55: 415-424. doi:10.1007/s00248-007-9287-1
- Syamala, M. and Ciba, C. (2017). Screening of Antagonistic Bacteria and Organic Amendments against Bacterial Soft Rot of Aloe vera Disease Incidence in the pot. *International Journal of Current Microbiology and Applied Sciences*. 6(12): 595-603. doi.org/10.20546/ijcmas.2017.612.072
- Toth.I.K., Bell, K.S., Holeva, M.C. and Birch, P.R.J. (2003). Soft rot Erwiniae: from genes to genomes. *Molecular Plant Pathology*. 4:17-30. doi:10.1046/j.1364-3703.2003.00149.x
- Weller, S.A., Elphinstone, J.G., Smith, N.C., Boonllam, N.C. and Stead, D.E. (2000) Detection of *Ralstonia solanacearum* strain with quantitative, mutiplex, real–time, fluorogenic PCR (TaqMan) assay, *Applied and Environmental Microbiology*. 66: 2853–2858.doi:10.1128/aem.66.7.2853-2858.2000