

NEW DISEASE REPORT

Pseudomonas viridiflava Causing Leaf Spot of Common Mallow in Iran

Seyed Zaman Hosseini¹ | Pejman Khodaygan¹  | Luisa Ghelardini²  | Srđan G. Aćimović³  | Esmail Basavand¹ 

¹Department of Plant Pathology, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran | ²Department of Agriculture, Food, Environment and Forestry Sciences and Technologies, University of Florence, Florence, Italy | ³Alson H. Smith Jr. Agricultural Research and Extension Center, Department of Plant Pathology, Physiology, and Weed Science, School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University, Winchester, Virginia, USA

Correspondence: Pejman Khodaygan (pkhodaygan@vru.ac.ir)

Received: 4 November 2024 | **Revised:** 22 November 2024 | **Accepted:** 11 December 2024

Keywords: common mallow (*Malva sylvestris* L.) | leaf spot disease | polyphasic identification | *Pseudomonas viridiflava*

ABSTRACT

In the spring 2018, a leaf disease including light and necrotic spots with irregular edges surrounded by chlorotic halos was detected on common mallow in Jiroft County (Kerman Province, Iran). A bacterium-forming cream-coloured and mucoid colonies on King's B agar medium was consistently isolated and was fitting of the traits of the genus *Pseudomonas*. Isolated strains caused spots on common mallow leaves in the pathogenicity test that was identical to natural field symptoms and belonged to *Pseudomonas viridiflava* based on phenotypic characteristics including LOPAT tests. Two representative isolates were further confirmed as *P. viridiflava* with 99% sequence identity after sequencing the 16S rRNA and *gyrB* genes. To our knowledge, this is the first report of *P. viridiflava* causing leaf spot disease on common mallow in Iran.

Common mallow (*Malva sylvestris*; Malvaceae) is a widely consumed edible–medicinal and cosmetic plant with a naturally wide distribution all around the world. In the spring 2018, a leaf disease characterised as light, necrotic spots (1–2 mm in diameter) with irregular edges surrounded by chlorotic halos were observed on common mallow plants (Figure 1A) in Jiroft county, Kerman province, Iran. Cream-coloured bacterial colonies were consistently isolated from the diseased leaf tissues (Basavand et al. 2021). The five isolates obtained caused soft rot of potato tubers, induced a hypersensitive reaction (HR) in tobacco leaves, and were negative for levan production, oxidase and arginine dihydrolase, indicating that they belonged to LOPAT group II, *Pseudomonas viridiflava*, defined by Lelliott, Billing, and Hayward (1966). All isolates were Gram negative, catalase positive and also hydrolysed gelatine and esculine. The isolates produced acid from fructose, mannitol and sorbitol but not from arabinose, cellobiose and tyrosine. Therefore, the isolated bacterial strains were tentatively identified as *P. viridiflava* (Schaad, Jones, and Chun 2001). In all tests, *P. viridiflava*

ATCC 13223 isolated from dwarf bean in Switzerland, was used as a control. The 16S rRNA (1200 bp) and the *gyrB* (570 bp) sequences of two representative isolates, that is, ZHA and ZHB (GenBank Accession Nos. [OQ891779](https://doi.org/10.26434/chemrxiv-2024-00000) and [OQ891780](https://doi.org/10.26434/chemrxiv-2024-00000); [OQ909931](https://doi.org/10.26434/chemrxiv-2024-00000) and [OQ909932](https://doi.org/10.26434/chemrxiv-2024-00000)), were amplified using previously reported PCR primers (Yamamoto et al. 2000; Weisburg et al. 1991). The isolates shared 99% sequence identity with *P. viridiflava* reference strains available in NCBI (National Center for Biotechnology Information). Pathogenicity tests were performed twice on common mallow leaves (three plants for each isolate) by spraying bacterial suspension (10^6 CFU/mL) of 24 h old cultures grown on plates of sucrose nutrient agar (nutrient agar containing 1% sucrose, SNA). Seven days after inoculation, leaf spots on inoculated leaves matched to those observed on naturally infected plants (Figure 1B), whereas control plants inoculated with sterile distilled water remained symptomless. Reisolated bacterial colonies were fluorescent under ultraviolet light on King's B agar medium and exhibited the same traits of the original cultures, thereby fulfilling Koch's postulates. To our knowledge,

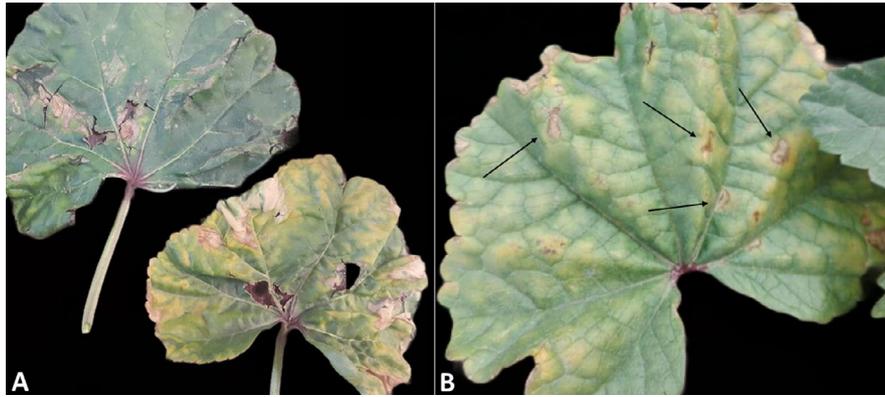


FIGURE 1 | (A) Naturally infected common mallow leaves caused by *Pseudomonas viridiflava* showing light, necrotic spots with irregular edges surrounded by chlorotic halos, (B) leaf spots induced artificially by spray inoculation of *P. viridiflava* strain ZHA on common mallow 7 days after inoculation.

this is the first report of *P. viridiflava* causing a disease of leaf spot of common mallow in Iran.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jph.70009>.

References

- Basavand, E., P. Khodaygan, H. Rahimian, V. Babaeizad, and H. A. Mirhosseini. 2021. "Pseudomonas syringae Pv. Syringae as the New Causal Agent of Cabbage Leaf Blight." *Journal of Phytopathology* 169: 253–259. <https://doi.org/10.1111/jph.12982>.
- Lelliott, R. A., E. Billing, and A. C. Hayward. 1966. "A Determinative Scheme for the Fluorescent Plant Pathogenic Pseudomonads." *Journal of Applied Bacteriology* 29: 470–489. <https://doi.org/10.1111/j.1365-2672.1966.tb03499.x>.
- Schaad, N. W., J. B. Jones, and W. Chun. 2001. *Laboratory Guide for the Identification of Plant Pathogenic Bacteria*. 3rd ed. St Paul, MN: American Phytopathological Society, APS Press.
- Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane. 1991. "16S Ribosomal DNA Amplification for Phylogenetic Study." *Journal of Bacteriology* 173: 697–703. <https://doi.org/10.1128/jb.173.2.697-703.1991>.
- Yamamoto, S., H. Kasai, D. L. Arnold, R. W. Jackson, A. Vivian, and S. Harayama. 2000. "Phylogeny of the Genus *Pseudomonas*: Intra-genetic Structure Reconstructed From the Nucleotide Sequences of *gyrB* and *rpoD* Genes." *Microbiology* 146: 2385–2394. <https://doi.org/10.1099/00221287-146-10-2385>.