



Common water-plantain, a new host of *Pseudomonas viridiflava* in rice fields in Iran

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The common water-plantain (*Alisma plantago-aquatica*), is a common perennial weed in the rice fields of northern Iran. During the summer of 2017, a leaf spot on common water-plantains was observed in the Mazandaran Province. The symptoms were small (50 to 100 mm²), round, and dark brown spots, which expanded with time, in a final stage leaves became completely necrotic and dried out. From these symptoms a green fluorescent bacterium was consistently isolated. Isolation and subsequent purification and biochemical profiling of causal organism were performed according to methods described by Borkar (2017). Upon isolation bacterial colonies on NSA medium were mucoid, circular, and cream-colored, 2 mm in diameter after 48 h growth. Ten isolates were negative in tests for oxidase, production of levan, hydrolysis of tween 80, urease, 3'-ketolactose, gas from glucose, phenylalanine deaminase, lecithinase, indole, arginine dihydrolase, H₂S from peptone, thiosulfate and cysteine but were fluorescent on King's B medium and were positive in tests for catalase, potato soft rot, nitrate reduction, RSS, NaCl 4 and 6% tolerance, tyrosinase activity, hydrolysis of casein, starch, esculin, gelatine, and arbutin. Litmus milk was turned alkaline and digested by isolates. Tested isolates used L-glutamine, D-galactose, D-tartrate, proline, L-tyrosine, succinic acid, malonate, D-xylulose, fructose, maltose, sucrose, L-valine, L-arabinose, fumarate, melibiose, glucose, mannitol, but not L-serine, L-tartrate, D-raffinose, D-lactose, D-cellobiose, sorbose, L-ornithine, L-arginine as carbon source for growth. In all of the tests, the type strain of *Pseudomonas viridiflava* (ATCC 13223) was used as a positive control and the isolates in this study were deposited in the Culture Collection of

Microorganisms in Vali-E-Asr University of Rafsanjan, Iran, under the numbers (ten isolates) VRU 1249 to VRU 1259. A pathogenicity test performed under greenhouse conditions by inoculation of a bacterial suspension of approximately 1×10^6 CFU/ml into mature leaves of common water-plantain plants yielded similar symptoms as observed in nature 10 days after inoculation. From these symptoms the pathogen was re-isolated and pathogenicity confirmed by Koch's postulates. For final identification, the partial 16S rRNA and *rpoD* genes from one representative isolate were amplified and sequenced (Rico et al. 2003; Weisburg et al. 1991), and sequences compared using the Blast software available at the NCBI database with other sequences in GenBank. The sequences of this isolate showed 100% similarity (*rpoD*: KY764288.1) and 99% (16S rRNA: MH793501) with those of the mentioned *P. viridiflava* strains. To our knowledge this is the first report of *P. viridiflava*, as a bacterial pathogen of common water-plantain in Iran.

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