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RAPID COMMUNICATION

First report of *Pseudomonas marginalis* causing tuber soft rot of potato in Iran

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Abstract

In 2016, potato tuber soft rot symptoms were observed in major potato production fields in Jiroft county (southern Kerman province, Iran). Bacteria, which appeared as white to creamy colonies, were isolated from diseased tissues and shown to be pathogenic on potato tubers after inoculation and re-isolation of the isolates. Based on the assessment of colony morphology, biochemical characteristics, and analysis of 16S rRNA and *gyrB* gene sequences, the pathogen was identified as *Pseudomonas marginalis*. To our knowledge, this is the first report of *P. marginalis* causing potato tuber soft rot disease in Iran.

Keywords: *gyrB*, potato (*Solanum tuberosum* L.), *Pseudomonas marginalis*, soft rot disease

Potato (*Solanum tuberosum* L.) is considered the third most significant crop globally and has been ranked first among non-cereal crops in terms of human consumption (Singh and Sandhu 2023). According to FAOSTAT data for 2022, China, India, Ukraine, the Russian Federation and the USA are the world's leading producers of potatoes (FAO 2022). In the same ranking, Iran is the 12th largest potato producer. Potatoes are susceptible to a wide range of diseases, of which the most serious are caused by bacterial pathogens. About seven bacterial diseases, including blackleg and soft rot (*Pectobacterium* spp., *Dickeya* spp., *P. marginalis*), brown rot, bacterial wilt and ring rot (*Ralstonia solanacearum, Clavibacter* spp.), common scab (*Streptomyces* spp.) and zebra chip (*Candidatus* Liberibacter), affect potatoes worldwide and cause severe damage, particularly to the tuber, which is the most economically important part of the potato plant (Li *et al*. 2007; Charkowski *et al*. 2020; Sadunishvili *et al*. 2020).

In April 2016, symptoms of tuber soft rot potato (cv. Sante) (Fig. 1A) were observed in commercial potato fields in Jiroft county (southern Kerman province, Iran), where potatoes are largely grown. Symptoms included rotting of the seed tuber and wilting of the stem and upper leaves. As the disease progressed, decaying tubers turned into a mushy, whitish, unpleasant-smelling pulp (Fig. 1B). Approximately 10% of plants were affected. Here, we identified the bacterial isolates causing tuber soft rot of potato in Iran using phenotypic characteristics and analysis of 16S rRNA and *gyrB* gene sequences.

Tubers were collected from 10 potato plants showing disease symptoms in five surveyed fields and taken to the laboratory. The suspected pathogen was isolated on sucrose nutrient agar medium (SNA, 1% sucrose), using the streak plate technique described by Li *et al*. (2007) and Basavand *et al*. (2021), with slight modifications. In brief, the naturally infected potato tubers were washed with tap water to remove excess soil. They were then surface disinfected in 70% ethanol for 2 minutes, rinsed in sterile distilled water (SDW), dried on filter paper and broken open just past the margin of the rot. Small parts at the infection margin

were removed aseptically and macerated in 1–2 ml of SDW in a Petri dish using a sterile surgical blade. After 25 minutes incubation at room temperature, 20 µl of the resulting suspension were streaked onto plates of SNA. The plates were incubated at 28°C in the dark for 2–5 days. Pure colonies, which were predominantly white to creamy, circular, slightly raised, and had smooth edges were consistently isolated from the samples. A total of five isolates of pure colonies were selected and used for further testing.

The five purified bacterial isolates were subjected to phenotypic and biochemical tests (Schaad *et al.* 2001), including the evaluation of features related to the LOPAT profile. The isolated strains were characterized as rod-shaped, Gram-negative and catalase-positive. They grew on nutrient agar at 35°C, hydrolyzed gelatin and starch, and produced fluorescent pigment on King's B medium. The LOPAT profile results were L+, /O+, /P+, /A+ and T –. Moreover, all isolates produced acid from sucrose, glucose, mannitol, mannose and salicin, but did not utilize cellobiose. Based on this phenotypic feature, we identified all isolated strains as being similar to rare *P. marginalis* strains reported in the literature (Li *et al.* 2007; Liyanapathiranage *et al.* 2023).

The pathogenicity of all five isolates was assessed using the methods described by Li *et al.* (2007). Two rinsed and surface sterilized tubers of potato (cv. Sante) were inoculated with 100 µl bacterial cell suspension (1×10^7 CFU · ml^{-1}) in SDW by using a sterile syringe at two positions on the tuber, 10 mm below the tuber surface, for each of the five isolates. Similarly, 10 control tubers were inoculated with SDW. The inoculated tubers were placed in sealed polythene bags and incubated at 25°C. The pathogenicity test was repeated twice, and yielded similar results. Rot symptoms appeared 7 days after inoculation with the bacterial suspension, and gradually developed inside the tubers (Fig. 1C). The negative control, inoculated with SDW had no symptoms. The bacteria isolated from the inoculated roots and tubers, which displayed symptoms, were found to be identical to the inoculum.

To confirm the identity of the pathogen causing soft rot of potato tubers, we analyzed the 16S rRNA and *gyrB* gene sequences of the two representative isolates, i.e., L1 and L4 (their selection was based on the identical phenotypic and biochemical characteristics among the bacterial isolates). Bacterial preparation and genomic DNA extraction followed previously described methods (Ausuble *et al.* 1992; Basavand *et al.* 2022). PCR was used to amplify partial sequences of the 16S rRNA (small subunit ribosomal RNA) and *gyrB* (DNA gyrase subunit B) genes using primer pair FD1/RD1 (Weisburg *et al.* 1991) and UP-1/UP-2r (Yamamoto and Harayama 1995), respectively. A 25 µl reaction mixture containing 12.5 µl of universal PCR Kit–Ampliqon® *Taq* DNA Polymerase Master Mix Red (Odense, Denmark), 6.5 µl of DNA-free water, 1 µl of each 10 µM primer and 4 µL of genomic DNA were used. The PCR conditions were: 4 min of predenaturation at 94°C, followed by 35 cycles of 1 min of denaturation at 94°C, annealing at 58°C (16S rRNA)

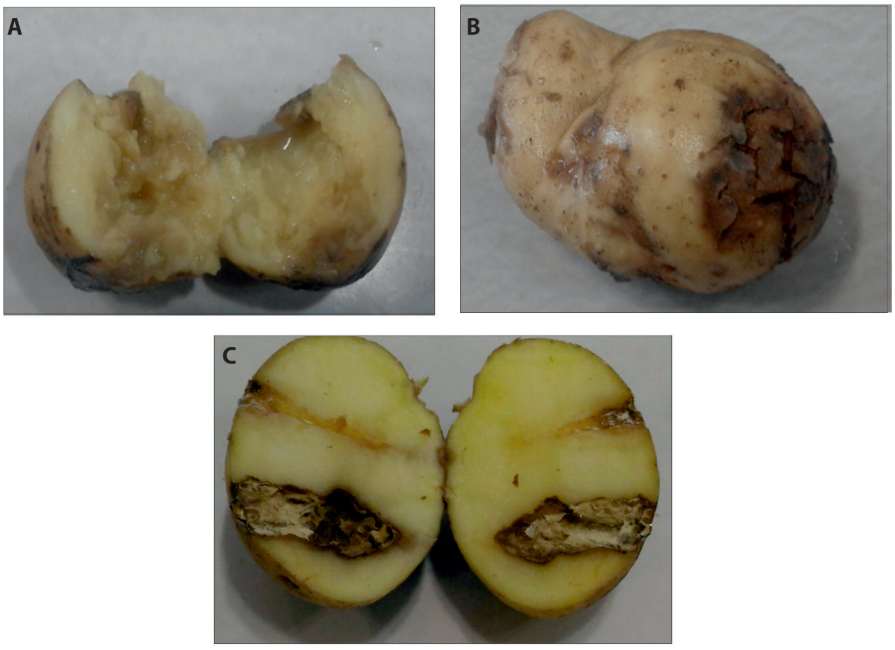

Fig. 1. Collected in the field, potato tuber with soft rot symptoms – A and rotted at later stages of the disease – B, from which the isolated casual bacteria were identified as *Pseudomonas marginalis*; C – rot developed on potato tubers 7 days post inoculation with *P. marginalis* strain L1

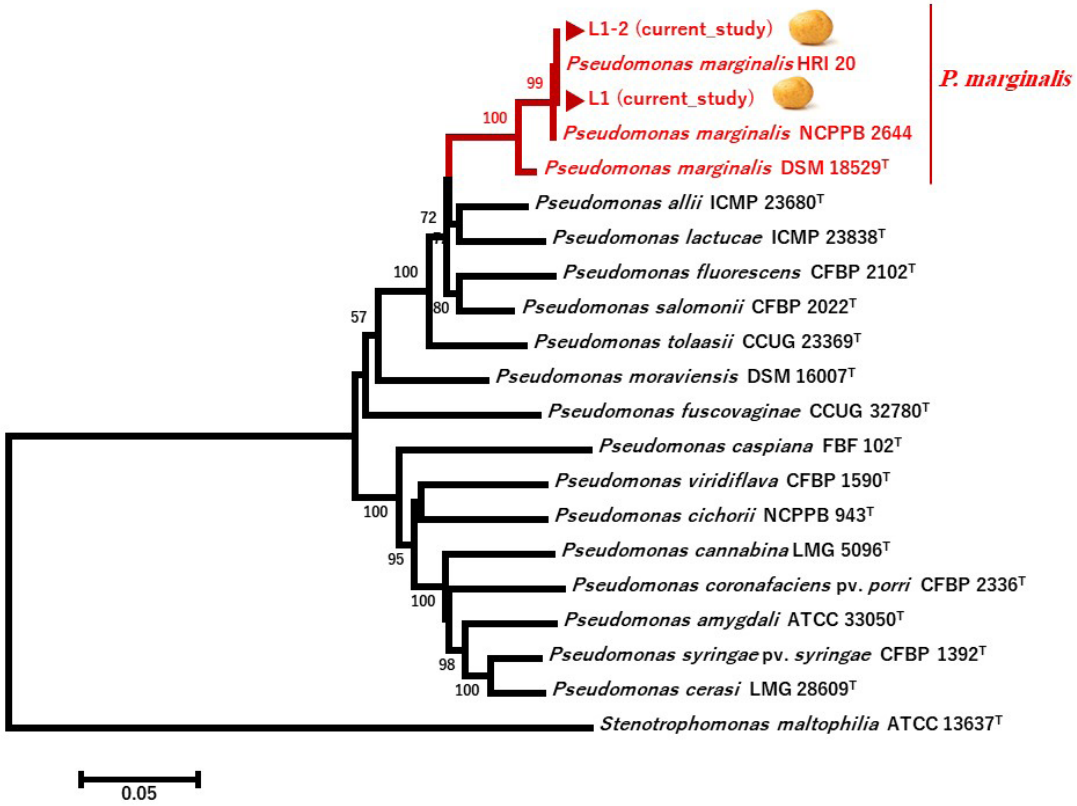

Fig. 2. Maximum likelihood phylogenetic tree based on an alignment using the *gyrB* gene sequences. The phylogenic analysis shows the position of potato isolates i.e., L1 and L4 among related strains of *Pseudomonas* spp. The tree was constructed using Kimura 2-parameter model with a gamma distribution and invariant sites (T93 + G + I). *Stenotrophomonas maltophilia* ATCC 13637T was used as the outgroup and bootstrap values (%) are marked on the branches. Bar lengths (0.05) indicate sequence dissimilarity. Bootstrap values calculated for 1000 replications are indicated

and 55°C (*gyrB*) for 1 min, 1 min of extension at 72°C; and then a final extension at 72°C for 10 min. The PCR products were analyzed using 1.5% agarose gel electrophoresis, and bands of the expected sizes (~1400 bp for 16S rRNA and 1200 bp for *gyrB*) were observed. The bands were purified and sequenced by the Macrogen sequencing service in Seoul, South Korea, using Sanger sequencing technology. The nucleotide sequences obtained were read and manually edited using FinchTV software (Geospiza, http//: www.finchtv.software.informer.com/1.4/), and compared using the BLASTn algorithm online in the GenBank with already deposited sequences in GenBank database. Blast searches revealed that the sequences of strains isolated from potato (GenBank accession no. PP373795, PP373796 for 16S rRNA; MZ346599, OK483346 for *gyrB*) shared 99-100% nucleotide identity to 16S rRNA and *gyrB* sequences of *Pseudomonas marginalis* in the GenBank (NCBI) database.

A phylogenetic dendrogram was constructed using the concatenated sequences that consisted of partial gene sequences of 16S rRNA and *gyrB*. The sequences of related *Pseudomonas* spp. were downloaded from GenBank and aligned using the ClustalW program implemented in BioEdit software (Hall 1999). The sequences were subjected to Modeltest 3.7 to determine the best-fitting evolutionary model (Posada and Crandall 1998). Neighbor-joining (NJ) and maximum-likelihood (ML) trees were reconstructed using MEGA7 (Kumar *et al.* 2016), applying the models and parameters set by Model test (Kimura 2-parameter model). A bootstrap analysis consisting of 1,000 replicates was conducted on the trees to assess the reliability of the generated cluster nodes. Both methods produced similar trees, and the phylogenetic dendrogram revealed that two strains isolated from potato belonged to *P. marginalis* (Fig. 2).

To the best of our understanding, this is the first documented case of *P. marginalis* causing soft rot disease in potatoes in Iran. *P. marginalis* commonly causes soft rot in multiple hosts and inhabits various environments, with an extensive host range. It can also infect economically important crops such as tomato, faba bean, cucurbits and maize (Sawada *et al.* 2023). To determine the current and future economic importance of *P. marginalis* in Iran, it is essential to assess the geographical distribution, the yearly rate of infection spread and the extent of damage caused by *P. marginalis* to potato and other hosts.

References

Ausuble F., Brent F.M., Kingestone R.E., Moor D.D., Smit J.A.,Seideman J.G., Struhl K. 1992. Current Protocols in Molecular Biology. Greene, Publishing Associates, Wiley Inter-science, New York, USA, 4648 pp.

Basavand E., Firouzianbandpey S., Rahimian H. 2022. Periwinkle leaf spots and stem lesions caused by *Xanthomonas campestris*. European Journal of Plant Pathology 164 (2): 167–176. DOI: https://doi.org/10.1007/s10658-022-02534-6

Basavand E., Khodaygan P., Rahimian H., Babaeizad V., Mirhosseini H.A. 2021. *Pseudomonas syringae* pv. *syringae* as the new causal agent of cabbage leaf blight. Journal of Phytopathology 169 (4): 253–259. DOI: https://doi.org/10.1111/jph.12982

Charkowski A., Sharma K., Parker M.L., Secor G.A., Elphinstone J. 2020. Bacterial diseases of potato. p. 351–388. In: "The Potato Crop" (Campos H., Ortiz O., eds). Springer, Cham.

FAO. 2022. Food and Agriculture Organization of the United Nations. [Available on: https://www.fao.org/faostat/en/#rankings/countries_by_commodity]

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41 (41): 95–98.

Kumar S., Stecher G., Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33 (7): 1870–1874. DOI: https://doi.org/10.1093/molbev/msw054.

Li J., Chai Z., Yang H., Li G., Wang D. 2007. First report of *Pseudomonas marginalis* pv. *marginalis* as a cause of soft rot of potato in China. Australasian Plant Disease Notes 2: 71–73. DOI: https://doi.org/10.1071/DN07029

Liyanapathiranage P., Avin F.A., Oksel C., Swiggart E., Gao Y., Baysal-Gurel F. 2023. First report of rusty root of *Panax quinquefolius* caused by *Pseudomonas marginalis* in Tennessee and the United States. Plant Disease 107 (7). DOI: https://doi.org/10.1094/PDIS-11-22-2563-PDN

Posada D., Crandall K.A. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics (Oxford, England) 14 (9): 817–818. DOI: https://doi.org/10.1093/bioinformatics/14.9.817

Sadunishvili T., Węgierek-Maciejewska A., Arseniuk E., Gaganidze D., Amashukeli N., Sturua N., Amiranashvili L., Kharadze S., Kvesitadze G. 2020. Molecular, morphological and pathogenic characterization of *Clavibacter michiganensis* subsp. *sepedonicus* strains of different geographic origins in Georgia. European Journal of Plant Pathology 158 (1): 195–209. DOI: https://doi.org/10.1007/s10658-020-02066-x

Sawada H., Fujikawa T., Satou M. 2023. Dismantling and reorganizing *Pseudomonas marginalis* sensu lato. Plant Pathology 72 (4): 654–666. DOI: https://doi.org/10.1111/ppa.13690

Schaad N.W., Jones J.B., Chun W. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. APS Press, Minnesota, USA, 410 pp.

Singh P., Sandhu A.S. 2023. Energy budgeting and economics of potato (*Solanum tuberosum* L.) cultivation under different sowing methods in north-western India. Energy 269: 126755. DOI: https://doi.org/10.1016/j.energy.2023.126755

Weisburg W.G., Barns S.M. Pelletier D.A., Lane D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173 (2): 697–703. DOI: https://doi.org/10.1128/jb.173.2.697-703.1991

Yamamoto S., Harayama S. 1995. PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. Applied and Environmental Microbiology 61: 1104–1109. DOI: https://doi.org/10.1128/aem.61.3.1104-1109.1995