ISSN 0026-2617, Microbiology, 2021, Vol. 90, No. 1, pp. 87–95. © Pleiades Publishing, Ltd., 2021.
Russian Text © The Author(s), 2021, published in Mikrobiologiya, 2021, Vol. 90, No. 1, pp. 100–109.

EXPERIMENTAL ARTICLES

Identification of *Pantoea* Phytate-Hydrolyzing Rhizobacteria Based on Their Phenotypic Features and Multilocus Sequence Analysis (MLSA)

A. D. Suleimanova[a], *, D. L. Itkina[a], D. S. Pudova[a], and M. R. Sharipova[a]

[a] *Kazan (Volga Region) Federal University, Kazan, 420008 Russia*

**e-mail: aliya.kzn@gmail.com*

Received May 3, 2020; revised August 21, 2020; accepted August 28, 2020

Abstract—Accurate strain identification within the *Pantoea* genus is difficult due to homologous recombination, which may affect the species boundaries. An integrated approach is presently the most effective one in determining the species of bacteria. Biochemical identification using the API20E system, phylogenetic analysis of the 16S rRNA gene sequences, and MLSA analysis based on partial sequences of the *fusA, pyrG, leuS, gyrB*, and *rpoB* genes showed that the soil phytate-hydrolyzing isolates belonged to the genus *Pantoea*, specifically to the species *Pantoea brenneri*. It was also established that phytate-hydrolyzing activity of the strains was accompanied by their ability to fix atmospheric nitrogen.

Keywords: *Pantoea*, identification, MLSA, API20E, nitrogen fixation

DOI: 10.1134/S0026261721010112

The genus *Pantoea* was originally described by the French scientist Gavini (Gavini et al. 1989) as consisting of two species: *Pantoea agglomerans* sp. nov and *Pantoea dispersa* sp. nov. Currently, this genus comprises 29 species (http://www.bacterio.net/pantoea.html). *Pantoea* species have been isolated from various environments, including soil, water, food stuffs, plants, animals, and humans (Chen et al., 2017). Some of the first isolates were plant pathogens, causing galls, wilting, soft rot, and necroses in a number of agricultural plants (Brady et al., 2008; Herrera et al., 2008). Contrariwise, other *Pantoea* isolates produce antimicrobial compounds and are widely used as commercial biologics (Johnson et al. 1993). For example, *P. ananatis* is able to decompose herbicides without forming toxic by-products and may be used for soil bioremediation (Pileggi et al., 2012). The species *P. agglomerans* was originally identified as an infectious agent for a wide range of plants. However, a number of strains of this species have the properties promoting plant growth and development, as well as biocontrol of plant pathogens (Dutkiewicz et al., 2016). The ubiquitous and universal character of *Pantoea* isolates, as well as their genetic flexibility, make this genus a perfect group both for investigation of the typical processes of adaptation and opportunism and for development of commercial products to be used in medicine and agriculture (Walterson and Stavrinides, 2015).

Phylogenetic analysis and comparison with other genera of the family *Erwiniaceae* revealed high diversity within the genus *Pantoea*. Biochemical heterogeneity within the genus hinders species identification of the strains. Accurate identification of members of the genus *Pantoea* is difficult, especially given the fact that homologous recombination and lateral gene transfer may affect the species boundaries. At present, bacterial species are identified using a comprehensive approach involving both genomic and phenotypic characteristics of the strains (Deletoile et al., 2009).

Application of the 16S pRNA gene sequences is an important tool for classification and systematization of *Pantoea* isolates. However, the 16S pRNA gene sequences exhibit low resolution at the intrageneric level (Gonzalez et al., 2013), preventing reliable identification of bacteria at the species and subspecies levels. Thus, species identification of new strains requires more accurate analysis involving such improved taxonomic methods as multilocus phylogenetic analysis (MLSA) (Gevers et al., 2005). Species identification of bacteria is presently based on analysis of the multilocus sequences of their housekeeping genes, such as *fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB*, which are used on a regular basis to specify the interspecies phylogenetic positions of *Pantoea* species (Brady et al., 2008; Deletoile et al., 2009; Palmer et al., 2017; Tambong, 2019). It has been established that the MLSA approach based on six genes (*leuS*, *fusA*, *gyrB*, *pyrG*, *rpo*B, and *rplB*) provides reliable differentiation