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Reclassification of subspecies of *Acidovorax avenae* as *A. Avenae* (Manns 1905) emend., *A. cattleyae* (Pavarino, 1911) comb. nov., *A. citrulli* Schaad et al., 1978) comb. nov., and proposal of *A. oryzae* sp. nov.

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Abstract

The bacterium *Acidovorax avenae* causes disease in a wide range of economically important monocotyledonous and dicotyledonous plants, including corn, rice, watermelon, anthurium, and orchids. Genotypic and phenotypic relatedness among strains of phytopathogenic *A. avenae* subsp. *avenae*, *A. avenae* subsp. *citrulli*, *A. avenae* subsp. *cattleyae* and *A. konjaci*, as well as all other *Acidovorax* species, including *A. facilis*, the type strain of *Acidovorax*, was determined. The 16s rDNA sequencing confirmed previous studies showing the environmental species to be very distant from the phytopathogenic species. DNA/DNA reassociation assays on the different strains of *A. avenae* revealed four (A, B, C, and D) distinct genotypes. Taxon A included six *A. avenae* subsp. *avenae* strains from corn that had a mean reciprocal similarity of 81%; taxon B included six *A. avenae* subsp. *avenae* strains from rice that had a mean reciprocal similarity of 97%; taxon C contained 11 *A. avenae* subsp. *citrulli* strains from cucurbits (cantaloupe, watermelon, and pumpkin) that had a mean reciprocal similarity of 88%, and taxon D contained four *A. avenae* subsp. *cattleyae* strains from orchids that had a mean similarity of 98%. The mean reciprocal relatedness between taxa A, B, C, and D was less than 70%. Sequence analysis of 16S rDNA and the 16S–23S rDNA internally transcribed spacer region, as well as AFLP analysis, revealed the same four taxa. All four were easily differentiated phenotypically from each other and from all other recognized *Acidovorax* species. Strains of *A. avenae* did not contain 3-hydroxyoctanoic acid, which was found in all other species. On the basis of these and previous genetic and phenotypic results, we propose an emendation of the species *A. avenae*. *A. avenae* subsp. *citrulli* (C strains) and *A. avenae* subsp. *cattleyae* (D strains) should be elevated to species rank as *A. citrulli* and *A. cattleyae*, respectively. We further propose a new taxon for the B strains, *A. oryzae* sp. nov. with FC-143^T = ICPB 30003^T = ICMP 3960^T = ATCC 19882^T as the type strain.
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Keywords: *Acidovorax*; Taxonomy; 16S and ITS sequencing; AFLP; Phenotypic tests; DNA/DNA reassociation; Emendation

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Introduction

Acidovorax avenae subsp. *avenae* (*Pseudomonas avenae*) and *A. avenae* subsp. *citrulli* (synonymous with *P. pseudoalcaligenes* subsp. *citrulli*) have emerged worldwide as serious pathogens on corn (*Zea mays* L.) [40] and watermelon (*Citrullus lanatus* (Thunb.) Matsumura and Nakai) [42], respectively. *Pseudomonas avenae*, originally described in 1909 as the causal agent of leaf blight of oats (*Avenae sativa* L.) [31], causes disease under conditions of high rainfall and high temperatures in numerous species of the family *Graminaceae*, including wheat (*Triticum aestivum* L.), finger millet (*Eleusine coracana* (L.) Gaertn.), Italian millet (*Setaria italica* (L.) Beauv.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), and proso millet (*Panicum miliaceum* L.) [4,9,32,33]. Additional hosts include tea (*Thea sinensis* L.), barley (*Hordeum vulgare* L.), mountain brome (*Bromus carinatus* Hook. & Arn.), rescue grass (*B. catharticus* Vahl), vasey grass (*Paspalum urvillei* Steud.), teosinte (*Zea mexicana* (Schrad.) Kuntze), and sugarcane (*Saccharum officinarum* L.) [2,13,17,49,50]. *P. alboprecipitans*, a pathogen of foxtail (*Chaetochloa lutescens* (Weigel) Stuntz) [36], was later shown to be synonymous with the oats and corn pathogen, *P. avenae* [40]. Additionally, the red stripe disease of sugarcane caused by *P. rubrilineans* [17] was shown to be synonymous with *P. avenae* [35]. More recently *A. avenae* subsp. *avenae* has emerged as a serious pathogen of rice (*Oryzae sativa* L.) [16,24,26,27,34]. The causal agent of bacterial stripe of rice also has been referred to as "[*P.*] *setariae*" [14] and *P. panici* [34], however, neither epithet has been validly published [56]. A pathogen of orchids (*Cattleya*, *Dendrobium*, *Oncidium,* and *Phalaenopsis*), *P. cattleyae*, was described in 1946 [1]. A pathogen of konjac (*Amorphophallus konjac* K. Koch.), *A. konjaci,* was described in 1983 [15]. All of these phytopathogenic bacteria were described previously as a heterogenous group of non-fluorescent, oxidase positive pseudomonads, which accumulated poly-β-hydroxybutyrate (PHB) and utilized D-arabinose [38].

Schaad et al. [41] described a non-fluorescent pseudomonad as the causal agent of a seedling blight of watermelon. The bacterium differed phenotypically from other known plant pathogenic pseudomonads and was classified as *P. pseudoalcaligenes* subsp. *citrulli* [41]. Later, Wall and Santos determined that the same organism caused a disease referred to as watermelon fruit blotch of mature watermelon fruit [42]. Additional hosts of *A. avenae* subsp. *citrulli* include melon/cantaloupe (*Cucumis melo* L. subsp. *melo* var. *cantalupensis* Naudin), cucumber (*Cucumis sativus* L.) [41], and pumpkin (*Cucurbita pepo* L.) [28]. The above non-fluorescent phytopathogenic pseudomonads together with *P. pseudoalcaligenes* subsp. *cattleyae* [1] and *P. pseudoalcaligenes* subsp. *konjaci* [15] were all shown to be part of the "acidovorans" DNA–rRNA homology group [7].

Based on DNA/DNA and DNA–rRNA homologies [5] and phenotypic assays, Willems et al. [55] proposed that the above "generically misnamed phytopathogenic pseudomonads" be transferred to the newly proposed genus *Acidovorax* [54]. Strains of *P. avenae* [*P. rubrilineans*] from corn, sugar cane, Indian shot (*Canna indica*), teosinte, finger millet, and "*P. setariae*" from rice were all classified as *A. avenae* subsp. *avenae*. Strains of *P. pseudoalcaligenes* subsp. *citrulli* from *Cucurbitaceae* hosts were classified as *A. avenae* subsp. *citrulli*; strains of *P. cattleyae* from orchids were renamed as *A. avenae* subsp. *cattleyae*; and strains of *P. pseudoalcaligenes* subsp. *konjaci* [15] from konjac were classified as *A. konjaci* [55]. Recently two new phytopathogenic species of *Acidovorax*, *A. anthurii* on anthurium (*Anthurium palmatum* (L.) G. Don) [11] and *A. valerianellae* on lambs' lettuce (*Valerianella locusta* (L.) Lat.) [12], as well as a denitrifying species, *A. caeni* from activated sludge [18], have been described.

The purpose of this study was to re-evaluate the phylogenetic relatedness among the above phytopathogenic *A. avenae* strains and between *A. avenae* and the phytopathogenic *A. konjaci*, *A. anthurii*, and *A. valerianellae*. A polyphasic taxonomic study revealed considerable differences among the different subspecies of *A. avenae* and between the other species of *Acidovorax*. Therefore, we propose a new species, *A. oryzae* for the rice strains of *A. avenae.* We also propose *A. avenae* subsp. *citrulli* and *A. avenae* subsp. *cattleyae* be elevated to species rank as *A. citrulli* comb. nov. and *A. cattleyae* comb. nov.

Materials and methods

Source of strains and confirmation of identity

All the strains used in this study were obtained from the International Collection of Phytopathogenic Bacteria (ICPB) maintained at the USDA, ARS Foreign Disease-Weed Science Research Unit (FDWSRU), Fort Detrick, MD or from other recognized culture collections (Table S-1). Each culture was streaked onto yeast extract-dextrose $CaCO_3$ (YDC) agar [23] and beige-tan colored, transparent, round, non-mucoid, convex colonies were retained. Cultures were maintained on YDC slants at room temperature and also archived at −80 °C [38].

Pathogenicity

Pathogenicity was determined for all *A. avenae* strains by inoculating 2–3 plants each of corn (cv. 'Iochief'),