Reclassification of *Enterobacter oryzophilus* and *Enterobacter oryzendophyticus* as *Kosakonia oryzihila* comb. nov. and *Kosakonia oryzendophytica* comb. nov.

Chun Yan Li,¹  Yankee Liang Zhou,¹ Jing Ji¹ and Chun Tao Gu²,³

¹Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin 150030, PR China
²College of Life Science, Northeast Agricultural University, Harbin 150030, PR China
³Synergetic Innovation Center of Food Safety and Nutrition, Northeast Agricultural University, Harbin 150030, PR China

The taxonomic positions of *Enterobacter oryzophilus* and *Enterobacter oryzendophyticus* were re-examined on the basis of concatenated partial *rpoB, atpD, gyrB* and *infB* gene sequence analysis. The reconstructed phylogenetic tree based upon concatenated partial *rpoB, atpD, gyrB* and *infB* gene sequences clearly showed that *E. oryzophilus* and *E. oryzendophyticus* and all defined species of the genus *Kosakonia* form a clade separate from other genera of the family *Enterobacteriaceae*, and, therefore, these species of the genus *Enterobacter* should be transferred to the genus *Kosakonia*. *E. oryzophilus* and *E. oryzendophyticus* are reclassified as *K. oryzihila* comb. nov. (type strain REICA_142T=LMG 26429T=NCCB 100390T) and *K. oryzendophytica* comb. nov. (type strain REICA_082T=LMG 26432T=NCCB 100390T), respectively.

Since 2013, 12 members (*Enterobacter nimpresseuralis, Enterobacter amnigenus, Enterobacter gergoviae, Enterobacter pyrimus, Enterobacter cowanii, Enterobacter radicincitans, Enterobacter oryzae, Enterobacter arachidis, Enterobacter helveticus, Enterobacter pulveris, Enterobacter turicensis* and *Enterobacter sacchari*) of the genus *Enterobacter* have been transferred to five novel genera (*Lelliottia, Parahibacter, Kosakonia, Franconibacter* and *Siccibacter*) by Brady et al. (2014) and Gu et al. (2014). *Enterobacter oryzophilus* and *Enterobacter oryzendophyticus* were proposed by Hardoim et al. in 2013 on the basis of 16S rRNA and *rpoB* gene sequence analyses, and their names were validly published in 2015 (Validation list no. 166; Oren & Garrity, 2015). A maximum-likelihood tree based on 16S rRNA gene sequences indicated that *E. oryzophilus* and *E. oryzendophyticus* were related phylogenetically to *E. arachidis, E. oryzae, E. radicincitans, Enterobacter cloacae* subsp. *cloacae, Enterobacter cloacae* subsp. *dissolvens* and *E. cowanii* (Hardoim et al., 2013). *rpoB* gene sequence analysis also showed that *E. oryzophilus* was related phylogenetically to *E. radicincitans, E. arachidis* and *E. cowanii* (Hardoim et al., 2013).

*E. arachidis, E. oryzae, E. radicincitans* and *E. cowanii* had already been transferred into a novel genus, *Kosakonia*, by Brady et al. (2013) on the basis of multilocus sequence analysis (concatenated partial *rpoB, atpD, gyrB* and *infB* gene sequence analysis). According to the results presented by Hardoim et al. (2013), *E. oryzophilus* and *E. oryzendophyticus* were probably members of the novel genus *Kosakonia*, so their taxonomic status was re-evaluated.

In the present study, the taxonomic status of *E. oryzophilus* and *E. oryzendophyticus* was re-examined on the basis of concatenated partial *rpoB, atpD, gyrB* and *infB* gene sequence analysis. Strains LMG 26429T and LMG 26432T were incubated aerobically at 30 °C on nutrient agar (pH 7.0) consisting of 0.5 % peptone, 0.3 % meat extract and 0.5 % NaCl.

The DNA for gene amplification was prepared using bacterial genomic DNA extraction kits (TIANGEN). The RNA polymerase β subunit (*rpoB*), DNA gyrase (*gyrB*), initiation translation factor 2 (*infB*) and ATP synthase β subunit (*atpD*) genes were amplified using the primers and protocol...
of Brady et al. (2008). Purification and sequencing (Sanger method) of PCR products were carried out by the Shenggong Company in Shanghai, China. The resulting sequences, together with those of related strains obtained from the GenBank database were aligned by using CLUSTALW (Thompson et al., 1994). Phylogenetic trees were reconstructed using the neighbour-joining method with the maximum composite likelihood model. Bootstrap analysis was performed, based on 1000 replicates. The MEGAS package (Tamura et al., 2011) was used for all phylogenetic analyses.

The reconstructed phylogenetic tree (Fig. S1, available in the online Supplementary Material) based upon concatenated partial rpoB, atpD, gyrB and infB gene sequences, of which GenBank/EMBL/DBJ accession numbers are listed in Table S1, clearly shows that *E. oryziphius* LMG 26429T and *E. oryzendophyticus* LMG 26432T form a clade with species of the genus *Kosakonia*, separate from other genera of the family Enterobacteriaceae, and, therefore, these species of the genus *Enterobacter* should be transferred to the genus *Kosakonia*. Strain LMG 26429T showed 92.3–97.7 % rpoB gene sequence similarities, 93.1–98.3 % atpD gene sequence similarities, 87.0–94.5 % gyrB gene sequence similarities and 86.4–96.8 % infB gene sequence similarities to strain LMG 26432T and the type strains of *Kosakonia cowanii*, *Kosakonia sacchari*, *Kosakonia arachidis*, *Kosakonia oryzae* and *Kosakonia radicincitans* (Table S2). Strain LMG 26429T exhibited relatively low gene sequence similarities with *E. cloacae*, which is type species of the genus *Enterobacter* (Table S2). As shown in Fig. S1, strain LMG 26429T was closely related to the type strains of *K. arachidis*, *K. oryzae* and *K. radicincitans*, having the highest gene sequence similarities (Table S2). Strain LMG 26432T showed 90.4–94.0 % rpoB gene sequence similarities, 92.9–97.3 % atpD gene sequence similarities, 85.9–90.4 % gyrB gene sequence similarities and 86.1–88.6 % infB gene sequence similarities to strain LMG 26429T and the type strains of *K. cowanii*, *K. sacchari*, *K. arachidis*, *K. oryzae* and *K. radicincitans* (Table S3). Strain LMG 26432T shared a relatively low infB gene sequence similarity (85.6 %) with *E. cloacae* (Table S3). As shown in Table S3, strain LMG 26432T exhibited relatively low gene sequence similarities with the type strains of all defined species of the genus *Kosakonia*. The highest sequence similarity (97.3 %, for the atpD gene) was found between strain LMG 26432T and *K. sacchari* LMG 26783T (Table S3).

Similarities between species of the genus *Kosakonia* on the basis of concatenated partial rpoB, atpD, gyrB and infB gene sequences were 89.8–97.5 % (Table S4). Concatenated partial rpoB, atpD, gyrB and infB gene sequence divergence between strains LMG 26429T and LMG 26432T and the type strains of phylogenetically related species showed that strains LMG 26429T and LMG 26432T represent two different species from all defined species within the genus *Kosakonia* (Tables S4). 96.5 % sequence similarity (Table S4) was found between strain LMG 26429T and *K. radicincitans* LMG 23767T, of which the DNA–DNA relatedness was 59 % (Hardoim et al., 2013). Strain LMG 26429T and *K. arachidis* KCTC 22375T, which had 63 % DNA–DNA relatedness (Hardoim et al., 2013), exhibited 96.0 % sequence similarity (Table S4). Although *K. sacchari* was not included in the study of Hardoim et al. (2013), low similarities (90.8 % and 92.0 %; Table S4) between strains LMG 26429T and LMG 26432T and *K. sacchari* LMG 26783T showed that strains LMG 26429T and LMG 26432T represent two different species from *K. sacchari*.

Hardoim et al. (2013) reported that the DNA–DNA relatedness values between *K. radicincitans* LMG 23767T and *K. oryzae* LMG 24251T, between *K. radicincitans* LMG 23767T and *K. arachidis* KCTC 22375T, and between *K. oryzae* LMG 24251T and *K. arachidis* KCTC 22375T were 79±6 % (higher than the result reported by Peng et al. in 2009), 66±17 % (higher than the result reported by Madhaiyan et al. in 2010) and 71±2 %, respectively, suggesting these three species probably had a close relationship. In the present study, four housekeeping gene sequences (rpoB, atpD, gyrB and infB) were available for all these three species, so their taxonomic status was re-examined by multilocus sequence analysis based upon concatenated partial rpoB, atpD, gyrB and infB gene sequences.

As shown in Table S4, similarity between *K. radicincitans* LMG 23767T and *K. oryzae* LMG 24251T based upon concatenated partial rpoB, atpD, gyrB and infB gene sequences was 97.5 %. Although the similarity was relatively high, *K. radicincitans* and *Kosakonia oryzae* can be clearly distinguished by fatty acid profiles and phenotypic features (Peng et al., 2009), and by enterobacterial repetitive intergenic consensus (ERIC)–PCR patterns and protein profiles analysed by MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) (Zhu et al., 2013). In the study of Zhu et al. (2013), a DNA–DNA relatedness study between *K. radicincitans* and *K. oryzae* was also performed. The AT% between *K. radicincitans* DSM 16656T and *K. oryzae* LMG 24251T was 11.1±1.0 (Zhu et al., 2013), indicating that they represent two different species (Wayne et al., 1987). Taking the above information into account, *K. radicincitans* and *K. oryzae* should be two different species. In future, an analysis of average nucleotide identity (ANI) based upon whole genome sequencing will provide a good verification of the taxonomic relationship of *K. radicincitans* and *K. oryzae*.

Similarities between *K. arachidis* KCTC 22375T and *K. radicincitans* LMG 23767T and between *K. arachidis* KCTC 22375T and *K. oryzae* LMG 24251T, based upon concatenated partial rpoB, atpD, gyrB and infB gene sequences, were 95.8 % and 95.6 % (Table S4), showing that *K. arachidis* represents a different species from *K. radicincitans* and *K. oryzae*. Distinction of *K. arachidis* and *K. radicincitans* was also supported by fatty acid profiles (Madhaiyan et al., 2010; Hardoim et al., 2013).

Phenotypic features that differentiate *E. oryziphius* and *E. oryzendophyticus* from all defined species of the genus *Kosakonia* are shown in Table 1.

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The description of *Kosakonia oryzendophytica* is identical to that proposed for *Enterobacter oryzendophytica* (Hardoim et al., 2013).

The type strain is REICA_082T (=LMG 26432T=NCCB 100390T). Phenotypic features that differentiate *Kosakonia oryzendophytica* from all defined species of the genus *Kosakonia* are shown in Table 1.

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**References**


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**Table 1. Phenotypic features that differentiate *E. oryziphilus* and *E. oryzendophylicus* from all defined species of the genus *Kosakonia***

<table>
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<th>3 (n=6)</th>
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</table>

*Data in parentheses refer to the results of the type strain.
†Data from Madhaiyan et al. (2010).*

On the basis of concatenated partial rpoB, atpD, gyrB and infB gene sequence analysis, *E. oryziphilus* and *E. oryzendophylicus* should be reclassified as *Kosakonia oryziphila* comb. nov. and *Kosakonia oryzendophylica* comb. nov. respectively.

**Description of *Kosakonia oryziphila* comb. nov.**

*Kosakonia oryziphila* [o.ry.zi’phi.la. L. fem. n. oryza rice; philus (from Gr. masc. adj. philos) friend, loving; N.L. fem. adj. oryziphila rice-loving].


The description of *Kosakonia oryzendophylica* is identical to that proposed for *Enterobacter oryzendophylicus* (Hardoim et al., 2013).

The type strain is REICA_082T (=LMG 26432T=NCCB 100390T). Phenotypic features that differentiate *Kosakonia oryzendophylica* from all defined species of the genus *Kosakonia* are shown in Table 1.

**Description of *Kosakonia oryzendophytica* comb. nov.**

*Kosakonia oryzendophytica* (o.ryz.en.do.phy’ti.ca. L. fem. n. oryza rice; Gr. pref. endo- within; Gr. neut. n. phytos plant; L. masc. suff. -icus suffix used with the sense of pertaining to; N.L. fem. adj. oryzendophylica within rice plant, pertaining to the original isolation from rice tissues).
Kosakonia radicincitans comb. nov., Kosakonia oryzae comb. nov. and Kosakonia arachidis comb. nov., respectively, and E. turicensis, E. helveticus and E. pulveris into Cronobacter as Cronobacter zurichensis nom. nov., Cronobacter helveticus comb. nov. and Cronobacter pulveris comb. nov., respectively, and emended description of the genera Enterobacter and Cronobacter.


Hardoim, P. R., Nazir, R., Sessitsch, A., Elhottová, D., Korenblum, E., van Overbeek, L. S. & van Elsas, J. D. (2013). The new species Enterobacter oryziphilus sp. nov. and Enterobacter oryzendophyticus sp. nov. are key inhabitants of the endosphere of rice. BMC Microbiol 13, 164.


