Complete genome sequence of *Kosakonia oryzae* type strain Ola 51<sup>T</sup>

Yuanyuan Li<sup>1†</sup>, Shuying Li<sup>1†</sup>, Mingyue Chen<sup>1</sup>, Guixiang Peng<sup>2</sup>, Zhiyuan Tan<sup>3*</sup> and Qianli An<sup>1*</sup>

**Abstract**

Strain Ola 51<sup>T</sup> (=LMG 24251<sup>T</sup> = CGMCC 1.7012<sup>T</sup>) is the type strain of the species *Kosakonia oryzae* and was isolated from surface-sterilized roots of the wild rice species *Oryza latifolia* grown in Guangdong, China. Here we summarize the features of the strain Ola 51<sup>T</sup> and describe its complete genome sequence. The genome contains one circular chromosome of 5,303,342 nucleotides with 54.01% GC content, 4773 protein-coding genes, 16 rRNA genes, 76 tRNA genes, 13 ncRNA genes, 48 pseudo genes, and 1 CRISPR array.

**Keywords:** Endophyte, *Kosakonia*, Nitrogen fixation, Plant growth-promoting bacteria

**Introduction**

*Enterobacter cowanii* [1], *E. radicincitans* [2], *E. oryzae* [3], *E. arachidis* [4], *E. sacchari* [5], *E. oryziphilus* [6, 7], and *E. oryzendophyticus* [6, 7] have been transferred into the novel genus *Kosakonia* of the family *Enterobacteriaceae* [8–10]. A novel species “*Kosakonia pseudosacchari*” [11] closely related to *K. sacchari* was recently proposed. With the exception of the type species *K. cowanii*, which was originally obtained from clinical samples [1], the other members of the genus *Kosakonia* are nitrogen-fixing bacteria associated with plants [2–6, 11] and commonly occur in the nitrogen-fixing bacterial community of some non-legume crops, such as rice [6] and sugarcane [12]. Some nitrogen-fixing *Kosakonia* strains are able to promote crop growth [12–14].

Strain Ola 51<sup>T</sup> (=LMG 24251<sup>T</sup> = CGMCC 1.7012<sup>T</sup>) is the type strain of the species *Kosakonia oryzae* and was isolated from surface-sterilized roots of the wild rice species *Oryza latifolia* grown in Guangdong, China [3]. Here we present the summary of the features of the *K. oryzae* type strain Ola 51<sup>T</sup> and its complete genome sequence, which provides a reference for resolving the phylogeny and taxonomy of closely related strains and the genetic information to study its plant growth-promoting potential and its plant-associated life style.

**Organism information**

**Classification and features**

*K. oryzae* strain Ola 51<sup>T</sup> is a Gram-negative, non-spore-forming, motile rod with peritrichous flagella (Fig. 1). It grows aerobically but reduces N<sub>2</sub> to NH<sub>3</sub> at a low pO<sub>2</sub>. It forms circular, convex, smooth colonies with entire margins on nutrient agar [3, 8]. It grows best around 30 °C and pH 7 (Table 1) [3]. *K. oryzae* Ola 51<sup>T</sup> has the typical biochemical phenotypes of the genus *Kosakonia*: positive for acetoin production (Voges-Proskauer test) while negative for indole production; positive for β-galactosidase and arginine dihydrolase while negative for lysine decarboxylase; positive for oxidation of arabinose, cellobiose, citrate, fructose, galactose, gluconate, glucose, glycerol, lactose, malate, malonate, mannitol, mannose, sorbitol, sucrose and trehalose (Table 1) [3, 8].

The 16S rRNA gene sequence of *K. oryzae* Ola 51<sup>T</sup> was deposited in GenBank under the accession number EF488759 [3]. A phylogenetic analysis of the 16S rRNA gene sequences from the strains belonging to the genus *Kosakonia* and *Escherichia coli* ATCC11775<sup>T</sup> (the type strain of the type species of the type genus of the family *Enterobacteriaceae*) showed that *K. oryzae* Ola 51<sup>T</sup> is most closely related to the strains belonging to the species *K. radicincitans* (Fig. 2) [3, 8–11].
**Chemotaxonomic data**

Whole-cell fatty acids were extracted from cells grown aerobically at 28 °C for 24 h on the TSA medium according to the recommendations of the Microbial Identification System (MIDI Inc., Delaware USA). The whole-cell fatty acid composition was determined using a 6890 N gas chromatograph (Agilent Technologies, Santa Clara, USA) and the peaks of the profiles were identified using the TSBA50 identification library version 5.0 (MIDI). *K. oryzae* Ola 51\(^T\) shows the typical cell fatty acid profile of the genus *Kosakonia* [8]. The major fatty acids are C\(_{16:0}\), C\(_{18:1}\) \(\omega 7c\), C\(_{16:1} \omega 7c/15:0\) iso 2OH, C\(_{17:0}\) cyclo, and C\(_{14:0}\) 3OH/16:1 iso I [8, 11].

**Genome sequencing information**

**Genome project history**

*K. oryzae* Ola 51\(^T\) was selected for sequencing based on its taxonomic significance. The genome sequence is deposited in GenBank under the accession number CP014007. A summary of the genome sequencing project information and its association with MIGS version 2.0 [15] is shown in Table 2.

**Growth conditions and genomic DNA preparation**

*K. oryzae* Ola 51\(^T\) was grown aerobically in liquid Luria-Bertani medium at 30 °C until early stationary phase. The genome DNA was extracted from the cells by using a TIANamp bacterial DNA kit (Tiangen Biotech, Beijing, China). DNA quality (OD260/OD280 = 1.8) and quantity (22 μg) were determined with a Nanodrop spectrometer (Thermo Scientific, Wilmington, USA).

---

**Table 1** Classification and general features of *Kosakonia oryzae* strain Ola 51\(^T\) according to the MIGS recommendations [15]

| MIGS ID | Property | Term | Evidence code* |
|---------|----------|------|----------------|
| **Classification** | Domain Bacteria | TAS [34] |
| | Phylum Proteobacteria | TAS [35] |
| | Class Gammaproteobacteria | TAS [36, 37] |
| | Order "Enterobacteriales" | TAS [38] |
| | Family Enterobacteriaceae | TAS [39, 40] |
| | Genus Kosakonia | TAS [8] |
| | Species *Kosakonia oryzae* | TAS [3, 8] |
| **Type strain**: Ola 51\(^T\) | | | TAS [3] |
| **Gram stain** | Negative | | TAS [3] |
| **Cell shape** | Rod | | TAS [3] |
| **Motility** | Motile | | TAS [3] |
| **Sporulation** | Non-sporulating | | TAS [3] |
| **Temperature range** | 10–40 °C | | TAS [3] |
| **Optimum temperature** | 28–37 °C | | TAS [3] |
| **pH range; Optimum** | 3.5–10; 6.0–8.0 | | TAS [3] |
| **Carbon source** | Arabinose, cellobiose, citrate, fructose, galactose, gluconate, glucose, glycerol, lactose, malate, maltose, manninitol, mannose, sorbitol, sucrose & trehalose | | TAS [3, 8] |
| **Habitat** | Plants | | TAS [3] |
| **Salinity** | 0 – 5% NaCl (w/v) | | TAS [3] |
| **Oxygen requirement** | Facultatively anaerobic | | TAS [3] |
| **Biotic relationship** | Free-living, endophytic | | TAS [3] |
| **Pathogenicity** | Not reported | | |
| **Geographic location** | Guangzhou, Guangdong, China | | TAS [3] |
| **Sample collection** | September 12, 2005 | | TAS [3] |
| **Latitude** | 23.1634171311 °N | | NAS |
| **Longitude** | 113.354469581°E | | NAS |
| **Depth** | 0.2 – 0.3 m below the surface | | TAS [3] |
| **Altitude** | 20 m | | NAS |

* Evidence codes – IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [41]
Resource. Sequencing was run on two SMRT cells and resulted in 124,997 high-quality filtered reads with an average length of 8,260 bp. High-quality reads were assembled by the RS_HGAP_Assembly.3 in the SMRT analysis v2.3.0. The final assembly produced 128-fold coverage of the genome.

Genome annotation
Automated genome annotation was done using the NCBI Prokaryotic Genome Annotation Pipeline [17]. Functional annotations were done by searching against the KEGG [18], InterPro [19], and COG [20] databases. Genes with signal peptides were predicted using SignalP [21]. Genes with transmembrane helices were predicted using TMHMM [22].

Genome properties
The genome of *K. oryzae* Ola 51T contains one circular chromosome (Fig. 3). The chromosome contains 5,303,342 nucleotides with 54.0% G+C content. The genome contains 4,926 predicted genes, 4,773 protein-coding genes, 105 RNA genes (16 rRNA genes, 76 tRNA genes, and 13 ncRNA genes), 48 pseudo genes, and 1 CRISPR repeats. Among the 4,773 protein-coding genes, 3,765 genes (78.88%) have been assigned functions, while 1,008 genes (21.12%) have been annotated as hypothetical or unknown proteins (Table 3). The distribution of genes into COG functional categories is presented in Table 4 and Fig. 3.

Insights from the genome sequence
The genome sequences of *K. cowanii* JCM 10956T, *K. radicincitans* DSM 16656T (=D5/23T) [23], *K. radicincitans* UMEnt01/12 [24], *K. radicincitans* YD4 [25], *K. sacchari* SP1T [26], “*K. pseudosacchari*” JM-387T [11], *K. oryzae* KO348 [27], and *Enterobacter* sp. R4-368 [28] which was close to *K. sacchari* SP1T [26] had been deposited in the GenBank database.

---

Table 2 Genome sequencing project information for *Kosakonia oryzae* strain Ola 51T

| MIGS ID   | Property                        | Term                                      |
|-----------|---------------------------------|-------------------------------------------|
| MIGS 31   | Finishing quality               | Finished                                  |
| MIGS-28   | Libraries used                  | PacBio 8–11 Kb library                    |
| MIGS 29   | Sequencing platforms            | PacBio RS II                              |
| MIGS 31.2 | Fold coverage                   | PacBio 128 X                              |
| MIGS 30   | Assemblers                      | HGAP Assembly.3 in SMRT analysis-2.3.0    |
| MIGS 32   | Gene calling method             | GeneMarkS+                                |
| Locus Tag |                                 | AWR26                                     |
| Genbank ID|                                 | CP014007                                  |
| GenBank Date of Release |                   | June 6, 2016                             |
| GOLD ID   |                                 | Gp0154734                                 |
| BIOPROJECT|                                 | PRJNA309028                               |
| MIGS 13   | Source Material Identifier      | LMG 24251T = CGMCC 1.7012T                |
| Project relevance |            | Taxonomy, agriculture, plant-microbe interactions |
The genome ANIs (Additional file 1: Table S1) between Ola 51\textsuperscript{T} and the other strains belonging to the genus Kosakonia were calculated using the Orthologous Average Nucleotide Identity tool \[29\]. The cut-off ANI value for species boundary was set at 95\% - 96\% \[30\]. The ANI value (95.85\%) between K. oryzae Ola 51\textsuperscript{T} and K. radicincitans DSM 16656\textsuperscript{T} is in the fuzzy zone 95\% - 96\%. The digital DDH value between Ola 51\textsuperscript{T} and DSM 16656\textsuperscript{T} calculated by the Genome-to-Genome Distance Calculator \[31\] with the Formula 2 is 66.2\%, below the 70\% cut-off value for species boundary. Moreover, Ola 51\textsuperscript{T} and DSM 16656\textsuperscript{T} were differentiated by metabolic phenotypes \[3, 11\] and ribosomal protein mass profiles \[5\]. Therefore, K. oryzae and K. radicincitans are closely related sister species.

Strain YD4 was closer to K. radicincitans DSM 16656\textsuperscript{T} than K. oryzae Ola 51\textsuperscript{T} on the phylogenetic tree based on the 16S rRNA genes (Fig. 2). However, the ANI value and the digital DDH value between YD4 and K. radicincitans DSM 16656\textsuperscript{T} is 95.56\% and 64.4\%, respectively, while between YD4 and K. oryzae Ola 51\textsuperscript{T} is 97.04\% and 74.3\%, respectively. Therefore, the strain YD4 belongs to K. oryzae but not K. radicincitans.
Strain KO348 was grouped with *K. sacchari* SP1\(^T\), Enterobacter sp. R4-368, and "*K. pseudosacchari*" JM-387\(^T\) on the phylogenetic tree based on the 16S rRNA genes (Fig. 2). The ANI value between KO348 and *K. oryzae* Ola 51\(^T\) is 84.04%. The strain KO348 thus does not belong to *K. oryzae*. The ANI value between KO348 and Enterobacter sp. R4-368 [27], *K. sacchari* SP1\(^T\), or "*K. pseudosacchari*" JM-387\(^T\) is 98.80%, 94.56%, and 94.05%, respectively. Therefore, KO348 and R4-368 belong to the same species, likely a novel species closely related to *K. sacchari* and "*K. pseudosacchari*".

*K. oryzae* Ola 51\(^T\) and YD4, *K. radicincitans* DSM 16656\(^T\) and UMENT01/12, these strains contain the most structural proteins (YscCJRSTUVN) of the type III secretion system, which is not widespread among the previously studied endophytic bacteria [32].

These plant-associated *Kosakonia* strains contain genes contributing to multiple plant growth-promoting activities. They all contain the *nif* gene cluster (*nifHDKT-YXENLISVWZMFLABQ*) for the Mo-Fe nitrogenase-dependent nitrogen fixation, the genes encoding indole-3-acetaldehyde dehydrogenase, aspartate aminotransferase, aromatic amino acid aminotransferase and phenylpyruvate decarboxylase for producing the phytohormone auxin, and the *budABC* genes for producing volatile acetoin and 2,3-butanediol which induce plant systemic resistance to pathogens [33]. In addition, *K. oryzae* Ola 51\(^T\) and YD4, and *K. radicincitans* DSM 16656\(^T\) and UMENT01/12 also contain the *anf* gene cluster (*anfHDKG*) for the Fe-Fe nitrogenase-dependent nitrogen fixation. In contrast, the clinical strain *K. cowanii* JCM 10956\(^T\) does not contain the *nif* gene cluster.

### Conclusions

The phylogeny of the members of the genus *Kosakonia* based on the 16S rRNA gene sequences is roughly in agreement with their overall genome relatedness. The complete genome sequence of *K. oryzae* Ola 51\(^T\) provides the reference genome for genomic identification of strains belonging to *K. oryzae*. Analyses of the overall genome relatedness indices (ANI and digital DDH values), easily and reliably show that *K. oryzae* and *K. radicincitans* are closely related sister species and that the strain YD4, which shows close 16S rRNA gene-based phylogeny to *K. radicincitans* and was classified into *K. radicincitans*, belongs to *K. oryzae*. As well as YD4, which is able to promote growth of the yerba mate plants in low-fertility soils [14], *K. oryzae* Ola 51\(^T\) contains both the *nif* gene cluster and the *anf* gene cluster for nitrogen fixation and genes contributing to production of auxin and volatile acetoin and 2,3-butanediol. Therefore, *K. oryzae* Ola 51\(^T\) may be able to promote plant growth. Genomic analyses also show that *K. oryzae* Ola 51\(^T\) and YD4 may have the type III and VI secretion systems and thus motivate us to study the functions of the type III and VI secretion systems in the interactions between beneficial *Kosakonia* bacteria and plants.

### Additional file

Additional file 1: Table S1. Average nucleotide identities (ANIs) between genomes of the strains belonging to the genus *Kosakonia*. (DOC 38 kb)
Abbreviations

ANI: Average nucleotide identity; DDH: DNA-DNA hybridization; SMRT: Single Molecule Real-Time

Funding

This work was supported by the National Natural Science Foundation of China (31711504, 31730052 and 31471449), Zhejiang Provincial Natural Science Foundation of China (LY14C010002), Science and Technology Planning Project of Guangdong Province (2014A030313459 and 2014A050503058), and the State Key Laboratory of Rice Biology, China.

Authors’ contributions

YL, SL and MC assembled the sequencing data and completed the genome analysis; GP did the microbiological studies and obtained the organism information; ZT and QA designed the study and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, China. 2College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China. 3College of Agriculture, South China Agricultural University, Guangzhou 510642, China.

Received: 1 September 2016 Accepted: 7 April 2017

Published online: 17 April 2017

References

1. Inoue K, Sugiyama K, Kosako Y, Sakazaki R, Yamai S. Enterobacter cowani sp. nov., a new species of the family Enterobacteriaceae. Curr Microbiol. 2000;41:417–20. PubMed http://dx.doi.org/10.1007/s002840010160.
2. Kämpfer P, Ruppel S, Remus R. Enterobacter radiocentris sp. nov., a plant growth promoting species of the family Enterobacteriaceae. Syst Appl Microbiol. 2005;28:213–21. PubMed http://dx.doi.org/10.1016/j.syapm.2004.12.007.
3. Peng G, Zhang W, Luo H, Xie H, Lai W, Tan Z. Enterobacter oxyae sp. nov., a nitrogen-fixing bacterium isolated from the wild rice species Oryza sativa. Int J Syst Evol Microbiol. 2009;59:1650–5. PubMed http://dx.doi.org/10.1099/ijs.0.06584-0.
4. Madhivanan M, Poonguzhali S, Lee J-S, Saravanan VS, Lee K-C, Santhanakrishnan P. Enterobacter arachidis sp. nov., a plant-growth-promoting diazotrophic bacterium isolated from rhizosphere soil of groundnut. Int J Syst Evol Microbiol. 2010;60:1559–64. PubMed http://dx.doi.org/10.1099/ijs.0.013664-0.
5. Zhu B, Zhou Q, Lin L, Hu C, Shen P, Yang L, An Q, Xie G, Li Y. Enterobacter sacchari sp. nov., a nitrogen-fixing bacterium associated with sugar cane (Saccharum officinarum L.). Int J Syst Evol Microbiol. 2013;63:2577–82. PubMed http://dx.doi.org/10.1099/ijs.0.045300-0.
6. Hardim PR, Nazir R, Sesstisch A, Ettová D, Kornblum E, van Overbeek LS, van Elas JD. The new species Enterobacter oxyphylus sp. nov. and Enterobacter oxyphyphus sp. nov. are key inhabitants of the endosphere of rice. BMC Microbiol. 2013;13:164. PubMed http://dx.doi.org/10.1186/1471-2180-13-164.
7. Oren A, Garrity GM. Validation list no. 166. Int J Syst Evol Microbiol. 2015;65:3763–7. PubMed http://dx.doi.org/10.1099/ijs.0.009632.
8. Brady C, Cleerwiek J, Venter S, Coutinho T, De Vos P. Taxonomic evaluation of the genus Enterobacter based on multilocus sequence analysis (MLSA): proposal to reclassify E. nimprissimilis and E. ammigenus into Lelliotia gen. nov. as Lelliotia nimprissimilis comb. nov. and Lelliotia ammigena comb. nov., respectively. FEMS Microbiol Lett. 2015;365:277–82. PubMed http://dx.doi.org/10.1111/1574-6968.12537.
9. Bergottini VM, Filippidou S, Junier P, Chain PS, Otegui MB, Zapata PD, Junier P. Genome sequence of Enterobacter radiocentris DSM16656T, a plant growth-promoting endophyte. J Bacteriol. 2012;194:5469. PubMed http://dx.doi.org/10.1128/JB.00239-15.
10. Chen M, Zhu B, Lin L, Yang L, Li Y, An Q. Complete genome sequence of Kosakonia sacchari type strain SP1T. Stand Genomic Sci. 2014;9:1311–8. PubMed http://dx.doi.org/10.4056/sigs.5779977.

Cronobacter helveticus comb. nov. and Cronobacter pulveris comb. nov., respectively, and emended description of the genera Enterobacter and Cronobacter. Syst Appl Microbiol. 2013;36:309–19. PubMed http://dx.doi.org/10.1016/j.syapm.2013.03.005.

Lin L, Li Z, Hu C, Zhang X, Chang S, Yang L, Li Y, An Q. Plant growth-promoting nitrogen-fixing enterobacteria are in association with sugarcane plants growing in Guangxi, China. Microbes Environ. 2015;27:391–8. PubMed http://dx.doi.org/10.1093/mee/mdv1277.

Copyright information

© The Author(s). Published by Springer Nature Limited.

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, China. 2College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China. 3College of Agriculture, South China Agricultural University, Guangzhou 510642, China.

Received: 1 September 2016 Accepted: 7 April 2017

Published online: 17 April 2017

References

1. Inoue K, Sugiyama K, Kosako Y, Sakazaki R, Yamai S. Enterobacter cowani sp. nov., a new species of the family Enterobacteriaceae. Curr Microbiol. 2000;41:417–20. PubMed http://dx.doi.org/10.1007/s002840010160.
2. Kämpfer P, Ruppel S, Remus R. Enterobacter radiocentris sp. nov., a plant growth promoting species of the family Enterobacteriaceae. Syst Appl Microbiol. 2005;28:213–21. PubMed http://dx.doi.org/10.1016/j.syapm.2004.12.007.
3. Peng G, Zhang W, Luo H, Xie H, Lai W, Tan Z. Enterobacter oxyae sp. nov., a nitrogen-fixing bacterium isolated from the wild rice species Oryza sativa. Int J Syst Evol Microbiol. 2009;59:1650–5. PubMed http://dx.doi.org/10.1099/ijs.0.06584-0.
4. Madhivanan M, Poonguzhali S, Lee J-S, Saravanan VS, Lee K-C, Santhanakrishnan P. Enterobacter arachidis sp. nov., a plant-growth-promoting diazotrophic bacterium isolated from rhizosphere soil of groundnut. Int J Syst Evol Microbiol. 2010;60:1559–64. PubMed http://dx.doi.org/10.1099/ijs.0.013664-0.
5. Zhu B, Zhou Q, Lin L, Hu C, Shen P, Yang L, An Q, Xie G, Li Y. Enterobacter sacchari sp. nov., a nitrogen-fixing bacterium associated with sugar cane (Saccharum officinarum L.). Int J Syst Evol Microbiol. 2013;63:2577–82. PubMed http://dx.doi.org/10.1099/ijs.0.045300-0.
6. Hardim PR, Nazir R, Sesstisch A, Ettová D, Kornblum E, van Overbeek LS, van Elas JD. The new species Enterobacter oxyphylus sp. nov. and Enterobacter oxyphyphus sp. nov. are key inhabitants of the endosphere of rice. BMC Microbiol. 2013;13:164. PubMed http://dx.doi.org/10.1186/1471-2180-13-164.
7. Oren A, Garrity GM. Validation list no. 166. Int J Syst Evol Microbiol. 2015;65:3763–7. PubMed http://dx.doi.org/10.1099/ijs.0.009632.
8. Brady C, Cleerwiek J, Venter S, Coutinho T, De Vos P. Taxonomic evaluation of the genus Enterobacter based on multilocus sequence analysis (MLSA): proposal to reclassify E. nimprissimilis and E. ammigenus into Lelliotia gen. nov. as Lelliotia nimprissimilis comb. nov. and Lelliotia ammigena comb. nov., respectively. FEMS Microbiol Lett. 2015;365:277–82. PubMed http://dx.doi.org/10.1111/1574-6968.12537.
9. Bergottini VM, Filippidou S, Junier P, Chain PS, Otegui MB, Zapata PD, Junier P. Genome sequence of Enterobacter radiocentris DSM16656T, a plant growth-promoting endophyte. J Bacteriol. 2012;194:5469. PubMed http://dx.doi.org/10.1128/JB.00239-15.
10. Chen M, Zhu B, Lin L, Yang L, Li Y, An Q. Complete genome sequence of Kosakonia sacchari type strain SP1T. Stand Genomic Sci. 2014;9:1311–8. PubMed http://dx.doi.org/10.4056/sigs.5779977.
27. Meng X, Bertani I, Abbruscato P, Piffanelli P, Liscastro D, Wang C, Venturi V. Draft genome sequence of rice endophyte-associated isolate Kosakonia oryzae KO348. Genome Announc. 2015;3:e00594–15. PubMed http://dx.doi.org/10.1128/genomeA.00594-15.

28. Madhaiyan M, Peng N, Ji L. Complete genome sequence of Enterobacter sp. strain RA-368, an endophytic N-fixing gammaproteobacterium isolated from surface-sterilized roots of Jatropha curcas L. Genome Announc. 2013;1:e00544–13. PubMed http://dx.doi.org/10.1128/genomeA.00544-13.

29. Lee I, Kim YO, Park S-C, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol. 2016;66:1–13. PubMed http://dx.doi.org/10.1099/ijs.0.000760.

30. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A. 2009;106:19126–31. PubMed http://dx.doi.org/10.1073/pnas.0906412106.

31. GGDC [http://ggdc.dsmz.de/distcalc2.php]. Accessed 9 Apr 2017.

32. Reinhold-Hurek B, Hurek T. Living inside plants: bacterial endophytes. Curr Opin Plant Biol. 2011;14:435–43. PubMed http://dx.doi.org/10.1016/j.pbi.2011.04.004.

33. Ryu CM, Farag MA, Hu CH, Wei HX, Paré PW, Klopper JW. Bacterial volatiles promote growth in Arabidopsis. Proc Natl Acad Sci U S A. 2003;100:4927–32. PubMed http://dx.doi.org/10.1073/pnas.0730845100.

34. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A. 1990;87:4576–9. PubMed http://dx.doi.org/10.1073/pnas.87.12.4576.

35. Garrity GM, Bell JA, Lilburn T. Phylum XIV. Proteobacteria phyl. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology, Volume 2, Part B. 2nd ed. New York: Springer; 2005. p. 1.

36. Garrity GM, Bell JA, Lilburn T. Class III. Gammaproteobacteria class. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology, Volume 2, Part B. 2nd ed. Springer: New York; 2005. p. 1.

37. Validation of publication of new names and new combinations previously effectively published outside the IJSEM. List no. 106. Int J Syst Evol Microbiol. 2005;55:2235-8. PubMed http://dx.doi.org/10.1099/ijs.0.64108-0.

38. Garrity GM, Holt JG. Taxonomic Outline of the Archaea and Bacteria. In: Garrity GM, Boone DR, Castenholz RW, editors. Bergey’s Manual of Systematic Bacteriology, Volume 1. 2nd ed. New York: Springer; 2001. p. 155–66.

39. Rahn O. New principles for the classification of bacteria. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung II. 1937;96:73–86.

40. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. Int J Syst Bacteriol. 1980;30:225–40. PubMed http://dx.doi.org/10.1099/00207713-30-1-225.

41. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene ontology: tool for the unification of biology. Nat Genet. 2000;25:25–9. PubMed http://dx.doi.org/10.1038/75556.

42. SINA Alignment Service [https://www.arb-silva.de/aligner/]. Accessed 9 Apr 2017.

43. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. PubMed http://dx.doi.org/10.1093/molbev/msr121.

44. Stothard P, Wishart DS. Circular genome visualization and exploration using CGView. Bioinformatics. 2005;21:537–9. PubMed http://dx.doi.org/10.1093/bioinformatics/bti054.