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RESOURCE ANNOUNCEMENT

Complete Genome Sequences of *Trifolium* spp. Inoculant Strains *Rhizobium leguminosarum* sv. *trifolii* TA1 and CC275e: Resources for Genomic Study of the *Rhizobium-Trifolium* Symbiosis

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Abstract

Rhizobium leguminosarum symbiovar *trifolii* strains TA1 and CC275e are nitrogen-fixing microsymbionts of *Trifolium* spp. and have been used as commercial inoculant strains for clovers in pastoral agriculture in Australia and New Zealand. Here we present the complete genome sequences of both strains, resolving their multipartite genome structures and allowing for future studies using genomic approaches.

Genome Announcement

Rhizobium leguminosarum is a species complex with five genospecies (gs) currently defined based on average nucleotide identity (ANI) (Kumar et al. 2015; Cavassim et al. 2020). Isolates of *R. leguminosarum* symbiovar *trifolii* engage in nitrogen-fixing endosymbiosis with *Trifolium* spp. (clover) (Kuykendall et al. 2015). Following a complex molecular dialogue, *R. leguminosarum* sv. *trifolii* colonizes symbiotic organs on the plant roots, i.e., nodules, wherein the microbes differentiate into organelle-like bacteroids capable of reducing atmospheric dinitrogen into ammonia for assimilation by the plant host (Poole et al. 2018). The *R. leguminosarum* sv. *trifolii–Trifolium* symbiosis is of significant economic importance as it introduces nitrogen into forage systems used for livestock production (Hoyos-Villegas et al. 2019). *R. leguminosarum* sv. *trifolii* CC275e, previously the commercial inoculant of *T. repens* in New Zealand, was replaced in 2005 with strain TA1, which is currently used for *T. repens* in pastoral agriculture in both Australia and New Zealand, because of ease of manufacturing (Delestre et al. 2015; Drew et al. 2012; Reeve et al. 2013). TA1 was isolated in the 1950s in Tasmania, Australia, from a root nodule of *T. subterraneum*; CC275e was isolated in Tasmania from a *T. repens* root nodule. Both strains form effective nodules on *T. repens*, but the two strains differ in their symbiotic properties on other agriculturally important clovers. TA1

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forms fully effective nodules on *T. subterraneum* but poorly effective nodules on *T. pratense*, while CC275e is fully effective on *T. pratense* but poorly effective on *T. subterraneum* (Delestre et al. 2015; Reeve et al. 2013).

Draft-quality genome assemblies are available for TA1 and CC275e (Reeve et al. 2013; Delestre et al. 2015). The fragmented nature of these assemblies poses problems for use in whole-genome studies utilizing Tn-seq (Perry et al. 2016) or RNA-seq (Green et al. 2019), as portions of their genome sequences may be unrepresented or duplicated. Additionally, *R. leguminosarum* sv. *trifolii* genomes are multipartite (Harrison et al. 2010), and this organization is not resolved in draft assemblies. We utilized Oxford Nanopore Technologies (ONT) long-read sequencing and hybrid assembly to resolve the complete genome sequences of TA1 and CC275e.

High–molecular weight DNA was isolated from stationary-phase tryptone yeast broth cultures grown at 28°C (Beringer 1974) using a phenol/chloroform extraction method (Meade et al. 1982). Sequencing libraries were prepared using the ONT rapid barcoding kit and

Table 1. Summary of *Rhizobium leguminosarum* sv. *trifolii* genomes

Contig	Size (bp)	GC%	Rh Group	Chromid	Conjugative	Mobilizable	Symbiotic	Accession
Strain TA1, genospecies gsC								
Chromosome	5,036,312	61.0						CP053205.2
pRltTA1A	498,418	58.6	Rh04	–	–	+	+	CP053209.2
pRltTA1B	611,070	61.0	Rh02	+	–	+	–	CP053208.2
pRltTA1C	662,289	60.6	Rh03	–	–	+	–	CP053207.2
pRltTA1D	805,040	60.7	Rh01	+	–	+	–	CP053206.2
Strain CC275e, genospecies gsA								
Chromosome	4,835,120	61.1						CP053439.1
pRltCC275eA	142,731	58.2	–	–	–	+	–	CP053445.1
pRltCC275eB	154,225	58.1	Rh06	–	+	–	+	CP053444.1
pRltCC275eC	260,097	61.1	Rh03	–	–	–	–	CP053443.1
pRltCC275eD	261,950	61.0	Rh05	–	–	–	–	CP053442.1
pRltCC275eE	492,830	61.4	Rh02	+	–	–	–	CP053441.1
pRltCC275eF	971,705	60.3	Rh01	+	–	–	–	CP053440.1

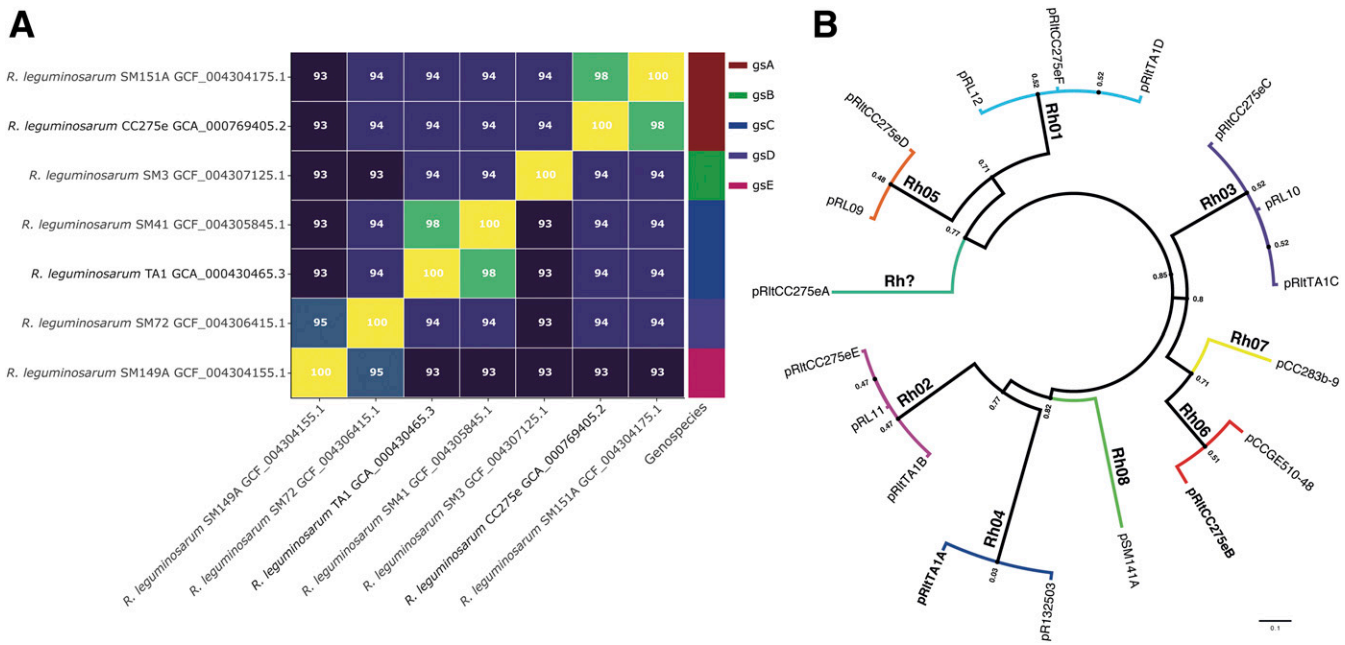

Fig. 1. Genospecies and plasmid Rh group assignments of TA1 and CC275e. **A,** Average nucleotide identity comparison of TA1 and CC275e genomes with representative strains of the *R. leguminosarum* sv. *trifolii* species complex outlined by Cavassim et al. (2020). **B,** Boot-strapped (1,000) neighbor-joining tree of RepABC protein alignment of TA1 and CC275e replicons and replicon Rh group representatives. Symbiotic plasmids of TA1 and CC275e are indicated in bold.

500 ng of DNA. Sequencing used a MinION and FLO-MIN106 flowcell (v9.4 Nanopore). Base-calling used guppy v3.4.1 and the template_r9.4.1_450bps_hac.jsn model (Wick et al. 2019). Median read lengths of the TA1 and CC275e ONT reads were 20,774 and 21,124 bp, respectively. Total data generated for TA1 and CC275e equated to 99× and 61× coverage, respectively. Hybrid assembly of long-read data and publicly available Illumina data for each genome (Delestre et al. 2015; Reeve et al. 2013) used a previously published pipeline (Perry et al. 2020). The completed genome sequences were deposited in the National Center for Biotechnology Information (NCBI) Genomes database and were annotated with the prokaryotic genome annotation pipeline (Tatusova et al. 2016).

The complete CC275e genome is 7,118,658 bp in size and contains a circular chromosome and six additional circular replicons (Table 1). CC275e belongs to gsA in the *R. leguminosarum* species complex (Fig. 1A). Alignment of the RepABC proteins from each replicon assigned five of the six replicons to replicon Rh groups (Fig. 1B; Table 1) previously defined by Cavassim et al. (2020). The symbiotic plasmid pRltCC275eB is the second smallest plasmid in the genome at 154 kb and carries a VirB/D4-type conjugative system.

The complete TA1 genome is 7,613,129 bp and contains one circular chromosome and four additional circular replicons (Table 1). The completed TA1 genome is 1.0 Mb smaller than the previous draft assembly, due to two large duplications in the previous assembly. TA1 was previously assigned to gsC (Kumar et al. 2015) and this was confirmed here (Fig. 1A). The additional four replicons in the TA1 genome were all assigned to Rh groups (Fig. 1B; Table 1). All non-chromosomal replicons in the TA1 genome harbor homologs of the conjugative relaxase *traA*, suggesting they are mobilizable; however, none carried genes coding for a conjugative pore, suggesting they are not self-transmissible. The symbiotic plasmid pRltTA1A is the smallest in the genome but is 3.2× larger than the symbiotic plasmid of CC275e, pRltCC275eB (Table 1).

In summary, the genome structures of both TA1 and CC275e are consistent with the finding of Cavassim et al. (2020), that the *R. leguminosarum* species complex consists of distinct genospecies, each of which carries a chromosome, two chromids, and a complement of plasmids with Rh groups characteristic of their genospecies.

Data availability. Long-read sequencing data, complete genome sequences, and annotations can be found under the NCBI Bioproject numbers PRJNA623952 and PRJNA623954 for *R. leguminosarum* sv. *trifolii* TA1 and *R. leguminosarum* sv. *trifolii* CC275e, respectively.

Author-Recommended Internet Resource

Hybrid-Assembly pipeline: https://github.com/BenjaminJPerry/HybridAssembly

Literature Cited

Beringer, J. E. 1974. R factor transfer in *Rhizobium leguminosarum*. J. Gen. Microbiol. 84:188-198.

Cavassim, M. I. A., Moeskjær, S., Moslemi, C., Fields, B., Bachmann, A., Vilhjálmsson, B. J., Schierup, M. H., Young, J. P. W., and Andersen, S. U. 2020. Symbiosis genes show a unique pattern of introgression and selection within a *Rhizobium leguminosarum* species complex. Microb. Genom. 6:e000351.

Delestre, C., Laugraud, A., Ridgway, H., Ronson, C., O'Callaghan, M., Barrett, B., Ballard, R., Griffiths, A., Young, S., Blond, C., Gerard, E., and Wakelin, S. 2015. Genome sequence of the clover symbiont *Rhizobium leguminosarum* bv. *trifolii* strain CC275e. Stand. Genomic Sci. 10:121.

Drew, E., Herridge, D., Ballard, R., O'Hara, G., Deaker, R., Denton, M., Yates, R., Gemell, G., Hartley, E., Phillips, L., Seymour, N., Howieson, J., and Ballard, N. 2012. Inoculating Legumes: A Practical Guide. Grains Research & Development Corporation, Kingston, Australia.

Green, R. T., East, A. K., Karunakaran, R., Downie, J. A., and Poole, P. S. 2019. Transcriptomic analysis of *Rhizobium leguminosarum* bacteroids in determinate and indeterminate nodules. Microb. Genom. 5:1-16.

Harrison, P. W., Lower, R. P. J., Kim, N. K. D., and Young, J. P. W. 2010. Introducing the bacterial 'chromid': Not a chromosome, not a plasmid. Trends Microbiol. 18: 141-148.

Hoyos-Villegas, V., O'Connor, J. R., Heslop, A. D., Hilditch, A., Jahufer, M. Z. Z., and Barrett, B. A. 2019. Rate of genetic gain for persistence to grazing and dry matter yield in white clover across 90 years of cultivar development. Crop Sci. 59: 537-552.

Kumar, N., Lad, G., Giuntini, E., Kaye, M. E., Udomwong, P., Shamsani, N. J., Young, J. P. W., and Bailly, X. 2015. Bacterial genospecies that are not ecologically coherent: Population genomics of *Rhizobium leguminosarum*. Open Biol. 5:140133.

Kuykendall, L. D., Young, J. M., Martínez-Romero, E., Kerr, A., and Sawada, H. 2015. Rhizobium. Pages 1-36 in: Bergey's Manual of Systematics of Archaea and Bacteria. Wiley, New York.

Meade, H. M., Long, S. R., Ruvkun, G. B., Brown, S. E., and Ausubel, F. M. 1982. Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn*5* mutagenesis. J. Bacteriol. 149: 114-122.

Perry, B. J., Akter, M. S., and Yost, C. K. 2016. The use of transposon insertion sequencing to interrogate the core functional genome of the legume symbiont *Rhizobium leguminosarum*. Front. Microbiol. 7:1873.

Perry, B. J., Sullivan, J. T., Colombi, E., Murphy, R. J. T., Ramsay, J. P., and Ronson, C. W. 2020. Symbiosis islands of Loteae-nodulating *Mesorhizobium* comprise three radiating lineages with concordant *nod* gene complements and nodulation host-range groupings. Microb. Genom. 6:mgen000426.

Poole, P., Ramachandran, V., and Terpolilli, J. 2018. Rhizobia: From saprophytes to endosymbionts. Nat. Rev. Microbiol. 16:291-303.

Reeve, W., Tian, R., De Meyer, S., Melino, V., Terpolilli, J., Ardley, J., Tiwari, R., Howieson, J., Yates, R., O'Hara, G., Ninawi, M., Teshima, H., Bruce, D., Detter, C., Tapia, R., Han, C., Wei, C.-L., Huntemann, M., Han, J., Chen, I.-M., Mavromatis, K., Markowitz, V., Ivanova, N., Ovchinnikova, G., Pagani, I., Pati, A., Goodwin, L., Pitluck, S., Woyke, T., and Kyrpides, N. 2013. Genome sequence of the clover-nodulating *Rhizobium leguminosarum* bv. *trifolii* strain TA1. Stand. Genomic Sci. 9:243-253.

Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E. P., Zaslavsky, L., Lomsadze, A., Pruitt, K. D., Borodovsky, M., and Ostell, J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 44:6614-6624.

Wick, R. R., Judd, L. M., and Holt, K. E. 2019. Performance of neural network basecalling tools for Oxford Nanopore sequencing. Genome Biol. 20:129.