

# 17<sup>th</sup> Australian Nitrogen Fixation Conference 2014 Proceedings

Adelaide, Australia

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### Example citation

Pipai, R. 2014. Biological nitrogen fixation by legume cover plants in oil palm plantations', in the 17<sup>th</sup> Australian Nitrogen Fixation proceedings, ed. Gupta V.V.S.R., Unkovich, M. and Kaiser, B. N. ASNF, Adelaide, pp. 19-21

ISBN 978-0-86396-000-0

# Similar strains of *Burkholderia* spp. nodulate the South African invasive legume *Dipogon lignosus* in New Zealand and Australian soils

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## Key Words

$\beta$ -Rhizobia, rhizobia-legume symbiosis

## Introduction

Brazil and South Africa are centres of diversity of *Burkholderia* spp. that nodulate legumes (Gyaneschwar *et al.* 2011; Beukes *et al.* 2013). The *nod* gene sequences of *Burkholderia* spp. capable of nodulating South African plants are clearly separated from those of *Burkholderia* spp. shown to nodulate South American plants. Where tested, the South African strains did not nodulate South American plants nodulated by *Burkholderia* spp. (Gyaneschwar *et al.* 2011).

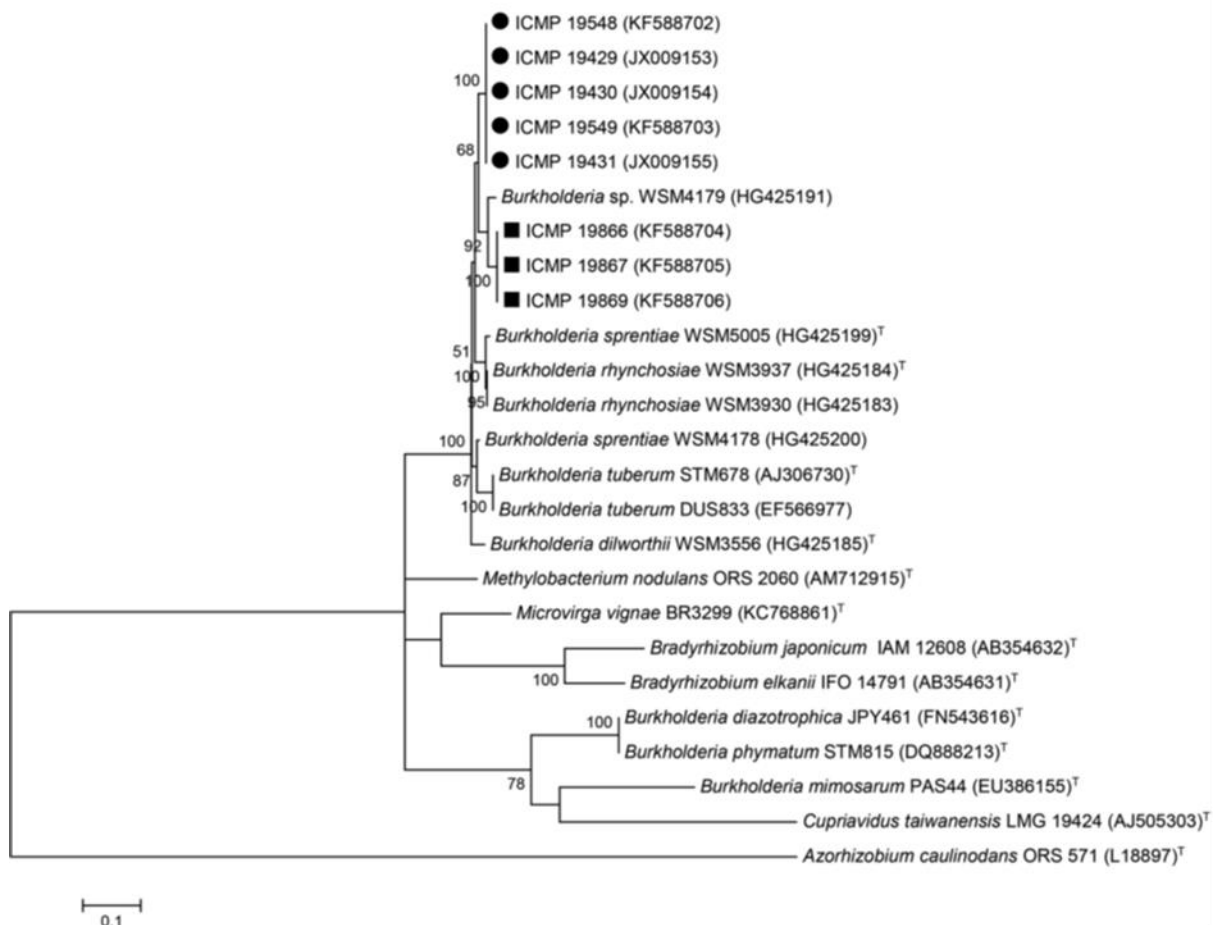
*Dipogon lignosus* is an herbaceous legume (tribe Phaseoleae) native to the Fynbos biome of the Cape of South Africa which has become invasive in the Australian-Pacific region (Lewis *et al.* 2005; Popay *et al.* 2010). Eight bacterial isolates which produced functional nodules on *D. lignosus* sampled at two field sites in NZ, were identified as *Burkholderia* sp. (Liu *et al.* 2014). Both 16S rRNA and *recA* gene sequences placed the eight *Burkholderia* isolates separate from previously described *Burkholderia* rhizobial species. *Burkholderia* isolates obtained from *D. lignosus* sampled in southwest Australia had identical 16S rRNA sequences (930 bp) to the New Zealand *D. lignosus* strain ICMP 19430. Here, we present the *nodC* gene sequences of the eight NZ isolates and argue that evidence is strong that these *Burkholderia* isolates originated in South Africa and were somehow transported with the plants from their native habitat to NZ and Australia.

## Methods

DNA was extracted from the bacterial cultures using the Genra Puregene DNA Purification Kit (Qiagen) following the protocol for gram-negative bacteria and the N-acetylglucosaminyl transferase nodulation protein C (*nodC*) gene sequenced (Liu *et al.* 2014). DNA sequences were aligned, and a maximum likelihood tree was constructed with 1000 bootstrap replications with partial deletion and an 80% coverage cut-off using MEGA5 software. All closely related strains and selected type strains were used in the trees. *Azorhizobium caulinodans* ORS 571 was used as an out-group. The tree was constructed using the MEGA5 software using the 'best' model (lowest Bayesian information score). This was the Tamura three parameter (T92) gamma distribution (+G) invariant sites (+I) model.

## Results and Discussion

The eight *Burkholderia* isolates were separated into two groups on the basis of their *nodC* sequences, one of five isolates from one field site and the other 3 isolates from another field site (Figure 1). The *nodC* sequences for the two groups showed 96.06% similarity (507 bp) to each other and clustered with *B. tuberum* STM678<sup>T</sup>, *B. rhynchosiae* WSM3937<sup>T</sup>, *B. sprentiae* WSM5005<sup>T</sup>, *B. dilworthii* WSM3556<sup>T</sup> and several other strains isolated from different plants and sites in the Cape Floristic Region (CFR) of South Africa (Figure 1; Liu *et al.* 2014). Strain ICMP 19430 was tested and shown to produce N<sub>2</sub>-fixing nodules on *Cyclopia subternata*, *Hypocalyptus sophoroides*, *Podalyria calyprata* and *Virgilia oroboides* previously reported to be nodulated by *Burkholderia* spp. isolated from legumes in the CFR (Liu *et al.* 2014). It did not nodulate *Mimosa pudica*, which is nodulated by *Burkholderia* spp. isolated from *Mimosa* and *Piptadenia* spp. in South America (Liu *et al.* 2014).



**Figure 1. Phylogenetic tree of *nodC* gene sequences (ca. 507 bp) of eight bacterial isolates from *Dipogon lignosus* sampled in NZ soils (●, ■). Genbank accession numbers are in parentheses. Numbers on branches are bootstrap per cent from 1000 replicates (shown only when > 50 %). Scale bar = 10% sequence divergence (one substitution per 10 replicates).**

## Conclusion

*Burkholderia* isolates from *D. lignosus* growing in Australia and NZ are a novel lineage of rhizobia closely related to *Burkholderia* spp. isolated from South African plants: it is likely that these novel *Burkholderia* originated in South Africa in association with *D. lignosus*.

## References

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