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CHARACTERISATION OF ROOT-NODULE BACTERIA ISOLATED FROM PERENNIAL SOUTHERN AFRICAN SPECIES OF LOTONONIS

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Introduction

Lotononis is a genus of approximately 150 shrubs, herbaceous perennials and annuals belonging to the subfamily Fabaceae (Van Wyk, 1991). They are distributed mainly in southern Africa, with some species extending throughout Africa, southern Spain, Turkey, south-eastern Bulgaria and part of the Arabian Peninsula to the north-west of the Indian sub-continent (Van Wyk, 1991). *Lotononis* species have shown potential as perennial pasture legumes that can be used to help reduce the risk of dryland salinity in southern Australian agricultural systems. Species in the section *Listia* in particular may be useful as pasture legumes as they are perennial, stoloniferous, and lack the poisonous cyanogenic or alkaloid compounds found in some species of *Lotononis. L. bainesii*, from the *Listia* section, has been shown to grow well in southern Australia (Roberts & Carbon, 1969) and will grow on acid, sandy soils (R. Yates, pers. comm.).

L. bainesii is nodulated by pink-pigmented root-nodule bacteria. Jaftha *et al.* (2002) characterised nine *L. bainesii* isolates and found them to be related to *Methylobacterium.* The genus *Methylobacterium*, often referred to as pink-pigmented facultative methylotrophs (PPFMs), are capable of growth on C_1 compounds such as formate and methanol as sole carbon sources. PPFMs are ubiquitous in the plant phyllosphere and rhizosphere, where they utilize methanol and other C_1 compounds that are the products of plant metabolism (Trotsenko *et al*, 2001). They can promote the germination or the growth of plants, probably because of their ability to synthesise auxins, cytokinins and other plant growth promoting substances (Holland & Polacco, 1994; Ivanova *et al*., 2000; Trotsenko *et al.*, 2001). However, until the paper by Sy *et al.* (2001), describing *Methylobacterium nodulans*, which was isolated from nodules of *Crotalaria* species found in Senegal, no *Methylobacterium* species had been known to nodulate legumes, or indeed to fix nitrogen.

The objectives in this study were to characterise root-nodule bacteria isolated from four species from the *Listia* section of *Lotononis* (*L. angolensis, L. bainesii, L. listii* and *L. solitudinis*) using a range of phenotypic and genetic techniques.

Results and Discussion

The *L. bainesii, L. listii* and *L. solitudinis* isolates (Group I) were highly homogeneous in their morphological and physiological properties. All were pink-pigmented and medium or medium-slow growers (Table 1). In contrast, the *L. angolensis* isolates (Group II) were either pale orange-pink or cream in colour, were fast growing and were noticeably more mucillaginous (Table 1). Group I isolates were able to utilise succinate and glutamate as sole carbon sources, but did not grow on L-arabinose, D-galactose, D-glucose or D-mannitol. Group II isolates were able to grow on all these substrates as sole carbon sources**.**

Sequencing of the 16S rRNA gene indicated that selected isolates from both Group I and Group II were related to *Methylobacterium*, although none of the strains tested were able to grow on methanol as a sole carbon substrate. In order to directly assess whether the isolates could metabolise methanol, a biochemical assay to measure methanol concentration in an inoculated liquid culture was performed. Methanol concentration (initially at 25mM) was determined by the oxidation of methanol to formaldehyde, followed by the colorimetric measurement of formaldehyde concentration by means of the Hantzsch reaction. The result for the xct9 strain showed that it neither grew on nor utilised

methanol (Figure 1). The results of a PCR amplification of *mxaF*, the gene that codes for the large subunit of methanol dehydrogenase, suggest the absence of this gene in these *Lotononis* isolates.

Table1. Host, growth rate and colony morphology of *Lotononis* isolates.

Isolate	Host	Growth Rate*	Colony Morphology
Group I Isolates			
WSM2597	*Lotononis bainesii*	Medium-slow	Circular, pink coloured, dry
WSM2599	*Lotononis bainesii*	Medium	Circular, pink coloured, dry
WSM2603	*Lotononis listii*	Medium	Circular, pink coloured, dry
WSM2660	*Lotononis listii*	Medium-slow	Circular, pink coloured, dry
WSM2666	*Lotononis listii*	Medium	Circular, pink coloured, dry
WSM2678	*Lotononis listii*	Medium-slow	Circular, pink coloured, dry
WSM2693	*Lotononis listii*	Medium	Circular, pink coloured, dry
WSM2799	*Lotononis listii*	Medium-slow	Circular, pink coloured, dry
WSM3032	*Lotononis solitudinis*	Medium-slow	Circular, pink coloured, dry
WSM3034	*Lotononis solitudinis*	Medium-slow	Circular, pink coloured, dry
WSM3035	*Lotononis bainesii*	Medium	Circular, pink coloured, dry
xct9	*Lotononis bainesii*	Medium-slow	Circular, pink coloured, dry
Group II Isolates			
CB1297	*Lotononis angolensis*	Fast	Circular, orange-pink, mucilaginous
CB1298	*Lotononis angolensis*	Fast	Circular, cream, mucillaginous
CB1299	*Lotononis angolensis*	Fast	Circular, cream, slightly mucillaginous
CB1321	*Lotononis angolensis*	Fast	Circular, pale orange-pink, mucillaginous
CB1322	*Lotononis angolensis*	Fast	Circular, orange-pink, mucillaginous
CB1323	*Lotononis angolensis*	Fast	Circular, orange-pink, mucillaginous
CB2406	*Lotononis angolensis*	Fast	Circular, orange-pink, mucillaginous

* 2-3 days = Fast 4-5 days = Medium 6-7 days = Medium-slow

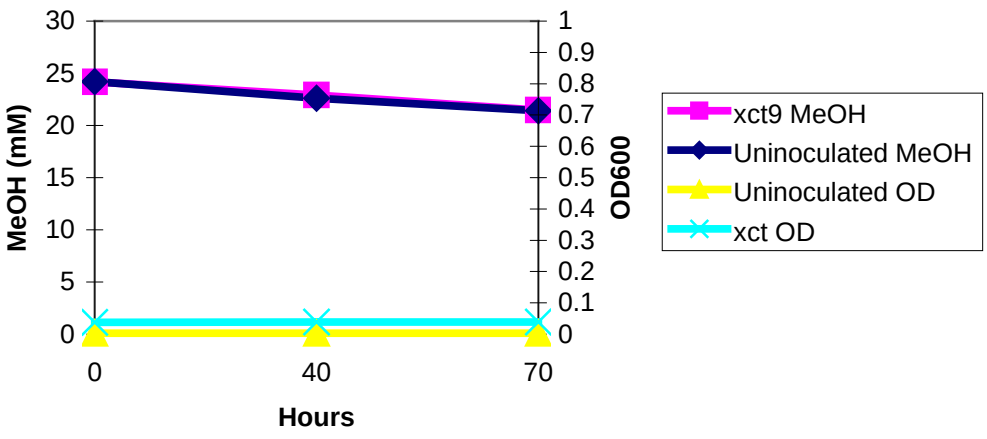

Figure 1. Methanol utilisation by xct9 compared with an uninoculated control. Shown are the OD_{600} and the concentration of methanol in supernatants of culture.

Nodulation on *L. angolensis*, *L. bainesii*, *L. listii* and *L. solitudinis* is of the lupin type and forms a collar around the root or hypocotyl. Cross-inoculation studies on *L. angolensis*, *L. bainesii* and *L. listii* suggest firstly that these members of the *Listia* section are highly specific in their nodulation requirements as they are nodulated only by these *Methylobacterium* strains. Secondly, different specificities exist within the *Listia* section, as the Group I isolates from *L. bainesii*, *L. listii* and *L. solitudinis* will all nodulate *L. bainesii* and *L. listii*, but not *L. angolensis*. Conversely, the Group II *L. angolensis* isolates effectively nodulate *L. angolensis*, but only form occasional small, white, ineffective nodules on *L. bainesii* and *L. listii*.

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