Is There Genetic Diversity in the ‘Leucaena Bug’ *Synergistes jonesii* Which May Reflect Ability to Degrade Leucaena Toxins?

Jagadish Padmanabha  
*CSIRO, Australia*

Michael J. Halliday  
*The University of Queensland, Australia*

Stuart E. Denman  
*CSIRO, Australia*

Carl K. Davis  
*CSIRO, Australia*

H. Max Shelton  
*The University of Queensland, Australia*

*See next page for additional authors*

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Jagadish Padmanabha, Michael J. Halliday, Stuart E. Denman, Carl K. Davis, H. Max Shelton, and Chris S. McSweeney

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Is there genetic diversity in the ‘leucaena bug’ Synergistes jonesii which may reflect ability to degrade leucaena toxins?

Jagadish Padmanabha A, Michael J Halliday B, Stuart E Denman A, Carl K Davis A, H Max Shelton A and Chris S McSweeney A

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Introduction

Leucaena leucocephala, a nutritionally rich forage tree legume, contains a non-protein amino acid, mimosine, which is degraded by ruminal bacteria to toxic metabolites 3,4-DHP and 2,3-DHP resulting in goitre-like symptoms in animals, severely restricting weight gain. Raymond Jones, et al., in the early 1980s, discovered the ‘leucaena bug’ in the rumen of goats in Hawaii that degraded these toxic DHP metabolites into non-toxic compounds (Jones and Lowry 1984) which was named Synergistes jonesii (Allison et al. 1992). Subsequently, a rumen inoculum containing S. jonesii was used as an ‘oral drench’ for cattle, kept in continuous culture (Klieve et al. 2013), a 16S rDNA (ATCC) in Queensland cattle as well as in cattle and other ruminants, internationally. These changes can appear as discrete mutations or ‘single nucleotide polymorphisms’ (SNPs) and may be correlated to their ability to degrade DHP, relative to the type strain.

Materials and Methods

Rumen fluid or faeces was collected from Australian cattle in Queensland and from cattle, sheep, goats, buffalos, native cattle and yak from Indonesia, Thailand, Vietnam, China and Brazil, mainly from local farmers. Microbial DNA was extracted from these samples and amplified with a set of 16S rDNA nested PCR primers which are specific for S. jonesii. PCR products positive for S. jonesii were then aligned against full-length S. jonesii 16S rDNA sequence for identification of SNPs.

Results

The nested PCR was able to detect S. jonesii in the majority of Australian cattle tested (Table 1). Overseas ruminants (cattle, buffalos, goats, sheep and yak), whether feeding on leucaena or not, had nested PCR detectable S. jonesii 16S rDNA sequences, suggesting that the ‘leucaena bug’ is indigenous to many of these animals (Table 1). In general, faecal samples failed to generate PCR products for S. jonesii from either Australian or international samples. Mutations, single nucleotide polymorphisms (SNPs), are distributed primarily at ‘hot-spots’ in bases corresponding to E. coli nucleotide positions 268 (C → T), 306 (A → G), 328 (G → A) and 870 (A → C) between bases 200-900 (~700 bp) of the S. jonesii ATCC 16S rDNA. Of these, ‘306’ & ‘870’ are almost always mutated when SNPs are detected; these 4 SNPs are present in the Queensland Department of Agriculture, Forestry and Fisheries (DAFF) inoculum which was provided to the farmers. The ‘268’ & ‘328’ are frequently present when good quality sequence reads are available (Table 1). Cattle from the University of Queensland, Gatton campus, had all 4 SNPs. In animals overseas, the very same SNPs (Table 1) were also distributed ranging from frequencies of 15% (for ‘870’ in Brazilian cattle) to 100% (all 4 SNPs in Vietnam cattle and goats). Among all the international samples analysed, only Jinnan cattle, Tibetan yak and Indonesian buffalos returned 100% identity with the type strain of S. jonesii. Interestingly, these buffalos were on 100% leucaena for 0.5-1 year and had high clearance of 3,4- and 2,3-DHP (data not shown). The Jinnan cattle and Tibetan yak were naïve to dietary leucaena. Other SNPs were spread along this fragment of the 16S rDNA whose frequencies were not consistent across animals, geographical regions or loci.

Conclusions

S. jonesii appear to be indigenous to the rumen across all types of ruminants and geographical regions tested. Classical SNPs are located in base positions 268, 306, 328 & 870. Their distribution is seen across all geographical regions and animal species; however, frequencies may vary. Other, minor mutations are distributed infrequently.
Table 1: Presence of SNPs in *S. jonesii* nested PCR positives (+ve) Australian (Qld) and international samples.

| Property/Country | Animals | Animals (n) | *S. jonesii* +ve | SNPs Frequency (%) |
|------------------|---------|-------------|------------------|--------------------|
|                  |         |             | n                | %                  | 268 'T' | 306 'G' | 328 'A' | 870 'C' |
| **Australia:**   |         |             |                  |                    |         |         |         |         |
| farms & Institutions |       |             |                  |                    |         |         |         |         |
| Lansdowne        | Cattle  | 7           | 5                | 71                 | 0       | 100     | 8       | 100     |
| Byrne Valley     | Cattle  | 8           | 7                | 88                 | 100     | 100     | 100     | IS      |
| Townsville       | Cattle  | 10          | 5                | 50                 | IS      | IS      | 100     | 100     |
| Mt. Garnet       | Cattle  | 5           | 3                 | 60                 | 0       | 100     | 0       | 100     |
| Murgon           | Cattle/Enrich | 2 | 2     | 100 | IS | 100 | 0 | 100 |
| UQ Gatton campus | Cattle  | 2           | 2                | 100                | 100     | 100     | 100     | 100     |
| DAFF Oral Drench | Rumen Culture | NA | NA | 100 | 100 | 50 | 100 | 50 | 100 |
| **Indonesia:**   |         |             |                  |                    |         |         |         |         |
| farms            |         |             |                  |                    |         |         |         |         |
| Provinces of NTB & NTT |       |             |                  |                    |         |         |         |         |
| Indonesia        | Goats   | 19          | 18               | 95                 | 89      | 89      | 85      | 90      |
|                |         |             |                  |                    |         |         |         |         |
|                |         |             |                  |                    |         |         |         |         |
| **Thailand:**   |         |             |                  |                    |         |         |         |         |
| farms & Khon Kaen Uni. |       |             |                  |                    |         |         |         |         |
| Thailand         | Goats   | 28          | 9                | 32                 | 30      | 100     | 30      | 100     |
|                |         |             |                  |                    |         |         |         |         |
|                |         |             |                  |                    |         |         |         |         |
|                |         |             |                  |                    |         |         |         |         |
| **Vietnam:**    |         |             |                  |                    |         |         |         |         |
| Can Tho Uni.    |         |             |                  |                    |         |         |         |         |
| Vietnam          | Cattle  | 6           | 1                | 17                 | 100     | 100     | 100     | 100     |
|                |         |             |                  |                    |         |         |         |         |
|                |         |             |                  |                    |         |         |         |         |
|                |         |             |                  |                    |         |         |         |         |
| **China:**      |         |             |                  |                    |         |         |         |         |
| Qinghai Tibetan Plateaux farms |           |             |                  |                    |         |         |         |         |
| China           | Jinnan cattle | 3 | 3     | 100 | 0 | 0 | 0 | 0 |
|                | Gansu sheep | 3 | 3     | 100 | 50 | 50 | 50 | 50 |
|                | Tibetan sheep | 3 | 2     | 67 | 50 | 50 | 50 | 50 |
|                | Yak       | 3 | 1     | 33 | 0 | 0 | 0 | 0 |
| **Brazil:**     |         |             |                  |                    |         |         |         |         |
| Sao Paulo Uni.  | Cattle   | 25          | 13               | 52                 | 54      | 69      | 61      | 15      |

* One *Sj* +ve animal had no SNPs

Two of the SNPs (306 & 870) are always present in the Queensland Department of Primary Industry oral drench, and the other two in <50% of sequences. Vietnamese animals and Gatton campus cattle had all 4 SNPs with 100% frequency. Only, Indonesian buffalos, Jinnan cattle and Tibetan yak sequences were identical to *S. jonesii* ATCC 16S rDNA sequence; these buffalos were on 100% leucaena and had high DHP clearances. The SNPs indicate genetic diversity at the species level which may be reflected in varying ability to degrade DHP. This study is ongoing.

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