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

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The XXII International Grassland Congress (Revitalising Grasslands to Sustain Our Communities) took place in Sydney, Australia from September 15 through September 19, 2013.

Proceedings Editors: David L. Michalk, Geoffrey D. Millar, Warwick B. Badgery, and Kim M. Broadfoot

Publisher: New South Wales Department of Primary Industry, Kite St., Orange New South Wales, Australia

Presenter Information

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

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Keywords: *Synergistes jonesii*, SNPs, 16S PCR, Leucaena, 2,3 & 3,4-DHP, rumen fluid, sequencing.

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, 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.* 2002) and supplied to farmers to dose cattle foraging on leucaena.

Studies on Queensland herds that received this oral drench showed that up to 50% of 44 herds grazing on leucaena had apparent subclinical toxicity based on high 3,4- and 2,3-DHP excretion in urine (Dalzell *et al.*, 2012). In another study by Graham *et al.* (2013), a 16S rDNA nested PCR showed that rumen digesta from 6 out of 8 properties tested had a variant DNA profile from *S. jonesii* ATCC 78.1 strain, which suggested a different strain of the bacterium.

It was postulated that either the continually cultured oral inoculum may have undergone genetic modification and/or that animals could harbor other DHP degrading bacteria or *S. jonesii* strains with differential DHP degrading potential (McSweeney *et al.* unpublished). The present study looks at changes in the 16S rDNA gene at the molecular level that may suggest divergence from the type strain *S. jonesii* 78.1 (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	50	100	50	100
Indonesia:	Goats	19	18	95	89	89	85	90
farms	Cattle	39	7	18	0	20	0	20
Provinces of NTB & NTT	Buffalo	7	5	71	0	0	0	0
Thailand: farms & Khon Kaen Uni.	Goats	28	9	32	30	100	30	100
	Buffalo	4	4	100	25	88	32	56
Vietnam: Can Tho Uni. farms	Cattle	6	1	17	100	100	100	100
	Goats	6	3	50	100	100	100	100
	Goats (+Leuc)	6	1	17	100	100	100	100
China: Qinghai Tibetan Plateaux farms	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. farm	Cattle	25	13	52	54	69	61	15

* One Sj +ve animal had no SNPs (IS= Insufficient sequences)

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.

Acknowledgement

We gratefully acknowledge the support of ACIAR

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