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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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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-DHP 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 of 8 farms 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 the continually cultured oral inoculum may have undergone genetic modification and/or animals could harbor other DHP-degrading bacteria or *S. jonesii* strains with differential DHP-degrading potential (C. 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 unpublished).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* ATCC 78.1 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 with their ability to degrade DHP, relative to the type strain.

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Materials and Methods

Rumen fluid or feces was collected from cattle in Queensland, Australia and from cattle, sheep, goats, buffalos, native cattle and yak from Indonesia, Thailand, Vietnam, China and Brazil, mainly owned by 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 rumen samples from 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, fecal samples failed to generate PCR products for *S. jonesii* from either Australian or international samples. Mutations (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 Queens-

land, Gatton campus, had all 4 SNPs. In animals overseas, the very same SNPs (Table 1) were also detected, ranging from frequencies of 15% (for '870' in Brazilian cattle) to 100% (all 4 SNPs in Vietnamese cattle and goats). Among all the international samples analyzed, 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-DHP 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 appears 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. Two of the SNPs (306 & 870) are always present in the DAFF 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 with the *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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Table 1. Presence of SNPs in *Synergistes jonesii* nested PCR positive (+ve) Australian (Qld) and international samples.

Country/Property	Samples from:	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[3]
Townsville	Cattle	10	5	50	IS	IS	100	100
Mt. Garnet	Cattle	5	3[2]	60	0	100	0	100
Murgon	Cattle/Enrich[1]	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 (Farms in NTB & NTT Provinces)	Goats	19	18	95	89	89	85	90
	Cattle	39	7	18	0	20	0	20
	Buffalo	7	5	71	0	0	0	0
Thailand (Farms & Khon Kaen University)	Goats	28	9	32	30	100	30	100
	Buffalo	4	4	100	25	88	32	56
Vietnam (Can Tho University 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-Tibet Plateau 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 (São Paulo University farm)	Cattle	25	13	52	54	69	61	15

[1]Enriched rumen digesta
[2]One Sj +ve animal had no SNPs.
[3]IS = Insufficient sequences.

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