

# L– and D–lactic acid producing bacteria of the equine gastrointestinal tract: identification and molecular characterisation

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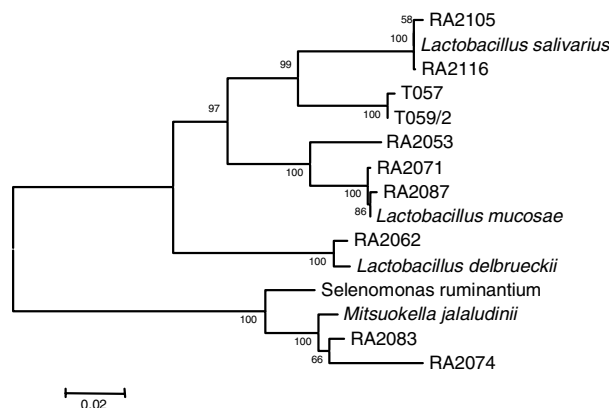
The contribution of lactic acid producing bacteria (LAB) to acidosis and laminitis in horses is well established. Stomach ulceration in horses is also thought to be associated with increases in the production of VFA and lactic acid in the stomach from the relevant LAB. To date, the majority of studies of LAB in the horse have focussed on *Streptococcus bovis* and *Streptococcus equinus* (Al Jassim and Rowe 1999). Little is known about other members of the LAB population of the equine gastrointestinal tract. In this paper we report on the identification and molecular characterisation of LAB not previously described from the equine gastrointestinal tract.

LAB were obtained from the stomach, small intestine, caecum, colon and rectum contents collected from both roughage fed and laminitis–induced horses. Isolates were initially cultured from serial dilutions ( $10^{-7}$  and  $10^{-8}$ ) of the contents on modified MRS media. On the basis of fermentation end products, 25 isolates were selected as LAB from a total of 68 isolates. Volatile fatty acid production and L– and D–lactate production were determined from pure cultures of each isolate. The 16S rDNA was amplified by PCR from genomic DNA of each isolate. The genetic diversity was initially determined by RFLP analysis with the restriction

enzymes *Mbo*I, *Hha*I and *Hin*fI. The PCR products were cloned into pGEM–T and sequenced. DNA sequence data were then used to characterise in more detail the selected LAB population.

All the selected isolates were able to produce L– and/or D–lactate in pure culture. DNA sequence analysis (Figure 1) of the 16S rDNA indicated that most of the isolates were very closely related to species from the genus *Lactobacillus*, including *L. salivarius*, *L. mucosae* and *L. delbrueckii*. Some isolates were very closely related to *Mitsuokella jalaludinii*, an organism recently isolated and characterised from the rumen of cattle (Lan *et al.* 2002). Characterisation of LAB from the equine gastrointestinal tract should contribute to our understanding and management of acidosis, ulceration of the stomach and laminitis.

- Al Jassim, R.A.M. and Rowe, J.B. (1999). Better understanding of acidosis and its control. *Recent Advances in Animal Nutrition in Australia* 12, 91–97.
- Lan, G.Q., Ho, Y.W. and Abdullah, N. (2002). *Mitsuokella jalaludinii* sp. nov., from the rumen of cattle in Malaysia. *International Journal of Systematic and Evolutionary Microbiology* 52, 713–718.



**Figure 1** Phylogenetic relationship of LAB isolates from the equine gastrointestinal tract, based on near complete 16S rDNA sequence data.