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Development, isolation and characterization of a new non-virulent mimosine-degrading *Klebsiella pneumoniae* strain from the rumen liquor of German steers by using IBT-Göttinger bioreactor

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Introduction

The tropical legume *Leucaena leucocephala* (leucaena) has many uses, including: a potential source of firewood and timber; for soil erosion control (Dijkman 1950); to provide shade; to enhance soil fertility; and as a nutritious forage for animal feed (Ruskin 1977). It is widely used as forage for cattle in tropical agriculture (Shelton 1998). In Myanmar, leucaena is used as a protein source in urea-molasses multi-nutrient blocks for ruminants (Ni Ni Maw et al. 2004).

However, the use of leucaena as ruminant feed is not without problems, because it contains mimosine, a toxic anti-nutritional factor limiting its use as animal feed. Jones (1981) reported the absence of toxicity when leucaena was fed to goats and cattle in Hawaii and Indonesia. According to the low dihydroxypyridine (DHP) in urine of those animals, it was assumed that they could degrade mimosine and DHP. Hawaiian goats, but not Australian goats, could degrade 3,4-DHP ruminally (Jones and Megarrity1983). Inoculation of susceptible animals with rumen liquor containing mimosine-degrading bacteria protected against DHP toxicity in ruminants (Jones and Lowry 1984).

For maintaining mimosine-degrading bacteria, the donor animals should be fed on leucaena continuously and it is expensive to maintain their veterinary care. Hence, we tried to develop mimosine-degrading ruminal bacteria using a fermenter, intending to produce a source of inoculum for the routine control of leucaena toxicosis in ruminants.

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Materials and Methods

The donor steer was from the Institute of Animal Physiology and Nutrition, Faculty of Agriculture, Georg-August University of Göttingen, Germany, and had never consumed leucaena leaves. Rumen liquor was obtained via a rumen cannula and immediately placed in a conical flask, flushed with carbon dioxide, stoppered, maintained under carbon dioxide, and frozen at 4 °C in a refrigerator for 2 weeks to kill the protozoa. Before subsequent fermentation, rumen liquor was thawed. Fermentation was commenced with 520 mL of rumen liquor.

The IBT-Goettinger Bioreactor was a modified form of that used by Böhnel and Zeggu (1993). The medium 98-5 for rumen microbes (Allison et al. 1990), without mimosine, was supplied continuously. Infusion of mimosine started at 25 mg/d, increasing by 25 mg of mimosine every second day for 16 days, when 200 mg/d was infused. Daily samples were taken and mimosine degradability of the sample was assessed by colorimetric assay. Purification of the mimosine-degrading bacterial strain was done from the samples showing mimosine degradability. After that, tests for biochemical properties and molecular identification were conducted. For proof that bacterial strains were non-pathogenic, a mouse bioassay was carried out to determine the lethal dose.

Results and Discussion

Although the rumen liquor of the donor steer was unable to degrade mimosine initially, infusing gradually increasing amounts of mimosine for 16 days (maximum of 200 mg mimosine/d) enabled the liquor to degrade mimosine (Figure 1). It is possible that some ruminal bacteria became adapted to degrading mimosine, as

found by Ruskin (1977). According to molecular identification, genotypic characteristics of this isolate were almost identical (94% similarity) to those of reference *Klebsiella pneumoniae* from GenBank entries held by the National Centre for Biotechnology Information (NCBI). Although this isolate is similar to pathogenic *K. pneumoniae*, it has some different biochemical properties (Biolog test). In this experiment, gradually increased amounts of mimosine infused into rumen liquor induced adaptation in the bacteria first, so they could degrade mimosine. Mice inoculated with the recommended dose of *K. pneumoniae* (3.3 x 10^8 CFU/mL) showed no clinical signs of klebsiellosis, as reported by Thakker et al. (2006).

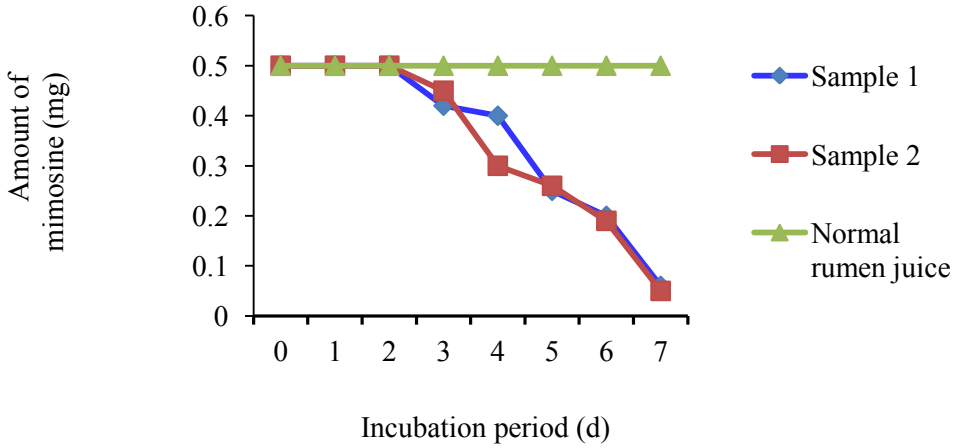

Figure 1. Effects of treatment with mimosine on the ability of rumen liquor to degrade mimosine. Sample 1 was taken on day 15 of treatment with mimosine, when dosage was 200 g/d; sample 2 was taken on day 16 of treatment.

Conclusion

This study has shown that treatment of rumen liquor with mimosine increases the ability of the bacteria to degrade mimosine. *Klebsiella pneumoniae* isolated from this experiment was effective at degrading mimosine. Studies are needed to compare the effectiveness of this bacterium in protecting animals fed leucaena, in comparison with *Synergistes jonesii*. An experiment to measure these parameters will be conducted in Myanmar.

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