

Isolation of ODAP-degrading bacteria from the sheep rumen

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ODAP, the major non-protein amino acid contained in *Lathyrus sativus*, causes adverse effects when fed to animals, especially monogastrics, but it is not clear whether any metabolism of the toxin can occur in ruminants. Rasmussen *et al.* (1995)⁽¹⁾ reported that after prolonged consumption of a non-lethal diet consisting of 50% *L. sylvestris*, which contained another non-protein amino acid, diaminobutyric acid (DABA), sheep successfully adapted and were subsequently capable of consuming a diet comprising 100% *L. sylvestris* without ill effects. This suggested that rumen microorganisms might contribute to this adaptation process. Using a similar argument, the rumen may also be a source of bacteria capable of metabolising ODAP.

A trial was conducted to isolate rumen microorganisms that have the potential to degrade ODAP contained in *L. sativus*. Rumen contents were obtained from sheep fed either on a *L. cicera* based diet (kindly provided by Dr. C. Hanbury, CLIMA, University of WA) or from sheep grazed on pasture and supplemented with lucerne chaff. Samples were transferred to an anaerobic hood (95% CO₂ and 5% H₂).

Enrichment and isolation of ODAP-degrading bacteria was carried out using a basal medium supplemented with ethanol extracts from *L. sativus*. The ODAP level in the concentrated extract was around 1300µg/ml. Aliquots (100µl) of crude rumen sample were inoculated into Hungate tubes containing 10ml basal medium plus extract. Everyday, 100µl of the cultured medium was collected and transferred to a fresh tube. After 7 days of consecutive subculture, samples from these tubes were streaked on nutrient agar plates. After 24 hours of incubation, single colonies were picked and inoculated into broth medium. After overnight growth, the purity of the cultures was checked and glycerol stocks were made. A total of 8 colonies were isolated, and 3 isolates that could degrade ODAP were identified by measuring ODAP differences in the medium before and after growth. Their ability to use ODAP as sole source of carbon and nitrogen was confirmed by viable counts before and after incubation in a minimal medium supplemented with pure ODAP.

Data show that 3 apparently different bacteria, morphologically cocci, were able to grow on ODAP, and degrade up to 46% of the ODAP in 24 hours. The common ruminal *Prevotella* species, *P. ruminicola* B14, did not degrade ODAP. Definitive identification will be carried out by 16S rDNA sequencing and biochemical studies. Further work will be aimed at analysing ODAP degradation products, determining the mechanism(s) involved in ODAP degradation, and ecological studies on the role of these bacteria in the rumen. Ultimately, it may be possible to transfer genes involved in ODAP degradation to other rumen bacterial species.

References

1. Rasmussen, M.A., Foster, J.G., Allison, M.J. (1995). In 'Poisonous Plants; Proceedings of the Third International Symposium', James, L.F., Keeler, R.F., Bailey Jr, E.M., Cheeke, P.R., Hegarthy, M.P. (Eds.). Iowa State University Press. Pp. 377-381.