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Isolation of Viruses For Bio-Control of Methanogenic Archaea From the Rumen

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Methane is produced by ruminants as a by-product of rumen fermentation, with enteric methane production attributed to the activity of populations of methanogenic archaea present in the rumen. Several strategies have been proposed for the control of these organisms, including the use of viruses (archaeaphage) that specifically infect and lyse archaeal host strains present in the rumen. Using this approach, archaeaphage could be used as a bio-control agent to directly reduce methanogen numbers or to help establish alternative rumen microbial populations, for example, reductive acetogens, which may out-compete methanogens for the hydrogen they require for growth. Currently, very few phage of ruminal archaea have been isolated and characterized, therefore methods have been optimized and experiments undertaken in order to obtain new archaeaphage isolates from the environment.

The method used for screening environmental samples and isolating new archaeaphage, was adapted from the soft agar overlay method developed in our laboratory for screening of bacteria for the production of archaeocins (Gilbert *et al* 2010). The method involves exposing environmental source material to potential host strains grown in confluent layers within Petri dishes and looking for clearing zones (plaques) within the confluent layer, produced as a result of lytic phage activity. Once a plaque is obtained, phage particles can be propagated *in vitro* and particle characteristics determined. Archaea used as potential hosts in phage isolation experiments include several strains of the methanogen species dominant in the rumen, *Methanobrevibacter*. Environmental source material used in phage isolation experiments include samples of bovine rumen fluid obtained from 2 cattle maintained on a Mitchell grass diet; effluent and effluent pond sludge samples from a commercial piggery where methane levels were being monitored; and influent and sludge samples obtained from a Brisbane waste-water treatment plant.

To date screening environmental source material has not produced distinct phage plaques on confluent layers of several strains of *Methanobrevibacter*. Therefore the methodology has been developed further to include additional steps for virus particle concentration, involving high speed centrifugation, and pre-enrichment of environmental source material prior to screening with the soft agar overlay method. Collection of alternative environmental source samples has also been undertaken, particularly samples which may be expected to contain either a variety of phage types, such as wastewater treatment plant material, or may be expected to contain populations of methanogenic archaea, for example, combined samples of bovine rumen fluid from a number of animals, on different dietary treatments, and samples from a wastewater treatment plant anaerobic digester.

Despite the lack of archaeaphage isolated to date, the existence of a phage integrated into the genome of *Methanobrevibacter ruminantium* M1 (Attwood *et al* 2008), indicates that such viruses do exist and will be cultivated *in vitro* once an environmental source is found and the correct conditions for growth are established.

Attwood G. T., Kelly W. J., Altermann E. H. and Leahy S. C. (2008) *Aust. J. Exp. Agric.* **48**, 83.
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