GENE AND MONOClonAL ANTIBODY PROBES FOR RUMEN MICROBIAL ANALYSES


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0.5-1% of a bacterial population.

**Strain identification by fingerprint analysis.**

Gene probes were used for bacterial strain identification by comparison of DNA hybridisation patterns from restriction digests and Southern transfers probed with species-specific gene probes. In these experiments, several *Selenomonas* gene probes were used to probe a set of restriction digests from each of at least 20 isolates purified from rumen samples of sheep that were maintained on different diets. The results of one set of digests (figure 2) show that fingerprint analyses can clearly distinguish between genetically identical and dissimilar isolates and we have identified 10 different strains of *Selenomonas* by this method.

**Gene probes for bacterial analysis in vivo.**

To determine whether gene probes could be used to enumerate bacterial populations in vivo, we inoculated mixed rumen cultures and the rumen of a sheep with a laboratory strain of *B. ruminicola*. We had previously established that
the B. ruminicola B14 gene probe was not able to detect the presence of any homologous DNA in the rumen. Rumen samples were incubated in Hungate tubes and inoculated with populations of B. ruminicola B14. After varying periods of time, samples were removed for DNA extraction and analysis. Dot blot assays showed that the introduced bacteria could be readily detected in the cultures and had a half life of approximately 9 hours (figure 3). Inocula of B. ruminicola B14 were also introduced into the rumen through a fistula and rumen samples were taken at regular intervals for DNA extraction and analysis. The results showed that the introduced cells could be detected in the rumen shortly after inoculation and mixing with the resident populations. However, in less than 3 hours, the numbers of B. ruminicola B14 had decreased to below the sensitivity of the assay. These numbers did not increase over the subsequent 3 days.

Monoclonal antibody probes for bacterial analysis

An alternative to the use of genes as probes for bacterial analysis is the use of antibodies. Cultures of B. ruminicola and S. ruminantium were used to raise polyclonal antiserum in rabbits and to immunise mice for the preparation of monoclonal
antibodies.

Polyclonal antisera was not sufficiently specific for serological analysis. However, a number of Butyriboles-specific and Selenomonas-specific monoclonal antibodies were isolated and characterised by competitive ELISA analysis. Each could readily detect the presence of $10^5$ specific bacteria in a population of $10^6$ total bacteria (figure 4). This sensitivity is 2 orders of magnitude greater than that obtained with gene probes. Immunofluorescence assays of bacteria spread directly on microscope slides were also established with the monoclonal antibodies.

**Gene v/s antibody probes**

Gene probes probably have the greatest potential for bacterial analysis because they have the specificity to distinguish between related strains and can be used to establish an unambiguous DNA fingerprint that can provide a benchmark or future identification of rumen isolates. Gene probes can also be used in enumerating particular strains in crude rumen cultures and in samples taken directly from the rumen. In contrast, serological assays using monoclonal antibodies are not strain-specific, but at the species level, these probes may be more useful than gene probes because of their increased sensitivity, particularly through immunofluorescence. An added advantage in quantitation using antibody probes is that no correction for the presence of plant DNA in the assay sample is required.

Gene probes therefore have their greatest potential in identifying bacterial species and establishing an unambiguous genotype. Antibody probes may be best used in quantitative studies of the rumen ecosystem.

*(Key Words: Gene Probes, Monoclonal Antibodies, Rumen Bacteria.)*

**Literature Cited**


