GENE AND MONOCLONAL ANTIBODY PROBES FOR RUMEN MICROBIAL ANALYSES

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Introduction

Detailed analyses of microbial interactions in the rumen have probably never been more important than now when developments in microbial genetics and molecular biology are leading to the design of new bacterial species for improving production efficiency in domestic ruminants. Unfortunately, identification and quantification of individual rumen bacterial species based on bacterial morphology is difficult. Even with volatile fatty acid or other metabolic analyses, identification of bacterial isolates can be ambiguous (Stahl et al., 1988). Gene and antibody probes may solve these problems. Because of their inherent specificity, gene probes have enormous potential for genotypic classification of new bacterial strains and for enumerating particular species in mixed rumen samples (Brooker et al., 1989).

Alternatively, rumen bacterial analyses may be achieved using serological techniques (Hazlewood et al., 1986, Ricke et al., 1988). Antibodies have the potential to distinguish between bacterial species based on structural differences in the outer lipopolysaccharide/protein cell envelope with the advantage over gene probes that they can be used in easy to operate ELISA's for rapid identification and quantification purposes. They can also be used in sensitive immunofluorescence microscopy assays for the direct identification of particular bacterial species. This paper describes the preparation of gene and monoclonal antibody probes for rumen bacterial analysis.

Materials and Methods

Purified rumen bacterial species *Bacteroides ruminicola and Selenomonas ruminantium* were grown anaerobically in Hungate tubes in brain heart infusion (BHI) medium as described previously (Attwood et al., 1988). Isolates from the rumen were spread on anaerobic BHI plates and individual colonies were picked for further culture and analysis.

Monoclonal antibodies were prepared by standard techniques and assayed in a plate ELISA where the target cells were washed thoroughly and bound to the ELISA plate by centrifugation and treatment with poly-L-lysine plus glutaraldehyde (unpublished). Antibody screening assays were based on the sandwich technique using alkaline phosphatase conjugated second antibody.

Results and Discussion

Gene probes for *Bacteroides* and *Selenomonas*

Genomic libraries for *Bacteroides* and *Selenomonas* were established in *Escherichia coli* (ED-8299) and recombinants were selected by inactivation of the lac phenotype. Colonies were picked at random for plasmid isolation and screening.

A series of clones that contained strain- or species-specific gene probes were selected by dot blot hybridisation to DNA from a variety of strains of *Bacteroides*, *Selenomonas* and other bacterial species. These included a *Bacteroides ruminicola* subspecies *brevis* B14-specific probe, several *Bacteroides* species probes, a *Selenomonas ruminantium* HD4-specific probe and several *Selenomonas* species probes. Sensitivity of the probes for bacterial analysis was established by a competitive dot-blot analysis in which target bacteria were mixed in varying proportions with a non-reactive bacterial species and total DNA was then extracted from each mixture. The amount of probe-specific DNA in each mixture was determined by quantification of hybridisation to the labelled gene probe and was represented graphically comparison with known bacterial numbers in the titration. The results of this type of titration experiment (figure 1) established that randomly picked single copy gene probes can detect complementary DNA present in a minimum of 2×10^7 cells even when these specific cells represent only

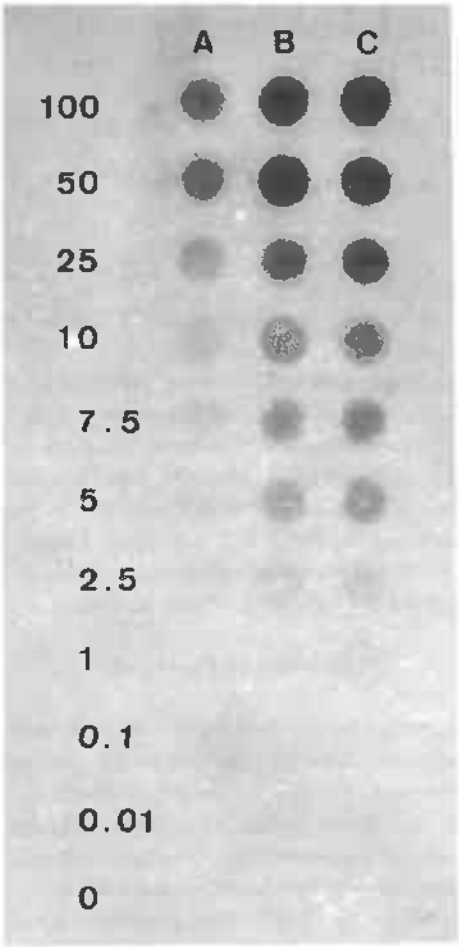

Figure 1. Sensitivity of *B. ruminicola* subsp. *brevis* B14 detection in a mixture of total ruminal microbial DNA.
Strain B14 cells were mixed in various proportions with total ruminal bacteria, and 1 (Lane A), 5 (Lane B) and 10 (Lane C)-ug samples of DNA from each mixture were hybridised to a radiolabelled pB·18 DNA probe. The numbers on the left represent the proportions of strain B14 cells in the mixtures.

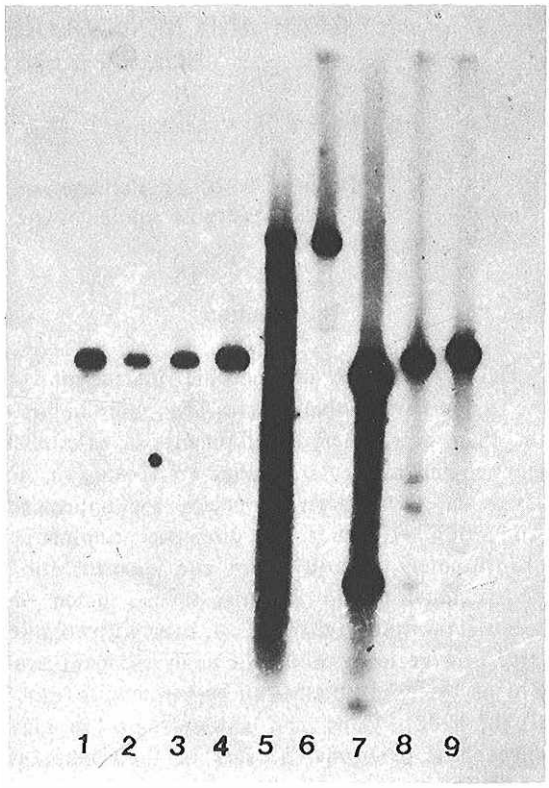

Figure 2. DNA fingerprint analysis of *S. ruminantium* isolates. DNA from isolates of *S. ruminantium* was digested with EcoR1, fractionated by ge' electrophoresis and *transferred to nylon membrane*. Transfers were hybridised with a radiolabelled *S. ruminantium* gene probe. Numbers represent separate rumen isolates.

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

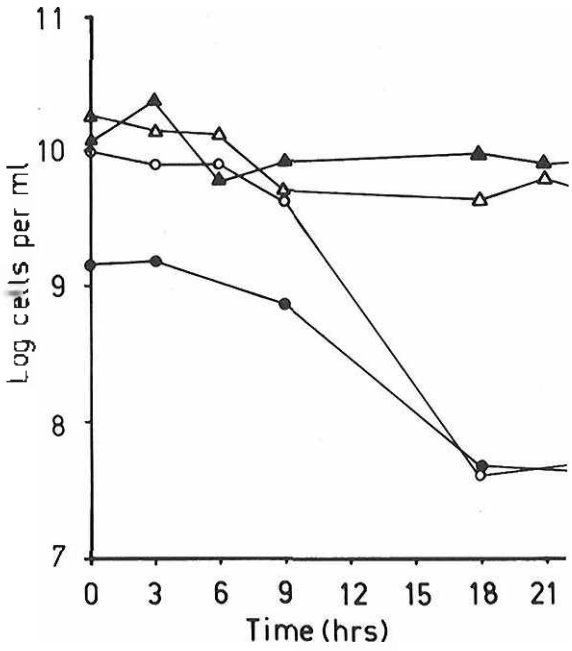

Figure 3. Survival of *B. ruminicola* subsp. *brevis* B14 in mixed culture *in vitro*. Strain B14 cells were inoculated into mixed cultures of ruminal bacteria at levels of 10% and 50%. Samples were taken at regular intervals for DNA analysis using a radiolabelled RNA hybridisation probe derived from clone B-18 and for cell number determinations. Data are expressed as equivalent cell numbers based on DNA standard curves. Symbols: ▲, total cell number 10% inoculum; △, total cell number 50% inoculum; ●, strain B14 cell number 10% inoculum; ○, strain B14 cell number 50% inoculum.

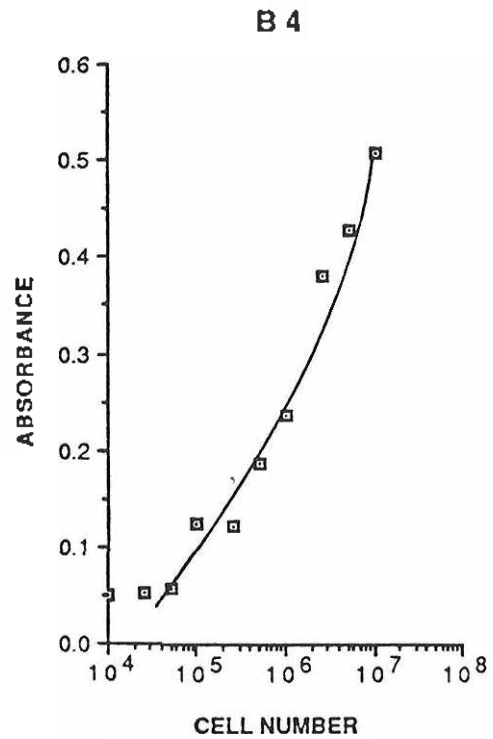

Figure 4. Competition ELISA for *B. ruminicola* subsp. *ruminantium*. ELISA plates, pretreated with poly L-lysine, were set up with varying proportions of *B. ruminicola* subsp. *ruminicola* cells in a total of 10^7 cells attached to each well. The non-specific competitor cells were *E. coli*. Cells were fixed with glutaraldehyde and assayed by ELISA using a *B. ruminicola* subsp. *ruminicola*-specific monoclonal antibody (B4). The results are expressed as absorbance at 405 nm.

antibodies.

Polyclonal antisera was not sufficiently specific for serological analysis. However, a number of *Bateroides*-specific and *Selenomonas*-specific mo - noclonal antibodies were isolated and characterised by competitive ELISA analysis. Each could readily detect the presence of 10^5 specific bacteria in a population of 10^8 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.)

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