Rumen Bacterial Isolation

In order to permit correction for microbial contamination, a bacteria sample must be collected. Nitrogen of microbial origin should be subtracted from the total N in the residue of the corresponding sample before mathematical analysis. Microbial contamination can be estimated by conducting purine analysis on the residue remaining in the lab and by knowing the purine: N ratio in bacteria collected from animals in which the samples were incubated. The procedures we follow in isolating bacteria from fresh ruminal contents are listed below.

Materials:

Storage container for rumen contents
Heavy-duty blender
Cheesecloth
250 ml centrifuge bottles
Centrifuge
Freeze-drier

Reagents:

Formalin-Saline Solution:

Dissolve 100 ml 37% (w/v) Formaldehyde (Aldrich Cat # BDH0500-4LP, FW 30.03, CAS # 50-00-0) + 9.00 g Sodium chloride (VWR Cat #BDH0286-500, FW 58.44, CAS #7647-14-5) in water to make 1 liter of solution. Store in refrigerator.

Saline Solution:

Dissolve 9.00 g of Sodium chloride (VWR Cat #BDH0286-500, FW 58.44, CAS #7647-14-5) in water to make 1 liter of solution. Store in the refrigerator.

Procedure:

Note: Do not use this method if you intend on running amino acid analysis on the samples. The formaldehyde will destroy some of the amino acids.

1. Thoroughly mix rumen contents and collect approximately 5 kg, collecting from all areas of the rumen.
2. Weigh 4 kg of rumen contents into a container and add 2 liters of formalin-saline solution. Mix well and incubate for 2-4 hours in the refrigerator. Samples may also be frozen at this point for later processing.
3. Blend sample in a large blender for 1 minute. If sample is too thick, add sufficient saline solution to blend smoothly.
4. Strain blended sample contents through cheesecloth (2-4 layers) and pour into 250 ml centrifuge bottles. Do not fill over two-thirds to prevent leaking.
5. Centrifuge at 500 x g (2,000 rpm; Rotor # JA 14) for 20 minutes to remove protozoa and feed particles.

6. Pour supernatant into clean 250 ml centrifuge bottles. When particles begin to loosen into supernatant, pour the remainder into a separate centrifuge bottle and spin at 500 x g for 20 minutes.
7. Centrifuge particle free supernatant at 30,000 x g (13,800 rpm; Rotor # JA 14) for 20 minutes to collect bacteria.
8. Carefully pour off supernatant and wash pellets with saline solution. Combine pellets and spin at 30,000 x g for 20 minutes.
9. Carefully pour off supernatant. Rinse pellet out of centrifuge bottles with deionized water into containers for freeze-drying. Freeze-dry and grind with waring blender or mortar and pestle for analysis.

Analyses:

Nitrogen: Nitrogen concentration should be determined for the original (non-incubated) material, for a representative sub-sample of the residue remaining in each bag, and for the bacteria sample.

Dry Matter: Dry matter concentration should be determined in the original (non-incubated) material and in the bacterial sample. Because calculations are done on an N basis, some work is saved by working on an as-is basis. Thus, one need not determine the dry matter concentration of the residue remaining in each bag.

Purines: Determine purine concentration in the bacteria sample and for a representative sub-sample of the residue remaining in each bag. For analysis of purine concentration, we follow the procedure described by Zinn and Owns (1986). (See purine procedure.)