Rumen Microbiology: Where are we and how did we get here?

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ADSA – ASAS meeting, Minneapolis 2006
Timeline – for rumen microbiology

- **19th Century**
  - Microbial role in rumen digestion recognized, bacteria and protozoa observed
  - Products --- acetic and butyric acid, CO2 and CH4 recognized
- **First ½ of 20th Century**
  - First isolations of cellulose digesting bacteria
  - Unsuccessful attempts to cultivate protozoa and the important bacteria
- **Second ½ of 20th Century**
  - Isolations and characterizations of predominant bacteria
  - Some of the protozoa cultivated in vitro
  - Fungi recognized
  - Interactions between microorganisms and with feedstuffs
  - Manipulations of mixed populations
- **Near end of 20th Century**
  - Molecular methods developed
    - Evolutionary-Phylogenetic relationships between species
    - Population characterization that does not depend upon culture
      - More about this later--------
Robert E. Hungate

(1906-2004)

- Hungate “The father of rumen microbiology”
- In May this year Am. Soc. Microbiol. held a symposium in celebration of the centennial year of birth of Robert E. Hungate
- “Studies on Cellulose Fermentation”
  - 1944 at U. of Texas isolated spore-former
  - 1947 at Wash State successful isolations of rod, *Fibrobacter succinogenes*.
- Hungate’s Methods
  - His “Roll Tube” method (1950 review)
  - simulation of the rumen habitat in-vitro
The Hungate tree (main branches)

- At Wash State Univ
  - Marvin Bryant
  - Richard McBee
  - Leroy Maki
  - Jose Gutierrez

- At U Cal Davis
  - Doug Wright
  - RTJ Clarke
  - Reg Moir
  - Henry Blackburn
  - MJB (Ben) Paynter
  - Tom Bauchop
  - Rudolph Prins
  - Khaled el-Shazly

- At U Cal Davis
  - Bob Mah
  - Paul H. Smith
  - Joan Macy
  - King-Thom Chung
## Marv Bryant’s collaborators (pub-med)

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Locations of research efforts in rumen microbiology

- Australia: Bauchop, Moir, Warner,
- Canada: Cheng, Forsberg, Forster, Teather, McAllister
- France: Fonty, Jounay
- New Zealand: Clark, Joblin, Wright
- The UK:
  - Coleman, Czerkawski, Eadie, Elsdén, Flint, Hobson, Orpin, Newbold, Theodorou, Stewart, Wallace,
- South Africa: Gilchrist, Kistner,
- Wash State-California: Hungate et al
- USDA Beltsville and U of Illinois: Bryant et al
- USDA labs –
  - Ames, Clay Ctr. Cornell, Peoria, Tx A&M, Wisc
- Universities:
  - Ga, K.ST, Ky, MI St, MN, NE, O St, Purdue, W Va, WY
Information needed to understand rumen ecosystem

- Kinds (identities) of microorganisms
  - Numbers (concentrations) of individual species
    - What is a species?
- Metabolic activities of individual species
- Metabolic activities of mixed populations
  - Rates -- in vivo and in vitro
- Interactions between groups of microorganisms
  - Cross feeding of nutrients, end-product removal, production of growth inhibitors, predation
- Interactions between microbes and feedstuffs:
  - Stratification of populations (biofilms on plant surfaces)
- Interactions between microorganisms and host animal
Rumen Microbial Communities

- Rumen – probably the best studied of any anaerobic gut habitat
- Complex ecosystem
  - Bacteria, protozoa, archaea, fungi
  - Open --- many niches
- Compartments:
  - Free, loosely associated to feed, rumen epithelial population
  - Tightly associated
- Micro-habitats --
  - Planktonic populations
  - Biofilms on diversity of plant sites
Comparisons of rumen populations

- Microscopic methods
  - Useful for protozoa
  - Of little value for bacteria – similar morphologies

- Culture based methods
  - Lack of good differential culture media
  - Non-differential media, grew predominant bacteria
    - Isolation of strains and characterization of these
    - Labor intensive - not suited for multiple samples

- Near end of 20th Century
  - Molecular methods developed
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Molecular methods for analysis of rumen populations

- **Advantages**
  - Population evaluations not limited by culture methods which miss part of populations and are very labor intensive
  - Samples may be frozen for later analysis
  - Comparisons with public databases possible
  - Results may point to important gaps in knowledge

- **Disadvantages**
  - Sequences aligned with uncultured species are (?)
  - Technology changing
  - Methodological limitations “now have >3,000 ruminal bacteria rRNA gene sequences archived, but we need hundreds of thousands of rRNA sequences for adequate characterizations of true microbial diversity” (Firkins and Yu)
Substrate based grouping of rumen bacteria

GENERALISTS: (16S rRNA probably in data bases)
- Pure cultures isolated on media designed for high counts
- Most numerous are carbohydrate fermenters
- Most of the predominant species have probably been identified – as to: taxon and function

SPECIALISTS: (16S rRNA probably not in data bases)
- Great diversity of micro ecologic niches/substrates (eg. plant secondary compounds)
- Selection during eons leads to great diversity
- This diversity lends stability to ecosystem
- Culture (colony formation) only on specialized media
- Probably only scratched the surface
Specialists: bacteria not isolated with usual non-selective media

- Methanogens:
- Anaerobic mycoplasmas
- Hyper-ammonia producers
  - amino acid degraders
- Succinate decarboxylating bacteria (Selenomonas?)
  - N.O. van Gylswyk --- 2 species
  - Weddington and Strobel --- succinate loss = synergism in co-culture with succinate producing *Fibrobacter*
- *Oxalobacter*: Oxalate degrading bacteria
- *Synergistes jonesii*: degrades toxic DHP
- *Denitrobacterium*: reduces toxic nitro-cpds
- *Wolinella succinogenes*: H$_2$ to reduce fumarate, NO$_3$
**Oxalobacter formigenes**

- **Gram** - rod, Obligate anaerobe
- **A Specialist** - Oxalate only energy source
  - selection of population based on oxalate supply
- \( ^{-}\text{OOCCOO}^- + \text{H}^+ \rightarrow \text{COO}^- + \text{HCOO}^- \)
  - products equimolar CO\(_2\) and formate
  - cell carbon from oxalate, acetate, CO2 and formate
  - proton consumed ---- proton gradient for ATP
- Widely distributed in gut habitats
  - (+) Pigs, horses, rabbits, wild rats, birds
  - (-) Not in dogs or cats (carnivores with short hind gut)
  - (+) in most humans
Oxalobacter significance in humans

- ~ 65 - 75% “normal” persons are colonized
  - fecal PCR test or cultural test - oxalate loss
- Populations at high risk Ca-oxalate kidney stones have lower colonization rates
  - persons with multiple episodes of stones
  - persons with enteric-hyperoxaluria (Crohn’s disease, IBD, JI by-pass surgery)
  - persons with high antibiotic exposure rates - eg. cystic fibrosis patients
Information needed to understand rumen ecosystem

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- Metabolic activities of individual species
- Metabolic activities of mixed populations
- Interactions between microorganisms
- Interactions between microbes and feedstuffs:
- Interactions between microbes and host animal
"The role of the infinitely small in nature is infinitely large"

- Louis Pasteur