PRIFYSGOL ABERYSTWYTH UNIVERSITY

Sefydliad y Gwyddorau Biolegol, Amgylcheddol a Gwledig
IBERS ABERYSTWYTH
Institute of Biological, Environmental and Rural Sciences

Use of new molecular biology techniques for the evaluation of zootechnical additives

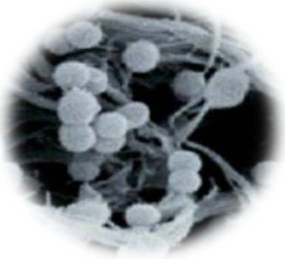
Jamie Newbold

The rumen

- Large and complex populations of micro-organisms.
- Essential for energy/ protein requirement.
- In balance in microbial community can lead to severe illness (acidosis, laminitis, liver abscesses, etc).
- Major contribution to GHG/ pollutant emissions

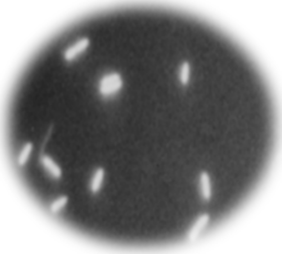
Bacteria

~300 species
10^{10} to 10^{11}cells/ml



Methanogenic Archaea

~6 species
10^{6} to 10^{8} cells/ml



Ciliate Protozoa

~40 species
<10^{5} cells/ml

Anaerobic Fungi

~30 species
<10^{5} cells/ml

Types of Rumen Microorganisms

BACTERIA

Ferment fiber, starches and sugars in feeds to VFA, H_2 and CO_2

Produce most of microbial cell protein, but also ferment feed proteins to VFA + NH_3

PROTISTS

Consume and ferment bacteria to VFA + NH_3

Sequester and ferment starch

Recycle N

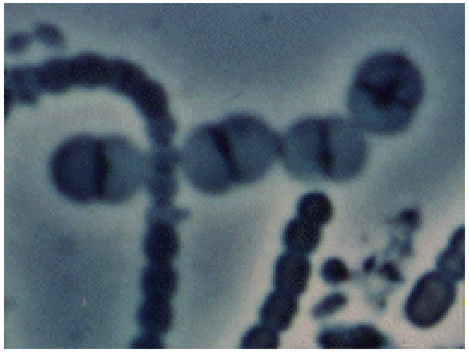
ARCHAEA

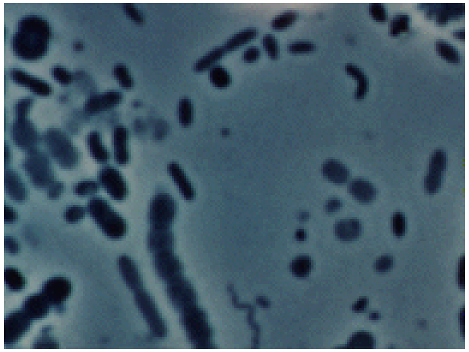
Convert H_2 and CO_2 to methane

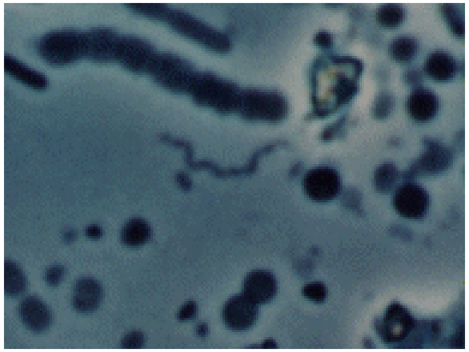
FUNGI

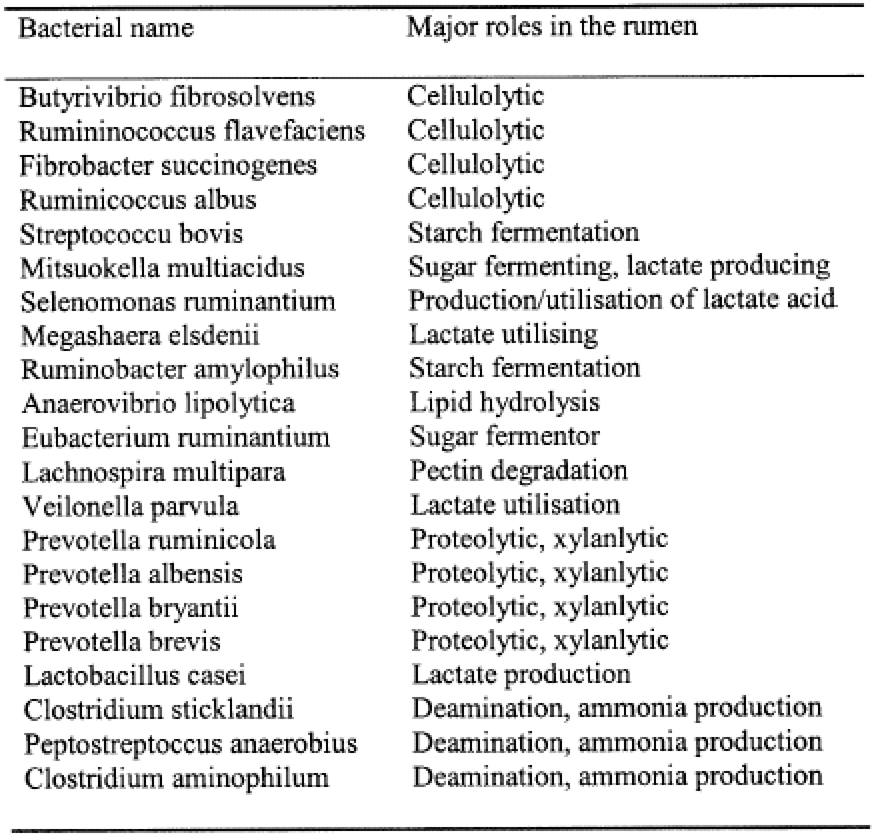
Assist in fiber digestion

Rumen bacteria





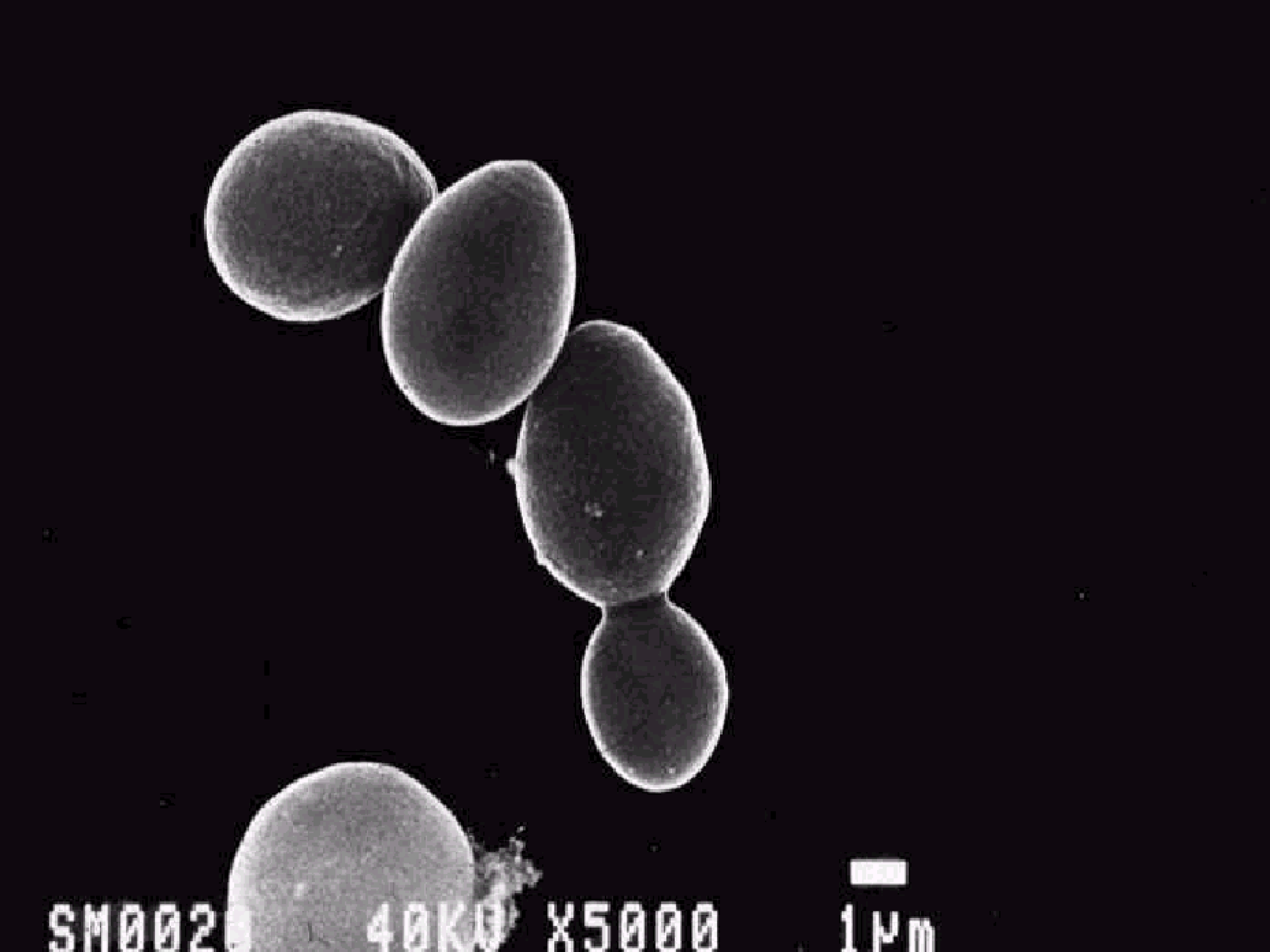


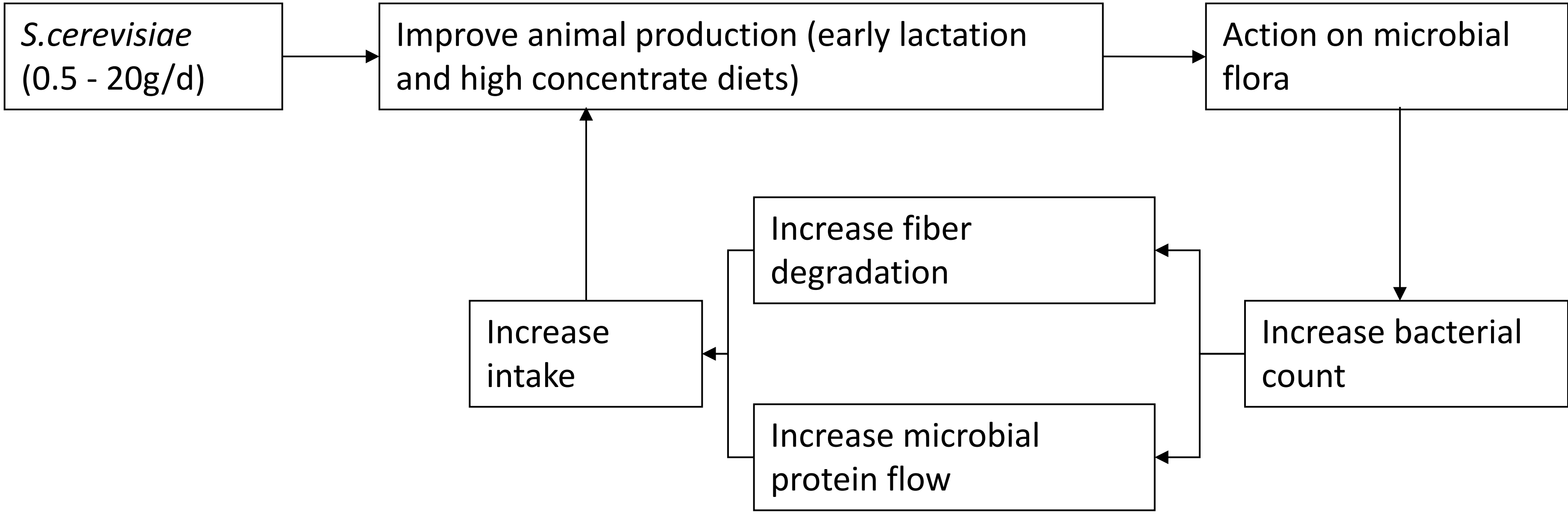


Bacterial name	Major roles in the rumen
Butyrivibrio fibrosolvens	Cellulolytic
Rumininococcus flavefaciens	Cellulolytic
Fibrobacter succinogenes	Cellulolytic
Ruminicoccus albus	Cellulolytic
Streptococcu bovis	Starch fermentation
Mitsuokella multiacidus	Sugar fermenting, lactate producing
Selenomonas ruminantium	Production/utilisation of lactate acid
Megashaera elsdenii	Lactate utilising
Ruminobacter amylophilus	Starch fermentation
Anaerovibrio lipolytica	Lipid hydrolysis
Eubacterium ruminantium	Sugar fermentor
Lachnospira multipara	Pectin degradation
Veilonella parvula	Lactate utilisation
Prevotella ruminicola	Proteolytic, xylanlytic
Prevotella albensis	Proteolytic, xylanlytic
Prevotella bryantii	Proteolytic, xylanlytic
Prevotella brevis	Proteolytic, xylanlytic
Lactobacillus casei	Lactate production
Clostridium sticklandii	Deamination, ammonia production
Peptostreptoccus anaerobius	Deamination, ammonia production
Clostridium aminophilum	Deamination, ammonia production

Manipulation of the rumen microbiome:

- Live yeast
- Defaunation


SM002
40KV
X5000
1μm



S.cerevisiae
(0.5 - 20g/d)

Improve animal production (early lactation and high concentrate diets)

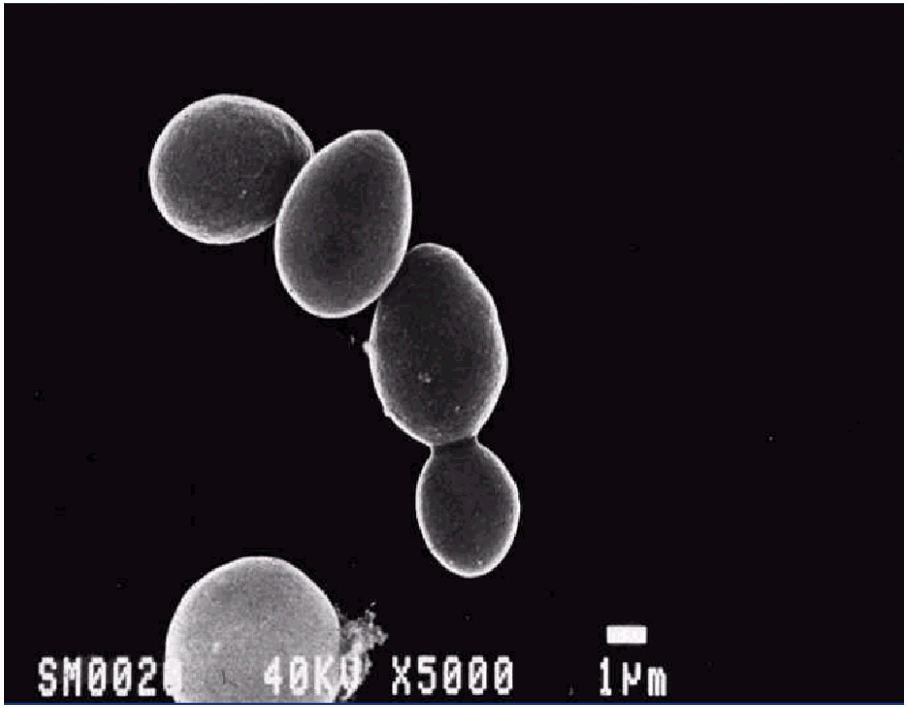
Action on microbial flora

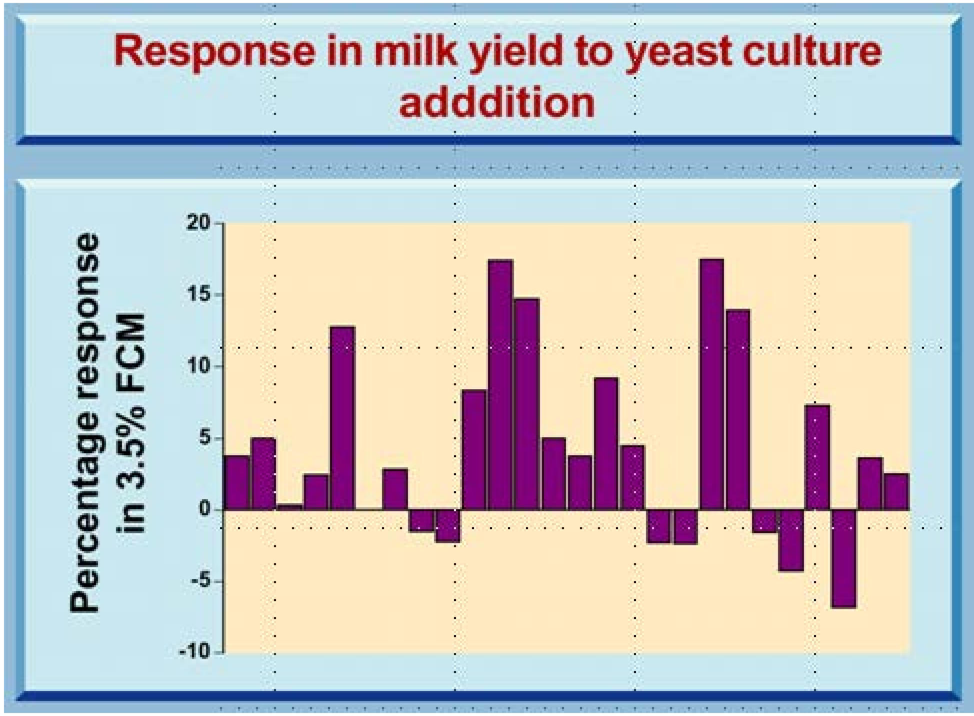
Increase bacterial count

Increase fiber degradation

Increase microbial protein flow

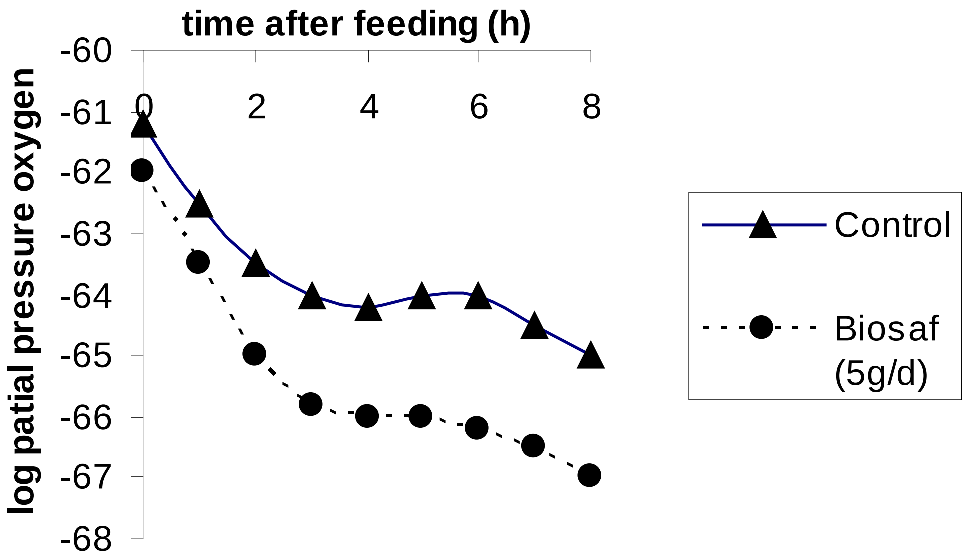
Increase intake



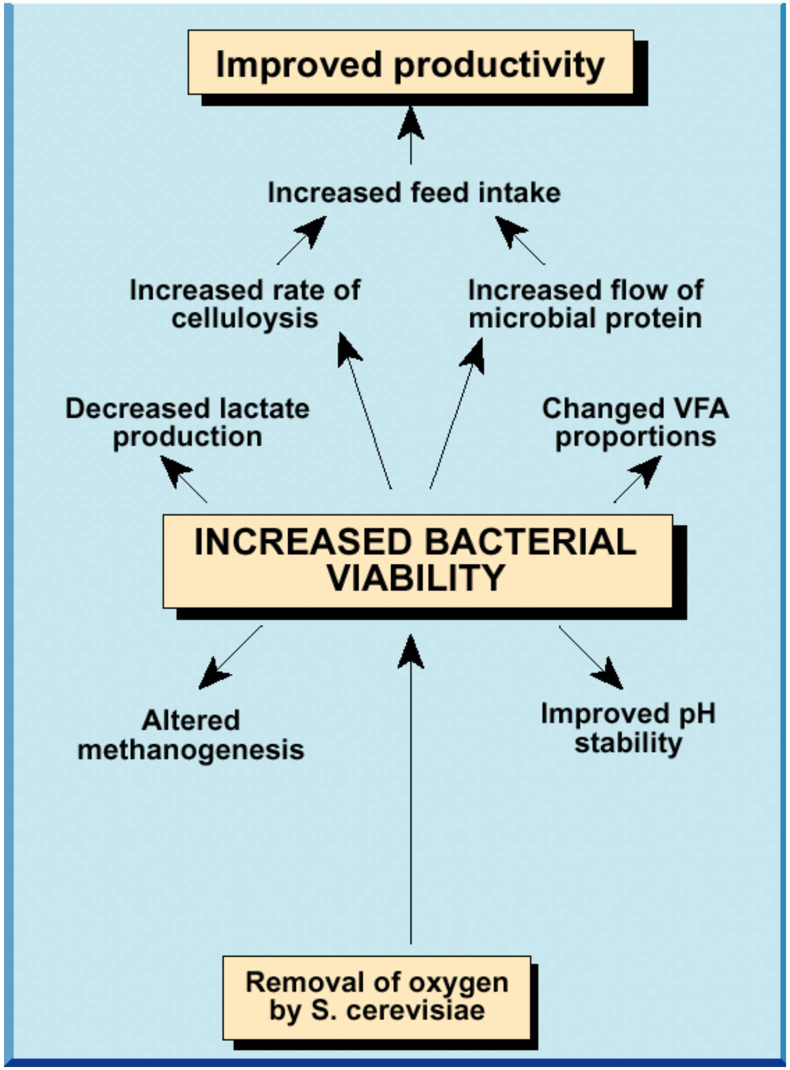


Response in milk yield to yeast culture adddition

The increase in bacterial activity is thought to be due to the removal of oxygen from the rumen



Rumen oxygen in lactating dairy cows fed a corn silage concentrate diet supplemented (●) or without (▲) 5 g /day yeast culture (Marden, 2007)



Improved productivity

Increased feed intake

Increased rate of celluloysis

Increased flow of microbial protein

Decreased lactate production

Changed VFA proportions

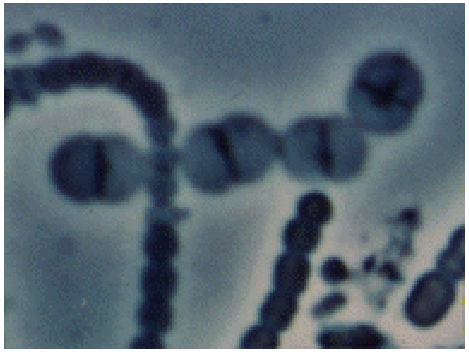
INCREASED BACTERIAL VIABILITY

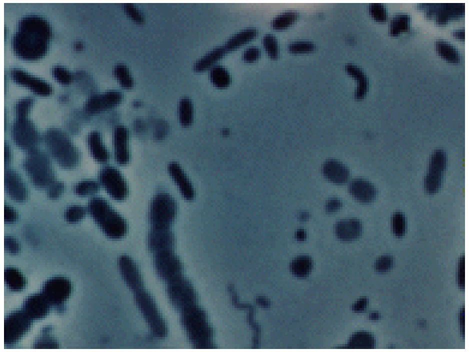
Altered methanogenesis

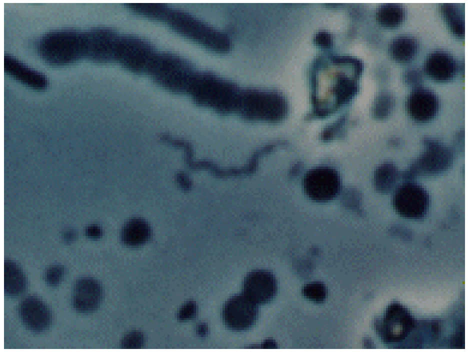
Improved pH stability

Removal of oxygen by S. cerevisiae

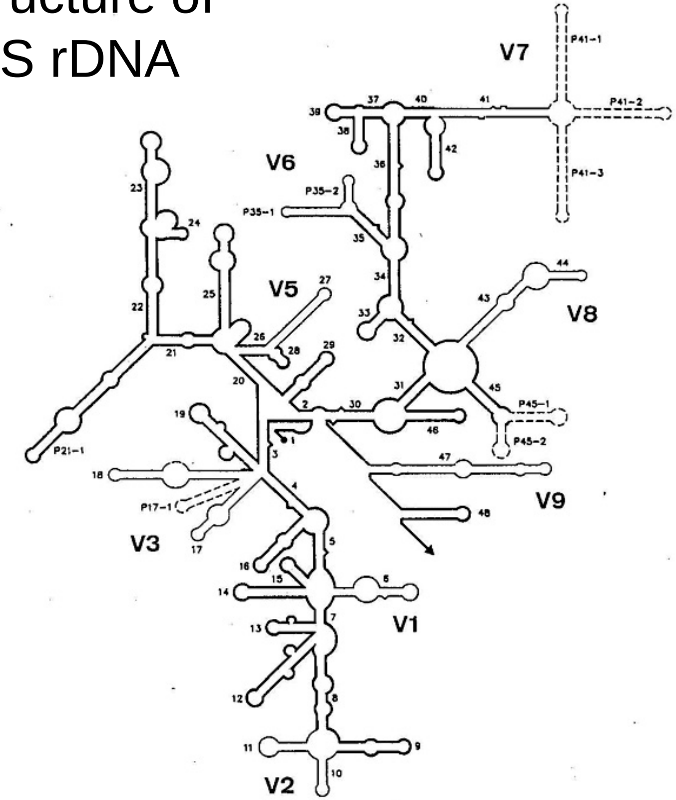
16S rRNA







Structure of
16S rDNA



Design of the animal trial (lactating cows)

3 x 3 Latin Square Design:

	A1	A2	A3
P1	L1	L0	L2
P2	L0	L2	L1
P3	L2	L1	L0

P : Period

C : Cows

L : Treatments:

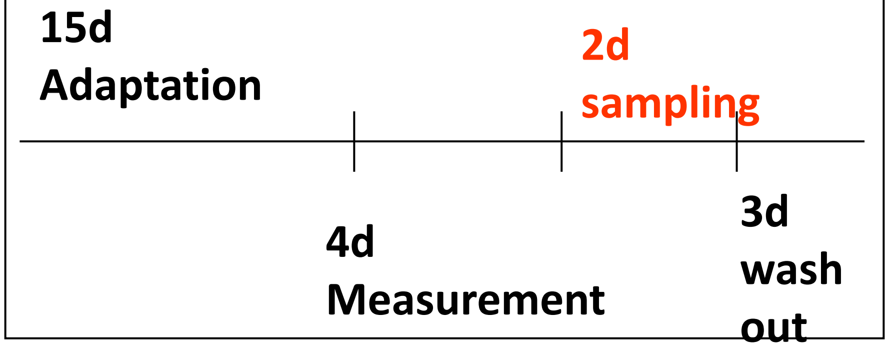
L0 = 0 ; L1 = 0.5g/d ; L2 = 5g/d

Live Yeast

Composition of the diet (acidogenic):

- 60% corn silage
- 30% concentrate
- 10% dehydrated alfalfa
- Minerals and vitamins

Period design



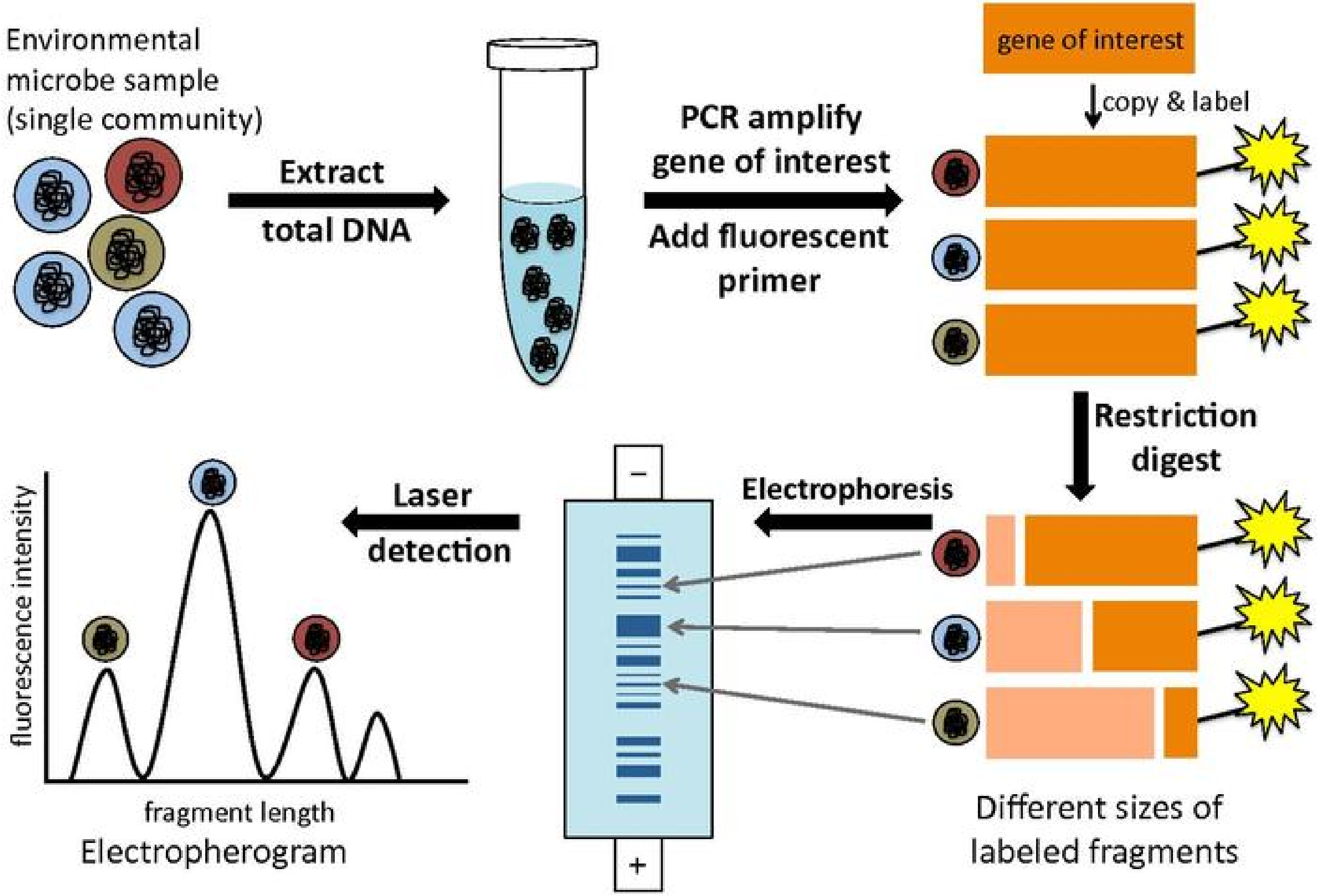
15d Adaptation

4d Measurement

2d sampling

3d wash out

T-RFLP: Terminal Restriction Fragment Length Polymorphism



Environmental microbe sample (single community)

Extract total DNA

PCR amplify gene of interest

Add fluorescent primer

gene of interest

copy & label

Restriction digest

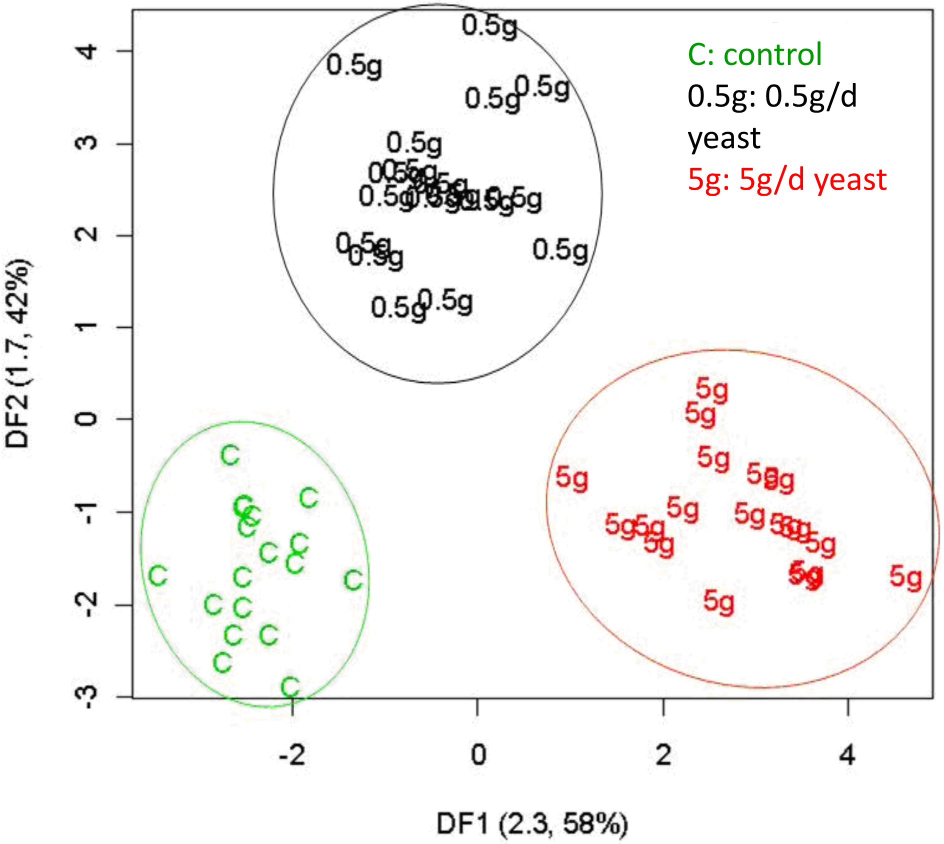
Different sizes of labeled fragments

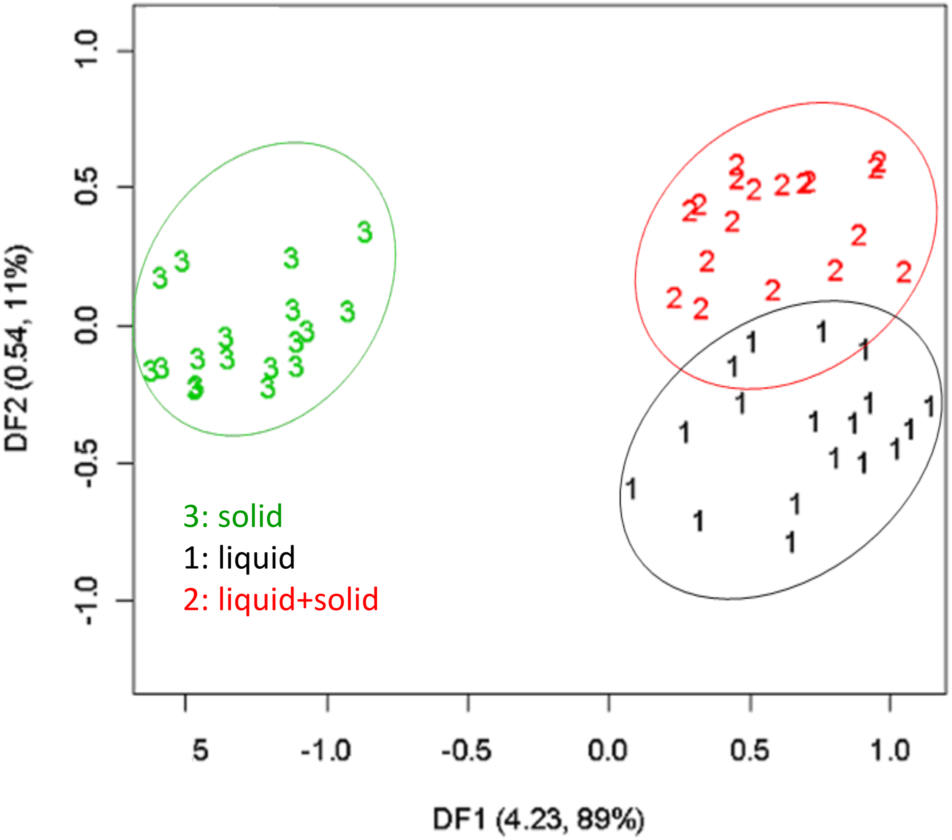
Electrophoresis

Laser detection

Electropherogram

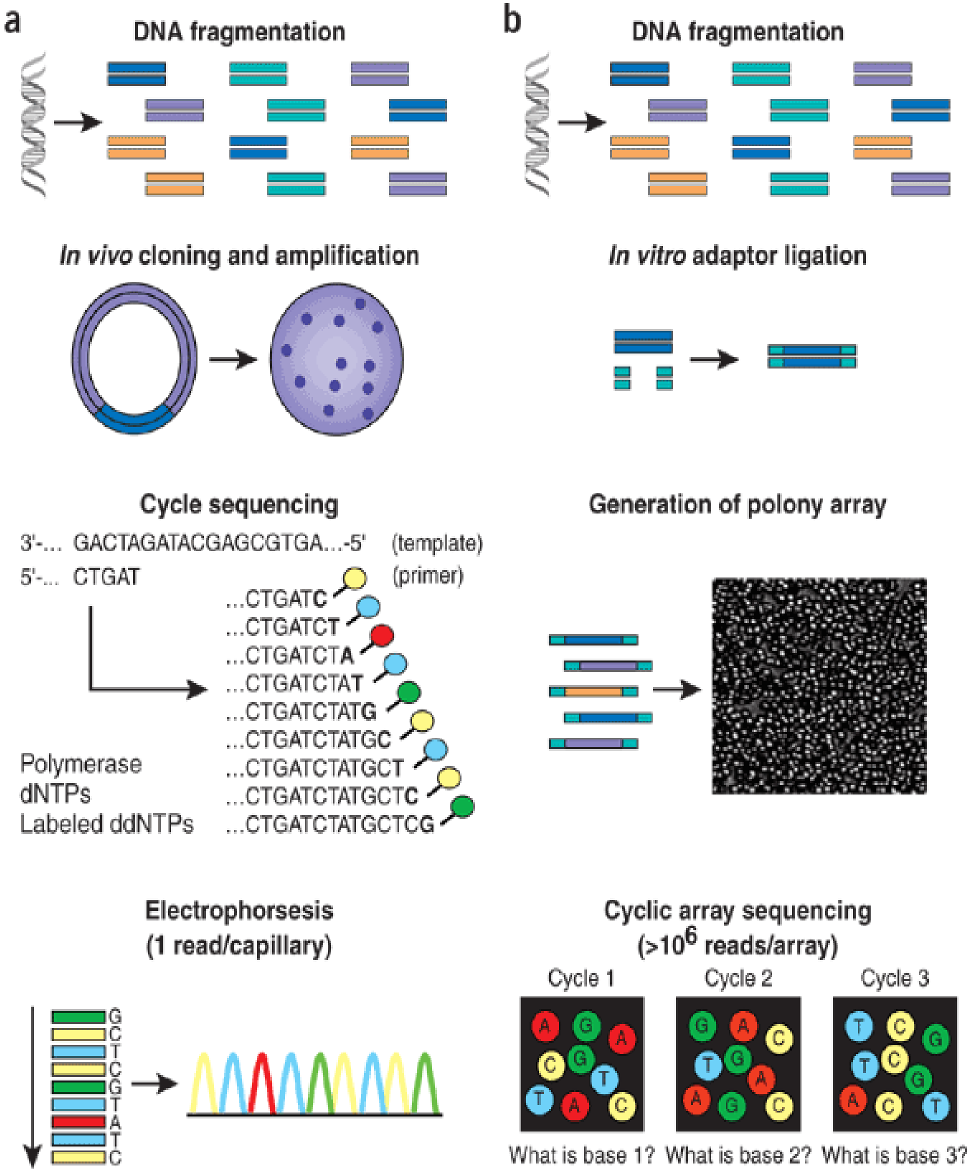
Linear Discriminant Analysis (LDA)





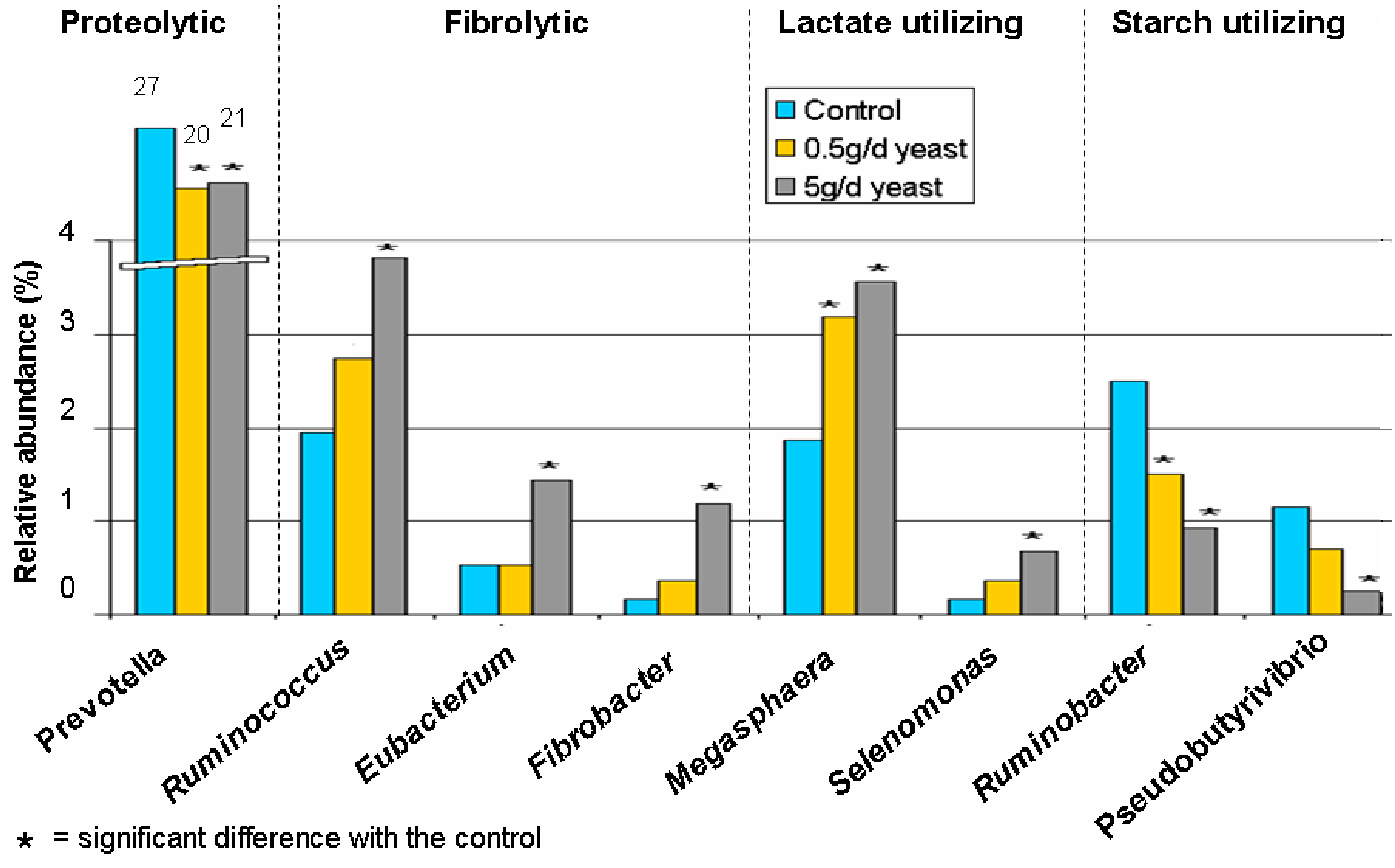
Sanger

Next Gen Seq



	Sanger	Next Gen
Sequencing samples	Single Clones	DNA libraries
Prep	Few, seq react, clean up	More complex
Data	One read per sample	Millions reads per sample

Distribution of bacterial groups and response to yeast treatment



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	A1	A2	A3
P1	L1	L0	L2
P2	L0	L2	L1
P3	L2	L1	L0

P : Period

C : Cows

L : Treatments:

L0 = CONTROL
L1 = SODIUM BICARBONATE
L2 = YEAST

450,000 sequences generated
350,000 sequences analysed (removed if inferior to 150nt and more than 3N)

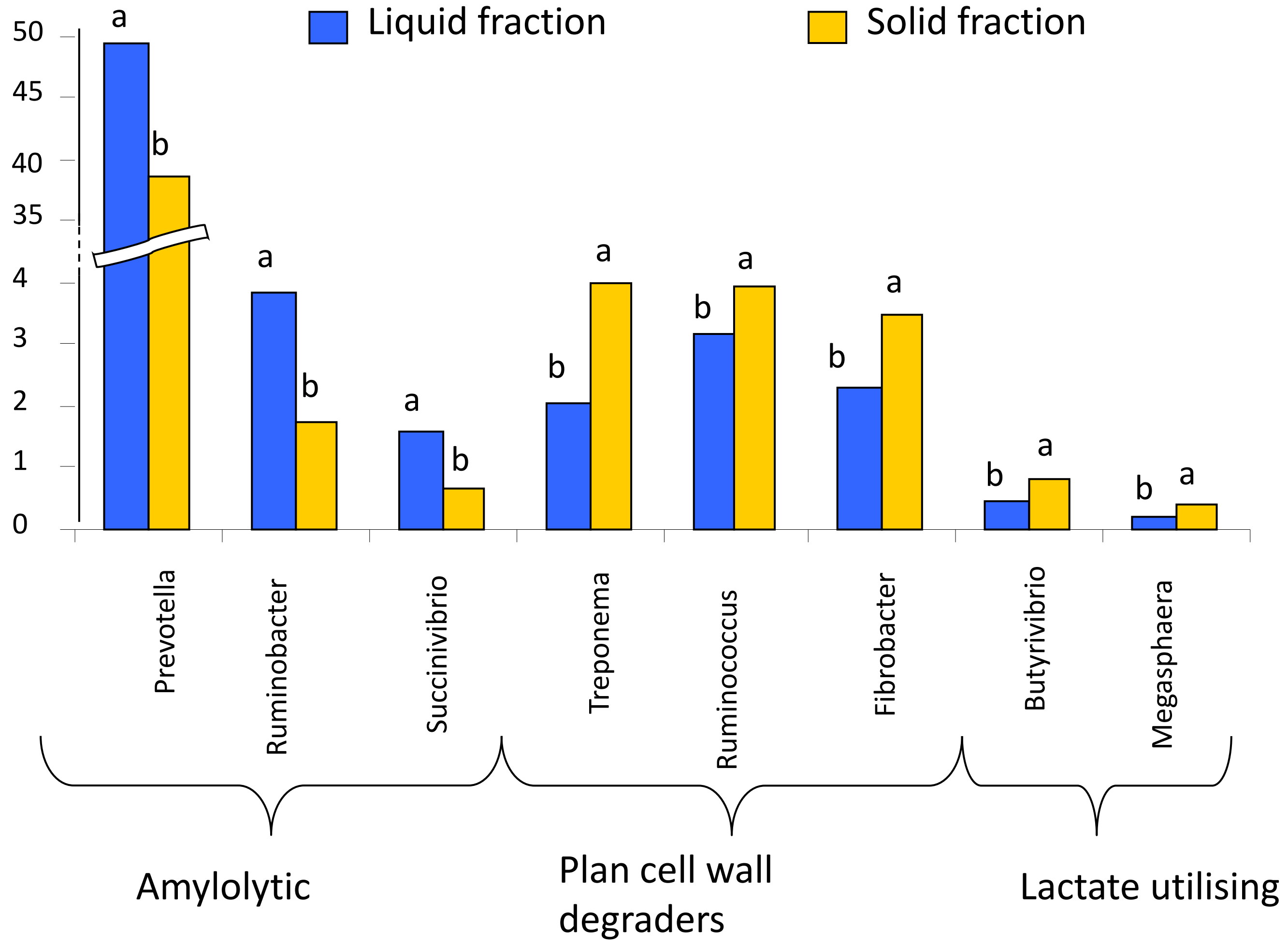
3 steps process

1. Pair wise alignment with a gut database of the same region that we sequenced (135,000 sequences)
   generated 9500 different matches

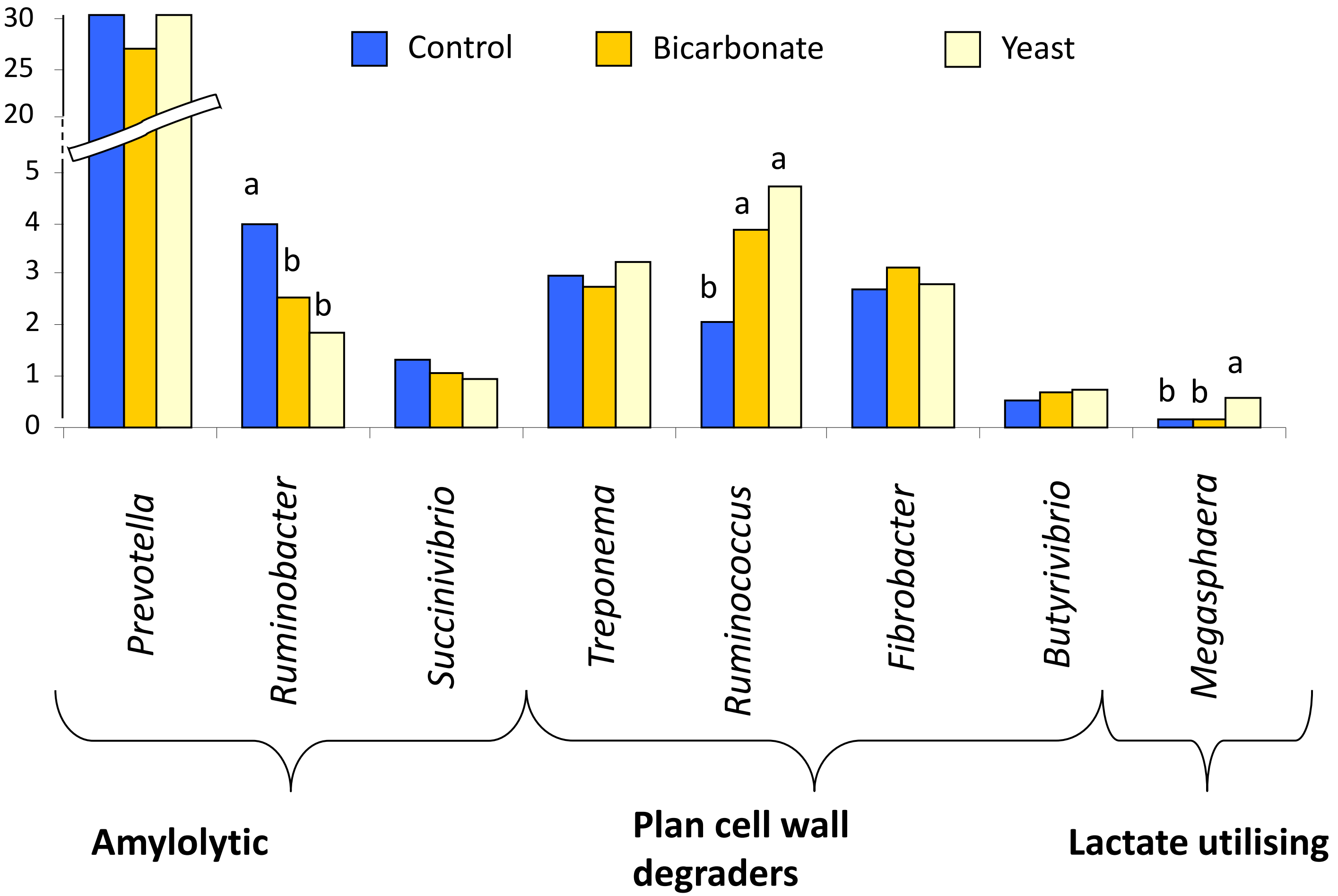
2. Recovered the full length of the sequence pair wise alignment of the 9500 matches against an isolate database (full length, with bacterium identified 110,000 sequences)

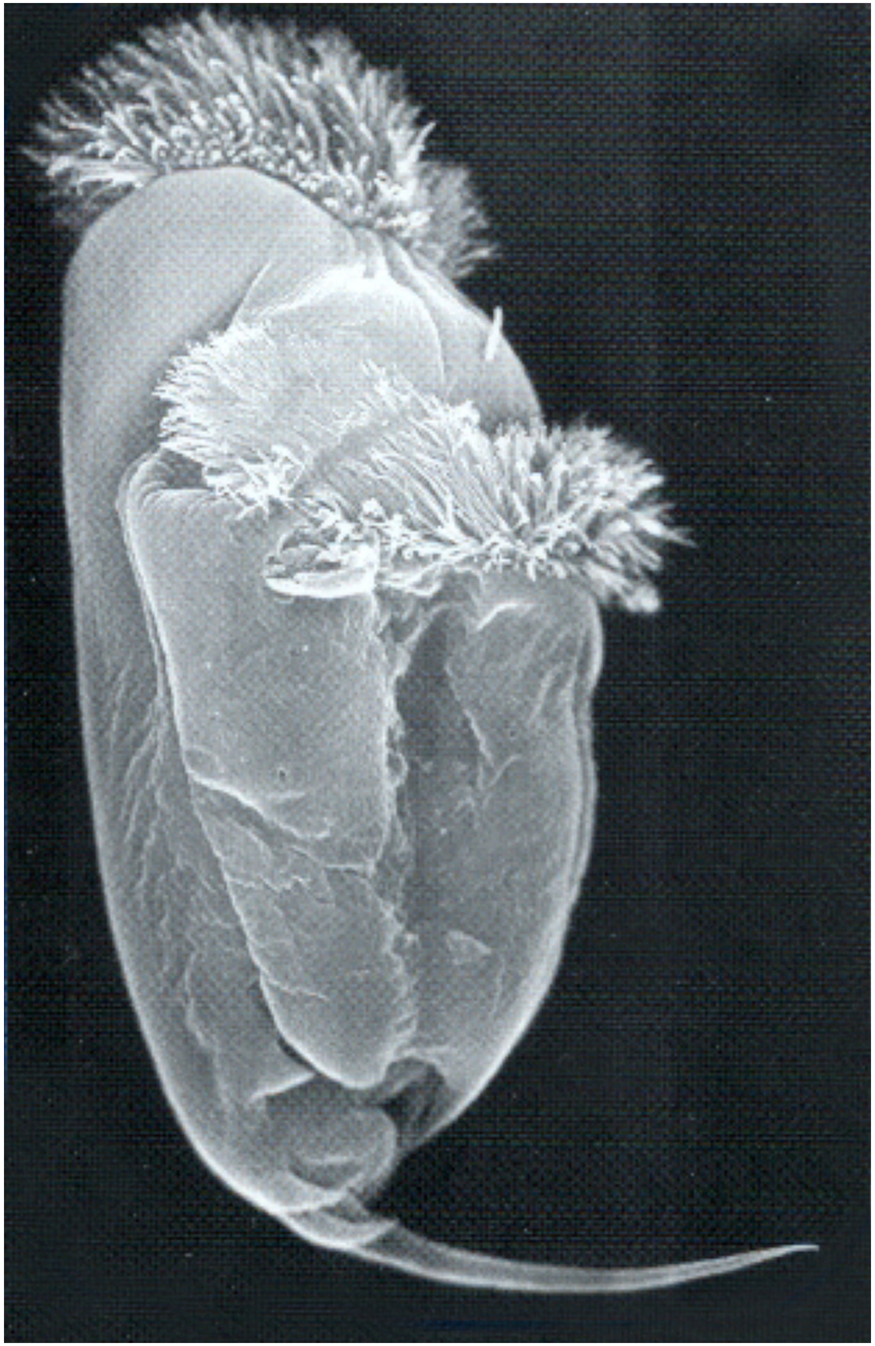
3. Report the bacterial composition (relative abundance in %)

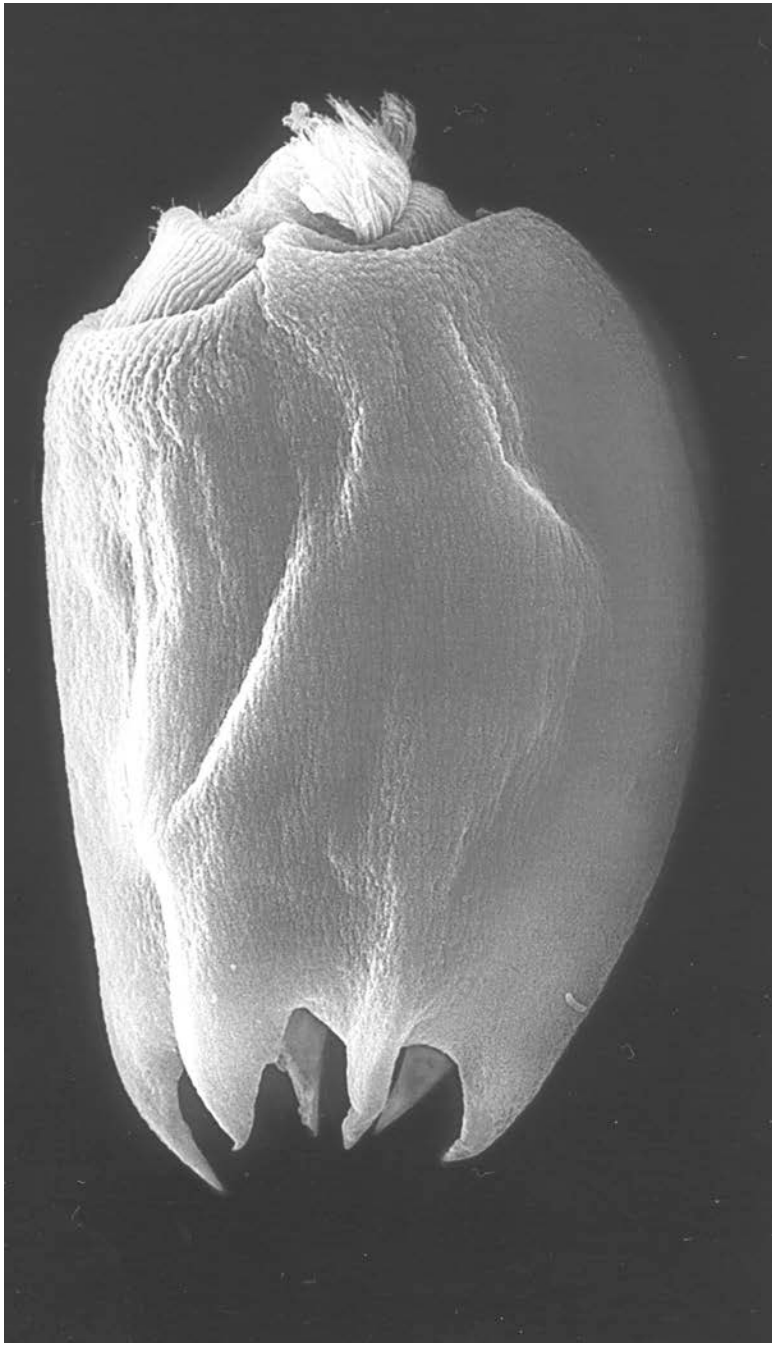
Bacterial composition at the genera level



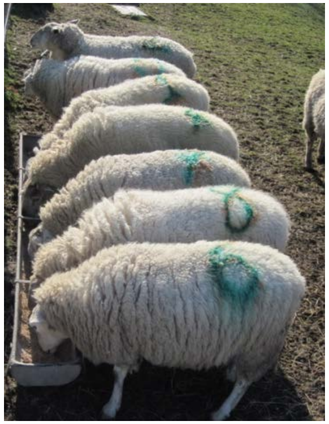
Bacterial composition at the genera level



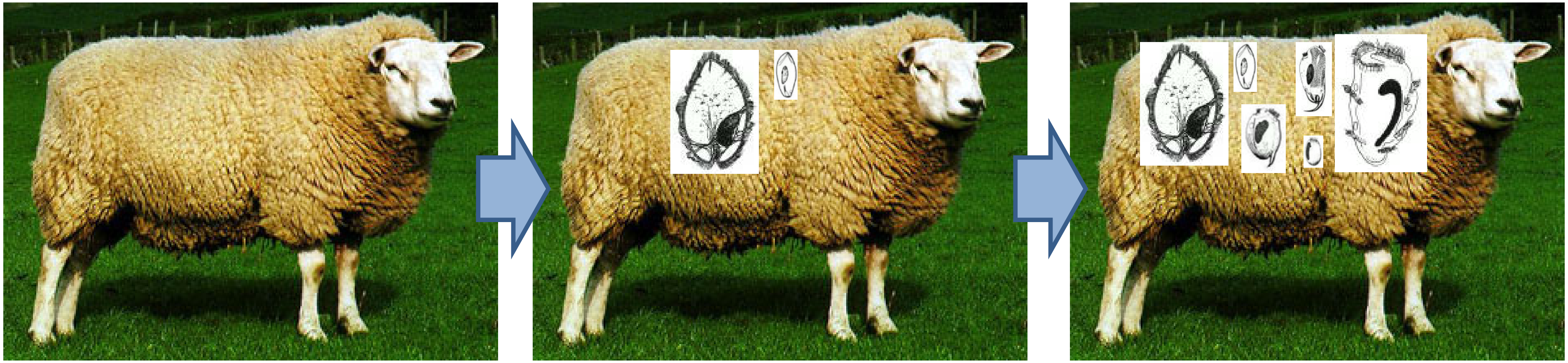




EXPERIMENTAL DESIGN



- **Animals:**8 adult Texel-crossbreed sheep (93 kg BW)
- **Treatments:**

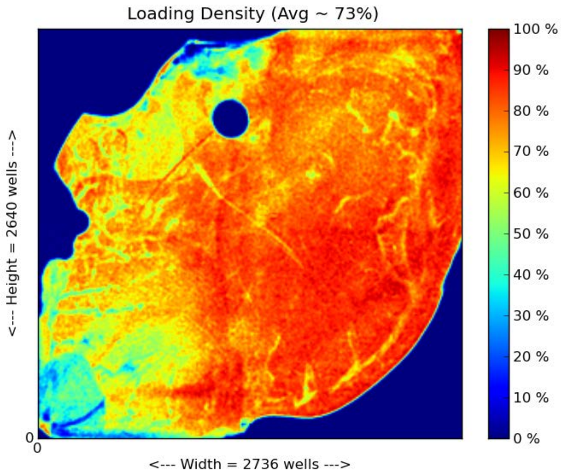


Protozoa-Free (P1)

Holotrich (P2)

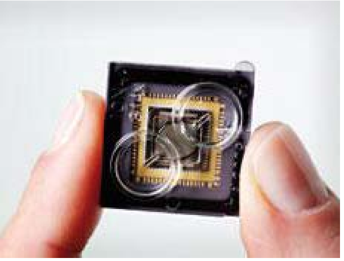
Total-Fauna (P3)

- **Diet:** DMI=1.6 kg DM/d (67% ryegrass hay, 33% ground barley)
- **Adaptation:** 3 months/period
- **Methane emissions** (4 days in respiration chambers)
- **Rumen fermentation and DNA studies**



Amplification V3 region 16srRNA

3.5 million raw sequences
Trimming and quality filtering by **MOTHUR**



Noise and quimeras removal and OTU table construction by **CD-HIT OTU**

Normalization of data by **Daisychopper**

864 OTU, 440664 seqs after normalization

Statistical analysis: **Genstat, Permanova, R**

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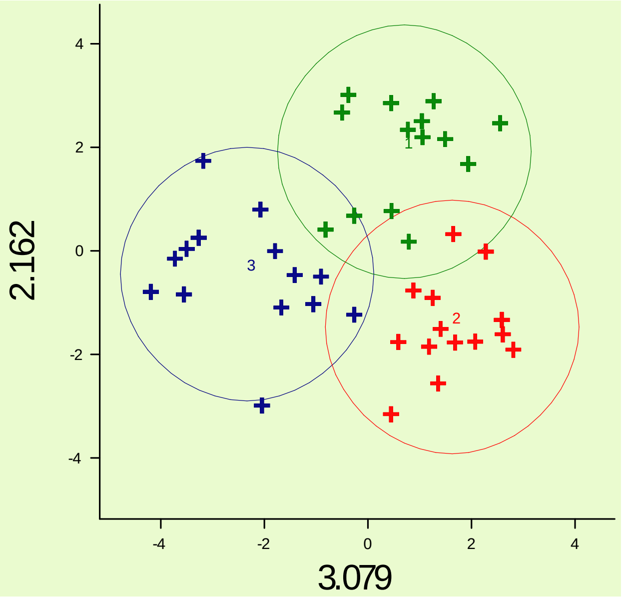
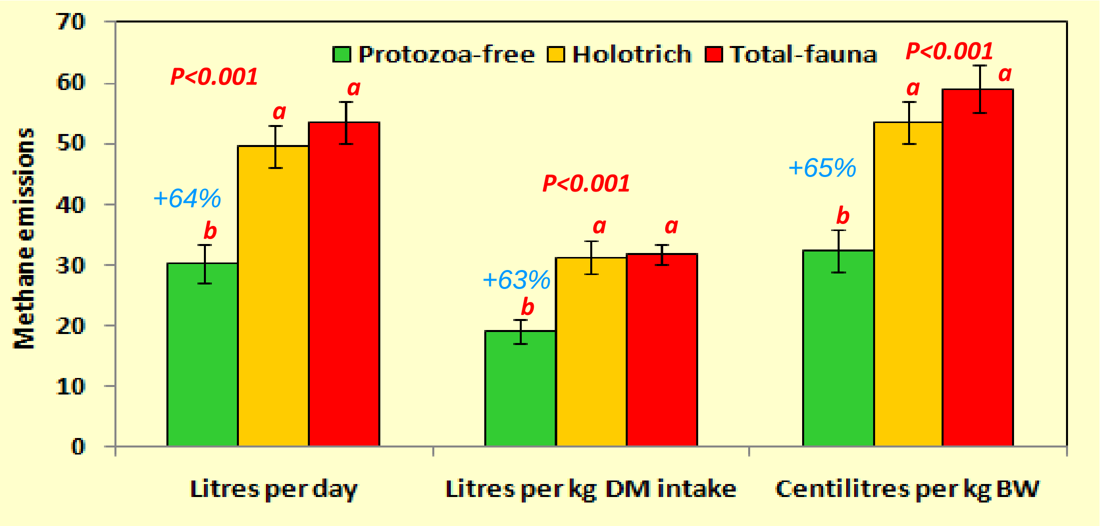
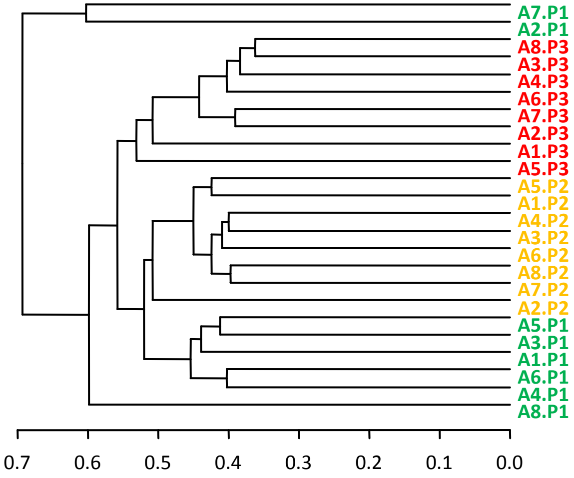


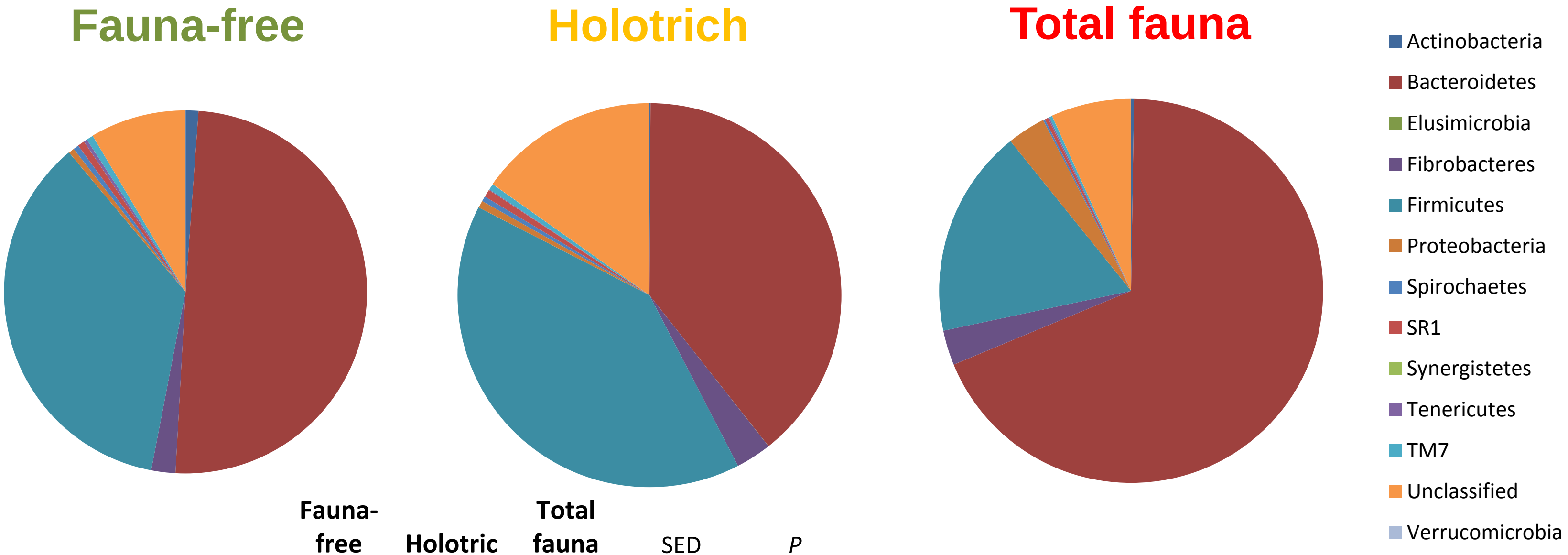




De la Fuente, G, Belanche, A, Moorby, JM and Newbold, C.J Influence of protozoal colonization on total bacterial communities in the rumen of sheep. *8th Joint Symposium RRI-INRA on Gut Microbiology. 17-20 June,2012. Poster*

Belanche, A, De la Fuente, G, Moorby, JM and Newbold, C.J Are methane emissions determined by the fermentation pattern or by the rumen microbiota? *8th Joint Symposium RRI-INRA on Gut Microbiology. 17-20 June,2012*

RESULTS



	Fauna-free	Holotric	Total fauna	SED	*P*
Actinobacteria	1.15A	0.09B	0.24B	0.304	**0.008**
Bacteroidetes	49.7B	39.3C	68.5A	3.74	**<.001**
Elusimicrobia	0.0041	0.0095	0	0.00575	0.283
Fibrobacteres	2.14	3	2.92	0.995	0.644
Firmicutes	35.9A	40.2A	17.5B	3.8	**<.001**
Proteobacteria	0.51B	0.6B	3.23A	0.949	0.019
Spirochaetes	0.489A	0.435A	0.159B	0.109	0.02
SR1	0.615	0.637	0.275	0.2163	0.208
Synergistetes	0.0034	0.00681	0.00068	0.00233	0.06
Tenericutes	0.304	0.057	0.123	0.1086	0.095
TM7	0.608	0.51	0.258	0.1568	0.106
Verrucomicrobia	0.003	0.037	0.042	0.0326	0.451
Unclassified	8.54B	15.17A	6.72B	1.259	**<.001**

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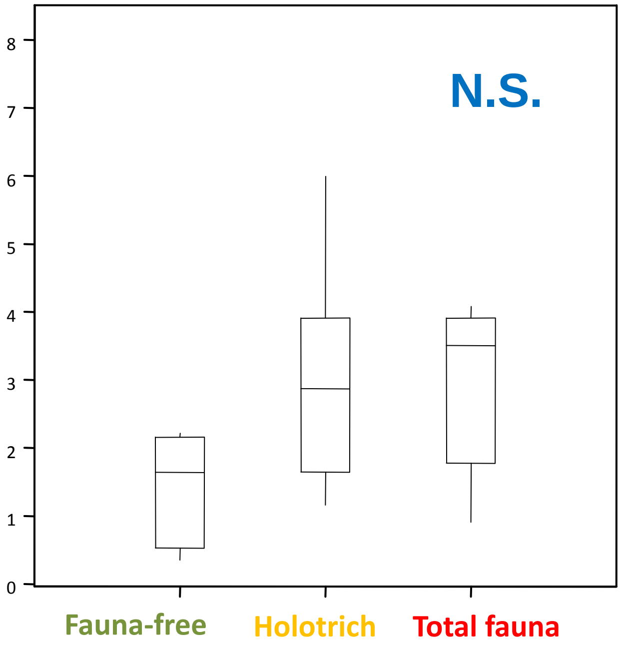
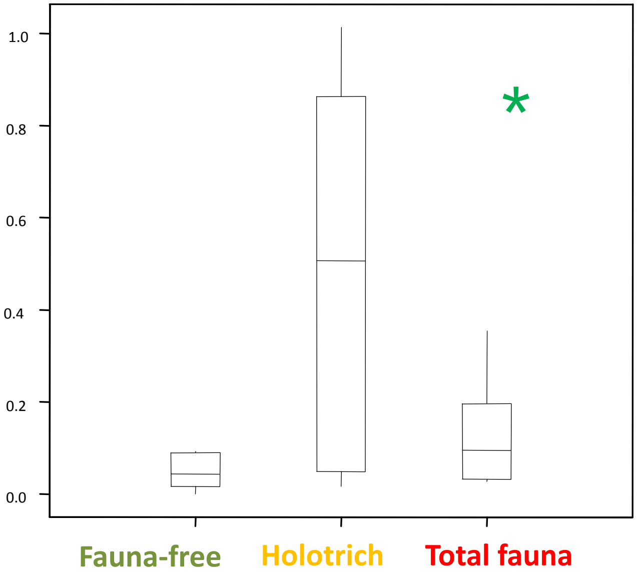
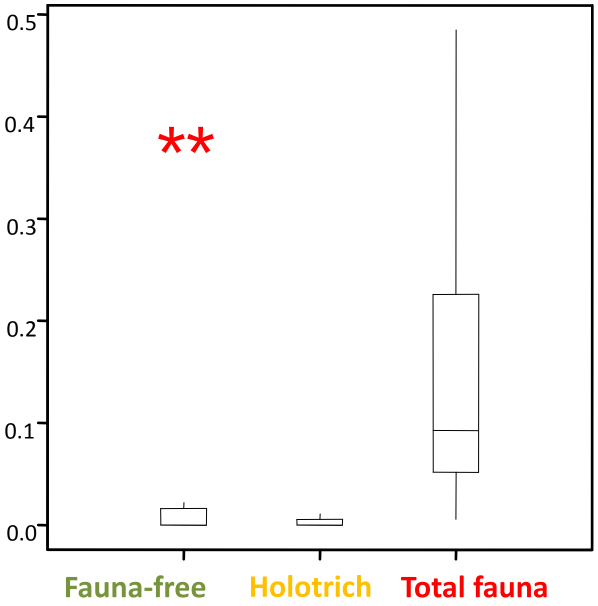
RESULTS



Fibrobacter



Ruminoccocus



Bacteroides

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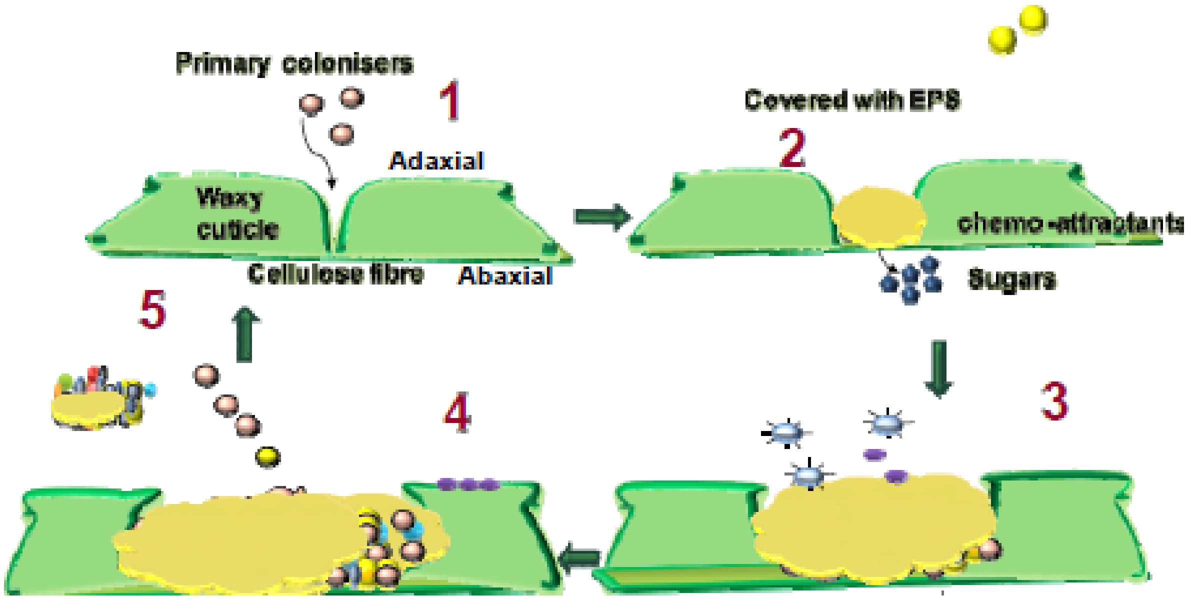
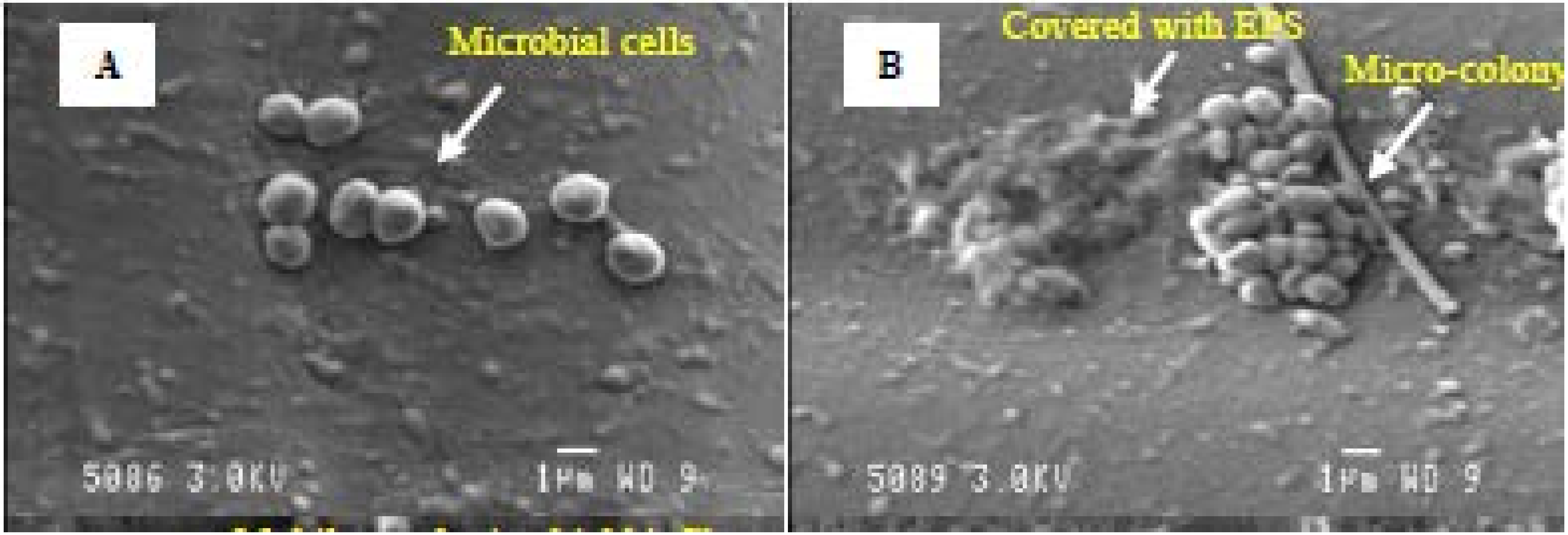

Primary colonisers
1
Adaxial
Waxy cuticle
Cellulose fibre
Abaxial
Covered with EPS
2
chemo-attractants
Sugars
3
4
5


A
Microbial cells
5006 3.0KV
1µm WD 9
B
Covered with EPS
Micro-colony
5009 3.0KV
1µm WD 9

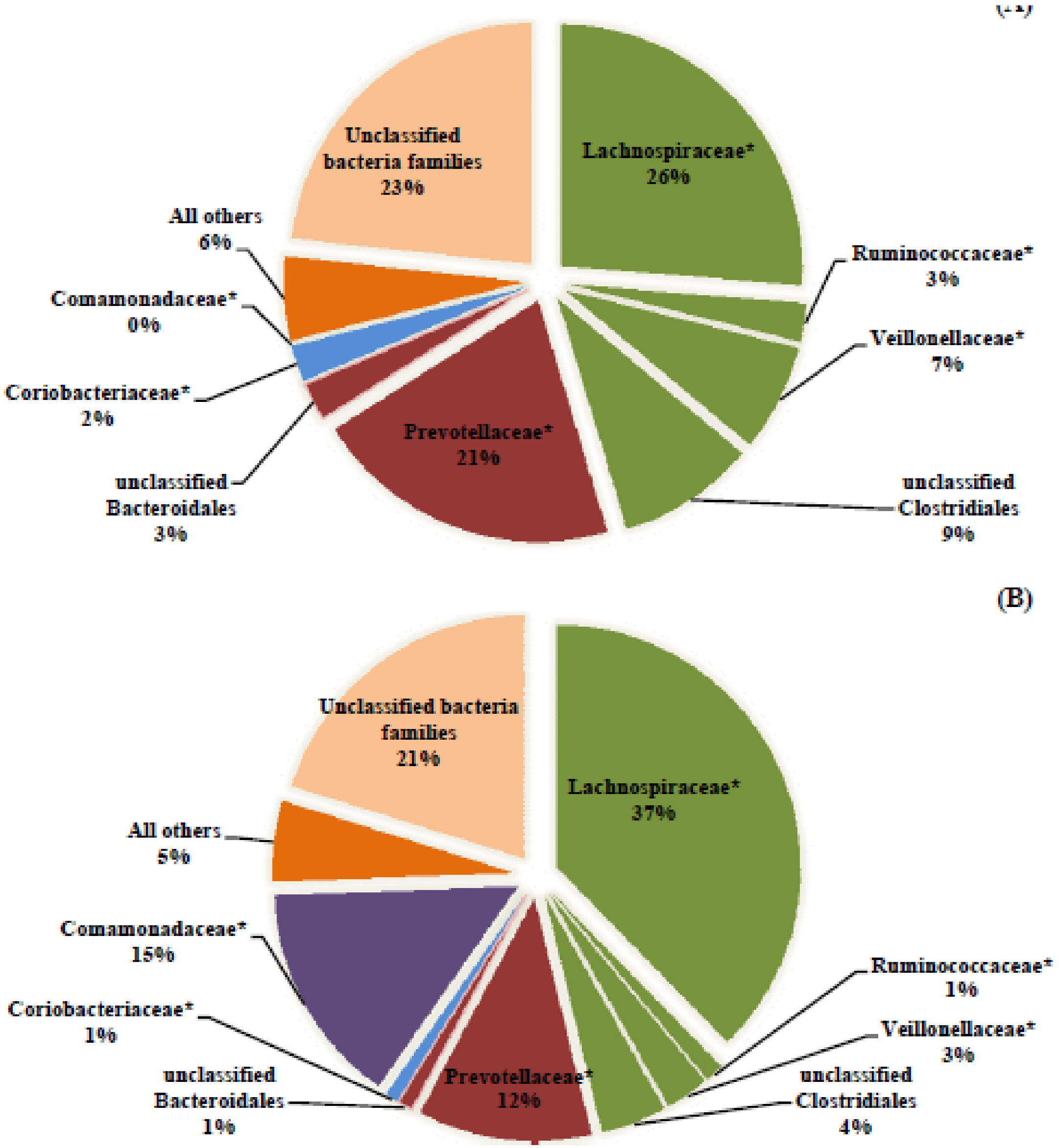


Figure 4.7 The results in percentage of assignments of families over 1 %, basing on 16S rRNA gene 454 pyrosequences. From **(A)** MC and **(B)** EPS communities at 2 h after anaerobic incubation. All others, grouping of the other observed families. The fraction pie colours represented the phylum which belong the assigned family: LGCGPB (green), CFB (red), HGCGPB (Blue), and Proteobacteria (purple). Ranks where sequences could not be assigned with a bootstrap confidence estimate above 90 % threshold are displayed under an artificial 'unclassified' taxon. * = significantly different at $P< 0.01$.

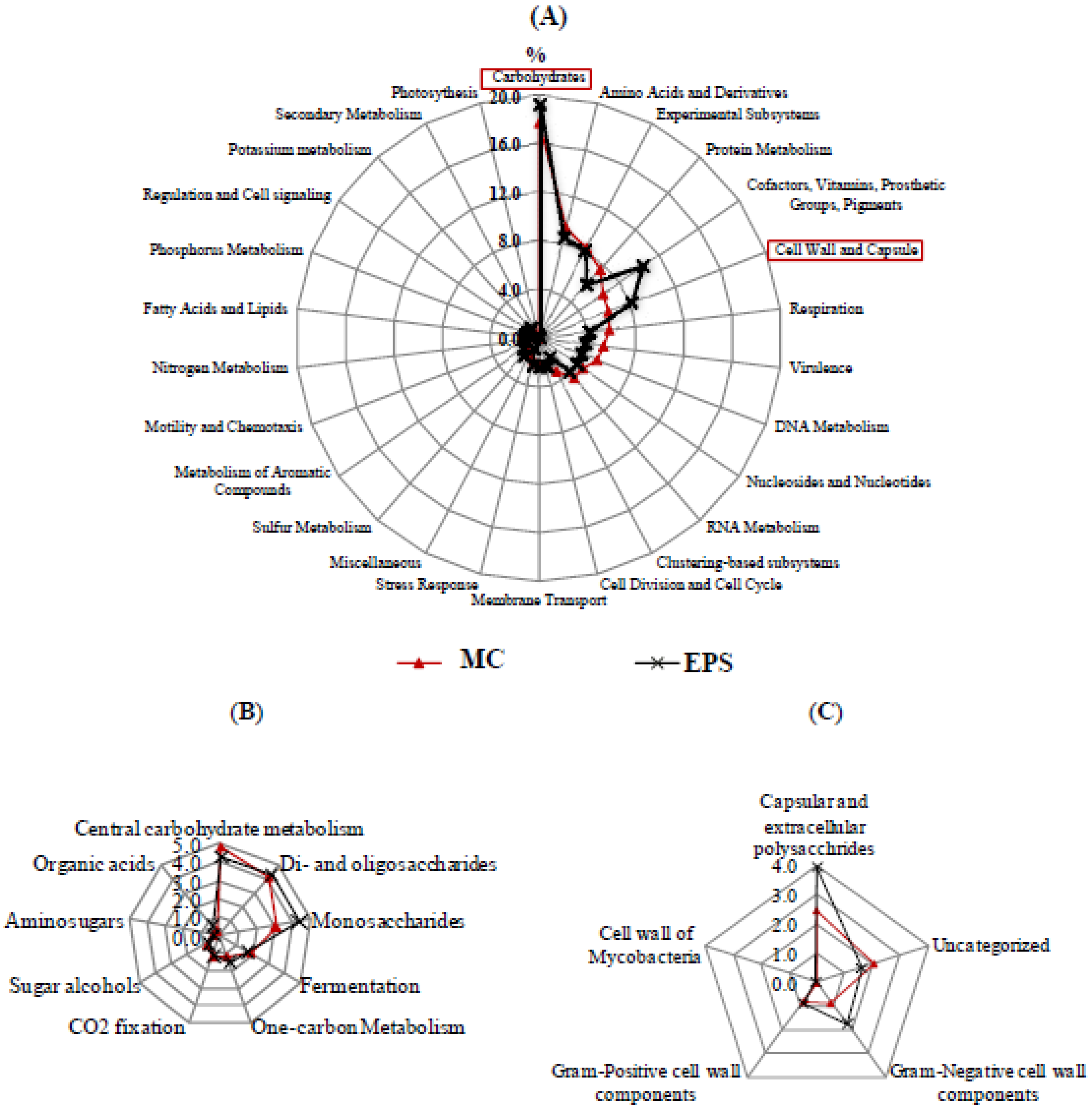
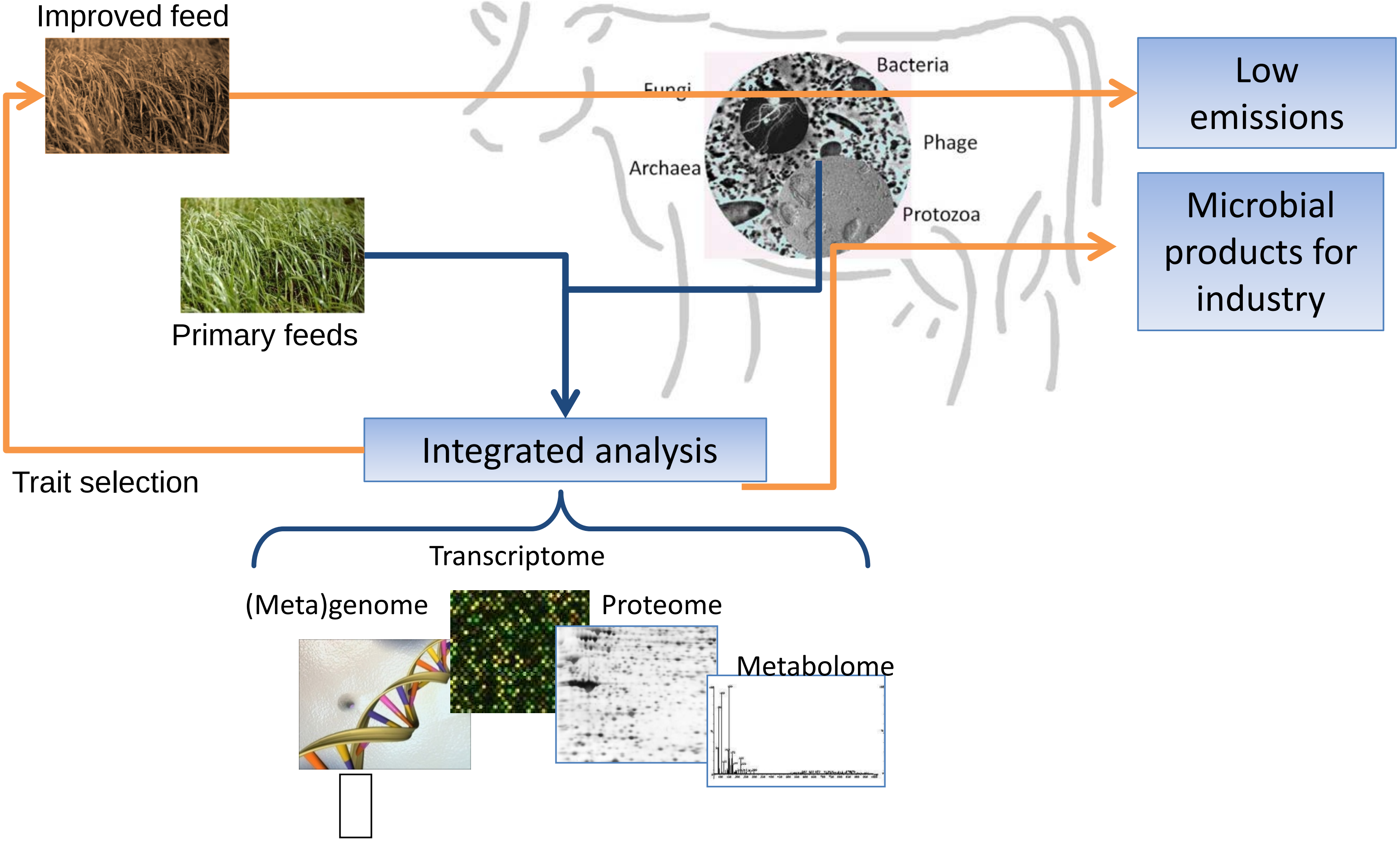


Figure 4.8 Genes connected to subsystems and their distribution in different categories from MC and EPS genomic 454 pyrosequences. **(A)** Comparison in percent of distribution patterns of subsystems between MC and EPS samples after 2 h anaerobic incubation. **(B)** The occurrence in percent of functional roles within the "Carbohydrates" subsystem. **(C)** The occurrence in percent of functional roles within the "Cell wall and capsule" subsystem. Black lines refer to EPS and red lines to MC DNA. EPS, extracellular polymeric substances. MC, attached microbial cells.

1. Apply next generation sequencing and –omics technologies at multiple platforms to collect data
2. Construct and utilise bioinformatic resources to increase depth of knowledge of functional rumen
3. Apply model to predict key forage traits and microbial community structures of complex systems



Improved feed

Primary feeds

Integrated analysis

Trait selection

Low emissions

Microbial products for industry

Transcriptome

(Meta)genome

Proteome

Metabolome

Thanks

- Neil McEwan
- David Yanez

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- Katherine Faulkner

- Helen Lloyd
- Pamela Heidt

- Eric Pinloche
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- Gabriel de la Fuente
- Kenton Hart
- Eli Saetnan
- Eva Ramos
- Toby Wilkinson

- Kirsty Douglas
- Kate Waddams
- Jolien Veneman

Questions