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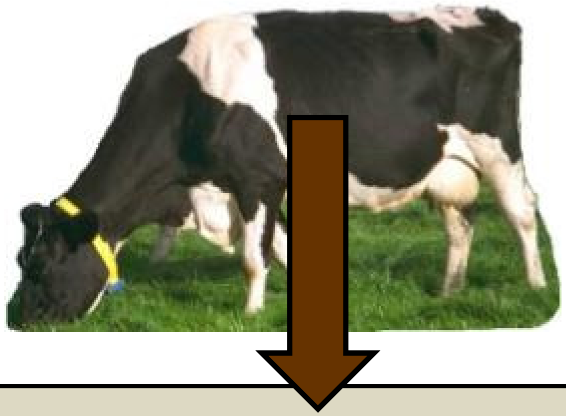
UNIVERSITY

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

Understanding the rumen metagenome

Jamie Newbold



Bacteria

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

Anaerobic Fungi

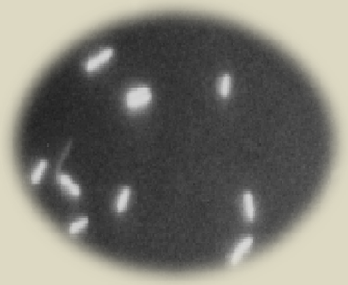
~30 species
$<10^{5}$ cells/ml

Ciliate Protozoa

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

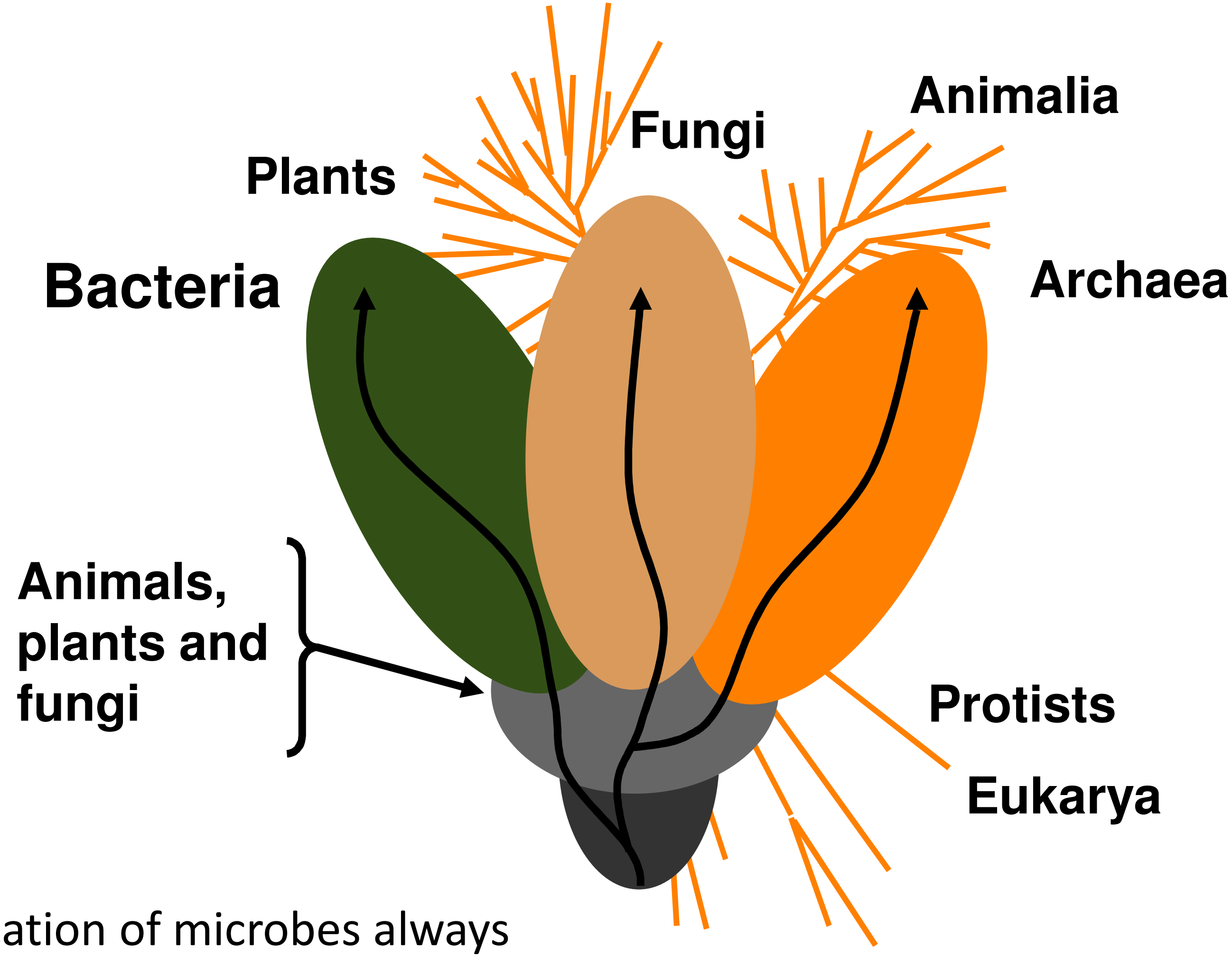
Methanogenic Archaea

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



The rumen microbiota is essential for ruminants to effectively utilise dietary material.

DISCOVERING THE WORLD OF THE “*UNCULTURED*”



Plants

Fungi

Animalia

Bacteria

Archaea

Animals, plants and fungi

Protists

Eukarya

Observation

- The direct identification of microbes always reveals that there are more microorganisms than those we can isolate as pure cultures

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:

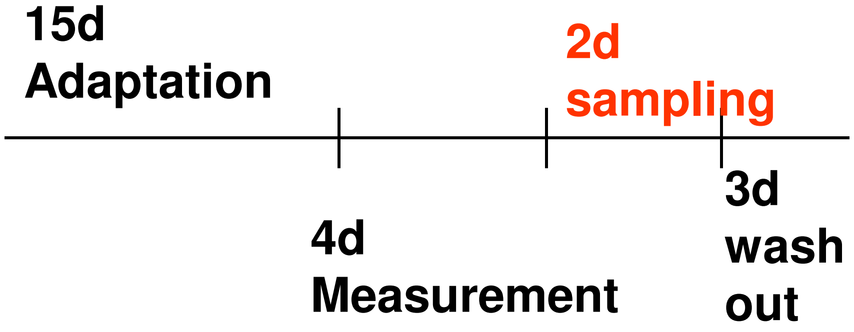
L0 = 0 ; L1 = 0.5g/d ; L2 = 5g/d

Biosaf Sc 47 : Lesaffre Feed additives

Composition of the diet (acidogenic):

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

Period design

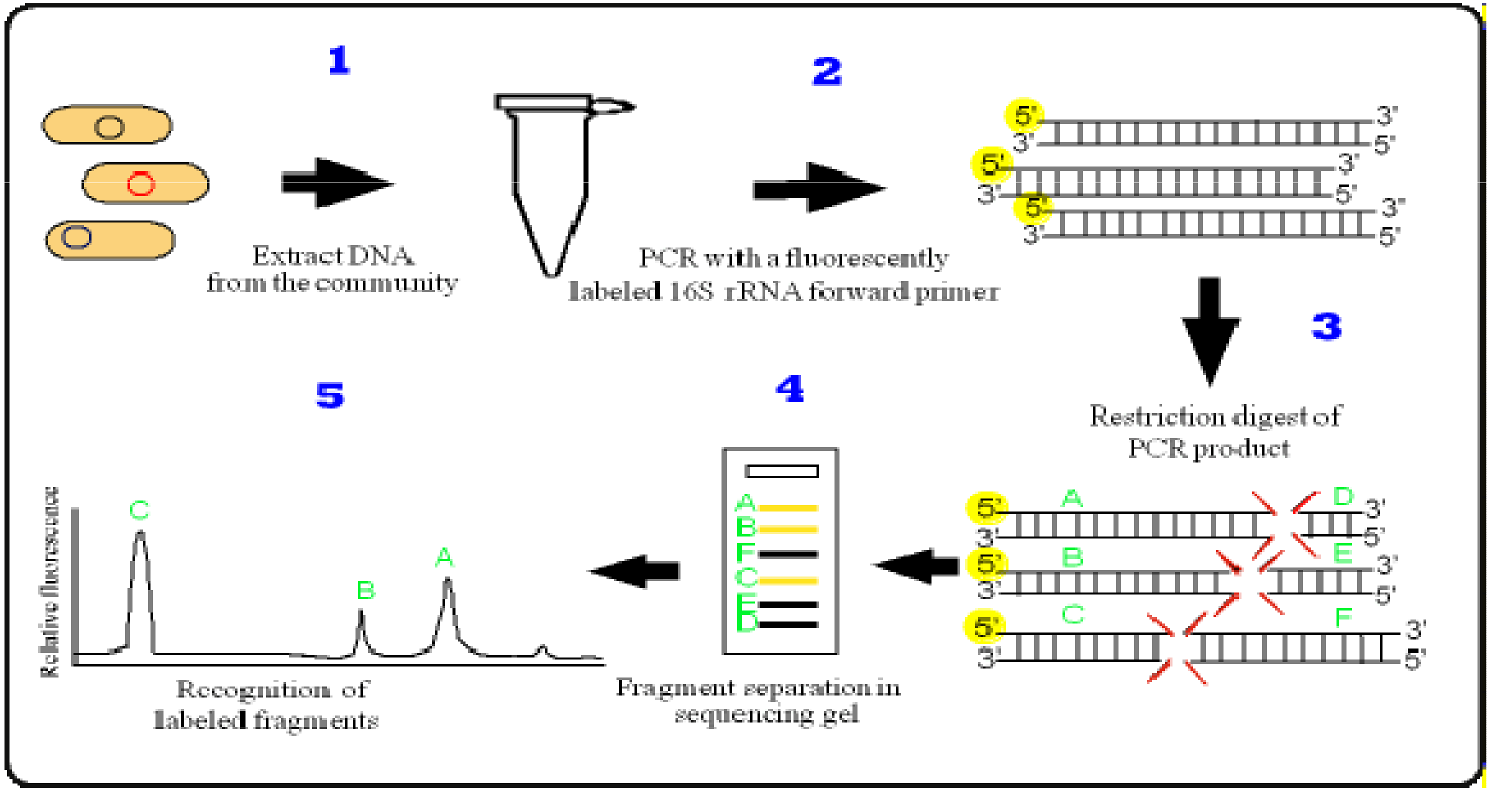

15d Adaptation

2d sampling

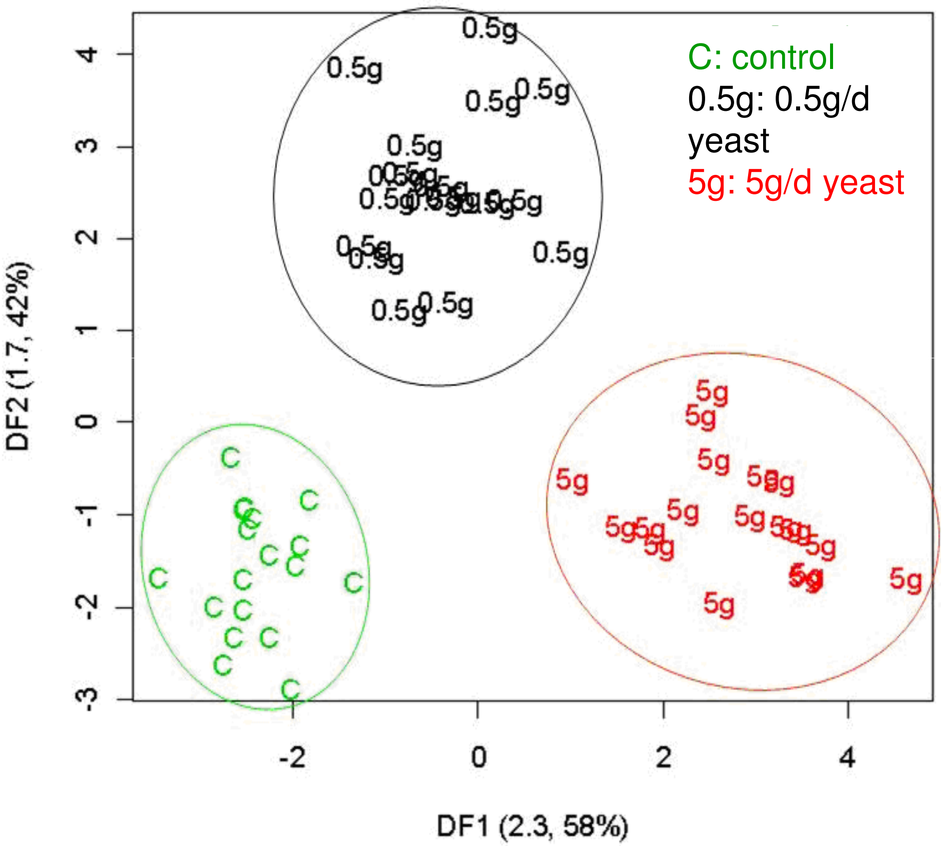
4d Measurement

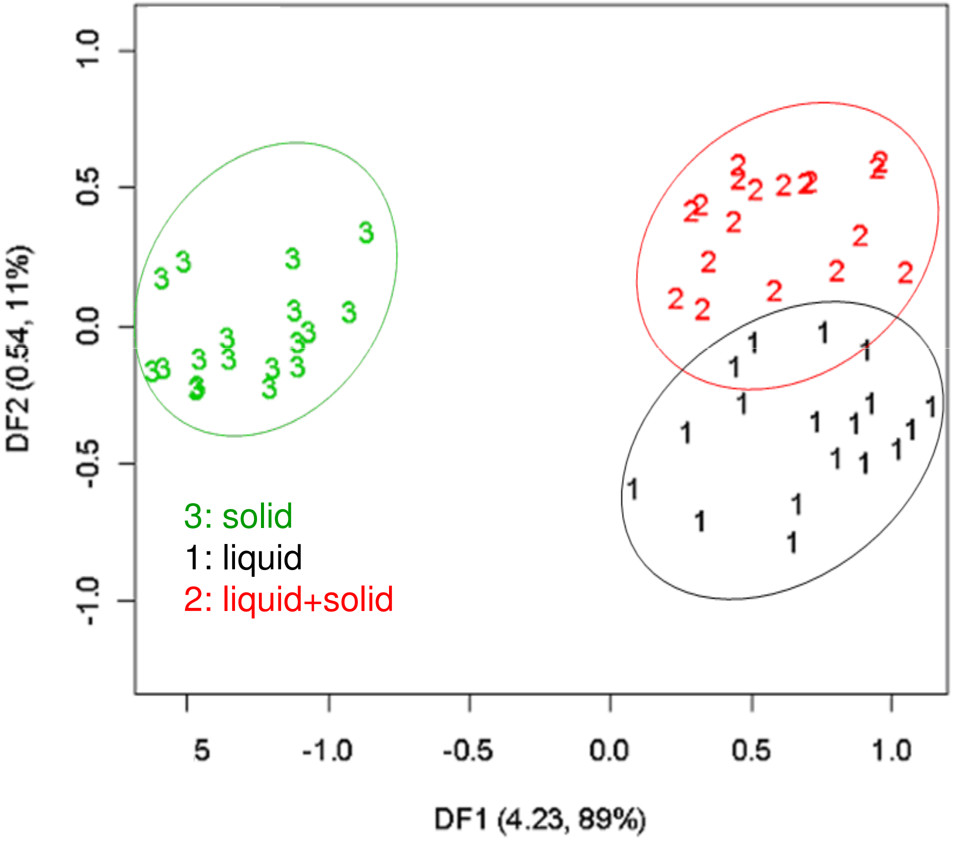
3d wash out

T-RFLP : Terminal restriction fragment length polymorphism

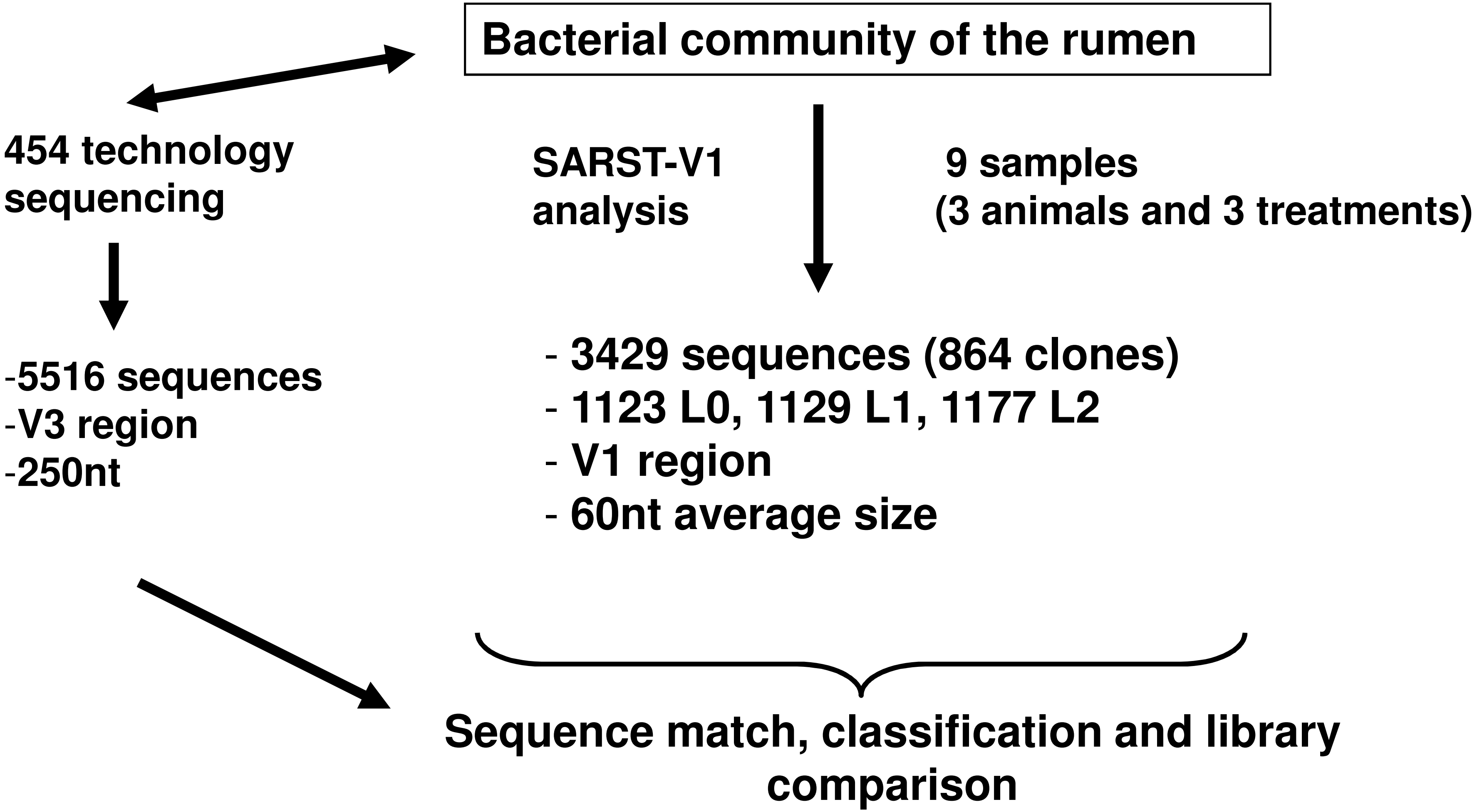


Linear Discriminant Analysis (LDA)





Analyse of the 16s rDNA sequences libraries



Bacterial community of the rumen

454 technology sequencing

-5516 sequences
-V3 region
-250nt

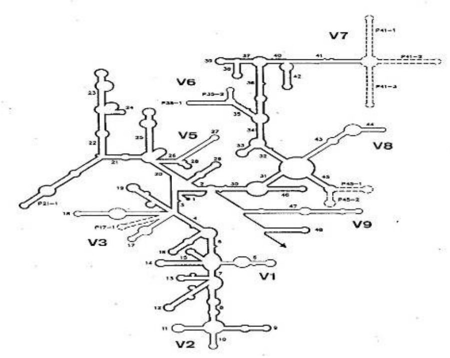
SARST-V1 analysis

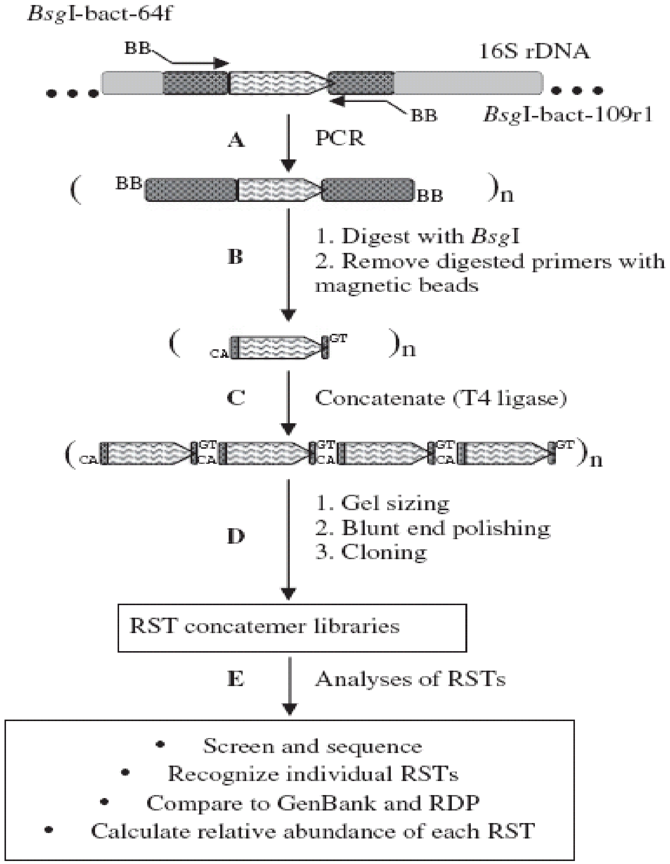
9 samples (3 animals and 3 treatments)

- **3429 sequences (864 clones)**
- **1123 L0, 1129 L1, 1177 L2**
- **V1 region**
- **60nt average size**

Sequence match, classification and library comparison

SARST-V1:serial analysis of ribosomal sequence tags





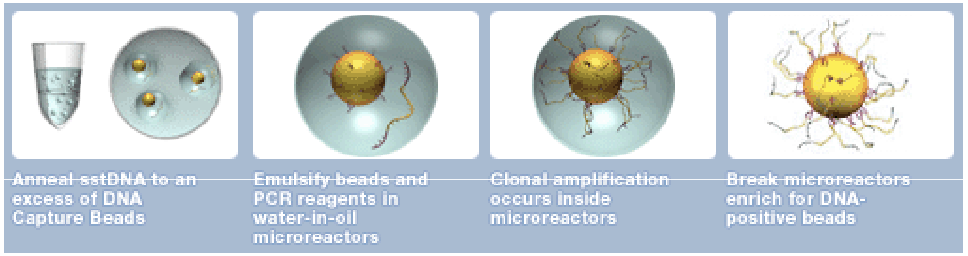
- A technique for the semi quantative analysis of complex bacterial populations (Yu and Morrisson, 2006).
- Each construct has circa 6-9 unique sequences we have sequenced 100 per animal
- Although small the recovered sequences allow phylogentic analysis

Pyrosequencing

5-100ng DNA

whole genome amplification

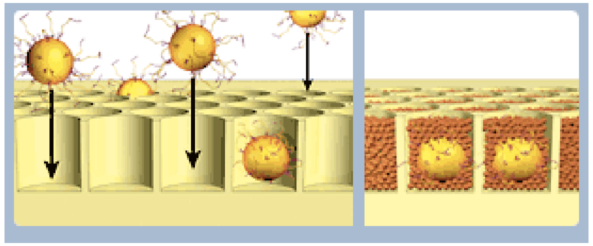
2-5 µg DNA

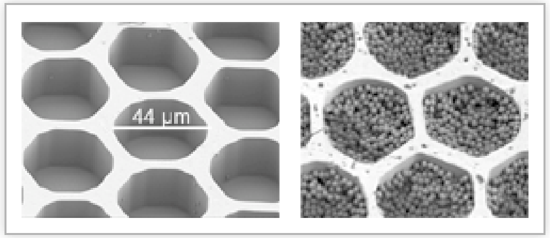

Anneal sstDNA to an excess of DNA Capture Beads

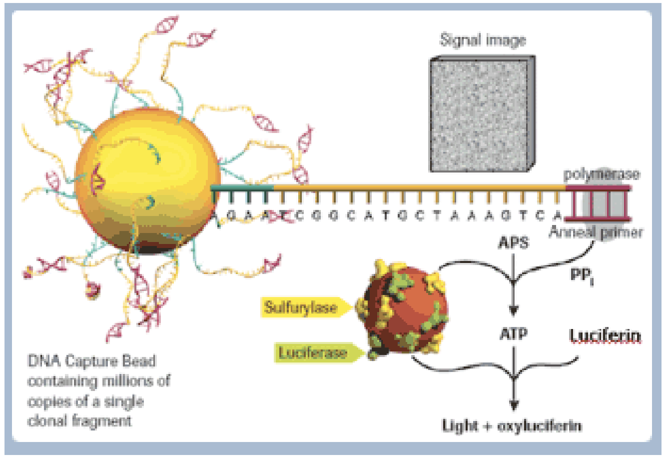
Emulsify beads and PCR reagents in water-in-oil microreactors

Clonal amplification occurs inside microreactors

Break microreactors enrich for DNA-positive beads

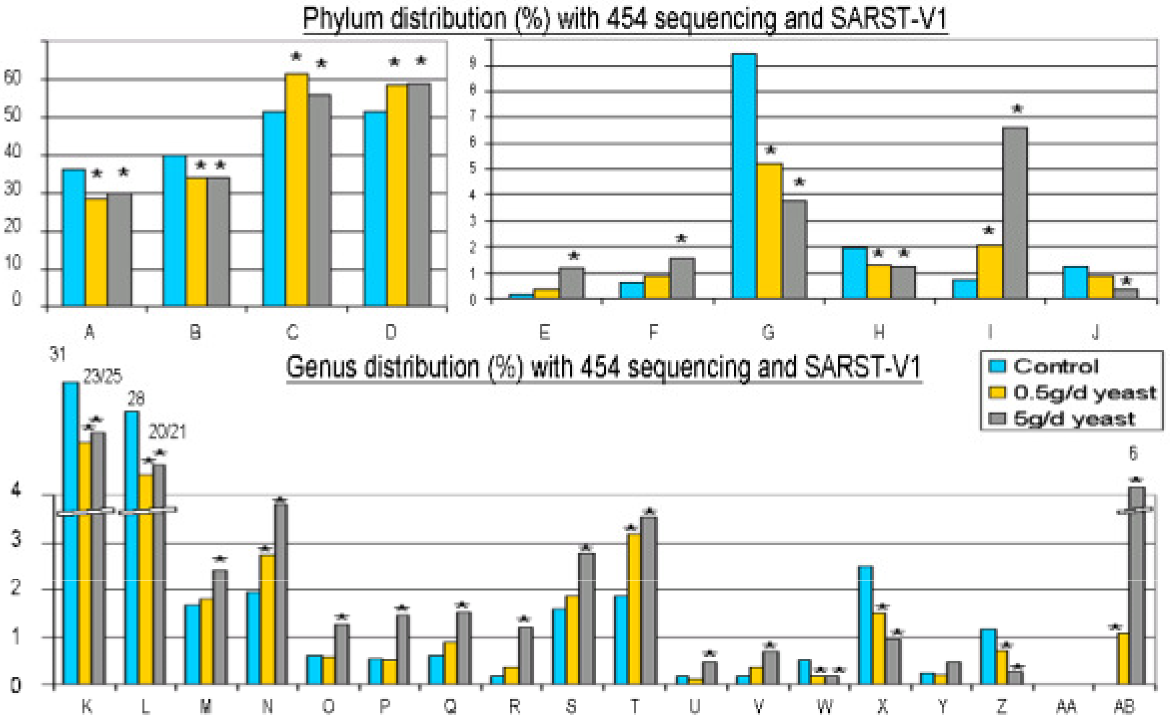




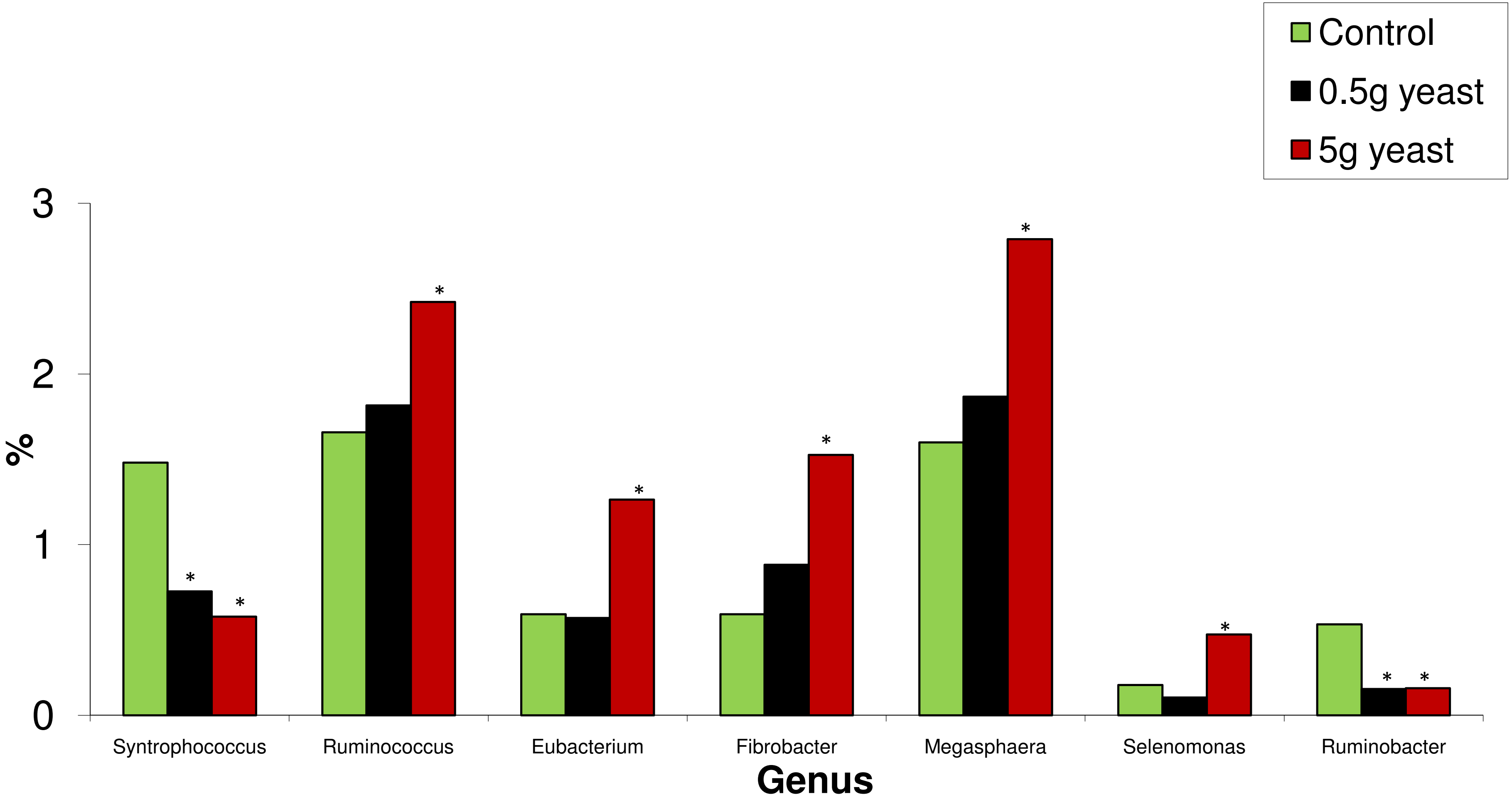


DNA Capture Bead containing millions of copies of a single clonal fragment

www.454.com



Phylum	**SARST**	**454**
Bacteroidetes	A	B
Firmicutes	C	D
Fibrobacteres	E	F
Proteobacteria	G	H
Actinobacteria	I	J
GENUS	**SARST**	**454**
Prevotella	K	L
Ruminococcus	M	N
Eubacterium	O	P
Fibrobacter	Q	R
Megasphaera	S	T
Selenomonas	U	V
Ruminobacter	W	X
Pseudobutyrivibrio	Y	Z
Bifidobacterium	AA	AB



* Significantly different from control

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 = CONTROL
L1 = SODIUM BICARBONATE
L2 = YEAST

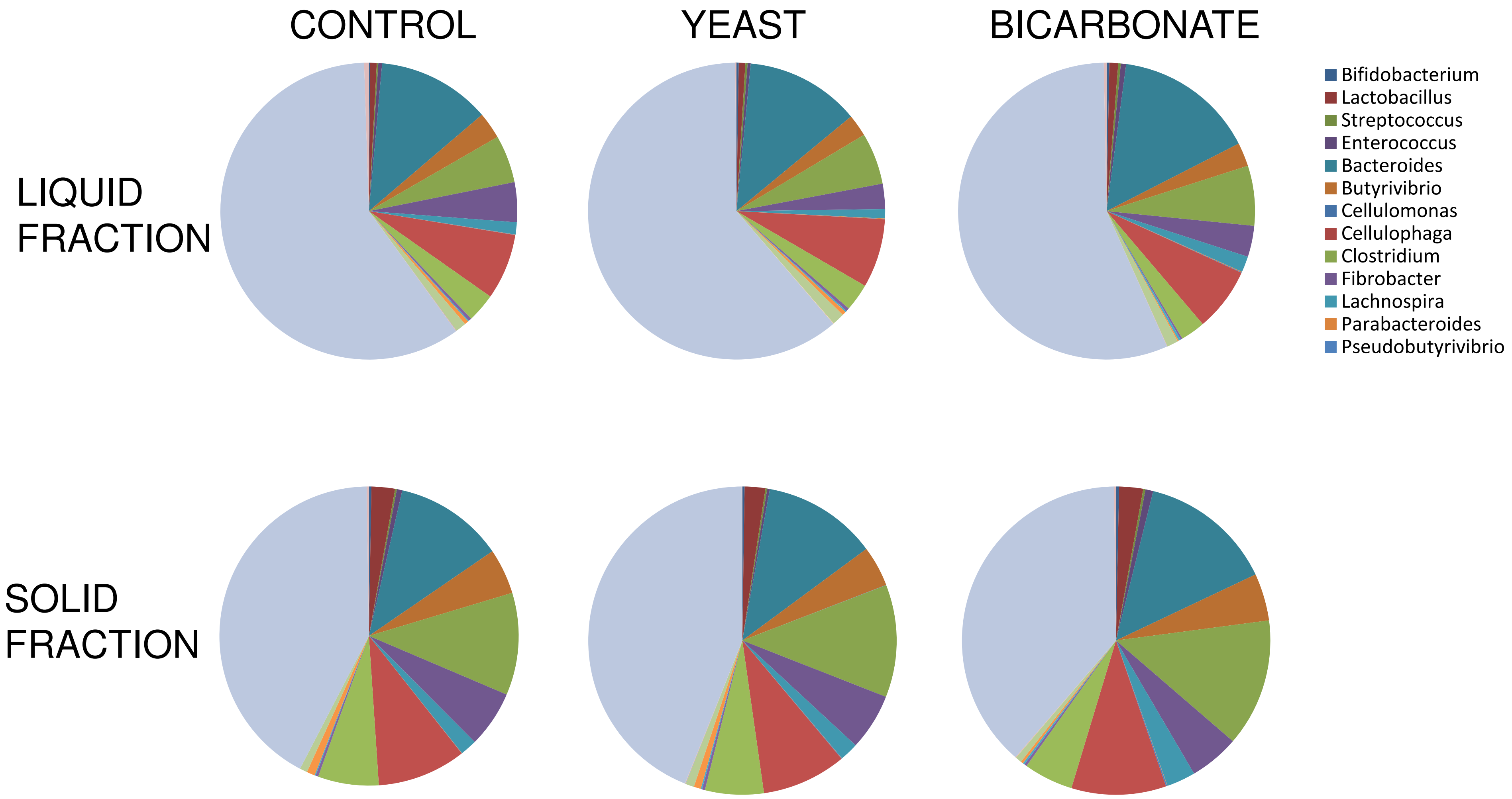
450,000 sequences generated
350,000 sequences analysed (removed if inferior to 150nt and more than 3N)

3 steps process

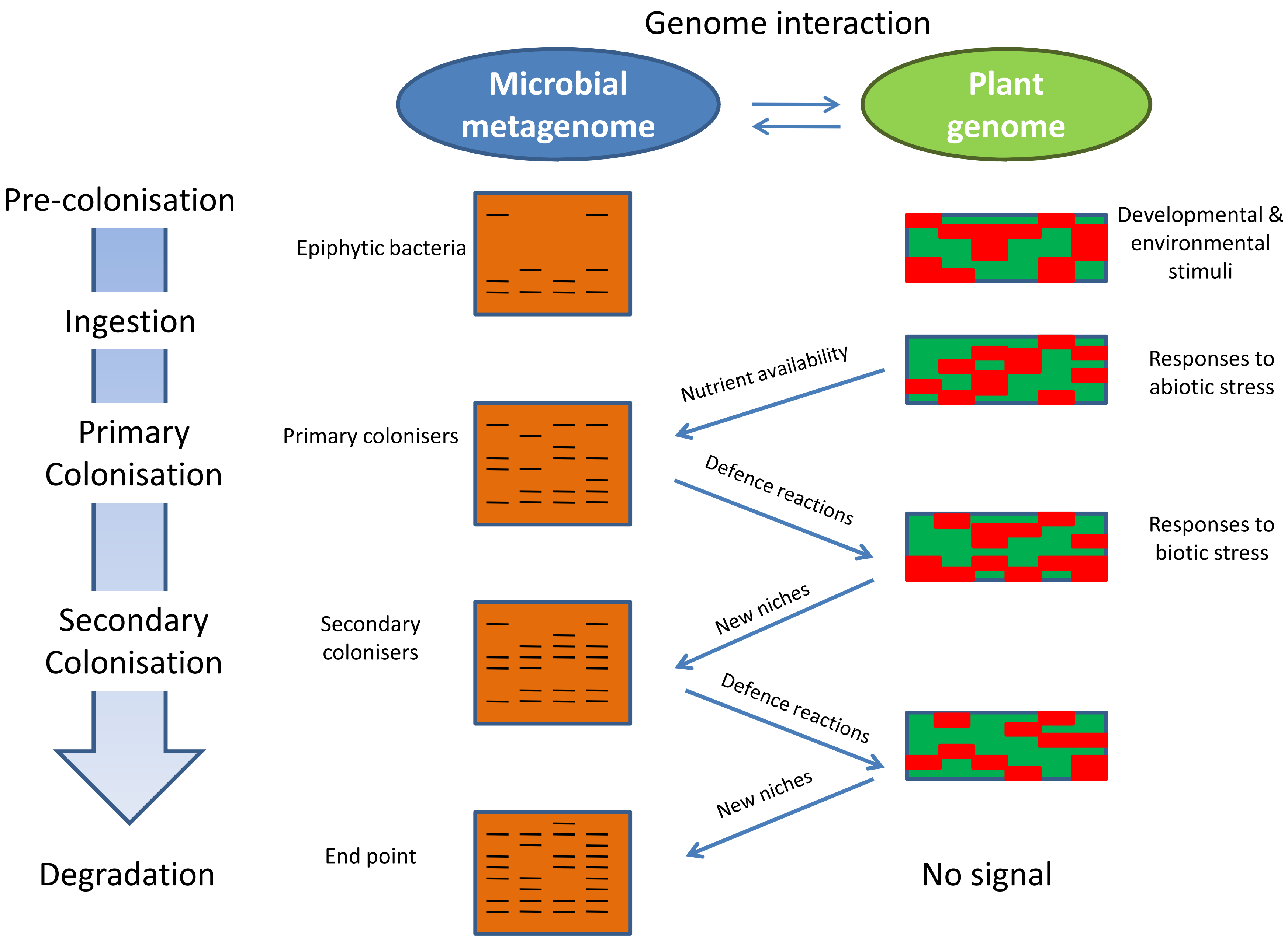
1. Pair wise alignment with a gut database of the same region that we sequenced (135,000 sequences)
   generated 9500 different matches

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)

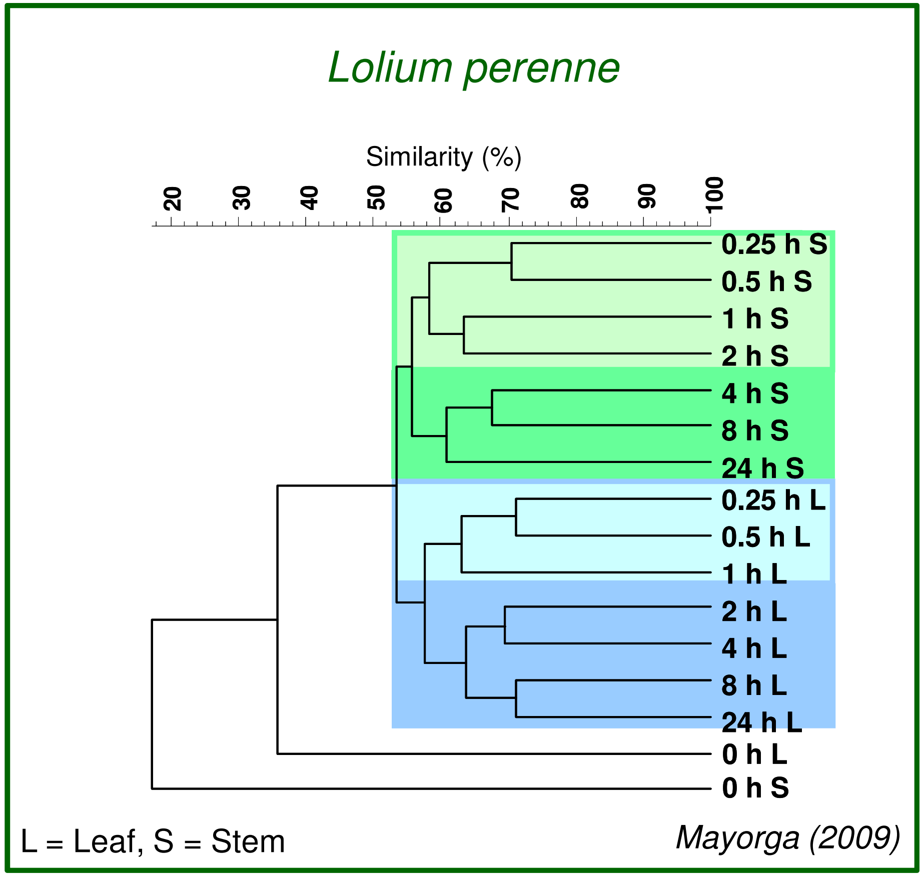
3. Report the bacterial composition (relative abundance in %)



	Liquid fraction			Solid fraction		
Metabolism	Control	Bicarbonate	Yeast	Control	Bicarbonate	Yeast
Acid production	0.95^{b}	1.47^{a}	1.06^{b}	2.2^{b}	2.45^{a}	1.84^{c}
Fibre degrading	24.14^{b}	27.14^{a}	24.46^{b}	32.13^{b}	35.52^{a}	33.09^{b}
Lactate user	1.39^{a}	1.27^{a}	1.62^{b}	1.3^{b}	0.92^{a}	1.4^{b}
Multi-substrate	39.37^{a}	38.89^{a}	42.86^{b}	26.03^{b}	24.38^{a}	28.4^{c}
Starch user	0.34^{b}	0.2^{a}	0.1^{a}	0.16^{a}	0.14^{a}	0.06^{b}


Genome interaction
Microbial metagenome
Plant genome
Pre-colonisation
Ingestion
Primary Colonisation
Secondary Colonisation
Degradation
Epiphytic bacteria
Primary colonisers
Secondary colonisers
End point
Developmental & environmental stimuli
Responses to abiotic stress
Responses to biotic stress
No signal
Nutrient availability
Defence reactions
New niches
Defence reactions
New niches

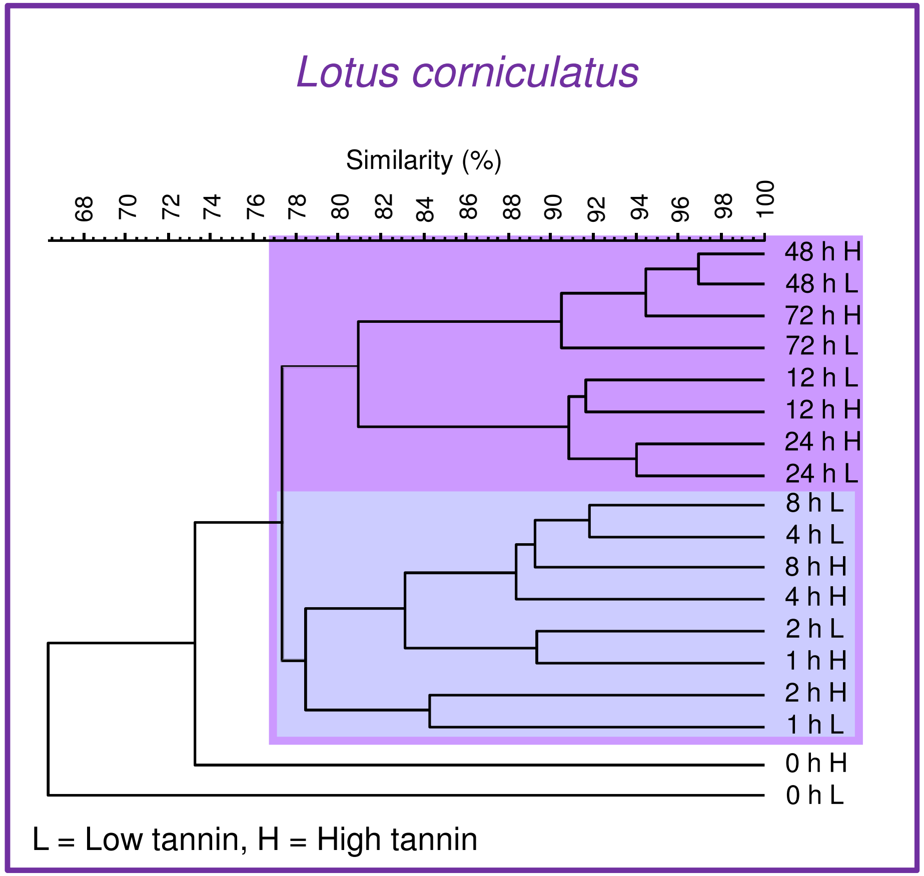
What affects colonising microbes?



Lolium perenne

L = Leaf, S = Stem

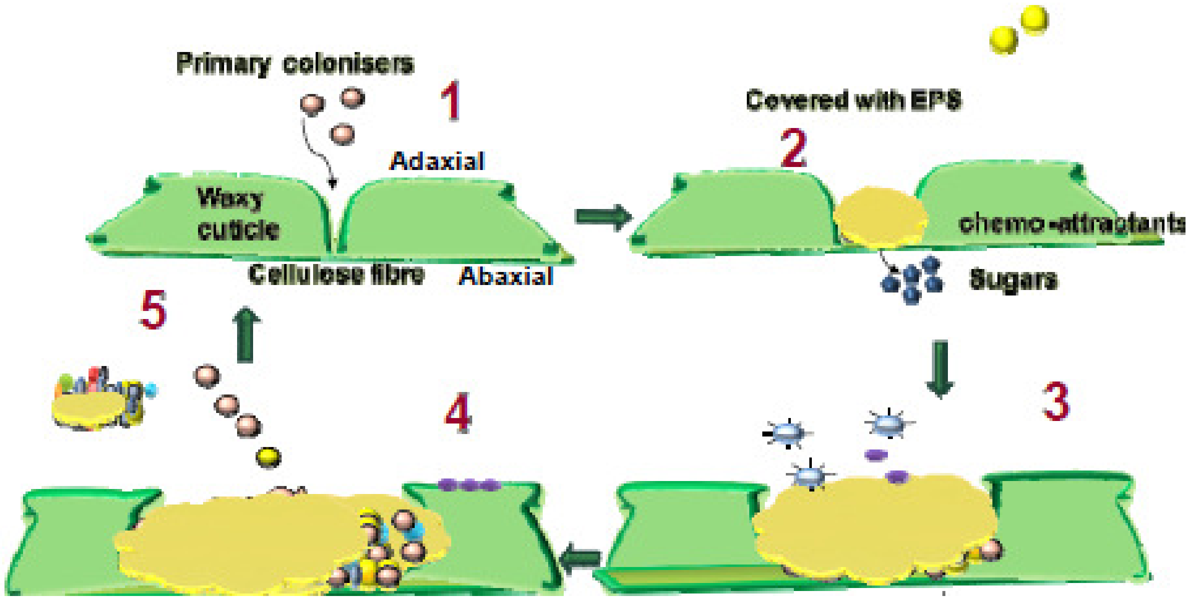
Mayorga (2009)

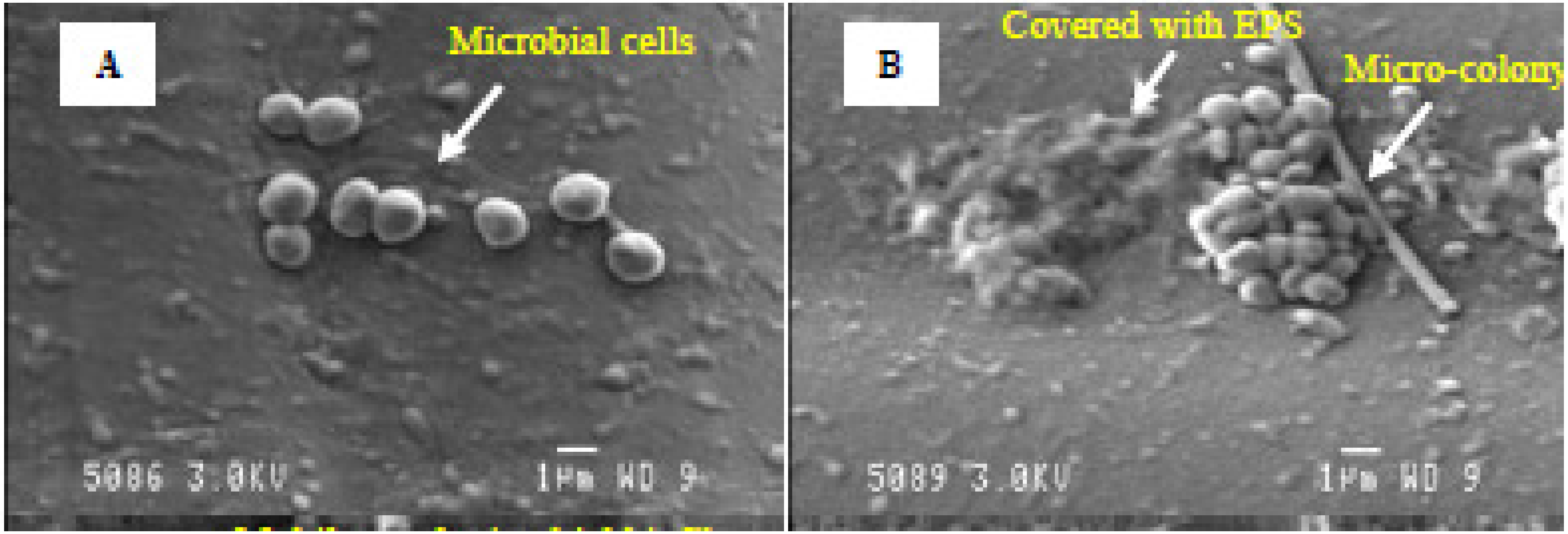


Lotus corniculatus

L = Low tannin, H = High tannin

Time but depends on the plant material.
Part of the plant material (leaf/stem/surface).


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


A
Microbial cells
5086 3.0KV
1μm WD 9
B
Covered with EPS
Micro-colony
5089 3.0KV
1μm WD 9

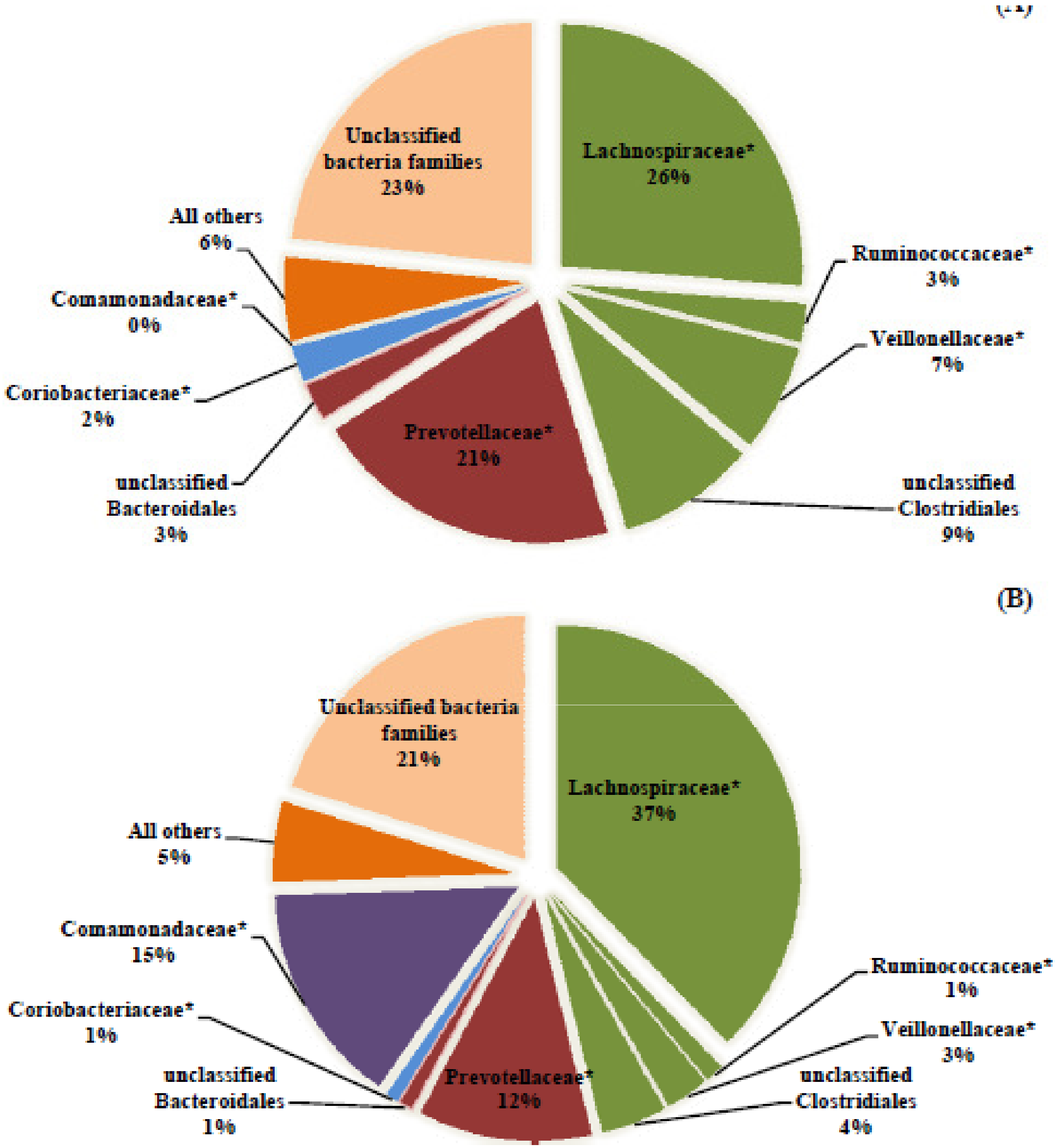
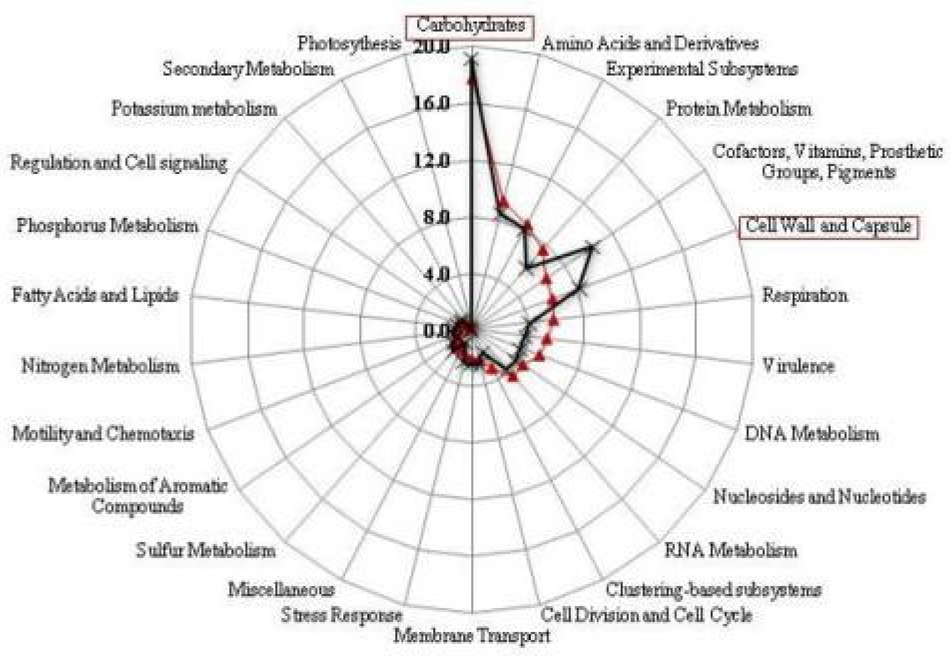


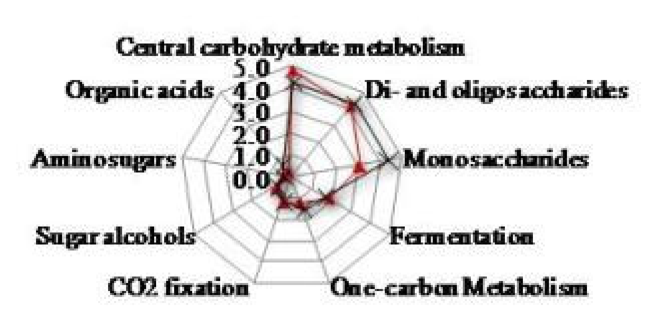
Figure 4.7 The results in percentage of assignments of families over 1 %, basing on 16S rRNA gene 454 pyrosequences. From **(A)** MC and **(B)** EPS communities at 2 h after anaerobic incubation. All others, grouping of the other observed families. The fraction pie colours represented the phylum which 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 $P < 0.01$.

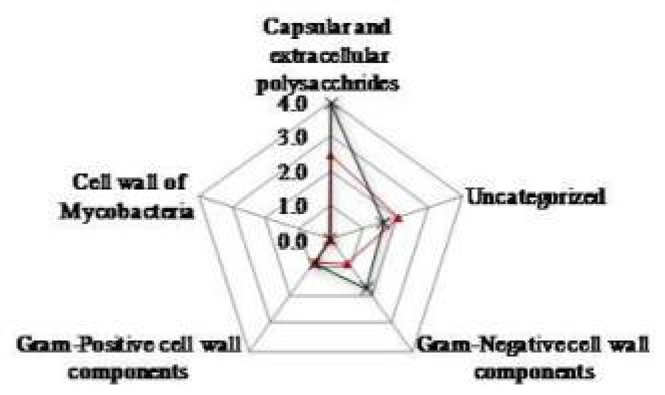
Microbial cell DNA & EPS DNA: Similar functionality?

Gene Subsystem Distributions

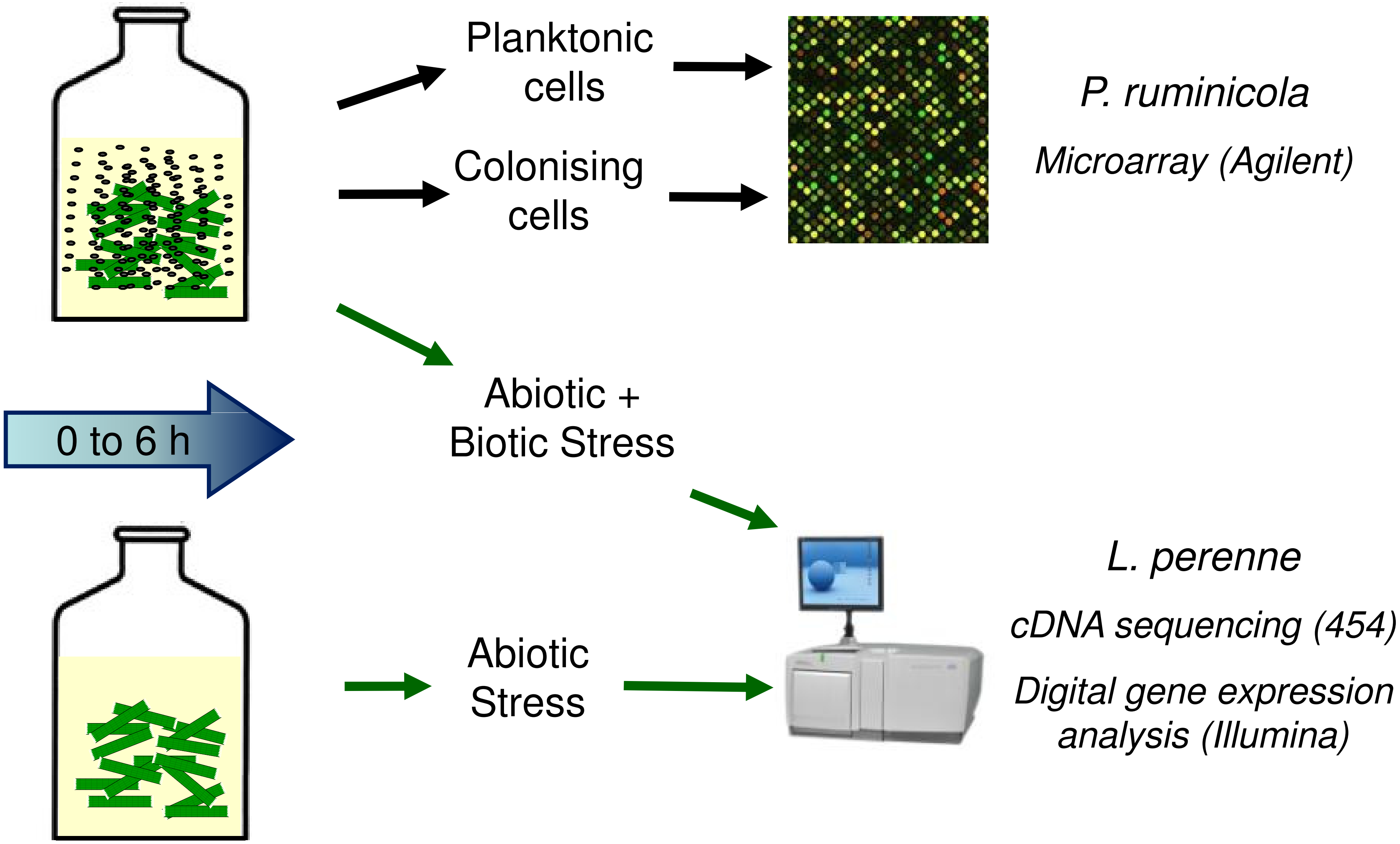
Microbial cells; EPS



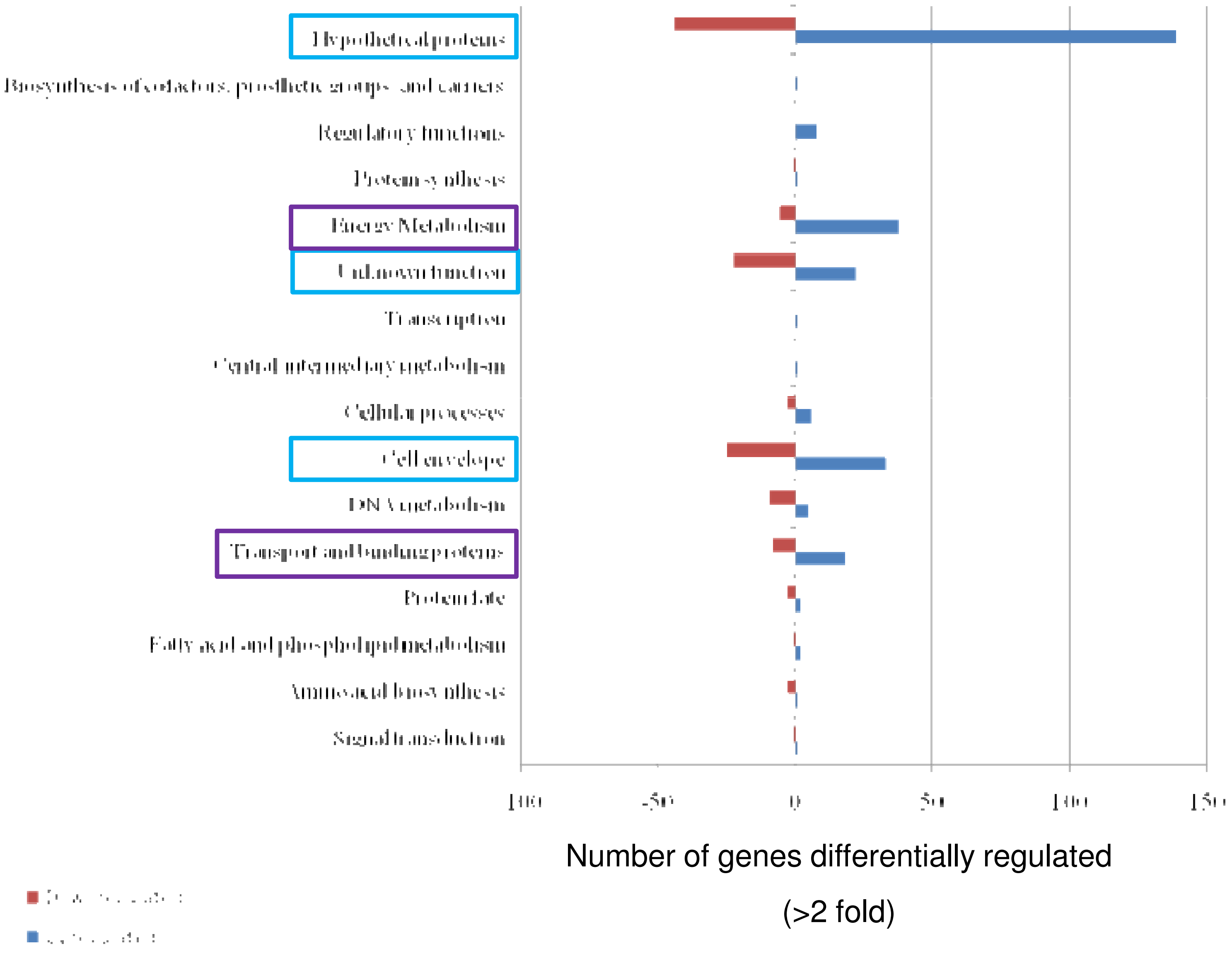




Broadly similar DNA functionality in EPS and microbial cells, even though differences in bacterial composition.


Planktonic cells
Colonising cells
P. ruminicola
Microarray (Agilent)
0 to 6 h
Abiotic + Biotic Stress
Abiotic Stress
L. perenne
cDNA sequencing (454)
Digital gene expression analysis (Illumina)

Planktonic cells (6 h) *vs.* inoculum (0 h)



RUMEN ENZYMES

EXPLORATION OF BOVINE RUMEN MICROFLORA

sampling ⇒ DNA extraction ⇒ library generation ⇒ screening, sequencing ⇒ characterize, optimize ⇒ apply

VOLUME 7 NUMBER 12 DECEMBER 2005 www.envmicro.com ISSN 1462-2912

environmental microbiology

New photosynthetic reaction centre organizations

Archaea mediating anaerobic oxidation of methane

New methopterin-linked C1 transfer genes

Special Issue
Metagenomics Part B

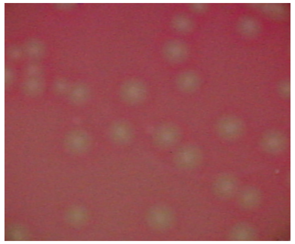
Guest editors:
Christa Schleper, Matthew Holden and
Juan Luis Ramos

Blackwell Publishing Blackwell Synergy

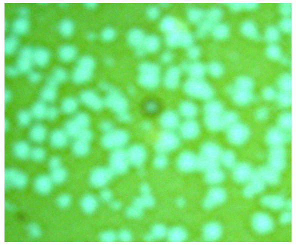
RESULTS:

- 13 novel esterases
- 9 endoglucanases
- 1 cyclodextrinase
- 1 laccase
- 25 glucosyl hydrolases

Cellulases



Esterases



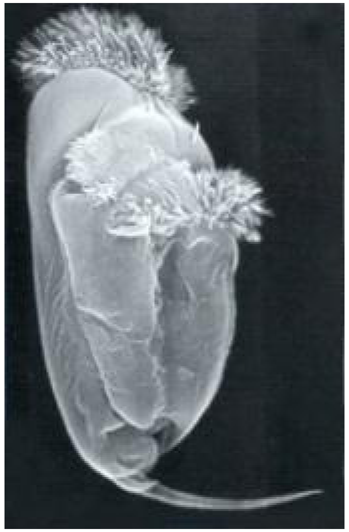
Novel hydrolase diversity retrieved from a metagenome library of bovine rumen microflora

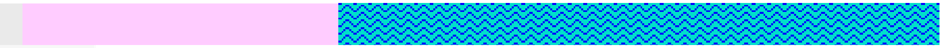
	Enzyme	MW	MWtotal	Subunits	pH	Temperature
ESTERASES	R.01	30956.71	92870	3	8.0-8.5	50
	R.13	31212.32	31212	1	8.0	50
	R.17	29649.20	237192	8	8.0	60
	R.27	28182.84	112728	4	8.0-9.5	60
	R.34	25811.36	25811	1	9.5	50
	R.35	6177.98	6200	1	10.0	70
	R.40	75502.00	453012	6	8.5-10.0	65
	R.41	34874.67	34800	1	7.5	50
	R.43	32961.62	32961	1	9.0	55
	R.44	74747.17	74748	1	4.0-11.0	70
	R.45	56508.72	113016	2	8.0-9.0	60
CELLULASES	RR.01-1	58301.81	233204	4	5.6	60
	RR.01-2	43010.25	172040	4	6.0	70
	RR.02-1	40221.46	160884	4	4.5	60
	RR.02-2	58686.32	234744	4	4.5	60
	RR.06	56508.72	113016	2	5.6-6.0	70
	RR.08	34555.39	69110	2	5.5	55
	RR.13	42035.36	42000	1	5.6	60
	RR.14	60652.05	181956	3	6.0	70
	RR.20	36535.99	36535	1	5.6-6.0	60
	RR.22	53243.82	53000	1	3.5-5.6	70
	RR.23	26832.10	27000	1	3.5-10.0	70

- **Highly efficient hydrolases**
- **Wide pH-profile**
- **High chemical stability**
- **High enantioselectivities**
- **New protein structures**

European Patent EP04015920.4.
European Patent EP04015680.4.
***Environ Microbiol* (2005) 7(12): 1996-2010**

Fibrolytic enzymes from *rumen protozoa*

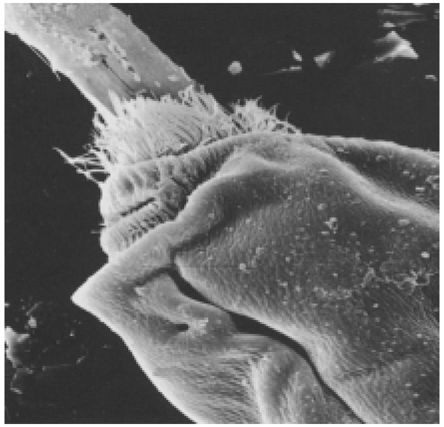


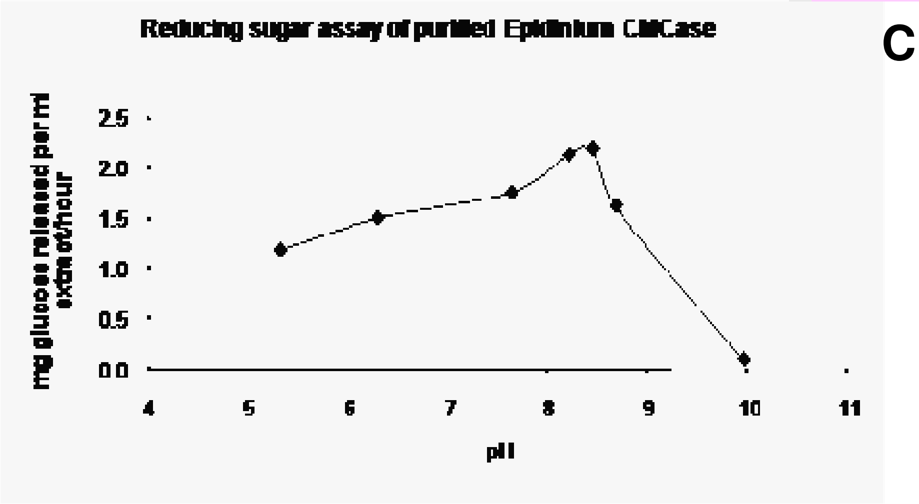


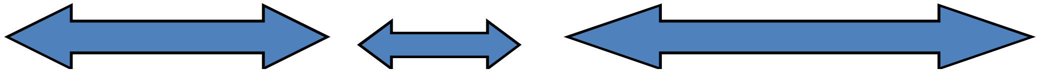
CD xylanase GH11

CD xylanase GH11

CBM22 **CD xylanase GH10**







ACID **NEUTRAL** **ALKALI**

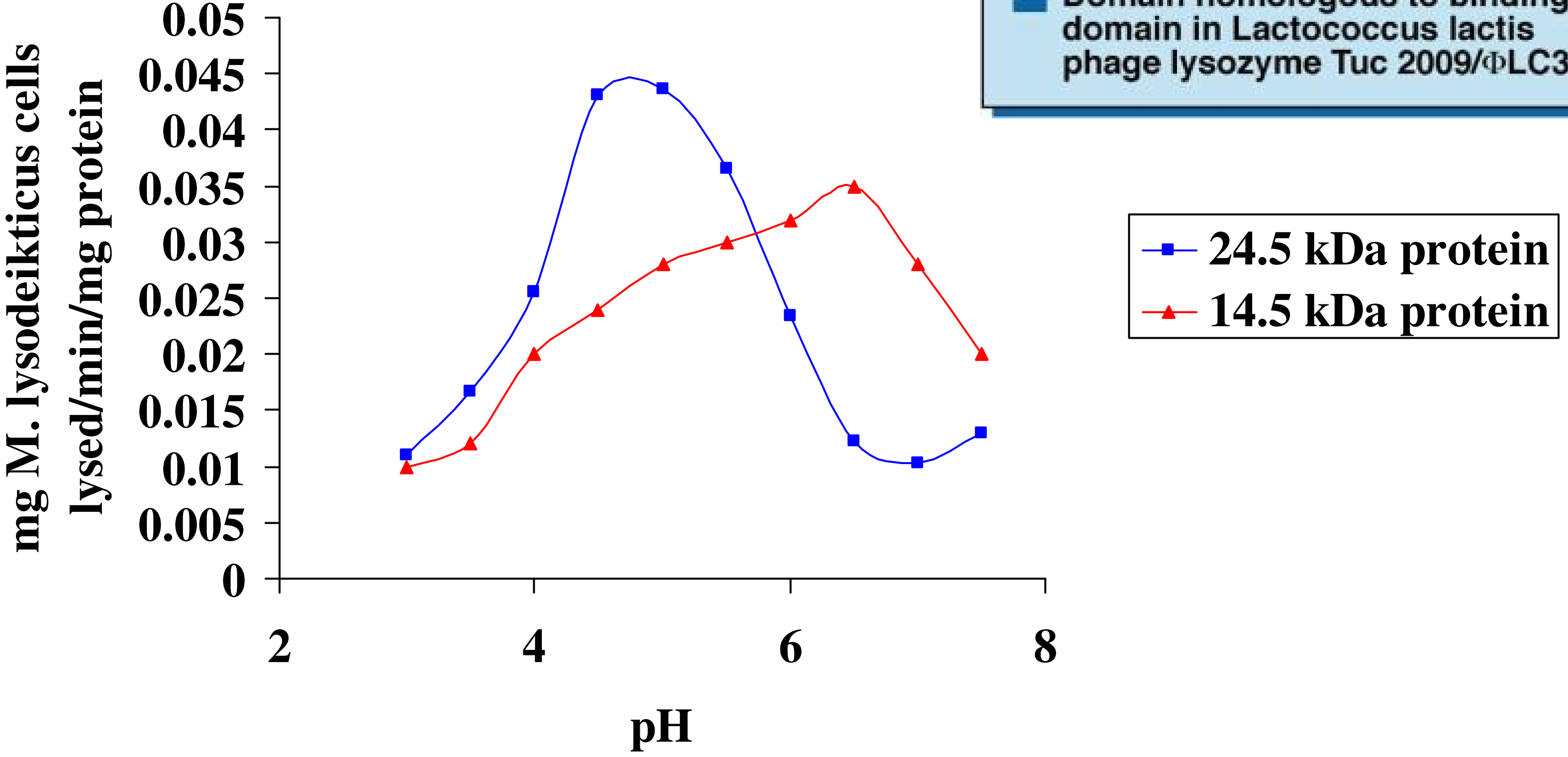
Lysozyme like protein cloned from E. caudatum

🟥 Signal peptide

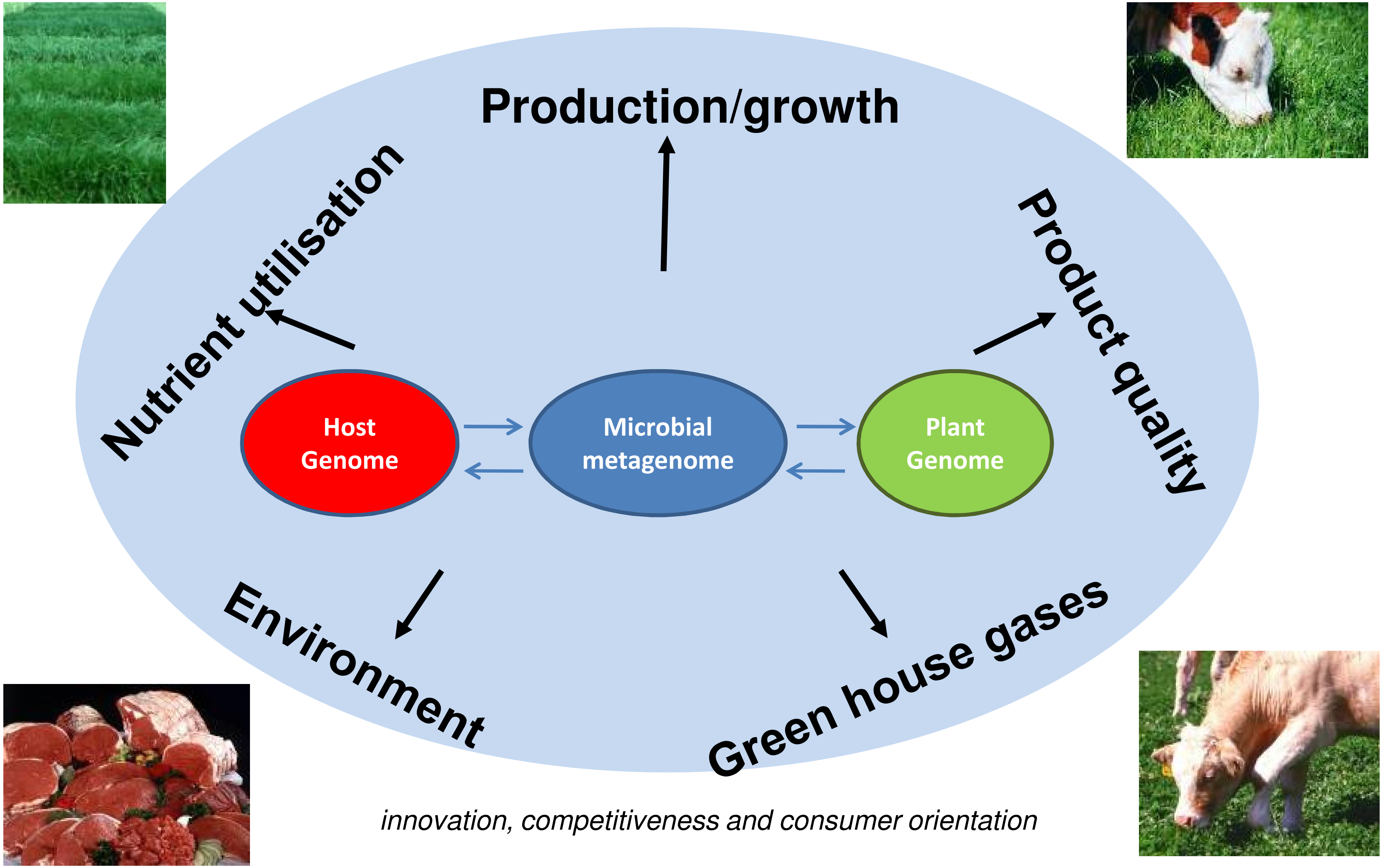
🟩 Putative catalytic domain

🟦 Domain homologous to binding domain in Lactococcus lactis phage lysozyme Tuc 2009/ΦLC3

Optimium pH of lysozymes from *E. caudatum*



Designing systems to produce food in a sustainable and efficient manner



Production/growth

Nutrient utilisation

Product quality

Host Genome

Microbial metagenome

Plant Genome

Environment

Green house gases

innovation, competitiveness and consumer orientation

Questions