Prospecting the rumen protozoa for lipolytic enzymes using metagenomic techniques.

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Presentation outline

- Context
- Aims
- Materials & Methods and Results
- Conclusions
- What next?

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Context

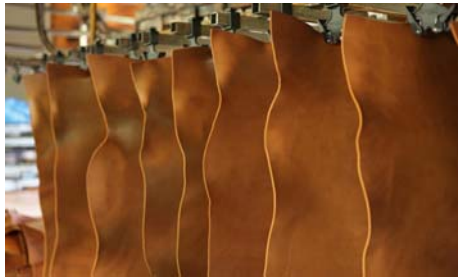
- The protozoa (and fungi) represent the rumen eukaryotome and account for up to 50% of microbial biomass – despite this they are frequently overlooked.

- Lipolytic activity is disputed in the rumen protozoa, and data shows they are unlikely to play a role in biohydrogenation.

- We now have more advanced metagenomic technology to probe the lipolytic capacity of the rumen protozoa.

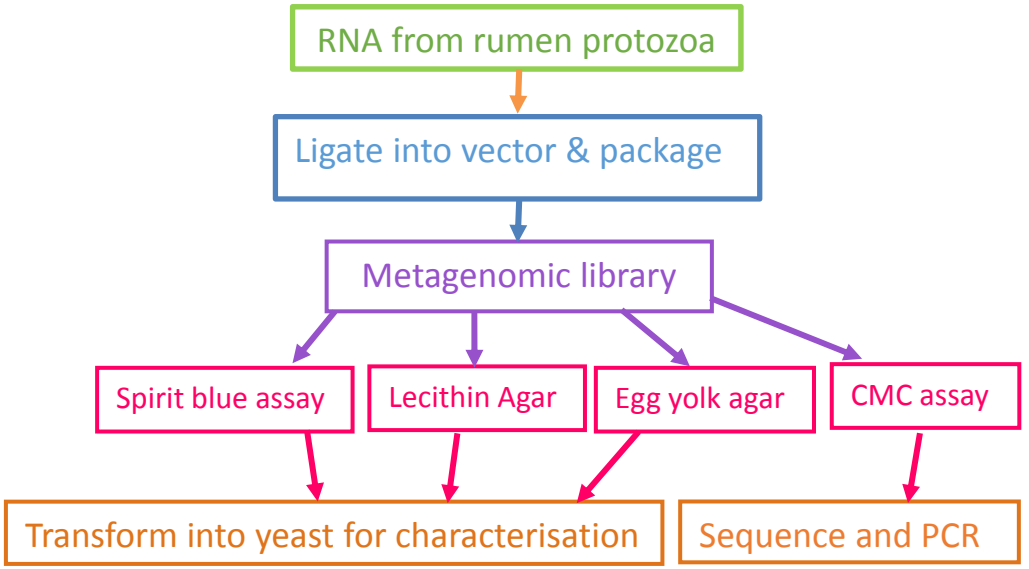
- The global market for industrial enzymes has boomed and is estimated to increase in value to 6.2 billion USD by 2020.

- Any new protozoal enzymes will be identified, characterised and screened for potential industrial applications, e.g. detergents/stain removers and food manufacture.



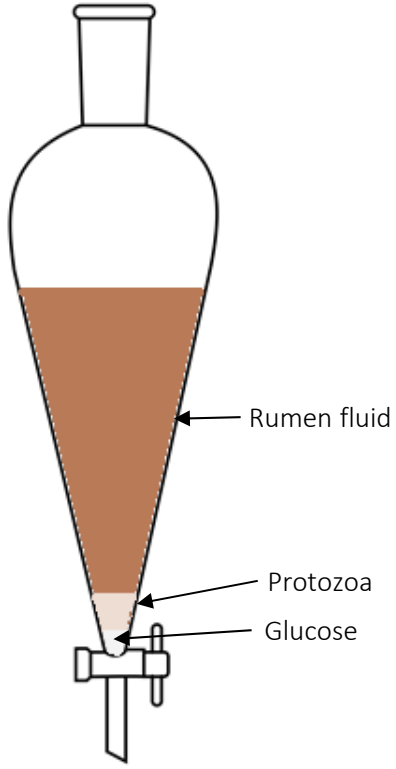
Project aims

- Construct a rumen protozoal metagenomic library.
- Screen for novel enzymes
- Screen current bacterial libraries for activity.
- Screening using sequence-based and function-based approach.

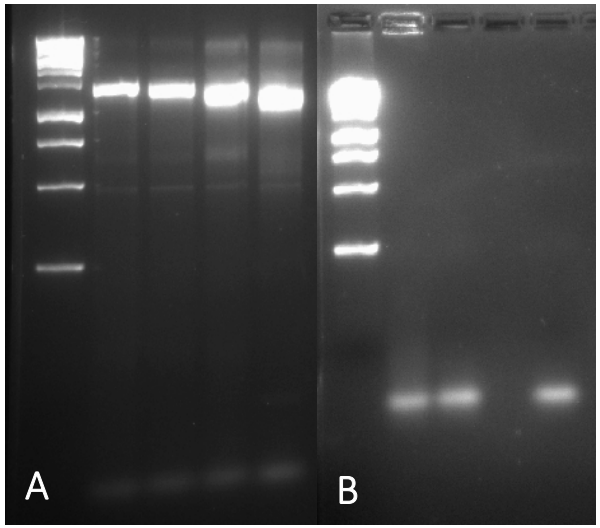


Materials and Methods

- Strained rumen fluid (3 L) was taken and pooled from three non-lactating, rumen-cannulated Holstein-Friesian cows.
- The protozoa were sedimented by addition of 0.5 g glucose per 1 L and siphoned off using the tap.
- The protozoa were then washed using centrifugation and Coleman's buffer.



- RNA was extracted using the FastRNA Pro™ Soil-Direct kit.
- Poly(A) Purist MAG kit was used to enrich polyadenylated mRNA (two rounds).
- RNA treated with Turbo DNase to eliminate gDNA.
- cDNA was generated with the SMART® cDNA library kit using LDPCR.

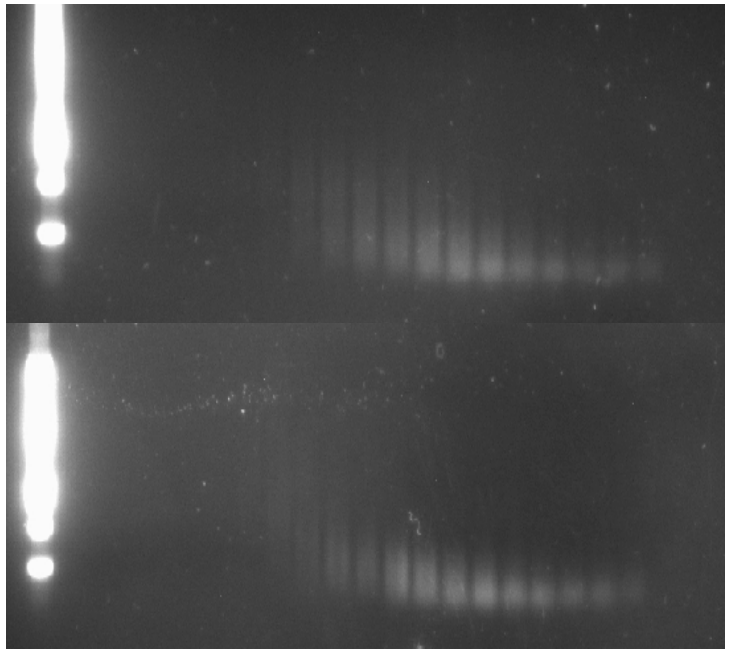


Bacterial PCR on RNA with 1 Kb ladder showing gDNA. B: Bacterial PCR after treatment with Turbo DNase with 1 Kb ladder.

- cDNA was digested using SfiI restriction enzymes to provide unidirectional cDNA.

- cDNA was size fractionated using CHROMA-SPIN-400 gel columns and the fractions containing the largest cDNA were selected.

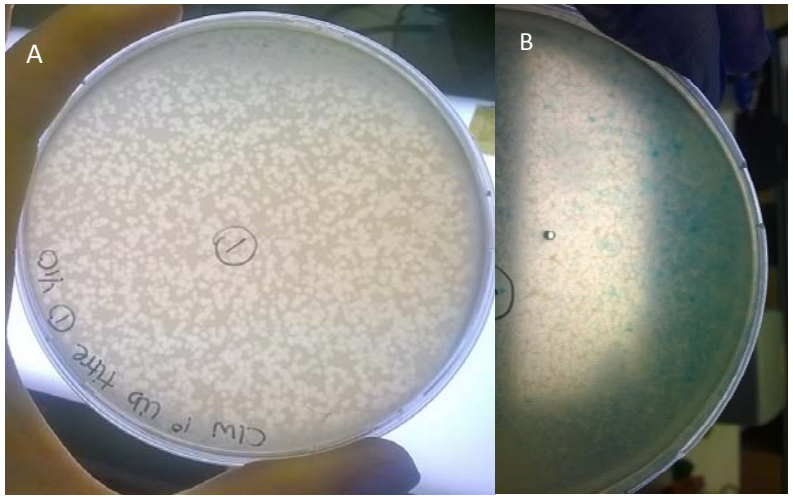
- Fractions were ligated into λTriplEx2 vector and packaged using MaxPlax Lambda extract.



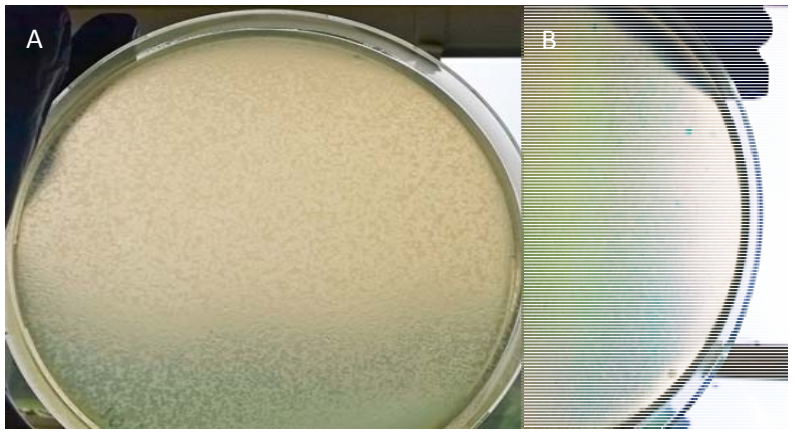
Size fractionated cDNA from two samples alongside a 1 Kb ladder.

- The primary library was evaluated using X-Gal and IPTG and showed a good ratio of recombinant (white): non-recombinant (blue) plaques, titre= 6.5 x 10^6 pfu/mL.

- The primary library was amplified and evaluated as before to ensure ratio was maintained, titre = 2.53 x 10^8 pfu/mL.

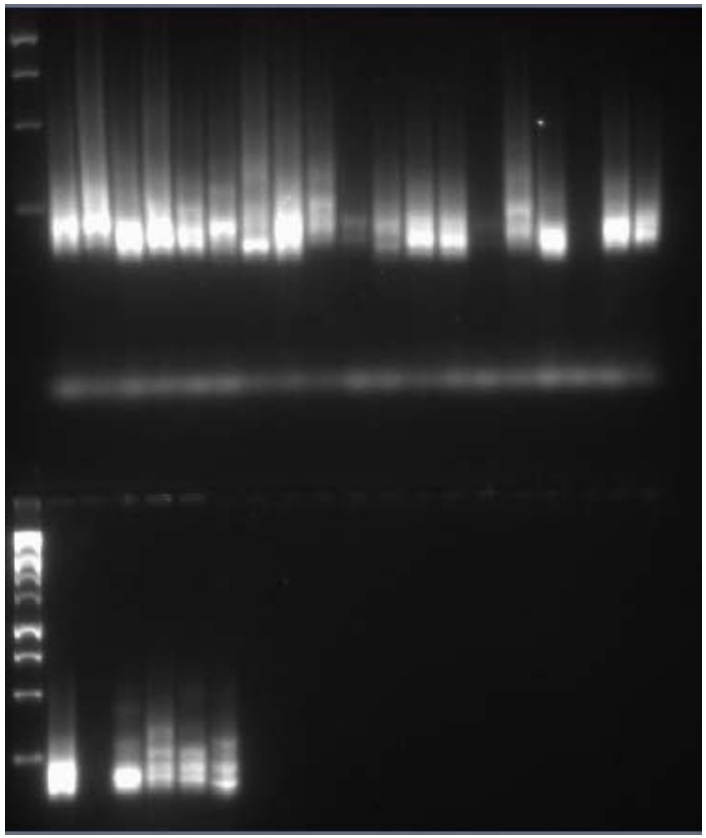


Primary library plated on *E.coli* lawn with X-gal and IPTG showing blue and white plaques (ratio approx. 1:100).



Amplified library plated on *E.coli* lawn with X-gal and IPTG showing blue and white plaques (ratio approx. 1:500).

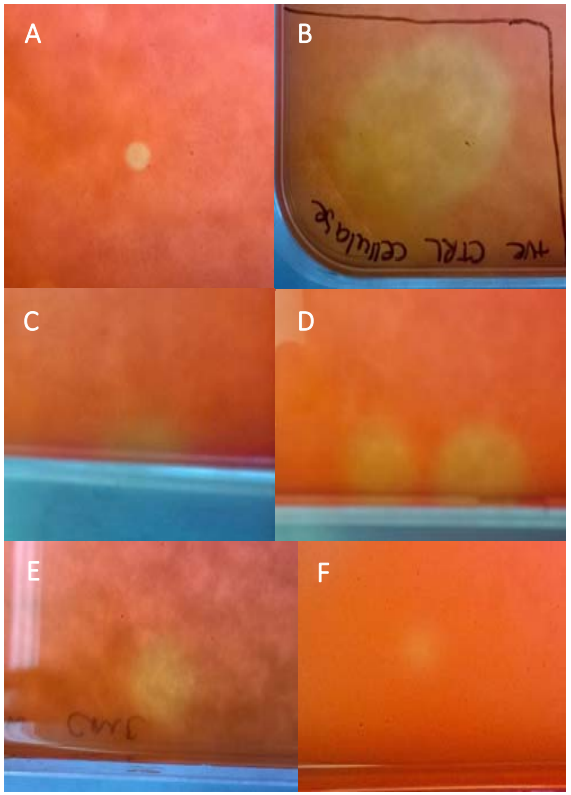
- The library inserts were characterised using vector encoded primers, PCR and agarose gel electrophoresis.
- Inserts varied in size.
- Inserts were excised, purified, Sanger sequenced and BLAST-ed. ~50% of inserts showed homology with known protozoal genes.



Insert characterisation using PCR with vector encoded primers and gel electrophoresis. 1Kb size standard in first lanes.

Preliminary screening results

- The amplified library was evaluated for cellulose activity using a carboxymethyl cellulose (CMC) assay.
- Six positive plaques were visualised.
- Sequencing is underway.



Amplified library plated on *E.coli* lawn with CMC over lay and post staining with congo red (primary assay).

Conclusions

- The protocol took A LOT of optimising!
- Size fractionation is VERY important.
- Assaying phage-based libraries on agar is difficult!
- A functional and representative phage-based library of the rumen protozoa has now been achieved!

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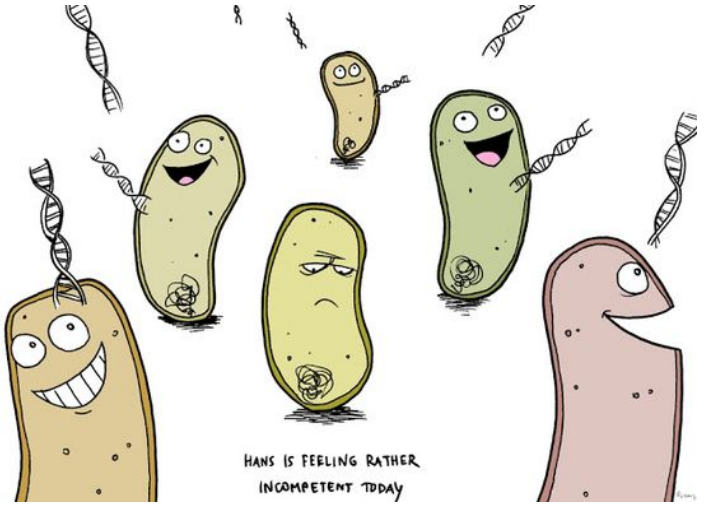

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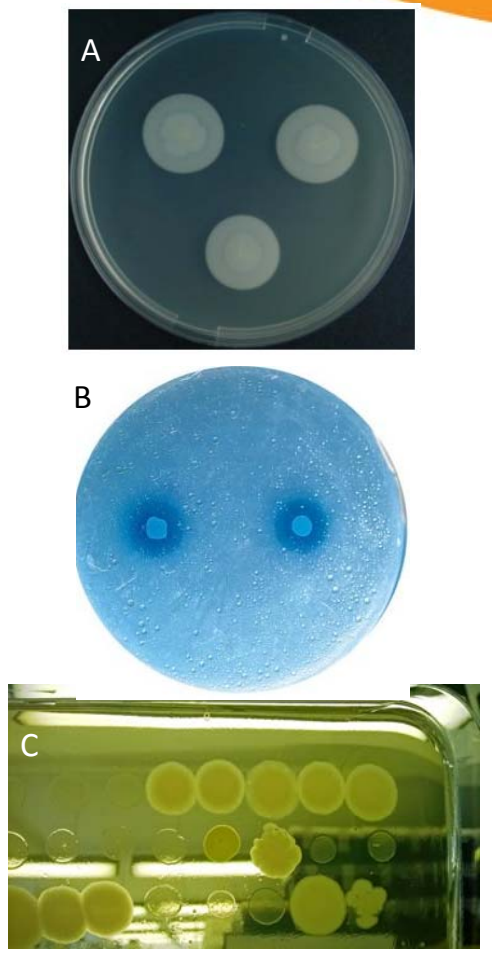
What next?

- Use egg yolk, Spirit Blue/Tributyrin and lecithin agar to screen for lipases.
- Sequence positive phagemids.
- PCR and transform identified lipase genes into yeast for characterisation.
- Investigate their role in the rumen.
- Applications in Industrial Biotechnology?



A: *Bacillus cereus* on egg yolk agar (positive). B: Staphylococcus aureus on Spirit blue agar (positive). C: Bacterial metagenomic library plated on lecithin agar.

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Dank je/Thank you for your attention!

I now welcome any questions you might have.

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