Prospecting the rumen protozoa for lipolytic enzymes using metagenomic techniques

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Protozoa, along with fungi, represent the rumen eukaryotome and account for up to 50% of microbial biomass; despite this, they are often overlooked. It is inferred that rumen protozoa play a role in lipid, protein and fibre metabolism, however, few of these enzymes have been identified and characterised. In order to capture the full functional capacity of the rumen protozoa, a metagenomic approach was selected and a phage-based metagenomic library constructed. Protozoal RNA was extracted from strained rumen fluid, reverse transcribed to cDNA, ligated into the λTriplEx2 vector and packaged into λphage (Clontech Laboratories Inc., USA). This protocol was refined and optimised to give a good ratio of recombinant: non-recombinant plaques (>9:1) and a large insert size (>800 bps). Inserts (n=50) were amplified using vector-encoded primers and sequenced (ABI 3130*xl*) with most inserts confirming similarity to other protozoal genes deposited in GenBank (Including *Entodinium, Epidinium* and *Epispathidium. sp)*. Cellulase assays conducted using carboxymethyl cellulose as a substrate coupled with post-staining using Congo Red. Approximately 30,000 plaques were screened in total and five positives identified. We are currently investigating the lipolytic potential within this metagenomic library to understand the role of the rumen protozoa in lipid metabolism.

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