A FUNCTIONAL METAGENOMIC ANALYSIS OF THE HUMAN GUT MICROBIOME TO IDENTIFY GENES INVOLVED IN OSMOTOLERANCE

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Background: More than 99% of microorganisms are unculturable using current methods. Metagenomics is a culture independent method of analysing a population of micro-organisms, their constituent genomes, phylogeny and ecology. The human distal gut is the highest density natural ecosystem known. This community contains more bacteria than there are cells in the human body and as such this ecosystem has a virtually limitless array of novel gene systems waiting to be discovered. Herein we outline a functional metagenomic approach, which led to the identification of a number of osmotolerance related loci.

Objectives:

1. Determine osmotolerance of host cells (E coli EPI300) containing fosmid harbouring metagenomic DNA from human gut microbiome compared to host cells lacking fosmid with inserted DNA.
2. Screen a metagenomic bank from the human gut to identify possible osmotolerant clones.
3. Identify genes from these clones that may be related to osmotolerance machanisms using a transposon mutagenesis strategy.

Methods:

1. NaCl growth curves were carried out to determine salt tolerance.
2. Over 20,000 clones were screened using QPix 2 XT robotic gridding machine.
3. Transposon mutagenesis carried out using a hyperactive Tn5 in vitro transposition system (EZ-Tn5).

Results:

1. Observed increased salt tolerance in host cells containing metagenomic DNA compared to host cells lacking DNA insert, indicating a gene(s) present in the metagenomic DNA contributes to increased osmotolerant phenotype.
2. We have identified a large number of proteins that function in a broad range of cellular activities.
3. Some of the proteins identified have not been shown to be involved in osmotolerance previously while others still represent hypothetical proteins and will prove interesting for further study.

Conclusions: Functional metagenomics can be utilized as a powerful tool in a relatively simple screening procedure to identify novel genes from an environment as complex and as vastly unexplored as the human gut microbiome.