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Poster 084

The Human Gut Microbiota

The human gut microbiota is a complex anaerobic ecosystem which is involved in a profusion of functions maintaining homeostasis in the gastrointestinal tract such as the metabolism of dietary fibers. Microbial fermentation of plant cell wall polysaccharides is now recognized as providing human health benefits but the fibrolytic community is still misunderstood, especially in the small intestine, because of its poor accessibility and sampling.

We focused here on the ileo-mucosal microorganisms, to examine their capabilities to degrade plant cell wall polysaccharides from dietary fibers using a functional metagenomic screening on a large insert metagenomic library. Glycoside hydrolase (GH) activities were detected using several polysaccharides as substrates.

CAZymes: Carbohydrate Active Enzymes

Plant cell-wall polysaccharides

Fibrolytic bacteria

CAZymes groups

Glycoside Hydrolases (GH)
Polysaccharide Lyases (PL)
Carbohydrate Esterases (CE)
GlycosylTransferases (GT)

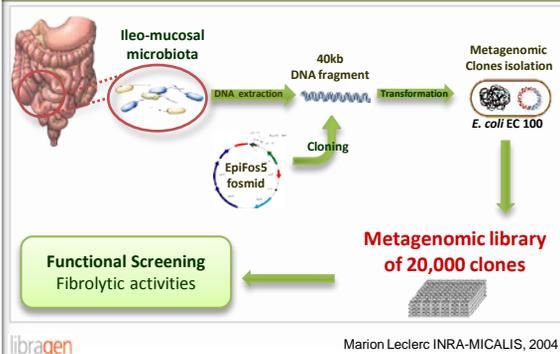
Oligosaccharides/Saccharides> SCFAs

133 GH families <http://www.cazy.org/>

81 families in the human gut microbiota = One of the richest source of CAZymes

Experimental proof of function of only 8 GH families (35 GH) from various ecosystems

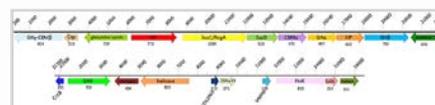
Metagenomic library construction



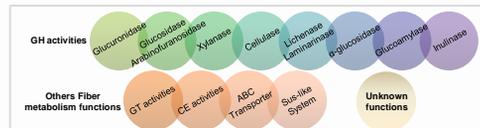
Bioinformatic Analyses

The large fragment DNA insert of bioactive clones was sequenced and the encoded enzymes deduced

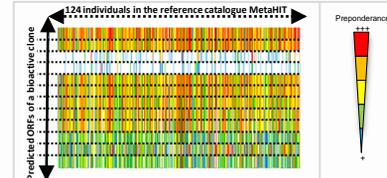
Genetic map of a bioactive clone



Predictive GH activities and associated proteins from bioactive clones



Preponderance of GH genes in the MetaHIT reference catalogue (Qin et al., Nature 2010)

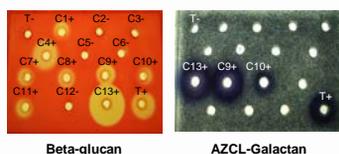


Functional screenings for GH activities

Bioactive clones revelation



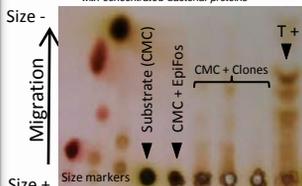
Validation of GH activity on concentrated bacterial proteins



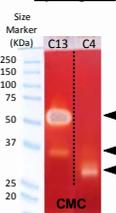
Positive clones (C+) → halo visualization on agar plates complemented by various substrates → Degraded substrate

Thin Layer Chromatography

Substrates incubated 4h at 37°C with concentrated bacterial proteins



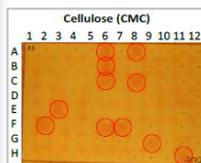
Zymogram



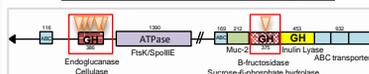
Screening of 20,000 clones → 21 positive clones
2 clones GH+ highlighted → positive on most than 10 substrates

Validation of the active genes

Screening after random transposition on various substrates

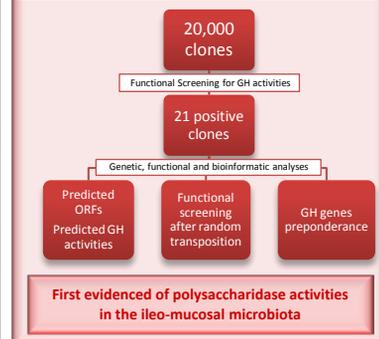


Identification of the active GH genes



Identification of targeted genes responsible for degradation activity

To summarize



Conclusion

- ✓ The screening of the 20,000 metagenomic clones of the ileo-mucosal library evidenced genes encoding proteins involved in the degradation and fermentation of plant cell wall polysaccharides, mainly GH families in the small intestine.
- ✓ Microorganisms in the upper part of the gastrointestinal tract are not only included in sugar metabolism but also in dietary fiber (polysaccharides) degradation.
- ✓ Functional metagenomic screening is a powerful tool to decipher major functions of the human gut microbiota
- ✓ GH genes evidenced in the microbiota might be used as good predictive markers of individual health status using quantitative metagenomics. (Le Chatelier et al., Nature 2013)