Isolation and functional characterization of hemi-cellulose breakdown enzymes from animal gut microbiome

Giang Le[1,2], Valeria Agamennone[1], Nico M. Van Straalen[1], Truong Nam Hai[2], and Dick Roelofs[1]

[1,] Vrije Universiteit Amsterdam, Molecular Cell Physiology, De Boelelaan 1085, 1081HV Amsterdam, the Netherlands;
[2,] Vietnam Academy of Science and Technology (VAST), Institute of Biotechnology , 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

Introduction

- Agricultural wastes lead to pollution and health problems.
- Biomass is a great bio-renewable carbon source for food, feed and biofuels. Hemi-cellulose is the second most abundance after cellulose.

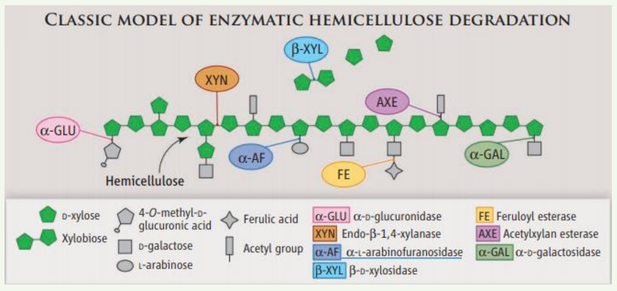


Figure 1: Hemi-cellulose degrading enzymes. (Reviewed by Berlin A, 2013)

- Investigate hemi-cellulose degredation in animals feeding on woods.
- Metagenomic tools have proven to be instrumental in identifying and isolating hemi-cellulose degrading genes from animal gut microbiomes
- Aim: Isolation and functional characterisation of arabinofuranosidase gene from gut microbiomes

Material and methods

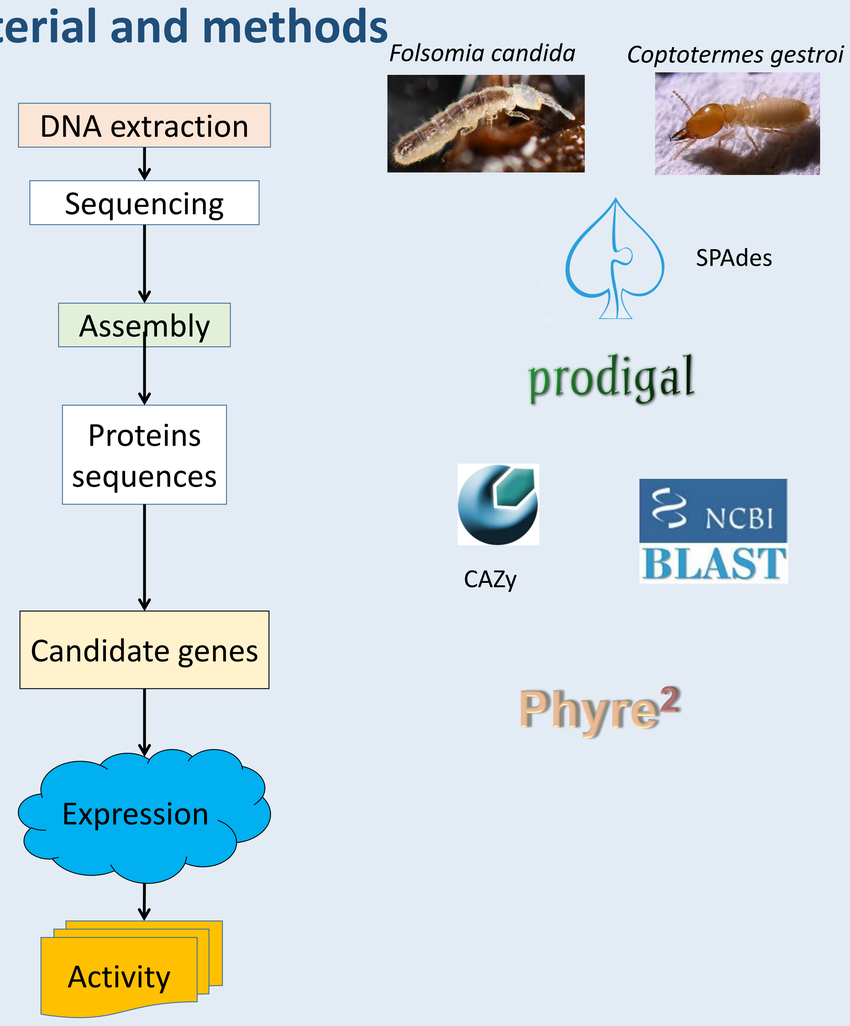
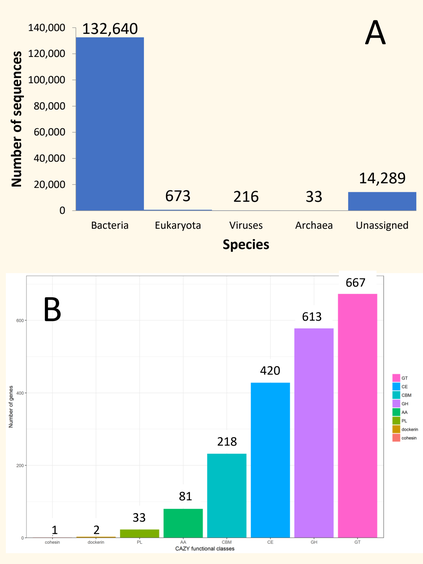


Figure 2: Pipeline for annotating, identifying and expressing genes with carbohydrate-related activity

- Metagenome sequences of springtail, termite and goat were generated.
- Screen gene annotations for potential hemicellulose degrading functions.
- Candidates are being cloned and expressed.
- Cloning performed in pET16 Expression vector. Protein is expressed in *E.coli* expression strain BL21-DE3 and Rosetta2.

Results



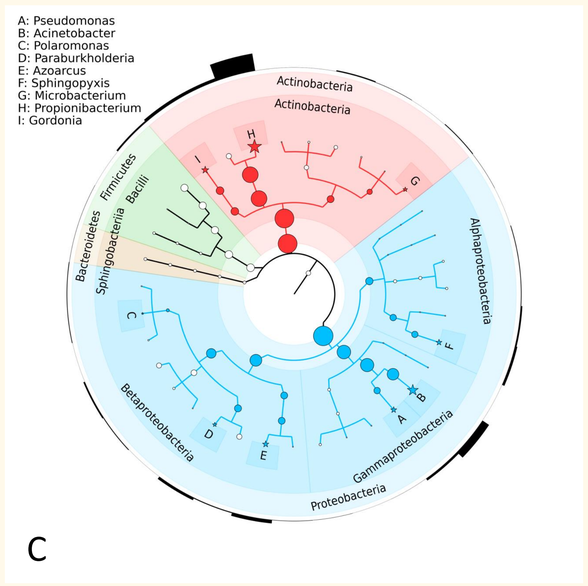


Figure 3: A: Annotated 147,851 genes: Majority map back to bacteria (90%)
B: Carbohydrate activity enzymes found in the metagenomes.
C: Taxonomic distribution of genus with CAZymes

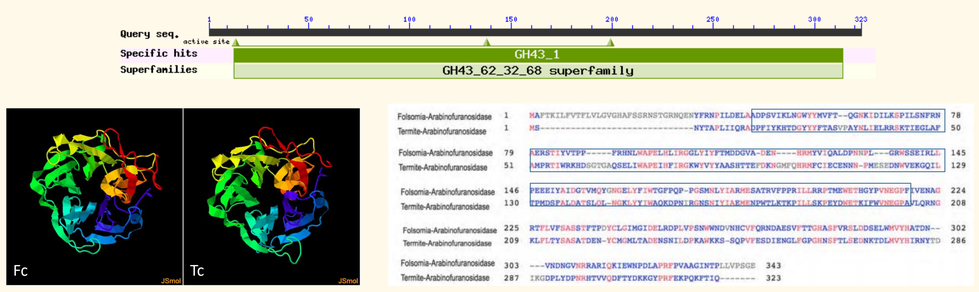


Figure 4: A: Blastp result of arabinofuranosidase gene.
A: 3D structure of the arabinofuranosidase enzymes from springtail (Fc) and termite (Tc).
B: The alignments of the two sequences with the conserved domains (boxes in alignment)

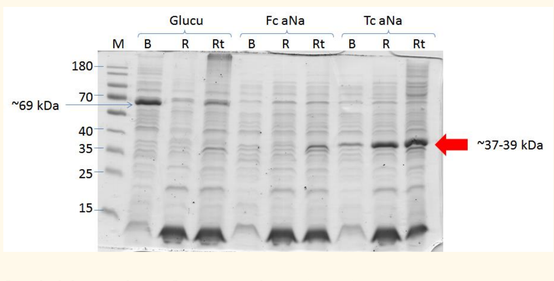


Figure 5: Expression of soluble arabinofuranosidase (aNa) gene in BL21-DE3 and Rosetta *E. coli*.
B: BL21, R: Rosetta2 : Soluble fraction Rt: Rosetta total fraction
Fc: Folsomia clone. Tc: Termite clone

Conclusion

- Two genetically divergent full length alpha N arabinofunosidase genes were isolated from termite and Folsomia metagenomic DNA
- Successful expression of proteins in soluble fraction.
- Future work: protein purification and activity assay (Megazyme kit)

Reference:

- Berlin A No Barriers to Cellulose Breakdown Science 342, 1454 (2013); DOI: 10.1126/science.1247697
- Do, T.H., Nguyen, T.T., Nguyen, T.N., Le, Q.G., Nguyen, C., Kimura, K., and Truong, N.H. (2014). Mining biomass-degrading genes through Illumina-based de novo sequencing and metagenomic analysis of free-living bacteria in the gut of the lower termite Coptotermes gestroi harvested in Vietnam. J. Biosci. Bioeng. *118*, 665–671.

Acknowledgements

This work is supported by a BE-Basic Foundation-FES grant (F07.003.07) from the Dutch Ministry of Economic Affairs, and by a grant from Institute of Biotechnology (IBT)