Biomimetic approach for developing lignocellulose valorization bioprocess using insect microbiome

Lucile Chatellard, Amandine Gales, Adèle Lazuka, Lucas Auer, Maider Abadie, Hélène Carrère, Jean-Jacques Godon, Michael O'Donohue, Guillermina Hernandez Raquet, Claire Dumas

Biomimetic approach for developing lignocellulose valorization bioprocess using insect microbiome

Lucile Chatellard[a], Amandine Gales[a], Adèle Lazuka[b,c], Lucas Auer[b,c], Maider Abadie[b,c] Hélène Carrère[a], Jean-Jacques Godon[a], Michael O'Donohue[b,c], Guillermina Hernandez-Raquet[b,c], Claire Dumas[b,c]

[a] *INRA, UR0050, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, Narbonne, F-11100, France*
[a] *(E-mail : godon@supagro.inra.fr)*

[b] *Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France*
[c] *INRA, UMR792, Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France*
[b,c] *(E-mail: cl_dumas@insa-toulouse.fr; hernandg@insa-toulouse.fr)*

Abstract
Insects are the world's tiniest, most efficient bioreactors known to transform lignocellulose. They have optimized their digestion in highly efficient systems. The proposed project aims to characterize the microbial and enzymatic consortia that allow efficient lignocellulose degradation. For this, the digestive microbiomes of five insects belonging to several orders (*Gromphadorrina potentosa*, *Ergates faber*, *Potosia cuprea*, *Gryllus bimaculatus*, *Locusta migratoria*) were placed in batch reactors, in physicochemical environment similar to their original medium. The microbiomes of selected insects were successfully maintained in bioreactors and their strategy for lignocellulose degradation was followed dynamically in semi-continuous reactors. Their ability to degrade lignocellulosic substrate as well as their microbial and enzymatic diversity allowed comparing the efficiency of these potential bioressources.
This research brings new information on microbial species and enzymatic deconstruction processes involved on lignocellulose degradation in natural environments; such information might be useful to produce biomimetic enzyme cocktails benefiting from millions of years of evolution.
Keywords
Biomimetism; insect guts; bioproducts; enzymatic activity; microbial diversity.

Over millions of years living organisms have optimized the digestion of a large variety of substrates resulting in highly efficient systems [1, 2]. The study of original strategies implemented in insect guts would be of interest for possible improvements of the biomass conversion in industrial processes [1, 2]. Until recently, the field of insect biology and biochemical engineering were far apart. Nevertheless, much of this knowledge is relevant to today's biorefinery challenge. The study of original strategies implemented in insect guts would be of interest to improve biomass conversion.
The aim of this work is to compare and taming the action of microbial and enzymatic consortia that allow efficient lignocellulose degradation. For this, with a biomimetic approach, the digestive microbiomes of five insects were implemented in batch reactors. The microbiome of three insect guts was selected for enrichment in semi-continuous reactors.

Their ability to degrade lignocellulosic substrate (wheat straw) as well as their microbial and enzymatic diversity was studied in order to compare the efficiency of these bioressources.

MATERIAL AND METHODS

Insect gut preparation

Insects were chosen based on their phytophagous or xylophagous diet and their ability to manipulate them in reactors during long term incubation. The digestive microbiomes of five insects belonging to several orders (*Gromphadorrina potentosa, Ergates faber, Potosia cuprea, Gryllus bimaculatus, Locusta migratoria*) were studied. Before dissection, the insects are anesthetized under CO_2 flow and surface sterilized with ethanol at 70%. Gut suspension was about 50g/L.

Batch tests

Three replicates of batch tests were realized in 120ml penicillin flask containing 10g /L of straw and inoculated with gut suspension. Wheat straw was ground in an impact mill (Ultrapez UPZ) and dry sterilized 20 minutes at 121°C. A fourth flask was prepared without straw as a control. Incubation was made at 30°C with shaking at 150rpm in an INOVA oven for 15 days. During the incubation, biogas, volatile fatty acid (VFA), ethanol and lactate production were monitored.

Semi continuous tests

Semi-continuous reactors were operated in an initial volume of 400mL with a straw concentration of 20 g/L. Three replicates (two with straw and one control without straw) were conducted under anaerobic conditions at 30°C, with controlled pH at 8, shaking at 300rpm. The duration for each test depended on the degradation kinetics.

Enzymatic activity tests

Enzymatic activities (cellulase, xylanase and β-glucosidase) were analyzed at T0 on solid (pellet) and liquid fraction (supernatant) in the different batch experiments inoculated with insect gut microbiomes.

Microbial diversity analysis

The bacterial communities of batch reactors were analyzed by the PCR–single strand conformation polymorphism (SSCP) technique (Wéry et al., 2008) targeting the highly variable V3 region of 16SrRNA gene. Illumina MiSeq sequencing was performed on V3-V4 regions following the instructions of Genomic and Transcriptomic platform of Toulouse (INRA, Auzeville, France).

A. SCREENING OF INSECT GUTS: BATCH REACTORS STUDY

Biogas

The biogas productions in batch flasks with wheat straw were significantly higher than the control reactors which contained only the digestive tracks. The biogas production was maximal for *Locusta migratoria* (12^{E-3}g COD of H_2) and for *Ergates faber* (8^{E-3}g COD of H_2), thought it was minimal for *Potosia cuprea* and *Gromphadorrhina portentosa*. A large part of biogas (between 30 to 50%) was composed of H_2 the rest being essentially CO_2.

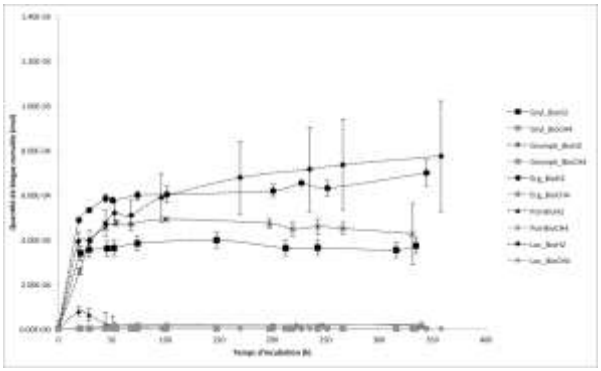

Figure 1: Biogas production (H_2 and CH_4 during batch experiment screening) (gDCO)

	VFA (mgCOD)		Metabolites (mgCOD)	
	T0	TF	T0	TF
Ergates	1.8	52.1	132.7	50.1
Potosia	2.8	175.1	212.9	0
Gromphadorrhina	5.8	140.0	50.6	67.7
Gryllus	12.6	131.0	145.4	66.9
Locusta	5.1	143.2	2.5	35.2

Table 1 : Total VFAs and metabolites productions

Fermentation products: VFA and metabolites

In the control flasks some VFAs were detected. Indeed, even if the initial VFAs concentration in control was low a production was observed probably due to the degradation of guts tissues (protein, fats). At T0, the metabolites remained in digestive tracks were lactate, acetate and ethanol. At the end of the fermentation, the VFAs produced were essentially acetate, propionate and ethanol. The initial concentration in ethanol was rapidly consumed in the case of *Ergates faber*, *Potosia cuprea* and *Gryllus bimaculus*.

Enzymatic activity

Our results showed that most of the studied enzymatic activities in microbiomes were linked to the solid fraction (except for xylanase activity in *Ergates faber*) and β-glucosidase in *Locusta migratoria* which were dominant in the liquid fraction. The xylanase activity was higher in Tf than in T0.

Microbial diversity

Microbial diversity analysis assessed by Illumina sequencing showed various diversity profiles, even at phylum scale, for the five insects considered. Globally, the dominant phyla were Proteobacteria, Firmicutes and Bacteroidetes.

Simpson diversity index (SI) which represents the variability of systems composition, showed a large diversity of *Potosia cuprea* microbiome with a SI of 0.02 (350 OTU). Conversely, SI of *Ergates faber* equaled 0.65 (66 OTU where the most abundant represent 78% of the community). Concerning T0 and Tf analysis, the SI was quite stable during the experiment.

B. CHARACTERIZATION OF INSECT GUTS: SEMI-CONTINUOUS REACTORS STUDY (*Gromphadorrhina potentosa, Potosia cuprea and Locusta migratoria*).

The screening experiments allowed to identify three insect guts displaying high lignocellulolytic activity; these insects were selected for a deeper analysis in semi-continuous reactors.

Biogas and VFAs productions

The results showed that biogas production (CO_2 and H_2) were comparable to that obtained in the batch experiments, however, some methane was detected in *Gromphadorrhina* and *Potosia* reactors. This difference could be attributed to the pH value which was quite higher in semi continuous experiments. The majority of VFA detected was acetate. At the end of the batch the maximal concentration in VFAs was obtained for *Locusta* followed by *Gromphadorrhina* and the minimum for *Potosia*.

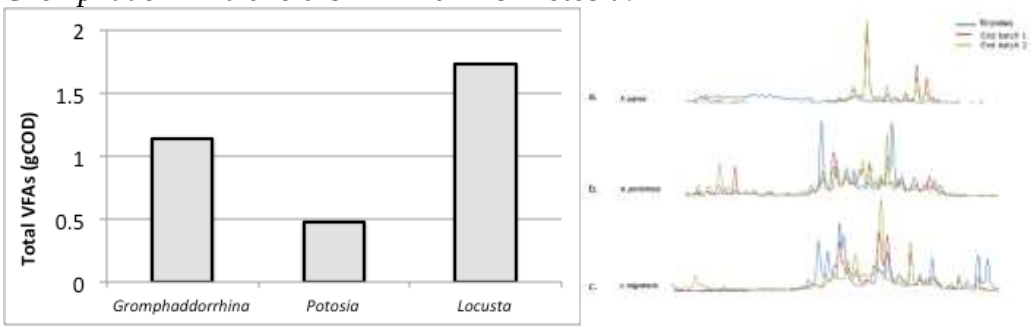

Figure 2: a) Total VFAs production during semi-continuous experiments (batch 1); b) SSCP profiles at T0 and the end of batch 1 for two replicates.

Microbial diversity

The community profiles (PCR-SSCP) from *Potosia* and *Gromphaddorrhina*, which showed similar performances, had similar community profiles and displayed the same evolution of the community composition. Indeed, the SSCP profile of the inoculum strongly changed after the first culture period probably due to the experimental conditions. The community profile is then maintained in the next reactors.

CONCLUSION

This research enabled the identification, characterization (microbial species and enzymatic activities) and taming of microbiomes form insect guts that synthesize enzymes interesting for lignocellulosic biomass deconstruction.

ACKNOWLEDGEMENTS

The authors thank 3BCar Institute for funding.

REFERENCES

[1] Martin, M. M. (1983). 75(3), 313–324.
[2] Godon, J.J. (2013), 6 (3), 1063-1081