MICROBIAL CONSORTIA FOR BIOWASTE MANAGEMENT – LIFE CYCLE ANALYSIS OF NOVEL STRATEGIES OF BIOCONVERSION (MICROWASTE)

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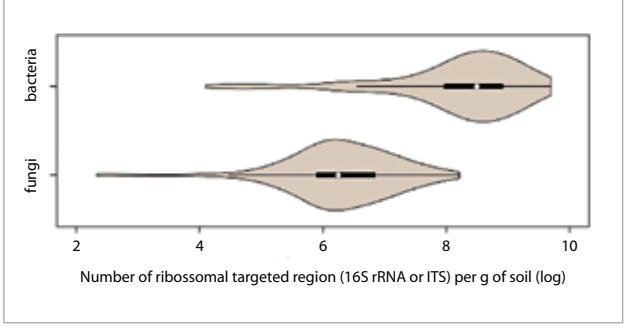

Figure 1. Frequency of quantification values for of Bacteria and Fungi in soils cultivated with sugarcane in distinct locations in Brazil and under differential agricultural managements

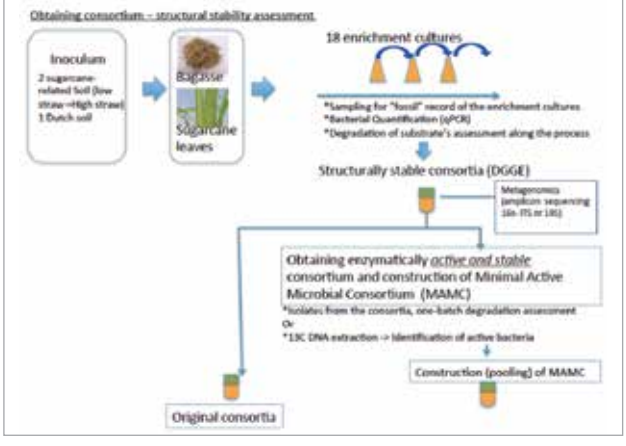

Figure 2. Experimental design for the enrichment and description of microbial consortia involved in the degradation of lignocellulosic material.

Biological waste from agricultural and other sources is a both nuisance and a source of biotechnological opportunities. The project MICROWASTE will develop and foster the understanding of microbial consortia involved in the degradation of the lignocellulosic matter present in agricultural biowaste (sugarcane remains [BR] and maize stalks [NL]). The two research groups have long track records of collaboration and are pioneers in the application of cultivation-independent tools to unravel the soil microbiota in terms of phylogeny and function. Moreover, they also pioneered studies on the interactions between bacteria and fungi in soils and biowaste processing. The current proposal links the activities at the NL partner, which address the key microorganisms of biodegradative consortia and their interactions, as well soil indicators of ecosystem services, with those in BR, which address the soil microbial communities in agricultural and natural ecosystems. Together, we will focus on interactions of key players in microbial consortia and their enzymes (e.g. cellulases, laccases, etc), next to their interactions. We will take a metagenomics approach, which will be combined with network analysis to pinpoint interactive species and key (novel) genes. These approaches will be also used to predict substrate degradability in soils, either with or without an added microbial consortium. Finally, the effect of consortia inoculation on ecosystem services will be assessed. The final outcome of the project will be (1) the availability of a stable microbial consortium which is robust due to its interactivity, with great potential to be applied for monomer production in reactors and in the field, (2) the assessment of the ecological gains of applying biowaste as opposed to the traditional way of burning or disposing it in soil, and (3) the prediction of the effects of biowaste incorporation on the soil processes.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Part I – Tracking microbial interactions in sugarcane fields

Study 1. Deep survey on microbial communities in soils used for sugarcane cultivation under distinct management

This study targets the spatial-temporal dynamics of microbial communities in soils used for sugarcane cultivation along the complete period of cultivation for sugarcane (4 years). We found a great support to develop this approach in the Brazilian Bioethanol Science and Technology Laboratory (CTBE). The results obtained in this first year are related with the quantification of bacteria and fungi in samples collected in the first and second years of cultivation. Data revealed that bacteria are more abundant than fungi in most of samples, and variations in these values were higher for fungi than bacteria. Distinctions among areas will be further addressed, when the complete dataset (with samples from times 0, 1, 2 and 3 years of sugarcane cultivation) are available.

Study 2. Combining the analysis of ribosomal gene sequences with metagenomics in soils samples from sugarcane fields

This step is developed to use data already generated in previous studies (also financed by FAPESP – process 2011/03487-2). The data was collected by sampling soils from sugarcane plantations along 10 distinct regions of the State of São Paulo, and further contrasting the microbial community composition with the environmental data.

At that moment a great dataset was generated, made of 95 soil samples subjected to archaeal and bacterial 16S rRNA gene sequencing, and 9 soils samples (those already showing more dissimilar ribosomal patterns) subjected to a deep sequencing for metagenomics analysis.

The exploration of this dataset was started in 2015, focusing the approaches in the description of microbial groups correlated with the degradation of lignocellulosic materials. It will be correlated the taxonomical analysis performed on ribosomal sequences, with a functional approach, obtained on the basis of the assessment of the metagenomes available from the targeted areas.

Part II – Hitherto a microbial consortia for efficient degradation of lignocellulosic materials

Here the focus is to obtain well-characterized and stable communities that are able to effectively degrade lignocellulosic material, depicting their interactions and ecological roles during the degradation process will be performed. The methodology includes a combination of cultivation techniques and molecular methods - metagenomics and metatranscriptomics.

The microbial consortium was obtained by sequential culturing of inoculant microbial communities (two sugarcane-related soils from Brazil) enriched with sugarcane residues (Bagasse and straw). Consecutive transfers ensured the stability of the communities. Degradability of the substrate was assessed by a gravimetric method. The next step will include meta-omics exploration of the stable consortia as well as isolation and characterization of strains in order to construct a Minimal Active Consortium (MAC) with high degradability properties.

MAIN PUBLICATIONS

Andreote FD, Gumiere T, Durrer A. 2014. Exploring interactions of plant microbiomes. *Scientia Agricola.* **71**: 528-539.

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Jimenez DJ, Korenblum E, van Elsas JD. 2014. Novel multispecies microbial consortia involved in lignocellulose and 5-hydroxymethylfurfural bioconversion. *Applied Microbiology and Biotechnology.* **98**: 2789-2803.

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