NOVEL MULTI-SPECIES MICROBIAL CONSORTIA INVOLVED IN LIGNOCELLULOSE AND 5-HYDROXYMETHYLFURFURAL BIOCONVERSION

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Studies on microbial consortium construction could be provide an important foundation for understanding the complex interactions (bacteria-fungi) of lignocellulosic degradation and can be a platform for production of plastics, or energy stored in biofuels (Hasunuma *et al*., 2013). In this study, we showed the successional microbial diversity of the two lignocellulolytic microbial consortia produced by us on either pretreated or untreated wheat tissue substrate. Also, we reported a novel, easy and fast method to detect oxidoreductase activity in the presence of 5-HMF. The two novel microbial consortia may constitute starting points to consolidated bioprocessing in the light of their possible capacities in the conversion of lignin, (hemi)cellulose, furanic compounds and cello-oligosaccharides (Jimenez *et al*., 2013).

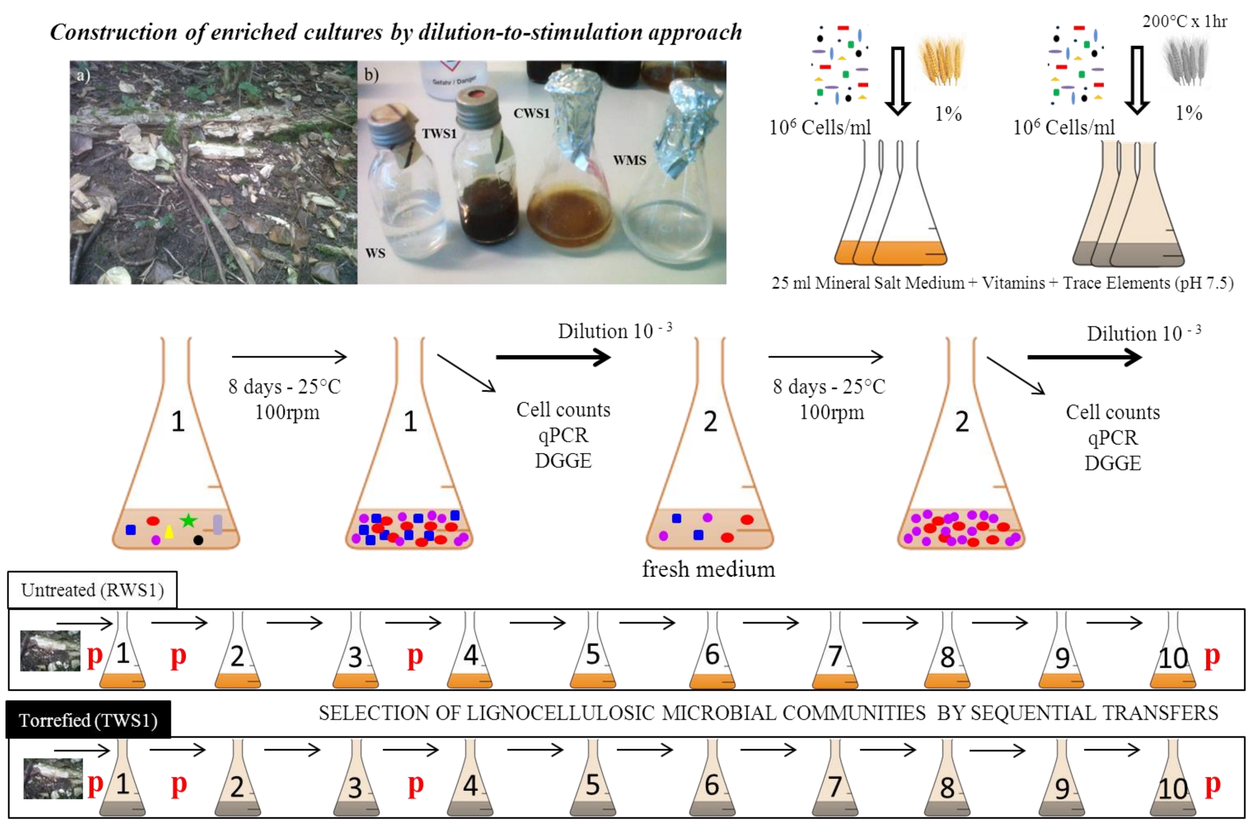


Fig. 1. Diagram of soil-microbial consortia construction by dilution-to-stimulation approach using untreated (RWS1) and pretreated (torrefied process) (TWS1) wheat straw as a carbon source. **p** means samples taken (cells from a forest soil as the inoculum (SS), and transfer T1, T3 and T10) to performed bacterial 16S rRNA gene / ITS1 region pyrosequencing (n=13) and total metagenome sequencing by Illumina (n=7). Two controls, i.e., one without substrate (WS) and the other without microbial source (WMS), were also set up (Jiménez *et al*., 2013).

RESULTS: Microbial composition of wheat straw degrading soil microbial-consortia (bacteria and fungi) -based on 454-pyrosequencing-

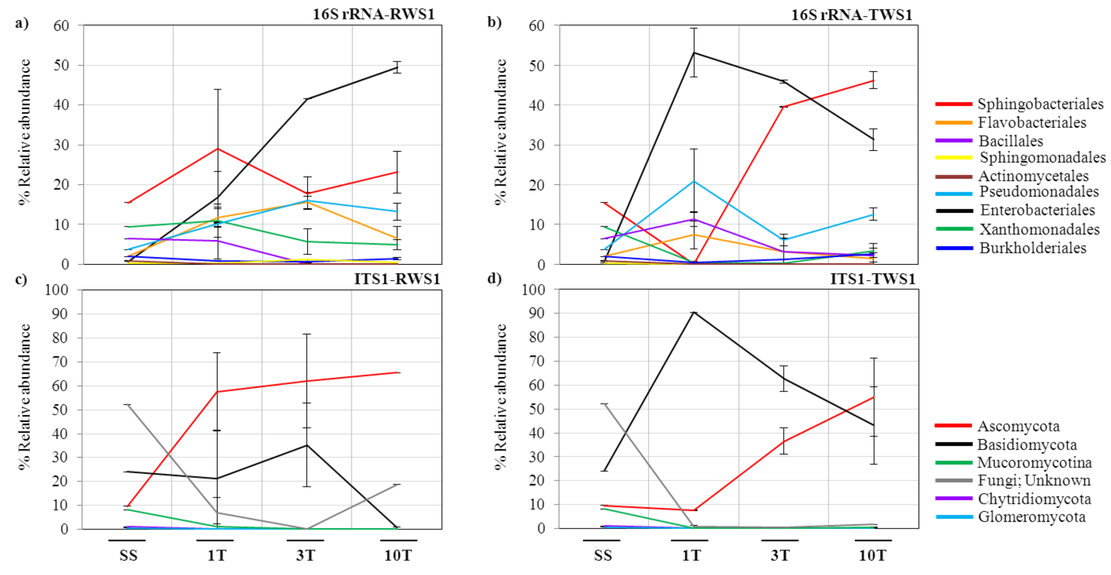


Fig. 2. Relative abundance (%) of the most abundant **a, b)** bacterial orders and **c, d)** fungal phylum members based on 1,400 (16S rRNA) and 550 (ITS1) sequences in the soil inoculum (SS) and in enriched cultures (RWS1 and TWS1) along the sequential-batches**.** The analyses were performed using (QIIME) software (Caporaso *et al*., 2010).

RESULTS: Structural composition and functional profile of wheat straw degrading soil microbial-consortia

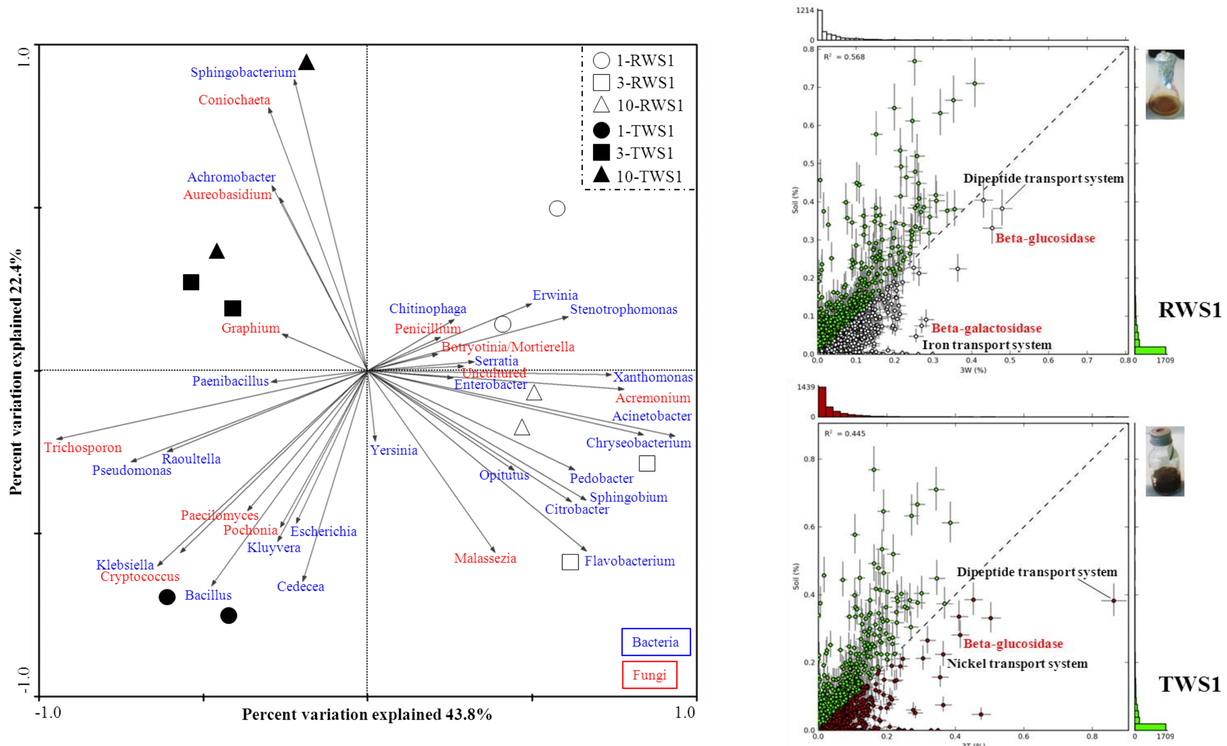
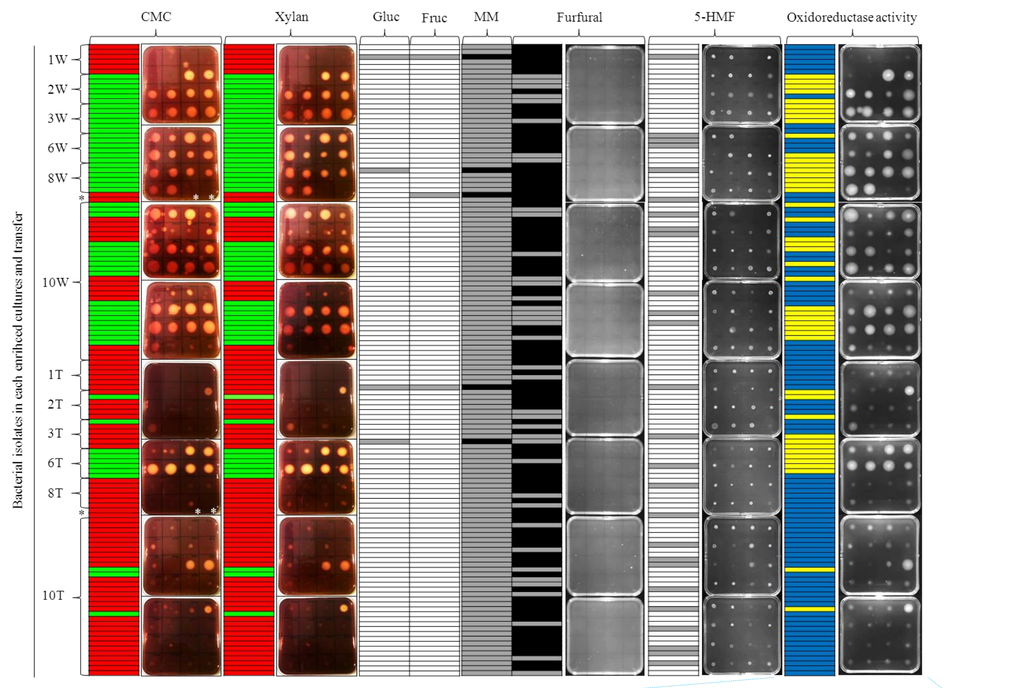
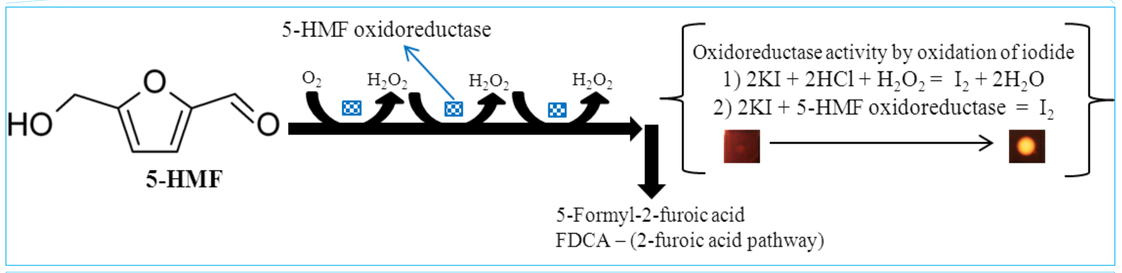


Fig. 3. Left: Principal Components Analysis (PCA) of the most abundant genus in the sequential-batches enriched cultures (RWS1 and TWS1). PCA was done using Canoco software v4.52 (Wageningen, The Netherlands). **Right:** Comparison of functional profile between Soil (green) vs RWS1 (white) and TWS1 (red) (3-transfer samples). Scatter-plot showing differences at function level ($p<0.05$). Enzymes involved in lignocellulose degradation are show in red letters.

RESULTS: Enzymatic screening of bacterial strains recovered from both multi-species consortia





1) Growth of bacterial strains on 5-HMF (7.5mM) agar
2) Flooding the colonies with Gram iodine
3) Production of a yellow halo – detection of extracellular 5HMF oxidoreductase activity

CONCEPT

i) Production of H_2O_2–oxidation of 2KI in acidic conditions–production of I_2 and change of color
ii) Production of a 5-HMF oxidoreductase (HmfH)–direct oxidation of 2KI–production of I_2 and change of color

Fig. 4. Functional screening of 124 bacterial strains recovered from RWS1 and TWS1 to detect (hemi)cellulolytic activity (CMC-ase and xylanase), growth in mineral medium (MMA) (without-substrate control), plus glucose (glu), fructose (fru) and furanic compounds (furfural and 5-HMF), and oxidoreductase activity in the presence of 5-HMF by iodine oxidation method (gray scale color plates). Labels- green and red: positive and negative (hemi)cellulolytic activity, respectively. Black, gray and white: negative, weak and positive growth, respectively. Yellow and blue: positive and negative extracellular oxidoreductase activity, respectively.

CONCLUSIONS

1. **Two novel wheat straw degrading soil microbial-consortia were constructed (Fig. 1)**
2. **The soil bacterial community was reshaped with reduction in diversity and expansion of *Enterobacteriales, Pseudomonadales, Flavobacteriales* and *Sphingobacteriales* (Fig. 2-3)**
2. ***Trichosporon, Coniochaeta, Pseudomonas and Klebsiella* were abundant in the TWS1 consortia (Fig. 3) and could be key players in the bioconversion of furanic compounds**
3. ***Klebsiella, Acinetobacter, Sphingobacterium, Flavobacterium* and *Acremonium* species are likely to be key players in the bioconversion of lignocellulose (Fig. 4).**
4. **Information about the metagenome showed enrichment of glycosyl hydrolases as well ABC transporters (Dipeptides/Nickel and Iron membrane transport systems)**

REFERENCES
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