Plant biomass-degrading microbial consortia

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SUMMARY

Recent developments have paved the way for the use of plant biomass as a source of sugars, which are useful for the production of renewable bio-based compounds (e.g. bioethanol, plastics and pharmaceutical-chemical intermediates). However, current methodologies for plant biomass saccharification are imperfect and so there is great interest in the development of processes that are based on novel enzymes. Lignocellulolytic microbial consortia constitute excellent sources for such novel enzymes. In this thesis, we performed an in-depth characterization of five soil-derived microbial consortia bred on wheat straw (RWS, TWS and WS1-M), switchgrass (SG-M) and corn stover (CS-M) under mesophilic and aerobic conditions. The data obtained indicated that different types of interactions (e.g. synergism and competition) and enzymatic machineries occur in the consortia. The plant biomass source, its complexity as well as the culture conditions all influenced the structures and diversities of the microbial communities that were obtained.

In chapters 2, 3 and 4, metataxonomic and metagenomic analyses of two wheat straw cultures (RWS and TWS) are reported. The two consortia were constructed by the dilution-to-stimulation approach. Forest soil served as the primary source of microorganisms and the consortial structures of three selected sequential batches were evaluated by amplicon pyrosequencing (bacterial 16S rRNA gene and fungal ITS1 region) as well as whole metagenome sequencing. In both systems a reshaping of the bacterial communities was found, with reductions in overall OTU richness and increases in the prevalence of particular members of the Enterobacteriales, Pseudomonadales, Flavobacteriales and Sphingobacteriales. With respect to fungal OTUs with biotechnological relevance, we detected members of the genera *Coniochaeta*, *Acremonium* and *Trichosporon*. The structure of the lignocellulolytic microbial consortia was strongly influenced by the nature of the substrate, i.e. treated versus untreated wheat straw. The metagenomic analyses revealed an overrepresentation of diverse carbohydrate transporters (ABC, TonB and phosphotransferases), two-component sensing and response systems and several genes encoding enzymes of the glycosyl hydrolase families GH2, GH43, GH92 and GH95 in the two consortia, as compared to the soil inoculum. The overrepresentation of genes for carbohydrate transporters and for proteins involved in lignocellulose degradation (e.g. β-xylosidases) is consistent with the hypothesis that enhanced lignocellulolytic degradation capacities are required in these consortia to allow growth on the complex plant materials (e.g. hemicellulose), next to capacities for transport of the products (e.g. xylose) into the cells. The total metagenomic assemblies encompassed around 32,000 contigs of ≥ 10 Kb. Thirteen contigs, containing 39 glycosyl hydrolase genes, were found to constitute novel (hemi)cellulose utilization loci with affiliation to sequences primarily found in the Bacteroidetes.

Two metagenomic libraries, generated from the RWS and TWS consortial DNAs, were constructed and screened for enzymes involved in hemicellulose deconstruction, using a novel mixture of different chromogenic substrates (chapter 5). Approximately 44,000 clones were screened yielding a total of 71 positives. Seven positive clones were selected for further sequence-based analyses. These analyses revealed eight genes that were predicted to encode enzymes of the glycosyl hydrolase families GH2, GH3, GH17 and GH53. In addition, functional analyses unveiled two clones that were predicted to encode novel thermo-alkaline

hemicellulases (an β-D-galactosidase and an β-D-xylosidase). The predicted proteins were traceable to *Klebsiella*-like species.

In chapters 6 and 7, we describe the characterization of the collective proteins (metasecretome) secreted by four consortia cultivated on wheat straw (RWS and WS1-M), switchgrass (SG-M) and corn stover (CS-M). Liquid chromatography-tandem mass spectrometry was used to analyze the RWS metasecretome following growth of the latter on wheat straw, xylose or xylan as a sole carbon sources. Moreover, we analyzed the metasecretomes of WS1-M, SG-M and CS-M using nine chromogenic polysaccharide hydrogels and three insoluble chromogenic biomass substrates. For RWS, 768 proteins were taxonomically and functionally classified. These proteins were mostly affiliated with *Sphingobacterium*-like consortium members (~50%). The most abundant clusters of proteins were predicted to be involved in polysaccharide transport and/or sensing (TonB depend receptors). In addition, proteins predicted to be involved in the degradation of plant biomass, i.e. endo-1,4-beta-xylanases (GH10), beta-xylosidases/alpha-L-arabinofuranosidases (GH43 and GH51), pullulanases (GH13) and alpha-L-fucosidases (GH95), were prominent. Based on the latter results, I suggest that the hemicellulose fraction of the used plant biomass supports, to a large degree, the growth of the lignocellulolytic consortia. The biggest contributors to the degradation process, for the RWS consortia, were members of *Sphingobacterium* and *Klebsiella*. Otherwise, the metasecretomics-based analyses of the WS1-M, SG-M and CS-M consortia showed the presence of enzymes able to deconstruct arabinan, arabinoxylan, xylan, beta-glucan, galactomannan and rhamnogalacturonan. Interestingly, these last consortial metasecretomes contained enzyme cocktails that enable us to produce oligosaccharides directly from wheat straw, sugarcane bagasse and willow (*Salix* spp.).

Based on all findings, I catalogue the microbial consortia that were produced as true microbial enzyme “factories”, which constitute excellent sources for the production of efficient enzyme cocktails for the pretreatment and saccharification of the lignocellulose moieties present in diverse raw materials. Overall, the findings yield a robust starting point for biotechnological exploration. For example, novel efficient enzyme cocktails can be concocted that can enhance the activity of currently-used cellulolytic enzymes in biorefining. Moreover, I also provide deep insight in the complexity of plant polysaccharide degrading capabilities of microbial consortia bred from forest soil.