

Target analysis of microbial lignocellulytic secretomes: a new approach to enzyme discovery

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Resumo

From both a fundamental and industrial biotech viewpoint understanding the deconstruction of lignocellulose in soil and compost is of central importance. In the natural environments microbial communities can efficiently degrade or modify lignin to enable the effective enzymatic hydrolysis of the polysaccharides present in plant cell walls. The aim of this proposal is to use metatranscriptomics and proteomics to determine gene - and protein - centred details to determine new mechanisms and improved methods of lignocellulose deconstruction in mixed microbial communities from composting wheat straw and sugar cane bagasse. Secreted proteins will be tagged, affinity purified and analyzed by LC-ESI-MS. In order to have a picture of the overall community dynamics in terms of species composition during the composting process DNA will be extracted for SSU rRNA profiling: Saccharification of the lignocellulose will be monitored and the lignin content of the straw or bagasse analyzed using FTIR spectroscopy and solid state NMR. Metatranscriptome analysis will be performed by preparing cDNA from samples taken at various time points from the lignocellulose enriched cultures, the cDNA will be sequenced using the Roche 454 GS FLX Titanium platform. The peptide sequences from the proteomics analysis will allow the identification of full and partial coding sequences in the library. These coding sequences will be cloned and expressed in established recombinant expression systems and the recombinant proteins screened for activity. (AU)

Matéria(s) publicada(s) na Agência FAPESP sobre o auxílio:
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PUBLICAÇÕES CIENTÍFICAS (8)

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