Exploring lignin degradation by termite-gut microbiota of a wood-feeding species, Nasutitermes ephratae

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manipulated. In this study, we report the development of a new Bifidobacteria Expression SysTem (BEST) allowing the expression and delivery of proteins of health interest. This BEST system is based on the broad host range plasmid pWV01 (able to replicate in both Gram-positive and Gram-negative bacteria), a stress-inducible promoter and two different signal peptides (SPs): one issued from the lactic acid bacterium (LAB) model *Lactococcus lactis*(SPExp4); and other from *Bifidobacterium longum*: SPBL1181. The functionality of BEST system was validated by cloning IL-10 cytokine and establishing the resulting plasmids, pBESTExp4:IL-10 and pBESTBL1181:IL-10, in different bifidobacteria species. We demonstrated that a recombinant strain of *B. bifidum* BS42 harboring pBESTBL1181:IL-10 plasmid efficiently secretes IL-10 and this secretion was significantly higher (7-fold) than its counterpart *B. bifidum* BS42 harboring pBESTExp4:IL-10 plasmid. We finally validated *in vivo* that recombinant *B. bifidum* secreting IL-10 under BEST system efficiently delivered the recombinant protein at mucosal surfaces and display anti-inflammatory properties in a murine model of colitis.

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Conversion of lignin into renewable chemicals is a major unsolved problem in the development of a biomass-based biorefinery. One of the objectives of the project Zero Waste Ligno-Cellulosic Bio-Refineries (ZELCOR) is the use of termites as models for the conversion of two types of recalcitrant by-products from lignocellulosic biomass (lignins and humins) into derived-fine chemicals.

Termite gut represents one of the more specific and unique microbial ecosystem. Indeed, termite gut microbiota consists mostly of novel lineages that have co-evolved or converged with their specific host. These microbial communities are very dense (up to 1011 cells/mL), diverse (6,000 phylotypes/mL), corresponding to many new lineages of mostly uncultivated bacteria that exclusively occur in this habitat. Furthermore, termites have a wide trophic diversity and by virtue of their gut microbial symbionts, they are the unique soil fauna able to degrade a broad range of organic matters, from wood to grass litter and soil.

Protobind 1000, grass alkali L from a mix of sarkanda and wheat, is one of model compound selected for investigating the conversion of lignin by termites.

Herein, we report on the fate of lignin upon passage of Protobind 1000 through wood-feeding gut system of living worker caste of N. ephratae and in gut homogenate-cultures. Protobind 1000, pyrolysis-GC-MS analysis was performed to determine the structure of Protobind 1000 and chemical modifications that occurred under experimental conditions. Furthermore, the potential of lignin oxidizing enzymes in the termite-guts was determined by FCA assay.

Vinylguaiacol and syringol were the predominant products in Protobind 1000. The signal of pyrograms of this substrate was different from those of termite guts. This confirms that pyrolysis-GC-MS is a suitable technique for determining the fate of lignin in termite-gut systems. Variations of the ratio S/G revealed that the structure of the lignin present in the gut was different from that derived from birch sawdust used as food for termites. The presence of phenolic compounds characteristics of Protobind 1000 indicated lignin ingestion by termites. The disappearance of some compounds, suggests that changes occurred in lignin structural. These changes were likely due to gut-microbiota. FCA assay showed that the gut microbiota of N. ephratae has enzymatic capability for bond cleavage releasing phenolic lignin compounds.