

436p Metabolic Engineering Bacteria for in Vitro Drug Metabolism

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Microbial models for human drug metabolism are being developed to replace many of the more expensive traditional methods such as the use of animal models or liver microsomes that contain the major drug metabolizing enzymes. There are several strains of bacteria that have been found to produce many of the same metabolites of different drugs as humans. One of these strains, *Actinoplanes* sp. ATCC 53771, is currently being used to produce seven metabolites of sirolimus, a macrolide immunosuppressant. This bacterium is not well characterized and has poor growth conditions, making it difficult to produce large quantities of the metabolites for further study. Actinomycetes are well known for containing cytochrome P450 systems, which leads us to believe that a cytochrome P450 enzyme in *Actinoplanes* sp. is responsible for the metabolism of sirolimus. We have performed growth curves under the conditions required for drug metabolism, and have identified at least seven different P450 gene fragments in *Actinoplanes* sp. One full P450 gene has also been identified, and we are currently working on characterizing it by expressing it in *E. coli*. A reduced CO-spectra assay will be used to examine folding and activity of the P450 protein and the folded protein will be assayed for sirolimus metabolism. The other P450 gene fragments are currently being extended to identify and clone other complete genes to be characterized.