Microbial Caffeine Junkies!
New Bacteria, Enzymes, and Genes

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ABSTRACT

Caffeine (1,3,7-trimethylxanthine) is a natural product commonly found in foods, beverages, and pharmaceuticals. It is also an environmental pollutant that is toxic to many insects and microbes and inhibits seed germination. Because of its wide use, caffeine enters the environment through human wastewater, decomposing plant matter, and solid and liquid wastes from coffee and tea processing plants. Interestingly, some bacteria have evolved the ability to utilize caffeine as a food source. However, the mechanism of bacterial caffeine degradation has not been established until now. We have isolated 14 strains of bacteria from soil that are capable of growing on caffeine as the sole carbon and nitrogen source. 

*Pseudomonas putida* CBB5 utilizes five novel enzymes belonging to the Rieske oxygenase family, NdmABCDE, to metabolize caffeine to xanthine *via* sequential N-demethylation. These enzymes are redox-dense proteins containing multiple [2Fe-2S] clusters, and are the first reported enzymes capable of N-demethylation of caffeine. We have also used a proteomic approach to identify NdmABCDE homologous enzymes in the bacterium *Pseudomonas* sp. CES.

Many of the methylxanthine metabolites produced in the caffeine N-demethylation pathway, such as paraxanthine (1,7-dimethylxanthine), 1-methylxanthine, 3-methylxanthine, and 7-methylxanthine, are high-value compounds used in the pharmaceutical industry. We have engineered over 40 strains of *E. coli* with various combinations of *ndmABD* to create these compounds from caffeine and theophylline (1,3-dimethylxanthine), a 200- to 5,000-fold increase in value. A strain of *E. coli* has also been “addicted” to caffeine by forcing it to utilize caffeine to synthesize its DNA. This new caffeine-addicted bacterial strain can be used as a biosensor to detect and quantify caffeine concentration in liquids.

These novel caffeine-degrading genes, enzymes, and microbes provide many potential applications, including (i) environmental remediation of coffee and tea waste and by-products, (ii) bio-decaffeination of caffeine, (iii) biosynthesis of pharmaceuticals, (iv) decaffeination of coffee and tea waste prior to fermentation, (v) development of caffeine biosensors, and (vi) regulation of synthetic gene networks using caffeine- or theophylline-responsive RNA switches.