A Systematic Study of the Caffeine Biosynthetic Enzymes in the Ilex Genus

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Yaupon Holly and Yerba Mate are two economically important, caffeine-producing holly (i.e., Ilex) species in America. Yaupon Holly is native to North America and Yerba Mate is native to South America. Yerba Mate contains three caffeine synthase (CS) enzymes, which catalyze the stepwise conversions from xanthine to caffeine. Unlike Yerba Mate, the caffeine biosynthetic pathways in Yaupon Holly were largely unknown. In addition to Yaupon Holly and Yerba Mate, other Ilex species were recently found to contain CS-like enzymes. However, these Ilex species do not produce caffeine. This project aims to conduct a systematic study of caffeine biosynthetic enzymes in the Ilex genus. Specifically, we identified and characterized several candidate CS genes from Yaupon Holly, as well as three CS-like genes from non-caffeine producing Ilex species, and compared them with known CS genes in Yerba Mate and tea plants. We found that Yerba Mate and Yaupon Holly both synthesize caffeine from the "xanthine - 3-methylxanthine - theobromine" route. We also discovered that CS-like enzymes from non-caffeine producing Ilex species have promiscuous activities towards different caffeine biosynthetic substrates. However, these enzymes have not fully evolved or diverged to complete a caffeine synthetic route. Our protein structure prediction showed that CS and CS-like enzymes in the Ilex genus have subtle structural differences, which may explain their differences in substrate preference and catalytic efficiency.

Awards Won:
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