Essential Region for 3-*N* Methylation in *N*-Methyltransferases Involved in Caffeine Biosynthesis

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The caffeine biosynthetic pathway is composed of three methylation steps, and *N*-methyltransferase catalyzing each step has high substrate specificity. Since the amino acid sequences among coffee 7-methylxanthosine synthase (*CmXRS1*), theobromine synthase, and caffeine synthase are highly homologous to each other, these substrate specificities seem to be determined in a very restricted region. The analysis of site-directed mutants for CmXRS1 that naturally acts at the initial step, *i.e.* 7-*N* methylation of xanthosine, revealed that the activity of 3-*N* methylation needs a histidine residue at corresponding position 161 in the CmXRS1 sequence. We succeeded in producing the mutant enzyme which can catalyze the first and second methylation steps in caffeine biosynthesis.

Key words: Coffee, Caffeine, *N*-Methyltransferase