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Analysis of the Function of the Yeast Mitochondrial C1-Tetrahydrofolate Synthase

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The trifunctional enzymes C1-tetrahydrofolate synthases catalyze the interconversion of three coenzyme forms of folic acid, a vitamin required by humans for the synthesis and metabolism of proteins and amino acids, nucleic acids and purines and pyrimidines and a vitamin. The yeast enzymes have been identified as the products of the ADE3 and MIS1 genes, and they have been shown to occur in the cytoplasm and mitochondria, respectively. The function of the product of the MIS1 gene is under question since the disruption of the MIS1 gene has no effect on the growth of yeast, although it has been assumed that the gene would be required for generation of the formyl donor for formylation of the initiator fMet-tRNA. The function of the MIS1 gene for mitochondrial isozyme will be discussed.

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A Thermostable Alkaline Protease from *Thermoactinomyces* sp. E79 and the DNA Sequence of the Encoding Gene

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A thermophilic *Thermoactinomyces* sp. E79 producing a highly thermostable alkaline protease was isolated from soil. The protease, produced extracellularly by *Thermoactinomyces* sp. E79 was purified by DEAE-Sephadex and Butyl-Toyopearl 650M column chromatography. The relative molecular mass was estimated to be 31,000 by SDS-PAGE. Enzyme activity was inhibited by PMSF, suggesting the enzyme to be a serine protease. The optimum temperature was 85°C and about 50% of the original activity remained after incubation at 90°C for 10 min in the presence of Ca²⁺. The optimum pH was 11.0 and the enzyme was fairly stable from pH 5.0 to 12.0. The gene for this protease was cloned in *E. coli* and the expressed intracellular enzyme was activated by heat treatment. Sequence analysis showed an ORF of 1,152 base pairs, coding for a polypeptide of 384 amino acids. The polypeptide was composed of a signal sequence (25 amino acids), a prosequence (81 amino acids), and a mature protein of 278 amino acids. The deduced amino acid sequence of mature protease had high similarity with thermitase and the extent of sequence identity was 76%.