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Amy K. Dadisman
Cleveland State University

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Purification and Crystallization Trials of the Dihydroorotase from Methanococcus jannaschii

College of Sciences and Health Professions

Student Researcher: Amy Dadisman

Faculty Advisor: Jacqueline Vitali

Abstract

Dihydroorotase is the enzyme that catalyzes the third step of the de novo biosynthesis of pyrimidines. M. jannaschii is a hyperthermophilic archaean that can serve as a model organism for research purposes. This experiment is a first step toward elucidating the structure of the dihydroorotase in M. jannaschii. The enzyme was purified by salting out and heating the solution and then putting the supernatant through cation exchange chromatography and hydrophobic interaction chromatography. Twenty-four conditions were tested to determine if a crystal of dihydroorotase could be formed. Two of these conditions led to preliminary crystal formation. These findings can be utilized to determine which other conditions to test to form a crystal. Once a crystal is formed, it can undergo X-ray crystallography to determine its structure. The structure of dihydroorotase in M. jannaschii can elucidate what structural characteristics allow this archaean to survive in extreme heat and provides further understanding of the diversity within the dihydroorotase family of proteins. It can also give further insight into this pathway in humans.