

Characterization of a Novel beta-Glucanase From the Hyperthermophile *Fervidibacter sacchari*, the Sole Isolate of an Ancient Bacterial Class

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Abstract

Although once speculated to be the oldest bacterial lineage, few studies have investigated candidate class “Fervidibacteria” since its discovery. *Fervidibacter sacchari* was isolated in our lab and is the only cultivated member of the class. It possesses a large and diverse cache of polysaccharide-degrading glycoside hydrolase (GH) enzymes and degrades diverse polysaccharides. One such GH belongs to family GH50, of which all are characterized members are classified as beta-agarases from predominantly mesophilic microbes. Here we describe heterologous expression of this GH50 in *E. coli* and its characterization using 3,5-dinitrosalicylic acid and 4-nitrophenol enzyme activity assays. We demonstrate that this GH possesses a novel activity for the family as a beta-glucanase with strong activity on beta-(1,4/1,3)-glucans, a pH optimum of ~7, and a temperature optimum of ~80 °C, making it the first characterized hyperthermophilic GH50. Creating a Lineweaver-Burk plot revealed a V_{\max} of 357.1 $\mu\text{M}/\text{min}$ and K_m of 12.6 mM. This project paves the way for characterizing other *F. sacchari* GHs and addresses a need for easily producible cellulolytic enzymes capable of degrading plant waste, a requirement for long-term space flight.