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## **Abstract**

*Fervidibacter sacchari* is an aerobic hyperthermophile within the phylum *Armatimonadota* that is capable of degrading various polysaccharides. Its genome encodes 117 glycoside hydrolases (GHs), including two from GH family 50 (GH50). In this study, one of these GH50 enzymes, Fsa11540Glu, was expressed, purified, and functionally characterized. A screen for GH activity against 12 substrates revealed activity on gellan gum,  $\beta$ -glucan, carboxymethyl (CM) curdlan, and agarose. Despite most GH50s being known as  $\beta$ -agarases, Fsa11540Glu was optimally active on gellan gum with low activity on agarose. The enzyme has a narrow temperature range of 70-90 °C with an optimal temperature of 80-90 °C and is thermostable up to 90 °C. Fsa11540Glu is bimodally active at pH 5.5 and 7 with the optimal activity at 5.5. ColabFold computed structure models (CSMs) along with electrophoresis predicted a monomer and identified two conserved glutamic residues Glu385 and Glu551 in the active site. Electrophoresis also confirmed that Fsa11540Glu has a molecular weight of ~137 kDa. Phylogenetic analysis of Fsa11540Glu and all other GH50 enzymes, revealed two distinct clades of enzymes from members of the class *Fervidibacteria*: North American and Asian (Japanese and Chinese), with Fsa11540Glu in the North American clade. It appears that Fsa11540Glu evolved vertically within the *Fervidibacter* genome or arose through duplication of a closely related GH50 family gene encoding enzyme Fsa16295Glu.