



INDUSTRIAL MICROBIOLOGY

Turning up the heat on biomass degradation

Cellulosic biomass can be used as a feedstock for biofuel production, but before it can be enzymatically or chemically hydrolyzed, it must undergo a pretreatment process involving high temperature coupled with extreme pH or steam explosion. Writing in *Nature Communications*, Graham *et al.* describe the identification of a highly stable hyperthermophilic archaeal cellulase that can degrade cellulosic material at temperatures above 100 °C.

Few archaea are known to be capable of degrading lignocellulose, and to date only a single hyperthermophilic archaeal species has been shown to grow on crystalline cellulose, at a temperature of 81 °C. To search for species that were able to degrade cellulose at even higher temperatures, the authors collected sediment from a 94 °C geothermal pool in northern Nevada, USA, and used

an enrichment strategy to identify an anaerobic consortium consisting of three archaeal species that could grow on a range of cellulose sources. By looking at the protein fraction that bound to the cellulose substrates, a number of active cellulases could be detected that ranged in size from 40 to 180 kDa. Metagenomic analysis of this consortium revealed 37 genes that encoded putative glycosyl hydrolases. The authors selected one of these putative cellulases, EBI-244, for further study.

EBI-244 is predicted to contain a glycosyl hydrolase family 5 domain and three domains that do not bear any sequence similarity to characterized proteins. The authors expressed EBI-244 in *Escherichia coli* and purified it for analysis. They found that the protein exhibits endoglucanase activity on a range of high-molecular-mass carbohydrate substrates

containing β -1,4-linked glucose monomers. This activity was maximal at 109 °C and was retained after boiling in detergent, after proteinase K treatment and at a pH range of between 3.5 and 8.0, suggesting that the enzyme is highly stable.

These findings represent a new benchmark for microbial degradation of lignocellulose biomass at high temperatures. Furthermore, the ability of EBI-244 to withstand such harsh conditions makes it a promising candidate for use in the production of biofuels, as it could minimize the cost and complexity of adjusting conditions between the pretreatment and hydrolysis processes.

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ORIGINAL RESEARCH PAPER Graham, J. E. Identification and characterization of a multidomain hyperthermophilic cellulase from an archaeal enrichment. *Nature Commun.* **2**, 375 (2011)