A xanthan-binding module from a multi-modular xanthanase of *Cohnella* sp. 56 VKM B-36720

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A xanthanase designated as *Csp*Xan9 was found in the new xanthan-degrading strain *Cohnella* sp. 56 VKM B-36720 by comparing the bacterial secretome from different media (lysogeny broth, glucose minimal medium, xanthan minimal medium). *Csp*Xan9 could efficiently degrade native xanthan, XLT-xanthan (lyase-pretreated xanthan)(1) and addtional xanthan derivatives lacking side chain substituents xan∆L, xan∆GL and xan∆FGL(2). The specific activity indicated that *Csp*Xan9 had a preference for substrates in the order XLT-xanthan, xan∆FGL, xan∆GL, xan∆L and native xanthan. The results of thin layer chromatography (TLC) showed that *Csp*Xan9 could putatively produce tetrasaccharides as primary end products from XLT-xanthan cleavage. Compared with a known xanthanase from *Paenibacillus nanensis*(1), the modular xanthanase *Csp*Xan9 had a different carbohydrate binding module at the C-terminal end. In order to investigate the function of this module, deletion derivative (*Csp*Xan9-C) lacking the non-catalytic domain was produced in recombinant *E. coli* strains. After protein purification, enzyme assays indicated that this deletion resulted in a slight increase of the specific activity on XLT-xanthan at 37 ˚C from 7.76 ± 0.74 U/mg (*Csp*Xan9) to 10.31 ± 0.23 U/mg (*Csp*Xan9-C), but no longer showed retarded mobility in native polyacrylamide gel electrophoresis (NAPAGE) containing different concentrations of XLT-xanthan. Considering that the C-terminus deleted enzyme retained hydrolytic activity on XLT-xanthan, various deletion variants containing single modules of this xanthanase were further expressed individually. However, NAPAGE analysis confirmed that only the C-terminal module is a xanthan-binding module, and it is highly specific to XLT-xanthan.

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2. Gansbiller M, Schmid J, Sieber V. In-depth rheological characterization of genetically modified xanthan-variants. Carbohydrate Polymer. 2019;213:236-46.