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B-glucosidases from *penicillium* and *Aspergillus* expression, purification and catalytic properties

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The enzymatic saccharification of polysaccharides from lignocellulose biomass is a crucial stage in production of second-generation biofuels (ethanol, butanol, etc.), bifunctional organic acids (lactic, fumaric) for biopolymer synthesis. Cellulose is converted to glucose under the synergistic action of at least three types of glycoside hydrolases (endoglucanases, exo-cellobiohydrolases and β-glucosidases). β-Glucosidase (BGL) is the key enzyme that completes the saccharification process, converting cellobiose to glucose. A novel bgl1 gene, encoding GH3 family β-glucosidase from *Penicillium verruculosum* (PvBGL) was cloned and heterologously expressed in *P. canescens* RN3-11-7 (niaD-) strain. BGL from *Aspergillus niger* (rAnBGL) was expressed in the same recipient host. Both BGLs were desalted and purified from cultural broth by AEX, HIC, and GF chromatography using columns packed with Bio-Gel P4, Source 15Q, Source15ISO, and Superdex75 for final polishing. SDS-PAGE followed by MALDI-TOF/TOF analysis of tripsin digests were used for purity control and proteins identification. Temperature and pH-optima for both ezymes were the same 65°C and pH value 4, 5-4, 6 respectively. The half-life time of rPvBGL and rAnBGL was 10 and 5 min respectively at 70°C. The activity of both enzymes decreases with the increase of degree of cellooligosaccharides polymerization (DP). Kinetic studies revieled differences between PvBGL and AnBGL. The catalytic efficiency of AnBGL was about 35% higher to compare PvBGL in p-NP-β-D-glucoside and cellobiose hydrolysis, while remains the same for 1,4-β-oligosaccharides with higher DP. AnBGL was more specific in hydrolysis of 1,4-β-cellooligosaccharides, while PvBGL was much more efficient in hydrolysis of 1,3-β- and mixed 1,3-β-/1,4-β-oligosaccharides.

Biography

Ivan N Zorov has completed his PhD at M V Lomonosov Moscow State University. He spent six years as Analytical and Marketing Manager in GC/LC/MS method development at Agilent/Hewlett-Packard. Currently, he is the Senior Scientist at MSU and the Federal Research Center of Biotechnology, Moscow. He has published more than 25 papers in reputed journals.

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