

TITLE: CLONING, EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF AN ENDOGLUCANASE GH7 FROM *Thermothielavioides terrestris*

AUTHORS: ALNOCH, R.C.^{1.}; SALGADO, J.C.S.^{2.}; ALVES, G.S.^{3.}; ALMEIDA, P.Z.^{3.}; WARD, R.J.^{2.}; SEGATO, F.^{4.}; POLIZELI, M.L.T.M.^{1,3.}

INSTITUTION: 1. Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras – Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil; 2. Departamento de Química, Faculdade de Filosofia, Ciências e Letras – Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil; 3. Departamento de Bioquímica, Faculdade de Medicina – Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil; 4. Departamento de Biotecnologia, Escola de Engenharia de Lorena – Universidade de São Paulo, Lorena, São Paulo, Brasil.

ABSTRACT:

Endoglucanases (EC 3.2.1.4) are important enzymes involved in the hydrolysis of the cellulose, acting randomly in the β -1,4-glycosidic bonds present in the amorphous regions of the polysaccharide chain. These biocatalysts have been classified into 16 glycosyl hydrolase (GH) families. GH7 family has a particular interest, since it may be act on a broad range of substrates, including cellulose, β -glucan, and xylan, an attractive feature for biotechnological application, especially in the renewable energy field. In the current work, an endoglucanase (GH7) gene from the thermophilic fungus *Thermothielavioides terrestris*, was cloned in the secretion vector pEXPYR and transformed into the high protein producing strain *Aspergillus nidulans* A773. Endoglucanase TtGH7 was expressed and purified by two chromatography steps, using an anion exchange (HiPrep Q FF) and size exclusion (Superdex 75 10/300 GL) columns integrated into an ÄKTA Purifier chromatography system. TtGH7 has a molecular weight of approximately 66 kDa, evidenced by SDS-PAGE. Circular dichroism confirmed the high β -strand content consistent with the canonical GH7 family β -jellyroll fold, also observed in 3D homology model of TtGH7. Biochemical characterization assays showed that TtGh7 was active over a wide range of pH values (3.5–7.0) and temperatures (45–70 °C), with the highest activity at pH 4.0, 65 °C. TtGH7 also was stable over a wide range of pH (3.5–9.0), maintaining more than 80% of its original activity after 24 h. The $K_{0.5}$ and V_{max} determined using low viscosity carboxymethylcellulose were 9.3 mg mL⁻¹ and 2.5 x 10⁴ U mg⁻¹, respectively. The results obtained in this work provide a basis for the development of applications of recombinant TtGh7 in the renewable energy field.

Keywords: *Thermothielavioides terrestris*, Endoglucanases, GH7 family, renewable energy.

Development Agency: The work was supported by the following: FAPESP (São Paulo Research Foundation, grants: 2014/50884, 2018/07522-6 and 2019/21989-7; Processes 2020/00081-4) and National Institute of Science and Technology of Bioethanol, INCT, CNPq (grant: 465319/2014-9).