

Structure and function of enzymes and auxiliary proteins from *Trichoderma*, active in cell-wall hydrolysis

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Auxílios(s) vinculado(s): [10/18849-4 - Purificação e caracterização de hidrolases de glicosídeos, produzidas pelo fungo filamentosso *Trichoderma harzianum*, com perspectiva de aplicabilidade dessas enzimas em coquetéis para hidrólise da biomassa e produção de bioetanol, AV.EXT](#)

Bolsa(s) vinculada(s): [13/15582-5 - Dinâmica molecular em múltiplas escalas de glicosídeo hidrolases e substratos lignocelulósicos, BP.PD](#)
[13/13766-1 - Estrutura e função de enzimas e proteínas auxiliares de *Trichoderma*, atuantes na hidrólise da parede celular, BP.TT](#)
[12/17298-0 - Apoio técnico ao grupo de biotecnologia molecular do Instituto de Física de São Carlos, IFSC/USP, BP.TT](#)
[+ mais bolsas vinculadas](#)

Convênio/Acordo de cooperação com a FAPESP: [CNPq - Temáticos - Pronex](#)

Publicação FAPESP sobre o auxílio: http://media.fapesp.br/bv/uploads/publicacoes/pasta_bioen_jun2012_70.pdf

Assunto(s): [Biologia molecular](#) [Fungos mitosporicos](#) [Trichoderma](#) [Enzimas](#)

Resumo

Lignocellulosic biomass, such as sugarcane bagasse, holds a promise of environmentally friendly bioenergy production in Brazil. However, enzymatic hydrolysis, currently considered a method of choice in biomass saccharification, is hampered by considerable cell-wall recalcitrance. To make this technology sustainable and cost effective, our comprehension of cellulose enzymatic hydrolysis should be significantly improved. Here we propose to conduct systematic structure-functional studies of *Trichoderma* cellulases and auxiliary proteins active in cell-wall degradation using a combination of X-ray protein crystallography, biophysical and biochemical studies, molecular dynamics simulations, statistical coupling analysis aligned with the site-directed mutagenesis and enzymatic assays aiming to obtain in-depth comprehension of cellulose hydrolysis. We plan to contribute toward structural analysis of *Trichoderma reesei* endoglucanases by solving a crystal structure of endoglucanase II (Cel5A), main enzymatically active, but structurally uncharacterized endoglucanase of this important industrial fungus. Moreover, we will contribute toward our knowledge of *Trichoderma* cellulases molecular organization by solving X-ray structures of main *Trichoderma harzianum* endo- and exoglucanases (primarily focusing on Cel7A and Cel5A) and by comparing them with the correspondent *T. reesei* enzymes. We also aim to structurally characterize swollenins, non-hydrolytic proteins, shown to enhance cellulose hydrolysis catalyzed by cellulases, and to study

thermodynamically its interactions with cellulose. In addition, we will construct chimeric enzymes by fusing of swollenin with the cellulases and will study enzymatic properties of such chimeras. Furthermore, we will conduct systematic molecular dynamics studies of the cellulases and swollenin, and investigate their flexibility by hydrogen deuterium exchange followed by mass spectrometry. Finally, we will use all these acquired knowledge to modify the proteins using site-directed mutagenesis aiming to better comprehend molecular basis of their function and to produce enzymes and their mixtures with enhanced hydrolytic properties. (AU)

Matéria(s) publicada(s) na Agência FAPESP sobre o auxílio:

[Aprimorar coquetéis enzimáticos é caminho para etanol celulósico](#)

PUBLICAÇÕES CIENTÍFICAS (16)

(Referências obtidas automaticamente do Web of Science e do SciELO, por meio da informação sobre o financiamento pela FAPESP e o número do processo correspondente, incluída na publicação pelos autores)

BERNARDINELLI, OIGRES DANIEL; LIMA, MARISA APARECIDA; REZENDE, CAMILA ALVES; POLIKARPOV, IGOR; DEAZEVEDO, EDUARDO RIBEIRO. [Quantitative C-13 MultiCP solid-state NMR as a tool for evaluation of cellulose crystallinity index measured directly inside sugarcane biomass.](#)

BIOTECHNOLOGY FOR BIOFUELS, v. 8, AUG 5 2015. Citações Web of Science: 0.

SEIKI KADOWAKI, MARCO ANTONIO; CAMILO, CESAR MOISES; MUNIZ, AMANDA BERNARDES; POLIKARPOV, IGOR. [Functional Characterization and Low-Resolution Structure of an Endoglucanase Cel45A from the Filamentous Fungus Neurospora crassa OR74A: Thermostable Enzyme with High Activity Toward Lichenan and beta-Glucan.](#) **MOLECULAR BIOTECHNOLOGY**, v. 57, n. 6, p. 574-588, JUN 2015.

Citações Web of Science: 0.

BERNARDES, AMANDA; TEXTOR, LARISSA C.; SANTOS, JADEMILSON C.; CUADRADO, NAZARET HIDALGO; KOSTETSKY, EDUARD YA.; ROIG, MANUEL G.; BAVRO, VASSILIY N.; MUNIZ, JOAO R. C.; SHNYROV, VALERY L.; POLIKARPOV, IGOR. [Crystal structure analysis of peroxidase from the palm tree Chamaerops excelsa.](#) **Biochimie**, v. 111C, p. 58-69, APR 2015. Citações Web of Science: 0.

MIOTTO, LIS SCHWARTZ; DE REZENDE, CAMILA ALVES; BERNARDES, AMANDA; SERPA, VIVIANE ISABEL; TSANG, ADRIAN; POLIKARPOV, IGOR. [The Characterization of the Endoglucanase Cel12A from Gloeophyllum trabeum Reveals an Enzyme Highly Active on beta-Glucan.](#) **PLoS One**, v. 9, n. 9 SEP 24 2014. Citações Web of Science: 0.

TSUCHIDA, JEFFERSON ESQUINA; REZENDE, CAMILA ALVES; DE OLIVEIRA-SILVA, RODRIGO; LIMA, MARISA APARECIDA; D'EURYDICE, MARCEL NOGUEIRA; POLIKARPOV, IGOR; BONAGAMBA, TITO JOSE. [Nuclear magnetic resonance investigation of water accessibility in cellulose of pretreated sugarcane bagasse.](#) **BIOTECHNOLOGY FOR BIOFUELS**, v. 7, SEP 10 2014. Citações Web of Science: 1.

NASCIMENTO, ALESSANDRO S.; MUNIZ, JOAO RENATO C.; APARICIO, RICARDO; GOLUBEV, ALEXANDER M.; POLIKARPOV, IGOR. [Insights into the structure and function of fungal beta-mannosidases from glycoside hydrolase family 2 based on multiple crystal structures of the Trichoderma harzianum enzyme.](#) **FEBS Journal**, v. 281, n. 18, SI, p. 4165-4178, SEP 2014. Citações Web of Science: 2.

CAMILO, CESAR M.; POLIKARPOV, IGOR. [High-throughput cloning, expression and purification of glycoside hydrolases using Ligation-Independent Cloning \(LIC\).](#) **Protein Expression and Purification**, v. 99, p. 35-42, JUL 2014. Citações Web of Science: 2.

MELLO, BRUNO LUAN; POLIKARPOV, IGOR. [Family 1 carbohydrate binding-modules enhance saccharification rates.](#) **AMB EXPRESS**, v. 4, APR 25 2014. Citações Web of Science: 0.

LIMA, MARISA A.; GOMEZ, LEONARDO D.; STEELE-KING, CLARE G.; SIMISTER, RACHAEL; BERNARDINELLI, OIGRES D.; CARVALHO, MARCELO A.; REZENDE, CAMILA A.; LABATE, CARLOS A.; DEAZEVEDO, EDUARDO R.; MCQUEEN-MASON, SIMON J.; POLIKARPOV, IGOR. [Evaluating the composition and processing potential of novel sources of Brazilian biomass for sustainable biorenewables](#)

[production.](#) **BIOTECHNOLOGY FOR BIOFUELS**, v. 7, JAN 18 2014. Citações Web of Science: 2.

LIMA, MARISA A.; OLIVEIRA-NETO, MARIO; KADOWAKI, MARCO ANTONIO S.; ROSSETO, FLAVIO R.; PRATES, ERICA T.; SQUINA, FABIO M.; LEME, ADRIANA F. P.; SKAF, MUNIR S.; POLIKARPOV, IGOR. [Aspergillus niger beta-Glucosidase Has a Cellulase-like Tadpole Molecular Shape INSIGHTS INTO GLYCOSIDE HYDROLASE FAMILY 3 \(GH3\) beta-GLUCOSIDASE STRUCTURE AND FUNCTION.](#) **Journal of Biological Chemistry**, v. 288, n. 46, p. 32991-33005, NOV 15 2013. Citações Web of Science: 4.

LIMA, LEONARDO H. F.; SERPA, VIVIANE I.; ROSSETO, FLAVIO R.; SARTORI, GERALDO RODRIGUES; DE OLIVEIRA NETO, MARIO; MARTINEZ, LEANDRO; POLIKARPOV, IGOR. [Small-angle X-ray scattering and structural modeling of full-length: cellobiohydrolase I from Trichoderma harzianum.](#) **Cellulose**, v. 20, n. 4, p. 1573-1585, AUG 2013. Citações Web of Science: 1.

DE ARAUJO, EVANDRO ARES; TOMAZINI, JR., ATILIO; SEIKI KADOWAKI, MARCO ANTONIO; MURAKAMI, MARIO TYAGO; POLIKARPOV, IGOR. [Crystallization and preliminary X-ray diffraction analysis of a new xyloglucanase from Xanthomonas campestris pv. campestris.](#) **Acta Crystallographica Section F**, v. 69, n. 6, p. 676-678, JUN 2013. Citações Web of Science: 0.

LIMA, MARISA A.; LAVORENTE, GABRIELA B.; DA SILVA, HANA K. P.; BRAGATTO, JULIANO; REZENDE, CAMILA A.; BERNARDINELLI, OIGRES D.; DEAZEVEDO, EDUARDO R.; GOMEZ, LEONARDO D.; MCQUEEN-MASON, SIMON J.; LABATE, CARLOS A.; POLIKARPOV, IGOR. [Effects of pretreatment on morphology, chemical composition and enzymatic digestibility of eucalyptus bark: a potentially valuable source of fermentable sugars for biofuel production - part 1.](#) **BIOTECHNOLOGY FOR BIOFUELS**, v. 6, MAY 9 2013. Citações Web of Science: 14.

DOS REIS, CAIO VINICIUS; BERNARDES, AMANDA; POLIKARPOV, IGOR. [Expression, purification, crystallization and preliminary X-ray diffraction analysis of Bifidobacterium adolescentis xylose isomerase.](#) **Acta Crystallographica Section F**, v. 69, n. 5, p. 588-591, MAY 2013. Citações Web of Science: 0.

PRATES, ERICA T.; STANKOVIC, IVANA; SILVEIRA, RODRIGO L.; LIBERATO, MARCELO V.; HENRIQUE-SILVA, FLAVIO; PEREIRA, JR., NEI; POLIKARPOV, IGOR; SKAF, MUNIR S. [X-ray Structure and Molecular Dynamics Simulations of Endoglucanase 3 from Trichoderma harzianum: Structural Organization and Substrate Recognition by Endoglucanases That Lack Cellulose Binding Module.](#) **PLoS One**, v. 8, n. 3 MAR 14 2013. Citações Web of Science: 6.

ROSSETO, FLAVIO R.; PUHL, ANA C.; ANDRADE, MAXUEL O.; POLIKARPOV, IGOR. [Crystallization and preliminary diffraction analysis of the catalytic domain of major extracellular endoglucanase from Xanthomonas campestris pv. campestris.](#) **Acta Crystallographica Section F**, v. 69, n. Part 2, p. 137-140, FEB 2013. Citações Web of Science: 0.