

Identification of a novel tailor-made chitinase from white shrimp *fenneropenaeus merguensis*

Azadeh Beygmoradi, Ahmad Homaei, Roohullah Hemmati, Jon Del Arco, Jesús Fernández-Lucas

Abstract

Fenneropenaeus merguensis (commonly named banana shrimp) is one of the most important farmed crustacean worldwide species for the fisheries and aquaculture industry. Besides its nutritional value, it is a good source of chitinase, an enzyme with excellent biological and catalytic properties for many industrial applications. In the present study, a putative chitinase-encoding cDNA was synthesized from mRNA from *F. merguensis* hepatopancreas tissue. Subsequently, the corresponding cDNA was cloned, sequenced and functionally expressed in *Escherichia coli*, and the recombinant *F. merguensis* chitinase (rFmCHI) was purified by His-tag affinity chromatography. The bioinformatics analysis of amino acid sequence of rFmCHI displayed a canonical multidomain architecture in chitinases which belongs to glycoside hydrolase family 18 (GH18 chitinase). Biochemical characterization revealed rFmCHI as a monomeric enzyme of molecular weight 52 kDa with maximum activity at 40 °C and pH 6.0. Moreover, the recombinant enzyme is also stable up to 60 °C, and in the pH range 5.0-8.0. Steady-state kinetic studies for colloidal chitin revealed K_M , V_{max} and k_{cat} values of 78.18 μM , 0.07261 $\mu M \cdot min^{-1}$ and 43.37 s^{-1} , respectively. Overall, our results aim to demonstrate the potential of rFmCHI as suitable catalyst for bioconversion of chitin waste.

Keywords

Marine organisms, Chitinolytic enzymes, Molecular cloning, Protein purification, Biochemical characterization

Referencias

S.M. Moss, D.R. Moss, S.M. Arce, D.V. Lightner, J.M. Lotz, The role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture, *J. Invertebr. Pathol.* 110 (2012) 247–250.

A. Homaei, Purification and biochemical properties of highly efficient alkaline phosphatase from *Fenneropenaeus merguensis* brain, *J. Mol. Catal. B Enzym.* 118

(2015) 16–22.

D.J. Vance, P.C. Rothlisberg, The biology and ecology of the banana prawns: *Penaeus merguensis* de Man and *P. indicus* H. Milne Edwards, *Adv. Mar. Biol.* 86

(2020) 1–139.

A. Beygmoradi, A. Homaei, R. Hemmati, P. Santos-Moriano, D. Hormigo, J. Fernandez-Lucas, Marine chitinolytic enzymes, a biotechnological treasure hidden in the ocean? *Appl. Microbiol. Biotechnol.* 102 (2018) 9937–9948.

H.P. Ramesh, R.N. Tharanathan, Carbohydrates—the renewable raw materials of high biotechnological value, *Crit. Rev. Biotechnol.* 23 (2003) 149–173.

Q.-S. Huang, X.-L. Xie, G. Liang, F. Gong, Y. Wang, X.-Q. Wei, Q. Wang, Z.-L. Ji, Q.-X. Chen, The GH18 family of chitinases: their domain architectures, functions and evolutions, *Glycobiology* 22 (2012) 23–34.

J. Zhang, Y. Sun, F. Li, B. Huang, J. Xiang, Molecular characterization and expression analysis of chitinase (Fcchi-3) from Chinese shrimp, *Fenneropenaeus chinensis*, *Mol. Biol. Rep.* 37 (2010) 1913–1921.

Y. Huang, F. Ma, W. Wang, Q. Ren, Identification and molecular characterization of a peritrophin-like gene, involved in the antibacterial response in Chinese mitten crab, *Eriocheir sinensis*, *Dev. Comp. Immunol.* 50 (2015) 129–138.

P.K. Dutta, J. Dutta, V. Tripathi, *Chitin and Chitosan: Chemistry, Properties and Applications*, 2004.

I. Hamed, F. Ozogul, J.M. Regenstein, Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): a review, *Trends Food Sci. Technol.* 48 (2016) 40–50.

A. Beygmoradi, A. Homaei, Marine microbes as a valuable resource for brand new industrial biocatalysts, *Biocatal. Agric. Biotechnol.* 11 (2017) 131–152.

P.E. Kidibule, P. Santos-Moriano, E. Jim´enez-Ortega, M. Ram´ırez-Escudero, M.

C. Limon, M. Remacha, F.J. Plou, J. Sanz-Aparicio, M. Fer´nandez-Lobato, Use of´ chitin and chitosan to produce new chitooligosaccharides by chitinase Chit42: enzymatic activity and structural basis of protein specificity, *Microb. Cell Fact.* 17 (2018) 47.

L. Hartl, S. Zach, V. Seidl-Seiboth, Fungal chitinases: diversity, mechanistic properties and biotechnological potential, *Appl. Microbiol. Biotechnol.* 93 (2012)

533–543.

R.S. Patil, V. Ghormade, M.V. Deshpande, Chitinolytic enzymes: an exploration, *Enzyme Microb. Technol.* 26 (2000) 473–483.

S.S. Paulsen, B. Andersen, L. Gram, H. Machado, Biological potential of chitinolytic marine bacteria, *Mar. Drugs* 14 (2016) 230.

T. Fukamizo, Chitinolytic enzymes catalysis, substrate binding, and their application, *Curr. Protein Pept. Sci.* 1 (2000) 105–124.

T. Ohno, S. Armand, T. Hata, N. Nikaidou, B. Henrissat, M. Mitsutomi,

T. Watanabe, A modular family 19 chitinase found in the prokaryotic organism *Streptomyces griseus* HUT 6037, *J. Bacteriol.* 178 (1996) 5065–5070.

A. Kasprzewska, Plant chitinases-regulation and function, *Cell. Mol. Biol. Lett.* 8

809–824.

F. Fusetti, H. von Moeller, D. Houston, H.J. Rozeboom, B.W. Dijkstra, R.G. Boot, J. M. Aerts, D.M. van Aalten, Structure of human chitotriosidase implications for specific inhibitor design and function of mammalian chitinase-like lectins, *J. Biol.*

Chem. 277 (2002) 25537–25544.

- M.R. Stam, E. Blanc, P.M. Coutinho, B. Henrissat, Evolutionary and mechanistic relationships between glycosidases acting on α - and β -bonds, *Carbohydr. Res.* 340 (2005) 2728–2734.
- A. Bendt, H. Hüller, U. Kammel, E. Helmke, T. Schweder, Cloning, expression, and characterization of a chitinase gene from the Antarctic psychrotolerant bacterium *Vibrio* sp. strain Fi:7, *Extremophiles* 5 (2001) 119–126.
- M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 72 (1976) 248–254.
- J.D. Thompson, Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, *Nucleic Acids Res.* 22 (1994) 4673–4680.
- S. Kumar, K. Tamura, M. Nei, MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment, *Brief. Bioinformatics* 5 (2004) 150–163.
- M. Biasini, S. Bienert, A. Waterhouse, K. Arnold, G. Studer, T. Schmidt, F. Kiefer, T. G. Cassarino, M. Bertoni, L. Bordoli, SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information, *Nucleic Acids Res.* 42 (2014) W252–W258.
- D. Case, T. Darden, T. Cheatham III, C. Simmerling, J. Wang, R. Duke, R. Luo, R. Walker, W. Zhang, K. Merz, AMBER 12, University of California, San Francisco, 2010, pp. 1–826, 2012, There is no corresponding record for this reference.[Google Scholar].
- R.L. Tellam, Protein motifs in filarial chitinases: an alternative view, *Parasitol.* Today 12 (1996) 291–292.
- Q. Zhu, Y. Deng, P. Vanka, S.J. Brown, S. Muthukrishnan, K.J. Kramer, Computational identification of novel chitinase-like proteins in the *Drosophila melanogaster* genome, *Bioinformatics* 20 (2004) 161–169.
- Z. Shen, M. Jacobs-Lorena, Evolution of chitin-binding proteins in invertebrates, *J. Mol. Evol.* 48 (1999) 341–347.

Y. Arakane, Q. Zhu, M. Matsumiya, S. Muthukrishnan, K.J. Kramer, Properties of catalytic, linker and chitin-binding domains of insect chitinase, *Insect Biochem.*

Mol. Biol. 33 (2003) 631–648.

M. Quentin, M. Ebbelaar, J. Derksen, C. Mariani, H. van Der Valk, Description of a cellulose-binding domain and a linker sequence from *Aspergillus* fungi, *Appl.*

Microbiol. Biotechnol. 58 (2002) 658–662.

M. Rechsteiner, S.W. Rogers, PEST sequences and regulation by proteolysis, *Trends Biochem. Sci.* 21 (1996) 267–271.

R. Sotelo-Mundo, E. Moran-Palacio, K. García-Orozco, C. Figueroa-Soto, M. Romo-Figueroa, E. Valenzuela-Soto, G. Yepiz-Plascencia, Kinetic characterization, expression and molecular modeling of a chitinase from the pacific white shrimp *Litopenaeus vannamei*, *J. Food Biochem.* 33 (2009) 246–259.

M. Kono, T. Matsui, C. Shimizu, D. Koga, Purifications and some properties of chitinase from the liver of a prawn, *Penaeus japonicus*, *Agric. Biol. Chem.* 54 (1990) 2145–2147.

X.-L. Xie, Q.-X. Chen, J.-C. Lin, Y. Wang, Purification and some properties of β -N-acetyl-D-glucosaminidase from prawn (*Penaeus vannamei*), *Mar. Biol.* 146 (2004) 143–148.

D. Koga, K. Mizuki, A. Ide, M. Kono, T. Matsui, C. Shimizu, Kinetics of a chitinase from a prawn, *Penaeus japonicus*, *Agric. Biol. Chem.* 54 (1990) 2505–2512.

F. Shojaei, A. Homaei, M.R. Taherizadeh, E. Kamrani, Characterization of biosynthesized chitosan nanoparticles from *Penaeus vannamei* for the immobilization of *P. vannamei* protease: an eco-friendly nanobiocatalyst, *Int. J.*

Food Prop. 20 (2017) 1413–1423.

R.S. Rasmussen, M.T. Morrissey, Marine biotechnology for production of food ingredients, *Adv. Food Nutr. Res.* 52 (2007) 237–292.

M. Hayes, B. Carney, J. Slater, W. Brück, Mining marine shellfish wastes for bioactive molecules: chitin and chitosan—part B: applications, *Biotechnol. J.:*

Healthcare Nutr. Technol. 3 (2008) 878–889.

K. Kurita, Chitin and chitosan: functional biopolymers from marine crustaceans, *Mar. Biotechnol.* 8 (2006) 203–226.

M. Revathi, R. Saravanan, A. Shanmugam, Production and Characterization of Chitinase from *Vibrio* species, A Head Waste of Shrimp *Metapenaeus dobsonii* (Miers, 1878) and Chitin of *Sepiella inermis* Orbigny, 1848, 2012.

D. Koga, Application of chitinase in agriculture, *J. Met. Mater. Miner* 15 (2005) 33–36.