# CHITIN DEACETYLASE OF *Bemisia tabaci* (HEMIPTERA: ALEYRODIDAE) AS NEW TARGET FOR PESTICIDE DISCOVERY

*Data de aceite: 01/12/2023*

**Ciro Pedro Guidotti Pinto**
Universidade de São Paulo Paulista (USP), Faculdade de Ciências Farmaceuticas de Ribeirão Preto. Av. Prof. Dr. Zeferino Vaz - Vila Monte Alegre, Ribeirão Preto - SP

**Narian Romanello**
Centro Universitário de Rio Preto UNIRP-Hospital Veterinário, Rodovia BR 153 s/n Km 69, Rua Halim Atique Doutor, SP

**ABSTRACT:** Chitin deacetylases are enzymes responsible for structuring chitin of chitin-producing organisms, such as insects. Chitin deacetylases are well studied in fungi and bacteria, but the canonical structure was just recently characterized for an insect, the silk moth *Bombyx mori* (Lepidoptera: Bombycidae) (PDB: 5ZNT; 5ZNS). The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a polyphagous insect that causes severe loses in agriculture every year and there is a lack of new and safer modes of action for controlling such pest. In the present study, the three-dimensional structure of *B. tabaci* chitin deacetylase was modeled discussed using as model the newly insect chitin deacetylase described. Firstly, by BLAST, the amino acid sequence of *B. tabaci* chitin deacetylase was obtained from GenBank according to its identity to *B. mori* chitin deacetylase. The three-dimensional model and quality estimation of *B. tabaci* chitin deacetylase was obtained by Swiss-Model tool. Active sites, chitin binding site and the overall structure of *B. tabaci* chitin deacetylase were observed to be in agreement to the crystal structure of *B. mori* chitin deacetylase. It is expected that three-dimensional model of chitin deacetylases may be valuable for rational pesticide design. Further, other molecular targets can be searched and studied with the same *in silico* tools.

**KEYWORDS:** *Bombyx mori,* whitefly, insect, polyphagous, three-dimensional.

## QUITINA-DESACETILASE DE *Bemisia tabaci* (HEMIPTERA: ALEYRODIDAE) COMO NOVO ALVO PARA DESCOBERTA DE PESTICIDAS

**RESUMO:** As quitina-desacetilases são enzimas responsáveis pela estruturação da quitina de organismos produtores de da mesma, como insetos. As quitina-desacetilases são bem estudadas em fungos e bactérias, mas em insetos, a estrutura

canônica foi apenas recentemente caracterizada para a mariposa-da-seda *Bombyx mori* (Lepidoptera: Bombycidae) (PDB: 5ZNT; 5ZNS). A mosca-branca *Bemisia tabaci* (Hemiptera: Aleyrodidae) é um inseto polífago que causa perdas severas na agricultura todos os anos e há falta de novos e mais seguros modos de ação para o controle dessa praga. No presente estudo, a estrutura tridimensional da quitina-desacetilase de *B. tabaci* foi modelada usando como modelo a nova quitina-desacetilase de insetos descrita. Primeiramente, por BLAST, a sequência de aminoácidos de *B. tabaci* quitina-desacetilase foi obtida do GenBank de acordo com sua identidade com a quitina-desacetilase de *B. mori*. O modelo tridimensional e a estimativa da qualidade da quitina-desacetilase de *B. tabaci* foram obtidos pela ferramenta Swiss-Model. Os sítios ativos, sítio de ligação na quitina e a estrutura geral da quitina-desacetilase de *B. tabaci* foram observados de acordo com a estrutura cristalina da quitina-desacetilase de *B. mori*. Espera-se que o modelo tridimensional de quitina-desacetilases possa ser valioso para o planejamento racional de pesticidas. Além disso, outros alvos moleculares podem ser pesquisados e estudados com as mesmas ferramentas *in silico*.
**PALAVRAS-CHAVE**: *Bombyx mori*, mosca-branca, inseto, polífago, tri-dimensional.

## INTRODUCTION

The chitin biosynthesis route is widely explored for pest management due to its exclusive effect to arthropods. Either insecticides or acaricides use active ingredients targeting chitin synthase, a transmembrane enzyme with the function of catalyzing bonds in the β-(1,4) position between subunits of the amino sugar GlcNAc, whose polymerization will result in the formation of chitin (Van Leeuwen, 2012). Inhibitors of chitin biosynthesis lead insects or mites to several symptoms such as failure in ecdysis, sterile eggs, degraded peritrophic membrane, disorders of the immunologic system, morphological malformation, stop on food consumption and death (Merzendorfer 2013, Nasr et al. 2010).

The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a widespread polyphagous insect that causes agricultural losses throughout all continents. This insect, beyond damaging plants by sucking the sap, is a vector of several phytopathogenic viruses, which makes it a threat to global food security (Chen et al. 2016). Buprofezin is the only commercial insecticide that acts directly on the chitin biosynthesis route of *B. tabaci*, and several cases of resistance have been reported (APRD, 2019). Therefore, different proteins from the chitin biosynthesis route may be interesting targets for exploration and discovery of new and safer pesticides.

Chitin deacetylase enzimes(CDA) belong to the family of carbohydrate esterase enzymes and their functions remain on catalyzing the removal acetyl groups from chitin, forming a polymer β-(1,4) D-glucosamine residues, playing an important role in formatting and modifying the chitin matrix (Tsigos et al. 2000, Liu et al. 2019a). Further, several microorganisms, such as bacteria and fungi, or animals, such as insects, express CDAs and it has been proposed to use such enzyme as a target for the design and discovery of antibiotics or pesticides (Brosson et al. 2005, Baker et al. 2007, Wang et al. 2010, Yang et

al. 2018, Pusztahelyi 2018, Tetreau and Wang 2019).

Recently, Liu et al. (2019a) reported two crystal structures of an insect CDA, the silkmoth *Bombyx mori* (Lepidoptera: Bombycidae), available in Protein Data Bank (https://www.rcsb.org/; PDB codes: 5ZNS and 5ZNT). Authors described two distinct proteins, one acting towards cuticle modifications and other modifying the peritrophic membrane. Three-dimensional structures of hemipteran CDA are not available and the structural knowledge of such protein would be interesting since it may be used as a target for novel pesticide development. New pesticide molecules are expected to be highly specific for the targeted protein and active in a selective way for the organism of interest (Sparks 2013). Thus, three dimensional models may help us to investigate such compounds by structure-based screening on chitin biosynthesis targets, which is a safer rote for exploring pesticides than nervous system (Schmitid et al. 2014, Liu et al. 2019b).

In this study, *B. tabaci* CDA (*Bt*-CDA) was modeled as an initial step for specific structure-based pesticide design. A CDA gene from *B. tabaci* is available in the GenBank (https://www.ncbi.nlm.nih.gov/; NCBI accession number: XP_018899356.1) as uncharacterized protein. Here, the homologous three-dimensional model of *B. tabaci* is characterized and, based on the crystal structure of *B. mori* CDA (*Bm*-CDA) as template, the canonical structure of the enzyme, *in silico* methods and the possibility of developing new pesticides, are discussed.

## MATERIAL AND METHODS

Firstly, BLASTp search was performed with *Bm*-CDA sequence (Protein Data Bank: 5zns) as query. In GenBank (NCBI) the amino acid sequence of *Bt*-CAD was found as an uncharacterized protein, but with a high identity (~90%; XP_018899356.1). The web-based server Swiss-Model was used for building and evaluating the quality of the three-dimensional model of *Bm*-CDA (Waterhouse et al. 2018). The CDA sequence of whitefly obtained from BLASTp was searched against the primary amino acid sequence contained in the Swiss-Model Template Library. Unsurprisingly, the template with the highest percentage of identity found was from *B. mori* (PDB code 5zns) at a resolution of 2.5 Å. For comparing amino acid sequences identity, primary structures were aligned by ClustaW and edited by BioEdit v.7.2.6 (Hall 1999). Finally, the generated three- dimensional model of *B. tabaci* was edited and analyzed with Discovery Studio 4.5 (Accelrys).

## RESULTS AND DISCUSSION

Amino acid sequences of *Bt*-CDA and *Bm*-CDA share high sequence identity, as compared in Fig. 1. There are 378 amino acid residues, sharing 340 identic, composing the primary structure of the protein (90%). The identity observed is an important feature that makes *B. mori* qualified as a satisfactory template for modeling *Bt*-CDA. Furthermore,

active sites, according the original template (PDB: 5zns) are also conserved, as highlighted in Fig. 1.

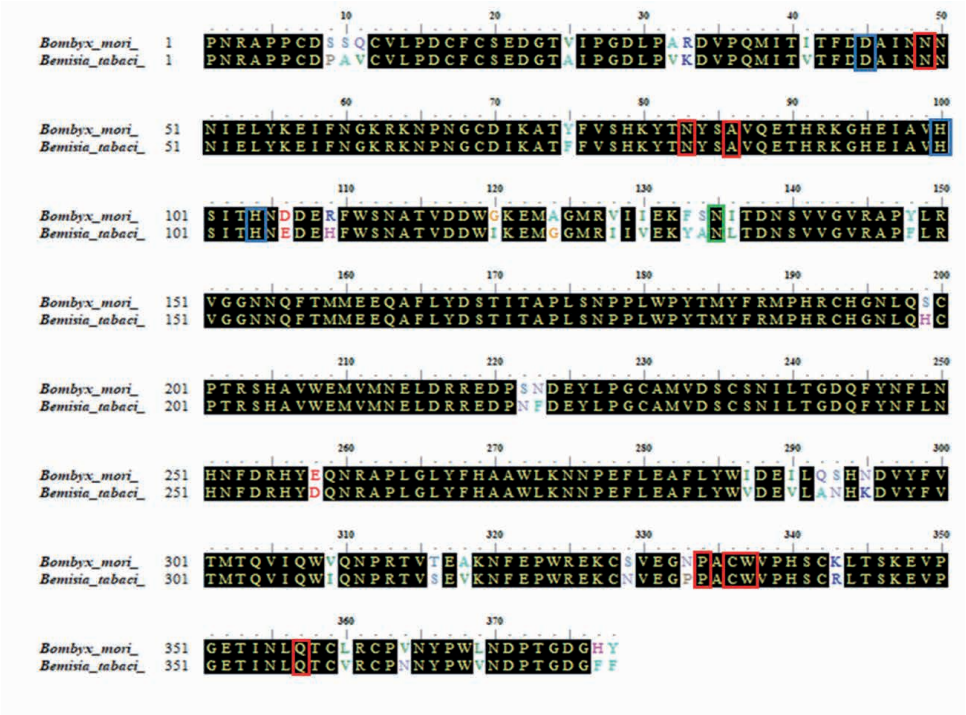


**Fig.1.** Aligned sequences of CDAs from *Bombyx mori* (PDB: 5zns) and *Bemisia tabaci* (GenBank: XP_018899356.1). Blue, red and green boxes denote active sites 1, 2 and 3, respectively.

The overall quality of the model was obtained by Ramachandran Plot, where 94.92% of the residues appeared to be in preferred regions, 4.81% in allowed regions and 0.27% are outliers (Suppl. Fig.1A). That means, most amino acids are placed in an adequate phi-psi distribution, consistent with right-handed α-helix, pointing as a good quality model. The Local Estimate Quality also was satisfactory, with most amino acid residues among 0.8 and 1.0, being the ideal above 0.6 (Suppl. fig. 1B). The Global Model Quality Estimation (GMQE), a parameter that reflects the expected accuracy of the model, was 0.98 out of 1. Further, the QMEAN-z, a scoring function that indicates the "degree of naiveness" of the built model was -0.39, which means a good quality model (BENKERT et al. 2010). Further, the compatibility of the model with its sequence was measured by Verify-3D.

The model of *Bt*-CDA was superposed on the structure of *Bm*-CDA, the only available structure of an insect CDA in PDB. An overview of the superposed structures shows that the 3D models are, overall, structurally similar (Fig. 2). Similar to the model of *B. mori*, described by Liu et al. (2019a), we found that the secondary structure of *Bt*-CDA is also a (β/α)7 barrel composed of seven parallel β-strands arranged in a barrel, surrounded by six

α-helix (Fig. 2).

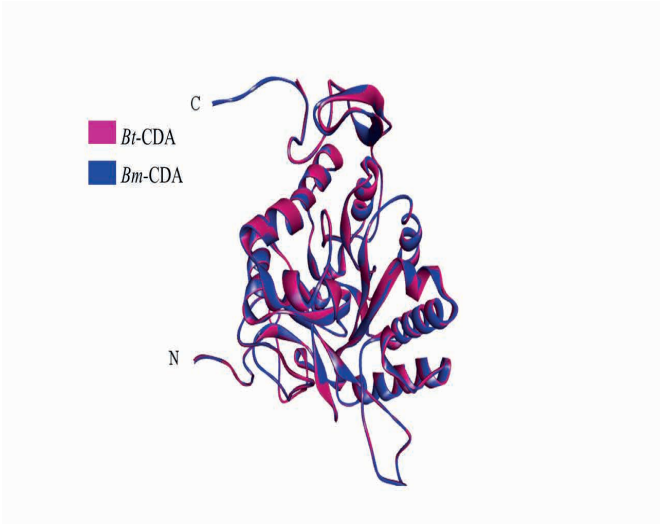


**Fig.2.** Overall superposed three-dimensional models of *Bt-CDA* and *Bm*-CDA colored magenta and blue, respectively. C and N represent C-terminus and N-terminus, respectively.

The structure of *Bt*-CDA is divided into N-terminus (1-23), chitin binding domain (24-122), lipo-protein receptor domain (123-161) and the catalytic domain (all amino acids from 162), according Figure 3, similar to the crystalized model of *B. mori* (Liu et al. 2019a).

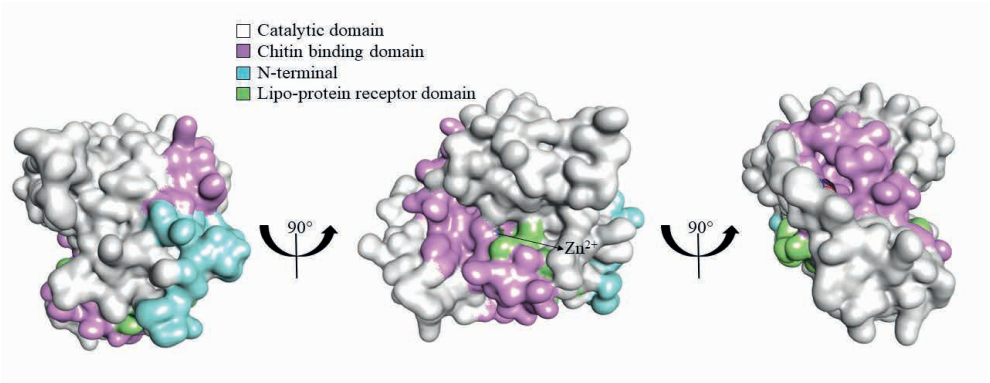


**Fig.3.** Surface graphic representing overall structure and domains of *Bt*-CDA. Domains were modeled based on crystalographed CDA of *Bombyx mori* (PDB: 5zns; Liu et al., 2019a).

The substrate binding site is composed of a Zinc ion surrounded for amino acid residues responsible for deacetylation of chitin. Amino acid residues that surround the zinc ion and compose the binding site of *Bt*-CDA are His 100/114/269, Asp 44/45, Ser 78, Arg 145, Ala 146, Pro 147 and Phe 148 (Fig. 4).

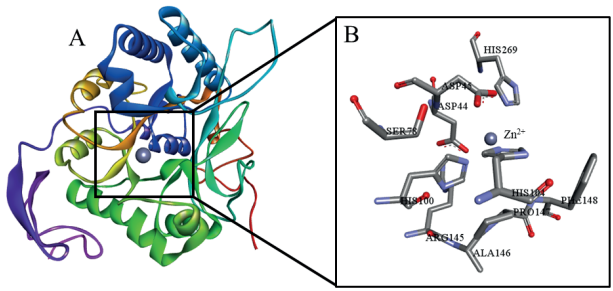


**Fig.4.** Chitin binding site of *Bt*-CDA composed by a zinc ion and surrounded by amino acid residues. A- ribbow overall structure; B- chitin binding site with zinc ion sorounded by amino acids.

Chitin pathways is an eco-friendly target to manage pests in crops, where biopesticides or selective molecules are used to suppress harmful insect populations (Liu et al. 2019b). Chitin deacetylase is a potential, but still unexplored, target in crop protection. This enzyme has important roles for structuring the integument, so it is found abundantly in ectoderm-derived tissues (Wu et al. 2019). Even so, these enzymes are also found in endoderm-derived chitinous tissues such as the peritrophic membrane of chewing insects (Liu et al. 2019a).

For instance, the artificial inhibition of CDA expression in chewing insects, provokes the reduction of leaf consumption index (Wu et al. 2019, Yu et al. 2016). The application of dsRNA for CDA inhibition in *Nilaparvata lugens* (Hemiptera: Delphacidae) nymphs caused mortality of up to 98% of insects (Xi et al. 2014). Bacteria also have carbohydrate esterase enzymes, the same family as the insect CDAs. Through virtual docking based on three-dimensional models, it was possible to elucidate effective inhibitors to such enzymes, providing the basis for the search for new molecules (Sarkar et al. 2017, Giastas et al. 2018).

Importantly, the amino acids that make up the active sites of *Bm*-CDA and *Bt*-CDA are conserved (Fig. 1), which somewhat hinders the search for taxon-specific compounds based on these sites. Even so, non-conserved allosteric sites can be sought for pesticide development. The spinosyn is an important example of the use of allosteric sites in pest control. Such molecules modulate allosteric nicotine receptor sites of postsynaptic neurons (Salgado and Saar 2004). Buprofezin is one of the few molecules available for controlling white fly targeting chitin pathway. Even though, due to misuse and the limited modes of action available, several cases of resistance are reported (APRD, 2021). This is why studies for searching for new modes of action should be carried out, as well as the awareness of companies, agronomists and farms for the rational use of insecticides and adopting non-chemical practices for pest control.

Designing specific inhibitors against the CDA of the targeted organism may be useful

to obtain an efficient inhibition. As 3D structure of an insect CDA was recently elucidated, the key for designing a structure-based inhibitor to act as insecticide became tangible. Although there are no CDA inhibitors for insect control, in this paper we propose this enzyme as a strong candidate for the development of new molecules. Once the canonical structure of this enzyme has been unraveled, the construction of new models and application of virtual docking against giant compound libraries became possible. Thus, it is expected that in the future new modes of action, increasingly safe for the environment and selective to natural enemies, will be investigated and available for pest control.

## REFERENCES


APRD. Arthropod Pesticide Resistance Database, online. Available in: <http://www.pesticideresistance.org/>. Access: march 15th, 202119.

Baker LG, Specht CA, Donlin MJ and Lodge JK (2007). Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*. *Eukaryotic cell*, *6*(5):855-867.

Benkert P, Biasini M, and Schwede, T (2011). Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics, 27(3): 343-350.

Brosson D, Kuhn L, Prensier G, Vivarès CP and Texier C (2005). The putative chitin deacetylase of Encephalitozoon cuniculi: a surface protein implicated in microsporidian spore-wall formation. FEMS Microbiol. Lett., 247(1): 81-90.

Chen W, Hasegawa DK, Kaur N, Kliot A, Pinheiro PV, Luan J, Stensmyr MC, Zheng Y, Liu W, Sun H and Xu Y (2016). The draft genome of whitefly *Bemisia tabaci* MEAM1, a global crop pest, provides novel insights into virus transmission, host adaptation, and insecticide resistance. BMC Biol., 14(1): 1-15.

Giastas P, Andreou A, Papakyriakou A, Koutsioulis D, Balomenou S, Tzartos SJ, Bouriotis, V. and Eliopoulos, E.E. (2018). Structures of the peptidoglycan N-acetylglucosamine deacetylase Bc1974 and its complexes with zinc metalloenzyme inhibitors. Biochemistry, *57*(5): 753-763.

Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* (Vol. 41, No. 41: 95-98). [London]: Information Retrieval Ltd., c1979-c2000..

Liu L, Zhou Y, Qu M, Qiu Y, Guo X, Zhang Y, Liu T, Yang J and Yang Q (2019). Structural and biochemical insights into the catalytic mechanisms of two insect chitin deacetylases of the carbohydrate esterase 4 family. J. Biol. Chem., 294(15): 5774-5783.

Liu X, Cooper AM, Yu Z, Silver K, Zhang J, and Zhu KY (2019). Progress and prospects of arthropod chitin pathways and structures as targets for pest management. Pestic. Biochem. Phys., 161: 33-46.

Merzendorfer H (2013). Chitin synthesis inhibitors: old molecules and new developments. Insect Sci., 20(2): 121-138.

Nasr HM, Badawy ME and Rabea EI (2010). Toxicity and biochemical study of two insect growth regulators, buprofezin and pyriproxyfen, on cotton leafworm *Spodoptera littoralis*. Pestic. Biochem. Phys., 98(2): 198-205.

Pusztahelyi T (2018). Chitin and chitin-related compounds in plant–fungal interactions. Mycology, 9(3): 189-201.

Salgado VL and Saar R (2004). Desensitizing and non-desensitizing subtypes of alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in cockroach neurons. J. Insect Phys., 50(10): 867-879.

Sarkar S, Gupta S, Chakraborty W, Senapati S and Gachhui R (2017). Homology modeling, molecular docking and molecular dynamics studies of the catalytic domain of chitin deacetylase from *Cryptococcus laurentii* strain RY1. Int. J. Biol. Macromol., 104: 1682-1691.

Schmidt T, Bergner A and Schwede T (2014). Modelling three-dimensional protein structures for applications in drug design. Drug Discov. Today, 19(7): 890-897.

Sparks TC (2013). Insecticide discovery: an evaluation and analysis. Pestic. Biochem. Phys., 107(1): 8-17.

Tetreau G and Wang P (2019). Chitinous structures as potential targets for insect pest control. *Targeting Chitin-containing Organisms*: 273-292.

Tsigos I, Martinou A, Kafetzopoulos D and Bouriotis V (2000). Chitin deacetylases: new, versatile tools in biotechnology. *Trends in Biotechnology*, *18*(7): 305-312.

Van Leeuwen T, Demaeght P, Osborne EJ, Dermauw W, Gohlke S, Nauen R, Grbić M, Tirry L, Merzendorfer H and Clark RM (2012). Population bulk segregant mapping uncovers resistance mutations and the mode of action of a chitin synthesis inhibitor in arthropods. PNAS, 109(12): 4407-4412.

Wang G, Maier SE, Lo LF, Maier G, Dosi S and Maier RJ (2010). Peptidoglycan deacetylation in *Helicobacter pylori* contributes to bacterial survival by mitigating host immune responses. Infec. Immun., 78(11): 4660-4666.

Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C Bordoli L and Lepore R (2018). SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res., 46(W1): W296-W303.

Wu JJ, Chen ZC, Wang YW, Fu KY, Guo WC and Li GQ (2019). Silencing chitin deacetylase 2 impairs larval–pupal and pupal–adult molts in *Leptinotarsa decemlineata*. Insect Mol. Biol., 28(1): 52-64.

Xi Y, Pan PL, Ye YX, Yu B and Zhang CX (2014). Chitin deacetylase family genes in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). Insect Mol. Biol., 23(6): 695-705.

Yang WJ, Xu KK, Yan X, Chen CX, Cao Y, Meng YL and Li C (2018). Functional characterization of chitin deacetylase 1 gene disrupting larval–pupal transition in the drugstore beetle using RNA interference. Comp. Biochem. Phys., 219: 10-16.

Yu R, Liu W, Li D, Zhao X, Ding G, Zhang M, Ma E, Zhu K, Li S, Moussian B. and Zhang J (2016). Helicoidal organization of chitin in the cuticle of the migratory locust requires the function of the chitin deacetylase2 enzyme (LmCDA2). J. Biol. Chem., *291*(47): 24352-24363.