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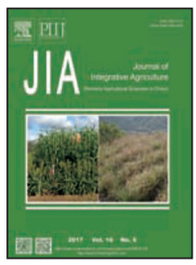

RESEARCH ARTICLE

High-level expression and purification of *Plutella xylostella* acetylcholinesterase in *Pichia pastoris* and its potential application

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Abstract

The acetylcholinesterase 2 (*AChE2*) cloned from *Plutella xylostella* was first successfully expressed in methylotrophic yeast *Pichia pastoris* GS115. One transformant with high-level expression of the recombinant AChE (rAChE, 23.2 U mL^{-1} in supernatant) was selected by plating on increasing concentrations of antibiotic G418 and by using a simple and specific chromogenic reaction with indoxyl acetate as a substrate. The maximum production of rAChE reached about 11.8 mg of the enzyme protein per liter of culture. The rAChE was first precipitated with ammonium sulfate (50% saturation) and then purified with procainamide affinity column chromatography. The enzyme was purified 12.1-fold with a yield of 22.8% and a high specific activity of 448.3 U mg^{-1}. It was sensitive to inhibition by methamidophos and pirimicarb, the calculated 50% inhibitory concentration (IC_{50}) values of the two pesticides were 0.357 and 0.888 mg L^{-1}, respectively, and the calculated 70% inhibitory concentration (IC_{70}) values were 0.521 and 0.839 mg L^{-1}, respectively. The results suggested that it has a potential application in the detection of pesticide residues.

Keywords: *Plutella xylostella*, acetylcholinesterase, *Pichia pastoris*, high-level expression

1. Introduction

Acetylcholinesterase (AChE, EC3.1.1.7) is a serine hydrolase essential for normal cholinergic neurotransmission and neuromuscular function in vertebrates and invertebrates. Because this enzyme is the primary target of organophosphate (OP) and carbamate (CA) insecticides and involves in insecticide resistance known as target-site insensitivity (Soderlund and Bloomquist 1990; Fournier and Mutero 1994; Zhu *et al.* 1996; Benting and Nauen 2004; Jiang *et al.* 2009; Khajehali *et al.* 2010), the insect AChE has been extensively used in development or *in vitro* selection of new AChE-targeting insecticides, biological detection of pesticide residues, and in pharmacological and toxicological fields (Pang 2006; Richter *et al.* 2006; Ilg *et al.* 2010; Lang *et al.* 2010; Pang *et al.* 2010; Lu *et al.* 2012).

Since the first insect *AChE* gene (*ace*) was sequenced from *Drosophila melanogaster* in 1986 (Hall and Spierer 1986), the cDNAs encoding *AChEs* have been cloned from more than 43 insect and acarus species (Shang *et al.*

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2007; Lu *et al.* 2012). Although *Drosophila* was confirmed to have only one *ace* gene (Myers *et al.* 2000), designated as *ace* orthologous gene (named as *ace2*), most other insects and ticks have recently been known to have two different *ace* genes (*ace1* and *ace2*) encoding for AChE1 and AChE2, respectively (Pang *et al.* 2010). These genes commonly correspond to the *Drosophila ace* paralogous and orthologous genes.

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a major pest of crucifers worldwide and causes economically significant damage on cruciferous crops (Talekar and Shelton 1993). Over the past years, OP and CA insecticides were extensively used to prevent its devastating outbreak. However, persistent insecticide usage has caused less sensitive AChE and insecticide resistance in some field populations throughout the world including South China (Baeka *et al.* 2005; Zhou *et al.* 2011).

Insensitive AChE caused by the mutation of *ace2* gene, which was regarded as the mechanism of OP and CA resistance, has been confirmed in some insects, including fruit fly (*Drosophila melanogaster*) (Mutero *et al.* 1994), Colorado potato beetle (*Leptinotarsa decemlineata*) (Zhu *et al.* 1996), house fly (*Musca domestica*) (Kozaki *et al.* 2001; Walsh *et al.* 2001), olive fruit fly (*Daeus oleae*) (Vontas *et al.* 2002), Australian sheep blowfly (*Calliphora vicina* Robineall) (Chen *et al.* 2001) and cotton aphid (*Aphis gossypii* Glover) (Li and Han 2004). Whereas, OP resistance studies and *AChE* gene cloning in *P. xylostella* suggested that an amino acid mutation (G227A) in AChE1 encoded by *ace1* was responsible for the AChE insensitivity to prothiofos (Baeka *et al.* 2005). Similarly, Lee *et al.* (2007) had proved that two amino acid mutations (A298S and G324A) in AChE1 were involved in target-insensitive resistance to prothiofos in *P. xylostella*. However, no mutations associated with resistance to OPs or CAs in *P. xylostella* have been identified in AChE2 (Ni *et al.* 2003) and no studies on the molecular biology of the AChE2 from the susceptible strain of *P. xylostella* have been published so far.

Effects of OP and CA insecticides on the AChE activity also have been examined *in vitro* with unpurified AChE, mainly extracted from homogenates of body tissue or whole body (Olson and Christensen 1980; Wang *et al.* 2002; Golombieski *et al.* 2008). However, the accuracy of the AChE assays was dramatically influenced, because this crude enzyme contains considerable amounts of contaminants, such as carboxylesterases, which can affect the measurement of AChE characteristics. Furthermore, the purification of the native enzyme from insects was time-consuming and the yield was low. To the best of our knowledge, however, the heterologous expression of the AChE from the susceptible strain of *P. xylostella* has not been reported yet. Therefore, it is essential to develop an efficient production procedure to prepare the pure AChE from *P. xylostella*.

In this study, we report the cloning, functional expression, purification and characterization of the recombinant *P. xylostella* AChE2 in *Pichia pastoris*.

2. Materials and methods

2.1. Insects, strains and plasmids

The susceptible strain of *P. xylostella* (L.) was established and maintained in the Key Laboratory of Natural Pesticide and Chemical Biology of the Ministry of Education of China at South China Agricultural University for years without exposure to any insecticides after collection in Gaoyao of Guangdong Province, China (Zhou *et al*. 2011). Strains used for experiments were reared on Chinese cabbage seedlings under the conditions of (25±2)°C, 16 h L:8 h D photoperiod, and 60–70% relative humidity.

Escherichia coli JM109 and *P. pastoris* strain GS115 were purchased from Invitrogen (Guangzhou, China) and used as the host strains for general cloning procedures and gene expression, respectively. Plasmid pPIC9K (Invitrogen, USA) was used to construct the expression vector of the *AChE* gene.

2.2. Identification, isolation and phylogenetic analysis of *AChE* gene from *P. xylostella*

Total cDNA from adult *P. xylostella* was prepared as previously described (Ilg *et al.* 2010). An internal fragment of *AChE* gene was amplified by PCR with the cDNA as template, two degenerate primers, 5′-TGGATHTAY GGNGGNGG-3′ and 5′-CCNGCNSWYTCNCCRAA-3′, were designed based on highly conserved amino acid sequences WIYGGG and FGESAG (Appendix A). The PCR products of the expected size (~280 bp) were cloned into pMD18-T vector (TaKaRa, China), and transformed into *E. coli* JM109 competent cells (TaKaRa). The inserted fragment was sequenced in both directions.

The 5′ and 3′ ends of the gene were obtained by rapid amplification of cDNA ends (RACE). The first strand of cDNA for 3′-RACE was synthesized with oligo dT-3 sites adaptor primer provided by the 3′-Full RACE Core Set (TaKaRa) according to the manufacturer's instructions, and the 3′-end fragment of *AChE* gene was amplified with gene specific forward primers GSPF1 (5′-TAGCGAGGAAGCGGCAG-3′), GSPF2 (5′-GCTTGTGGGATCAGCAGCTG-3′), and the 3′-RACE adaptor primer 5′-CTGATCTAGAGGTACCG GATCC-3′.

5′-RACE was performed using the 5′-RACE System Kit (Invitrogen) according to the manufacturer's protocol. The