Pesticide Biochemistry and Physiology 131 (2016) 18–23

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Development of an antibody-based diagnostic method for the identification of *Bemisia tabaci* biotype B

Ji Hyeong Baek [a,1], Hye Jung Lee [b,1], Young Ho Kim [c], Kook Jin Lim [d], Si Hyeock Lee [e,f], Bum Joon Kim [d,*]

[a] College of Pharmacy and Research Institute of Pharmaceutical Science, Gyeongsang National University, 501 Jinju-daero, Jinju 660-701, Republic of Korea
[b] Graduate Program for Nanomedical Science, Yonsei University, 134 Shinchon-dong, Seodaemun-Gu, Seoul 120-110, Republic of Korea
[c] Department of Entomology, Kansas State University, Manhattan, 66506, KS, USA
[d] Proteometech Inc., 15-1 Yonhee-dong, Seodaemun-gu, Seoul 120-110, Republic of Korea
[e] Department of Agricultural Biotechnology, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Republic of Korea
[f] Research Institute for Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Republic of Korea

ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form 21 December 2015
Accepted 8 February 2016
Available online 9 February 2016

Keywords:
Bemisia tabaci
Whitefly
Biotype
Protein biomarker
Carboxylesterase

ABSTRACT

The whitefly *Bemisia tabaci* is a very destructive pest. *B. tabaci* is composed of various morphologically undistinguishable biotypes, among which biotypes B and Q, in particular, draw attention because of their wide distribution in Korea and differential potentials for insecticide resistance development. To develop a biotype-specific protein marker that can readily distinguishes biotypes B from other biotypes in the field, we established an ELISA protocol based on carboxylesterase 2 (COE2), which is more abundantly expressed in biotypes B compared with Q. Recombinant COE2 was expressed, purified and used for antibody construction. Polyclonal antibodies specific to *B. tabaci* COE2 [anti-COE2 pAb and deglycosylated anti-COE2 pAb (DG anti-COE2 pAb)] revealed a 3–9-fold higher reactivity to biotype B COE2 than biotype Q COE2 by Western blot and ELISA analyses. DG anti-COE2 pAb exhibited low non-specific activity, demonstrating its compatibility in diagnosing biotypes. Western blot and ELISA analyses determined that one of the 11 field populations examined was biotype B and the others were biotype Q, suggesting the saturation of biotype Q in Korea. DG anti-COE2 pAb discriminates *B. tabaci* biotypes B and Q with high specificity and accuracy and could be useful for the development of a *B. tabaci* biotype diagnosis kit for on-site field applications.

1. Introduction

Today, more than 1550 species of whitefly are known to compose the Aleyrodidae family, the only member of the superfamily Aleyrodoidea [1,2]. The most notorious whitefly species, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), has a wide host range and utilizes more than 500 plant species as host [3], and is one of the most widely distributed agricultural pests in the world. This polyphagous pest directly damages plants by feeding on them and indirectly damages them by transmitting more than 100 different plant virus species [4]. Recently, tomato cultivars in Korea have been damaged by tomato yellow leaf curl virus (TYLCV), which can be transmitted by the whitefly [5]. All of the whitefly-transmitted geminiviruses (WTGs), including TYLCV, are transmitted by biotype B, whereas other biotypes are unable to transmit certain geminiviruses [6].

The biotypes of *B. tabaci* have been classified according to various ecological, physiological, and genetic factors, including their different traits with respect to host range, host-plant adaptability, feeding ability, and plant virus-transmission capability [7]. To date, at least 24 populations have been labeled as specific biotypes [8,9], and biotypes B and Q are most widely observed in Korea [10]. The *B. tabaci* nomenclature has been controversial for many decades [11]. Here, we use the 'biotype' nomenclature system.

Biotypes B and Q have shown intrinsically different insecticide resistance profiles. Biotype B has been reported to be resistant to most insecticides used for *B. tabaci* control [12,13]. In contrast, the resistance to pyriproxyfen is associated with biotype Q rather than biotype B [14,15]. In addition, biotypes B and Q vary in their developmental rates [16] and in behaviors such as mating interactions [17]. These intrinsic differences between biotypes B and Q suggest that some genetic and physiological factors differ between the biotypes. Therefore, rapid and accurate diagnosis of biotypes B vs. Q is a crucial element in the proper management of this serious pest.

Many researchers have attempted to develop a protocol for the rapid identification of *B. tabaci* biotypes. Since the B and Q biotypes, however, are morphologically indistinguishable, the biotype determination primarily depends on molecular diagnostics. Recent molecular diagnostic methods for distinguishing biotypes B and Q include polymerase chain reaction (PCR) and the sequencing of various marker genes, including mtCOI, 16s rRNA [18] and nuclear ITS region [19]. Additional

* Corresponding author.
E-mail address: bjkim@proteometech.com (B.J. Kim).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.pestbp.2016.02.003
0048-3575/

methods include PCR-restriction fragment length polymorphism (PCR-RFLP) [20], random amplified polymorphic DNA analysis (RAPD-PCR) [21], real-time PCR [22] and microsatellite DNA analysis [23]. These methods, however, should be performed in the laboratory and require a relatively long time commitment.

Protein-based diagnostic tests are not only simple and rapid but can also be developed as an on-site diagnostic kit. Thus, in a previous study, we implemented a two-dimensional electrophoresis (2DE)-based proteomic approach to search for a protein marker that distinguished the two *B. tabaci* biotypes. We found that carboxylesterase 2 (COE2) in biotype B was expressed >5-fold higher than in biotype Q and the expression level of COE2 could be employed as a biotype-specific marker [24]. In fact, genetic polymorphisms among the *B. tabaci* biotypes were first reported as an explanation for various esterase banding patterns [25]. Because the esterase banding patterns varied among biotypes, this protein was used as a biotype determination marker [17]. In particular, biotype B generally exhibited higher esterase activity than other biotypes [21,26]. Among these esterases, only 1 esterase (named E0.14) has been regularly detected under various conditions, and biotype B was initially defined by the presence of E0.14 [25]. Kang and colleagues [24] determined that E0.14 is COE2 and that this esterase is involved in *B. tabaci* pyrethroid and organophosphate insecticide resistance [24]. In this study, we generated polyclonal antibodies (pAb) against COE2 and evaluated their compatibility for the development of a *B. tabaci* biotype diagnostic kit.

2. Material and methods

2.1. Generation of recombinant COE2 (rCOE2)

The partial *coe2* (from Phe70 to Ile486) gene was cloned into the pET30a(+) expression vector and then transformed into *Escherichia coli* BL21 competent cells. His-tagged rCOE2 was induced for 3 h by the addition of 0.5 mM IPTG. The inclusion body (IB) of rCOE2 was washed three times with 10 mM Tris–HCl (pH 8.0) containing 0.5% Triton X-100 and 100 mM NaCl, and the IB was then solubilized with 8 M urea in 20 mM Tris–HCl (pH 8.0). The solubilized IB containing rCOE2 was subjected to diethylaminoethyl (DEAE)-sepharose ion exchange chromatography at pH 7.4. The eluted protein fractions were collected and analyzed by SDS-PAGE. The fractions that eluted with a linear gradient of 50–150 mM NaCl were pooled and applied to a Ni^{2+}-nitrilotriacetic acid (NTA) affinity column. The target protein was eluted with a gradient of 10–250 mM imidazole, and its purity was verified by SDS-PAGE and image analysis using ImageJ [27]. To confirm the purified rCOE2, the target protein band was excised from the Coomassie blue-stained gel and digested in the gel with trypsin. The eluted peptides and the intact rCOE2 were analyzed via matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. MALDI-TOF mass spectrometry was carried out on a MICROFLEX analyzer (Bruker Daltonics, Germany) in positive reflection mode. The mass peaks were calibrated using FlexAnalysis (Bruker Daltonics, Germany) and were searched with NCBI Database using the MASCOT database search algorithms, allowing oxidation, propionamide and one of missed trypsin cleavages. Peptide tolerance was 0.2 Da or 200 ppm.

2.2. Production of COE2 pAb

An anti-COE2 pAb was produced by AbClon (Seoul, Korea) using the purified rCOE2 as the target. A total of two rabbits were immunized once with 50 μg of rCOE2 and three times with 20 μg of rCOE2. The anti-COE2 serum was deglycosylated (DG anti-COE2 pAb) by endoglycosidase according to the manufacturer's protocol (PNGase F; SMAnalytical, Incheon, Korea). The DG anti-COE2 pAb was purified using a spin column (Viaspin 5000WMCO; Sartorius, Goettingen, Germany).

2.3. Immunoassay of anti-COE2 pAb

2.3.1. Bemisia tabaci

Eleven *B. tabaci* field populations were collected in the Goyang, Pyeongtaek (Pyeongtaek-1 and -2), Jinju, Suncheon, Buyeo, Miryang, Gurye, Changnyeong, Yesan, and Gochang regions of Korea in 2009 and 2010. Nine biotype reference populations were used as described previously [28], including five biotype B populations (two collected in Beijing, China; two collected in Crete island, Greece; and one maintained in the National Academy of Agricultural Science, Korea) and four biotype Q populations (one collected from Chungyang, Korea; one collected in Beijing, China, and two collected in Crete island, Greece). The biotypes of the 9 reference populations were confirmed using the mitochondrial marker [29] as well as the biotype-specific PCR of partial COE2 genomic DNA [28]. The greenhouse whitefly, *Trialeurodes vaporarium* Westwood, 1856 was collected in Chungyang, Korea. The ants, *Formica japonica* Motschulsky, 1866 were collected in Seoul, Korea. The greenhouse whitefly and the ants were used as negative controls.

2.3.2. Protein extraction

Adults of the 20 populations (5 biotype B populations, 4 biotype Q populations and 11 field populations) were homogenized in lysis buffer (2 μl per fly; 0.1% Triton X-100 in 0.2 M Tris–HCl, pH 8.2) with a plastic pestle. Following centrifugation at 13,000 × *g* for 15 min, the supernatant was used for Western blot and ELISA analyses. Crude greenhouse whitefly and ant proteins were also extracted using the same protocol.

2.3.3. Western blot analysis using anti-COE2 pAb

To confirm the specificity and detection limit of the anti-COE2 pAb, rCOE2 and crude *B. tabaci* proteins from the B and Q biotypes were used for Western blot analysis. rCOE2 and the *B. tabaci* crude proteins were separated on a 12% SDS-PAGE gel and transferred to a nitrocellulose (NC) membrane. The membrane was blocked with 5% skim milk (0.05% Tween-20 and 5% skim milk in PBS). The anti-COE2 serum was diluted (1:100,000) in 5% skim milk and applied to the membrane overnight at 4 °C. The membrane was then washed three times with PBST (0.05% Tween-20 in PBS). Horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (1:10,000 dilution) (Jackson Immuno Research, West Grove, PA) was added to the membrane and incubated for 2 h at 37 °C. The membrane was then washed as described above, and the proteins were detected using the enhanced chemiluminescence (ECL) kit (Pierce™ ECL Western Blotting Substrate; Pierce Biotechnology, Rockford, IL) or tetramethylbenzidine (TMB) (Promega, Madison, WI). The eleven filed populations were analyzed, and the biotype diagnoses were conducted using five adults from each population and the protocol described above.

2.3.4. ELISA analysis with anti-COE2 pAb

A direct sandwich ELISA was performed to confirm that the anti-COE2 pAb is useful as a biotype-specific biomarker and determine the biotype of eight field populations. A 96-well maxisorp plate (Nunc, Roskilde, Denmark) was pre-coated with anti-COE2 pAb (10 μg/ml in 100 mM sodium carbonate, pH 9.6) overnight and blocked with 1% BSA and 5% sucrose diluted in PBS for 4–8 h at room temperature. The crude proteins extracted from five adults (approximately 3 μg) for each of nine biotype references and nine field populations, respectively, were diluted in sample dilution buffer (0.15 M sodium phosphate buffer, pH 7.4, containing 0.3% gelatin, 0.1% sodium azide, and 0.02 mg/ml bromocrezole purple) in a final volume of 100 μl. Additionally, 3 μg of crude *T. vaporarium* and *Myrmica sulcinodis* proteins were extracted and diluted. Protein extracts were then incubated for 1 h in 96-well microtiter plates pre-coated with antibodies. The detection antibodies included biotinylated DG anti-COE2 pAb (1:600 dilution in antibody dilution buffer, 0.15 M sodium phosphate buffer, pH 7.4, containing 5% BSA, 0.1% Tween 20, and 0.1% sodium azide) and biotinylated intact