# Molecular cloning, recombinant protein expression and purification of a novel carboxylesterase from *Liposcelis bostrychophila*

Osamu Ishibashi*

Laboratory of Biological Macromolecules, Graduate School of Agriculture, Osaka Metropolitan University, Sakai 599-8531, Japan

*To whom correspondence should be addressed. E-mail: ishibos1@omu.ac.jp

Keywords: *Liposcelis bostrychophila*, booklouse, carboxylesterase, insecticide, synergist

## Summary

Booklice are tiny insect pests commonly found in an indoor environment and characterized by extremely high proliferation ability. Booklouse contamination in stored foods causes serious food loss; therefore, they are recognized as so-called stored-food pests. Furthermore, since booklice in house dust have been reported to be a potent allergen, it should be also considered that the accidental ingestion of booklice possibly causes allergic symptom. Therefore, safe extermination of booklice would contribute to the solution of both food-loss and allergic concerns. Organophosphates (OPs) are compounds generally used as harmless insecticides; nonetheless, the cases of OP poisoning are sometimes reported worldwide. Therefore, considering usage for stored food products, OP doses should be low as far as possible. It is known that carboxylesterase-mediated inactivation of OPs decreases the efficacy of OPs, suggesting that an inhibitor of carboxylesterase could be a synergist to reduce the dose of OP. Herein, I report the molecular cloning and characterization of a novel carboxylesterase highly expressed in a representative species of indoor booklice, *Liposcelis bostrychophila*.

## Introduction

Losses in the food chain from production to harvest, storage, distribution, processing, sales, and consumption have recently been recognized as a problem because they not only cause possible food shortages but also indirectly contribute to global warming. It was reported that global food loss and waste during 2010–2016 were estimated to equal 8–10% of total anthropogenic greenhouse gas

emissions and cost about 1 trillion USD per year [1]. Further, it is estimated that more than 40% of global food loss is "post-harvest loss" [1]; thus, countermeasures to address this issue are an urgent task. Biotic post-harvest food losses include the infestation of microorganisms, pests, and rodents. Pests, which include some insects and mites, occasionally infest food products, *e.g.*, grains and cereals, during storage and distribution. Particularly, insect pests, including cockroaches, ants, molds, warehouse beetles, and booklice (psocids), are considered a nuisance and cause huge losses in the grains [2]. The amounts of stored grain lost due to insect infestation are estimated to be 5% to 10% in developed countries and as much as 35% in developing countries [3]. Among the insect pests, booklice did not attract much attention for a long period because solid evidence as to quantitative and qualitative food losses caused by them was missing. However, the status of booklice in terms of food pests began to change in the late 1980s as booklouse infestation in stored grains in diverse locations worldwide was reported [4]. Further, Kučerová reported a quantitative data that the average weight of grain samples (broken wheat kernels) infested with booklice decreased by 9.7% after 3 months of infestation [5]. These reports clearly indicate that food loss caused by booklouse infection should be seriously considered.

Booklice are tiny insects measuring 1-4 mm in length. The proliferation of booklice is extremely rapid. Therefore, once the outbreak of booklice occurs, it is extremely difficult to get rid of them. Booklice include numerous species, and some of them are often found in food factories and warehouses. Booklice generally prefer to feed on molds; thus, they can infest stored foods not only directly but also indirectly through food-infesting molds. Among the booklouse species, *Liposcelis* (*L.*) *bostrycophila*, *L. decolor*, *L. entomophila* and *L. paeta* are common food-infesting booklice. Particularly, *L. bostrychophila* is a representative species of indoor booklice, which are commonly found in food facilities, as well as ordinary houses; therefore, it has been studied more commonly than the other species [5].

In addition, it has recently been reported that *L. bostrychophila* in house dusts are an allergen to cause allergic asthma [6,7], suggesting that accidental ingestion of foods infested with booklice may induce allergic symptoms. Although it is possible to suppress the proliferation of booklice by maintaining proper temperature and humidity, providing an environment suitable for food storage is sometimes difficult for various reasons such as cost issues. Thus, safe extermination of booklice using less harmful insecticides would contribute to the solution of food-loss problems, which could lead to the achievement of SDGs. A lot of effective and less harmful insecticides have been developed so far. However, their effectiveness to booklice is not sufficient to extirpate booklice,

which is attributed to their extremely high proliferative capacity and the emergence of insecticide-resistant booklice [8]. Of insecticides, acetylcholinesterase (AChE) inhibitors such as organophosphates (OPs) have been reported to exert a relatively high anti-booklouse effect [9]. Therefore, the usage of high-dose OPs may potentially extirpate booklice. However, OPs should be carefully used for food control, as cases of OP poisoning often occur even though OPs are considered to have low toxicity to humans. In addition, as with many other insects, acquisition of resistance to OPs has been reported for booklice [10]. So far, several mechanisms underlying the acquisition of resistance to OPs have been documented [11,12], which includes carboxylesterase (CE)-mediated OP inactivation in the insect body. Notably, Correy *et al*. showed that a specific inhibitor of α-carboxylesterase (αE7), which was designed based on its protein structure, significantly suppressed OP degradation, and increased the efficacy of OPs, indicating that the combination of OPs and CE inhibitors possibly enables to extirpate booklice at lower insecticide concentrations [13].

Herein, we report the molecular cloning of a novel CE expressed at a high level in *L. bostrycophila*, designated LBCE1, and the generation of recombinant LBCE1 protein with an esterase activity.

## Methods

### Analysis of the RNA sequencing data of *L. bostrychophila*

Reads obtained by our RNA sequencing analysis performed previously, which are available from Sequence Read Archives (NCBI) under the identifier DRR086994, were subjected to contig construction as previously described [7]. The predicted open reading frame in these contigs were then analyzed using the BLAST tool to identify possible homologous proteins in the NCBI protein database.

### PCR-based molecular cloning of *L.bostrychophila* carboxylesterase 1 (LBCE1)

Primers used to amplify a region encompassing the predicted coding sequence of LBCE1 are as follows. First, total RNA extracted from *L. bostrychophila* bodies were subjected to reverse transcription using a PrimeScript RT Master Mix (Perfect Real Time) (Takara-bio, Japan) and the LBCE1-reverse primer. This was followed by PCR using KOD-plus2 DNA polymerase (Takara) and the primers for LBCE1. The PCR products were purified, inserted in pGEM-Teasy (Promega),

and subjected to Sanger sequencing to verify their nucleotide sequences. This was then followed by the subcloning of the PCR product into the expression vector pET22b to construct pET22b-LBCE1.

**Expression and purification of LBCE1 using an *E. coli* expression system**

*E. coli* cells of the strain BL21 (DE3) were transformed with pET22b-LBCE1, and the expression of LBCE1 tagged with 6x His at the N-terminus was induced by culturing the cells in an autoinduction medium, OvernightExpress TB medium (Sigma), for 8 h. Since the expressed protein was shown to be secreted or leaked into the culture medium at a detectable level, the His-tagged LBCE1 was purified from the culture medium as follows. First, 100 ml of the culture medium was concentrated and buffer-changed to the equivalation buffer (20 mM phosphate, 500 mM NaCl, pH7.4) using an Amicon Ultra-15 filtration unit (10-kDa cutoff) (Merck-Millipore). The concentrate was then applied to an affinity column filled with TALEN Metal Affinity Resin (Takara-bio), washed with the equivalation buffer, and resin-bound proteins were eluted with the same buffer containing 10 mM imidazole.

**LBCE1 activity assay**

The purified His-tagged LBCE1 was assayed for esterase activity using *p*-nitrophenol acetic acid as a substrate as described earlier [14]. The hydrolysis of the substrate was monitored by measuring $OD_{450}$.

**Western blotting**

The purified His-tagged LBCE1 was subjected to SDS-PAGE, transferred to a nylon membrane, and probed with the anti-His tag monoclonal antibody (Clone 6C4) (MBL, Tokyo, Japan).

## Results and Discussion

**Identification of a novel carboxylesterase homologue in *L. bostrychophila***

First, we searched for contigs that encompass full ORFs coding for CE homologues in *L. bostrychophilla*. Several contigs were identified to have ORFs encoding proteins that exhibit significant homology with CEs registered in the NCBI databese. Of these contigs, one contig designated Comp59220 encompasses an ORF encoding a CE-homologous protein consisting of 555 amino acids (Figure 1). The Comp59220-encoded protein exhibits significant identities with CEs

from various insects, with the highest (47.2%) identity with EF4 esterase from *Pediculus humanus* (human louse) (Table 1); therefore, it was designated *L. bostrychophila* carboxylesterase 1 (LBCE1). Of proteins registered in the Protein Data Bank (PDB), a database for the three-dimensional

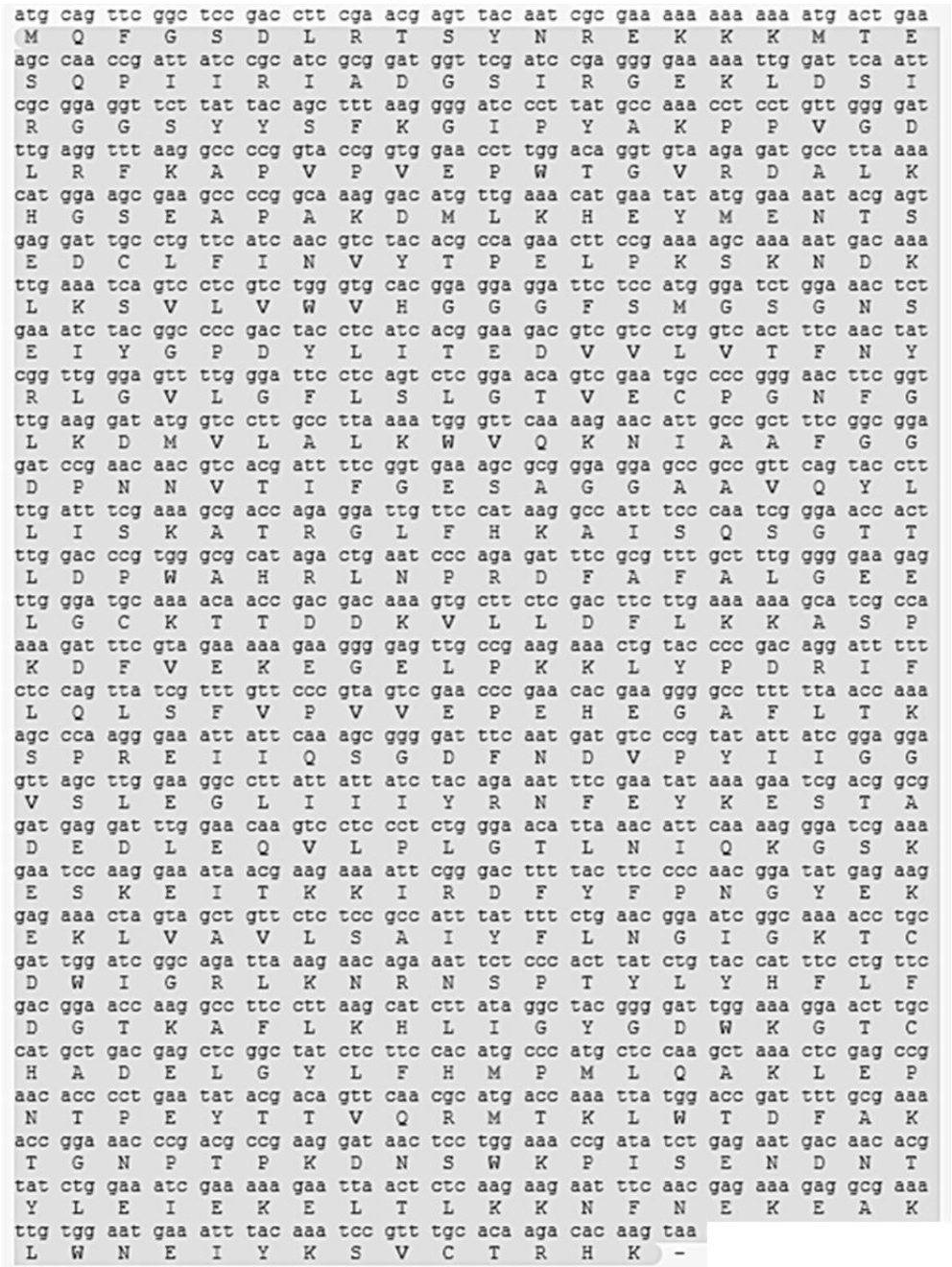


**Figure 1** Nucleotide sequences of the longest ORF of Comp59220 and deduced amino acid sequence

structural data of large biological molecules such as proteins, LCαE7, a CE from *Lucilia cuprina*, exhibits the highest (30%) identity with LBCE1. LCαE7 was previously reported to be a CE that potentially inactivates OPs, which leads to the increased OP resistance of *Lucilia cuprina*. Consistently, it was shown that the use of *in silico*-designed inhibitors of LCαE7 as synergists significantly increased the efficacy of OPs. Therefore, in this study, we focused on LBCE1 as a potential target of OP synergists.

**Table 1** BLAST search hits identified as LBCE1-homologous proteins in the NCBI protein database

| Description | Query Cover | E value | Per. Ident | Accession |
|---|---|---|---|---|
| Esterase FE4 precursor, putative [Pediculus humanus corporis] | 96% | 5e-171 | 47.19% | XP_002424560.1 |
| esterase E4 isoform X2 [Cryptotermes secundus] | 94% | 2e-153 | 45.49% | XP_023716388.1 |
| esterase E4 isoform X1 [Cryptotermes secundus] | 94% | 4e-153 | 45.67% | XP_023716379.1 |
| esterase E4-like isoform X2 [Zootermopsis nevadensis] | 95% | 5e-152 | 45.32% | XP_021912901.1 |
| esterase E4-like isoform X1 [Zootermopsis nevadensis] | 95% | 2e-150 | 44.49% | XP_021912894.1 |
| esterase FE4 [Cryptotermes secundus] | 94% | 8e-148 | 44.07% | XP_023716405.1 |
| uncharacterized protein LOC111869379 [Cryptotermes secundus] | 94% | 9e-147 | 45.80% | XP_023716645.1 |
| uncharacterized protein LOC111869395 [Cryptotermes secundus] | 96% | 2e-142 | 45.12% | XP_023716667.1 |
| PREDICTED: venom carboxylesterase-6 [Amyelois transitella] | 94% | 7e-142 | 44.48% | XP_013183727.1 |
| venom carboxylesterase-6 [Manduca sexta] | 95% | 5e-141 | 43.68% | XP_030024687.1 |
| venom carboxylesterase-6-like [Aphantopus hyperantus] | 95% | 2e-139 | 42.91% | XP_034834181.1 |
| venom carboxylesterase-6-like [Hyposmocoma kahamanoa] | 95% | 3e-138 | 43.48% | XP_026315216.1 |
| venom carboxylesterase-6 [Bicyclus anynana] | 95% | 6e-138 | 42.75% | XP_023935921.1 |

**Enzyme assay of the recombinant LBCE1**

The *E. coli expression* system yielded 30 μg of the recombinant His-tagged LBCE1 after purification (Figure 2A and 1B). The esterase activity of this enzyme was examined using a *p*-nitrophneol acetic acid as a substrate. As shown in Figure 2C, the substrate was shown to be hydrolyzed in a time-dependent manner, indicating that this protein is has an esterase activity.

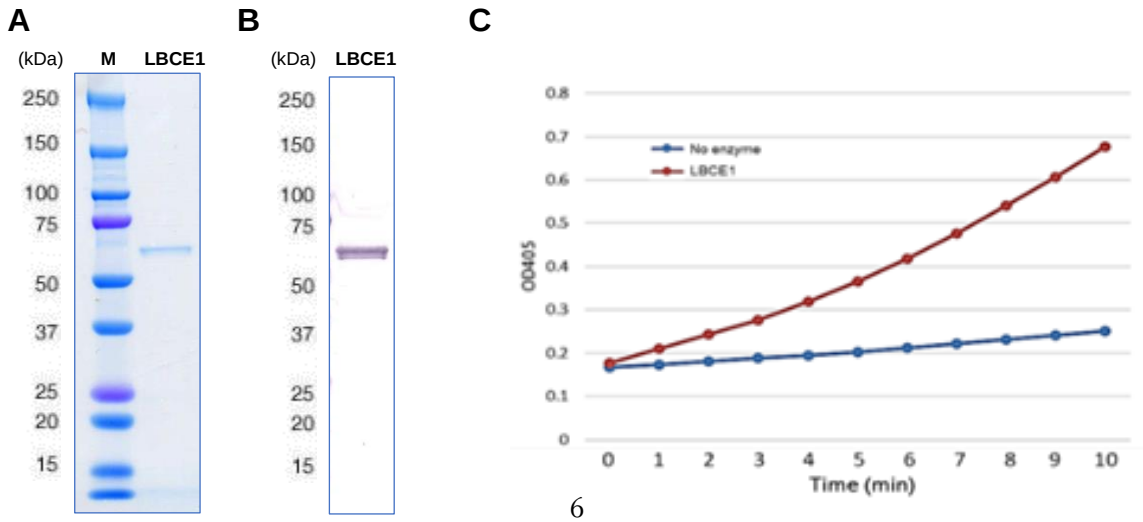

**Figure 2** (A) SDS-PAGE of the purified His-tagged LBCE1. (B) Western blotting of the purified His-tagged LBCE1 using an anti-His tag antibody. (C) Time-dependent hydrolysis of *p*-nitrophenol acetic acid by the recombinant LBCE1. As a control, the substrate was also incubated without LBCE1. M: Size marker.


## Acknowledgement

This work was supported by the Tojuro Iijima Foundation for Food Science and Technology.


## Conflict of interest

The authors declare no conflict of interest.

## References


1. Mbow C, Rosenzweig C, Barioni LG, Benton TG, Herrero M, Krishnapillai M, Liwenga E, Pradhan P, Rivera-Ferre MG, Sapkota T, Tubiello FN, Xu Y. 2019: Food Security. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.
2. Kumar D, Kalita P. Reducing Postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries. *Foods* 2017;6:8.
3. Campbell JF, Arthur FH, Mullen MA. Insect management in food processing facilities. *Adv Food Nutr Res*. 2004;48:239-95.
4. Nayak MK, Collins PJ, Throne JE, Wang JJ. Biology and management of psocids infesting stored products. *Annu Rev Entomol*. 2014;59:279-97.
5. Kučerová Z. Weight losses of wheat grain caused by psocid infestation (Liposcelis bostrychophila: Liposcelididae: Psocoptera). *Plant Protect. Sci*. 2002;38:103-107.
6. Fukutomi Y, Kawakami Y, Taniguchi M, Saito A, Fukuda A, Yasueda H, Nakazawa T, Hasegawa M, Nakamura H, Akiyama K. Allergenicity and cross-reactivity of booklice (*Liposcelis bostrichophila*): a common household insect pest in Japan. *Int Arch Allergy*

*Immunol*. 2012;157:339-348.

7. Ishibashi O, Sakuragi K, Fukutomi Y, Kawakami Y, Kamata Y, Sakurai M, Nakayama S, Uchiyama H, Kobayashi H, Kojima H, Inui T. Lip b 1 is a novel allergenic protein isolated from the booklouse, *Liposcelis bostrychophila*. *Allergy* 2017;72:918-926.
8. Clemmons EA, Taylor DK. Booklice (Liposcelis spp.), Grain Mites (Acarus siro), and Flour Beetles (Tribolium spp.): 'Other Pests' Occasionally Found in Laboratory Animal Facilities. *J Am Assoc Lab Anim Sci.* 2016;55:737-743.
9. Collins PJ, Nayak MK, Kopittke R. Residual efficacy of four organophosphate insecticides on concrete and galvanized steel surfaces against three liposcelid psocid species (Psocoptera: Liposcelidae) infesting stored products. *J Econ Entomol*. 2000;93:1357-1363.
10. Ding W, Zhao ZM, Wang JJ, Tao HY, Zhang, YQ. The relationship between resistance to controlled atmosphere and insecticides of Liposcelis bostrychophila Badonnel (Psocoptera:Liposcelididae). *Scientia Agricultura Sinica*. 2004;37:1308–1315.
11. Khan S, Uddin MN, Rizwan M, Khan S, Uddin MN, Rizwan M, Khan W, Farooq M, Sattar Shah A, Subhan F, Aziz F, Rahman KU, Khan A, Ali S, Muhammad M. Mechanism of Insecticide Resistance in Insects/Pests. *Polish Journal of Environmental Studies*. 2020;29:2023-2030.
12. Boyer S, Zhang H, Lempérière G. A review of control methods and resistance mechanisms in stored-product insects. *Bull Entomol Res*. 2012;102:213-229.
13. Correy GJ, Zaidman D, Harmelin A, Carvalho S, Mabbitt PD, Calaora V, James PJ, Kotze AC, Jackson CJ, London N. Overcoming insecticide resistance through computational inhibitor design. *Proc Natl Acad Sci USA*. 2019;116:21012-21021.
14. Ashour MB, Gee SJ, Hammock BD. Use of a 96-well microplate reader for measuring routine enzyme activities. *Anal Biochem*. 1987;166:353-360.