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Short communication

Immune function against bacteria of chitin deacetylase 1 (*EcCDA1*) from *Exopalaemon carinicauda*

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ABSTRACT

Chitin deacetylase (CDA, EC 3.5.1.41), belonging to a family of extracellular chitin-modifying enzymes, can catalyze the deacetylation of chitin. In this study, the full-length cDNA sequence encoding chitin deacetylase 1 (*EcCDA1*) was obtained from*Exopalaemon carinicauda*. The complete nucleotide sequence of *EcCDA1* contained a 1611 bp open reading frame (ORF) encoding EcCDA1 precursor of 536 amino acids. The domain architecture of the deduced EcCDA1 protein contained a signal peptide, a chitin-binding peritrophin-A domain (ChtBD2), a low-density lipoprotein receptor class A domain (LDLa) and a Polysacc_deac_1 domain. *EcCDA1* mRNA was predominantly expressed in the gills. The expression of *EcCDA1* in the prawns challenged with *Vibrio parahaemolyticus* and *Aeromonas hydrophila* changed in a time-dependent manner. The expression of *EcCDA1* in the prawns challenged with *V. parahaemolyticus* was up-regulated at 12 h ($p < 0.05$), and significantly up-regulated at 24 h and 48 h ($p < 0.01$), and then returned to the control levels at 96 h post-challenge ($p > 0.05$). At the same time, the expression in *Aeromonas*-challenged group was significantly up-regulated at 12, 24 and 48 h ($p < 0.01$) and returned to the control levels at 120 h post-challenge ($p > 0.05$). Then, *EcCDA1* was recombinantly expressed in *Pichia pastoris* and the purified recombinant EcCDA1 could not inhibit the growth of *V. parahaemolyticus* or *A. hydrophila*, which indicated that the CDA1 may play its biological activity in immune defense by deacetylation from chitin.

1. Introduction

Chitin, one of the most important biopolymers in nature, is mainly produced by fungi, arthropods and nematodes. In arthropods, their cuticles can form an exoskeleton to keep pace with body growth due to the presence of chitin and sclerotized proteins [1]. In addition, their growth and morphogenesis are strictly dependent on the capability to remodel chitin-containing structures [1]. Chitin-related enzymes play fundamental roles in chitin metabolism and they can be divided into three main categories, based on their functions to synthesize chitin (chitin synthases), to enzymatically alter chitin by deacetylation (chitin deacetylase, CDA) and to degrade chitin by hydrolytic process (chitinases and N-acetylglucosaminidases) [2]. CDAs (EC 3.5.1.41) are secreted proteins belonging to a family of extracellular chitin-modifying enzymes and they can hydrolyze the acetamido group in the N-acetylglucosamine units of chitin and chitosan [3]. CDA was first discovered from extracts of *Mucor rouxii* and it could convert nascent chitin into chitosan [4,5].

At present, a lot of CDA genes have been obtained in the species of Arthropod, especially in insects [3,6–10]. In insects, CDAs can convert chitin into chitosan, the N-deacetylated form of chitin, which influenced the mechanical and permeability properties of structures such as the cuticle and peritrophic matrices [11]. At present, a family of genes encoding chitin deacetylase (CDA)-like proteins in insects had been identified in the annotated genome sequences and the number of CDA genes was five to nine depending on the species [7]. All of the insect CDAs could be clustered into five major groups [6]. In *Helicoverpa armigera*, it is reported that the downregulation of a midgut-specific CDA-like protein as a possible mechanism to reduce susceptibility to baculovirus by decreasing peritrophic membrane (PM) permeability [12]. However, there was only one CDA gene reported in crustaceans, that is, CDA cDNA (named *PmCDA1*) cloned from the gills of black tiger shrimp, *Penaeus monodon* [13]. *PmCDA1* was reported to be distinctly highly expressed in the gills of shrimp and the authors thought that gills in shrimps served as the predominant site for the formation of hemocyte nodules during injection of foreign particles and accumulation of viable bacteria during infection, suggesting its significant role in shrimp defense [13]. As we know, there is no model animal in Crustacean to be

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Table 1
Primers mentioned in the paper.

Primers	Sequences (5′-3′)	Sequence information
RT-EcCDA1F	AGAAGAAGAGGGTCGTCAAGC	Real-time PCR
RT-EcCDA1R	GACTCATCGGCACAGTCAAAT	Real-time PCR
18S-F	TATACGCTAGTGGAGCTGGAA	Real-time PCR
18S-R	GGGGAGGTAGTGACGAAAAAT	Real-time PCR
9k-EcCDA1F	GCGAATTCCATCATCACCATCACCACGAAATAGTGAAACGCCAGGCGGCCAC	Construct the expression vector, introducing a restriction enzyme site for *EcoR* I and a 6 × His-tag
9k-EcCDA1R	GCGCGGCCGCTTAGAAGTAACCCTCTCCCAAAGGATC	Construct the expression vector, introducing a restriction enzyme site for *Not* I
5′AOX1	GACTGGTTCCAATTGACAAGC	Confirm the insert target gene
3′AOX1	GCAAATGGCATTCTGACATCC	Confirm the insert target gene

Note: F and R stand for forward primers and reverse ones, respectively.

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1    GTGAACGGTTGGGTCGGAGAGTCACCATGACAAGTATACGAGTTGCGGCGATAGCCCTTGCCCTCCTTGGCATCGCATCCGGAGAAATAGTGAAACGCCAGGCGGCCACCGTAAGCGAAC 120
                                M  T  S  I  R  V  A  A  I  A  L  A  L  L  G  I  A  S  G  E  I  V  K  R  Q  A  A  T  V  S  E

121  CCACTGAAGATGAGGCCGATGCCTTCACCAAAGAGTTGTGTCGAGACAAGGGCGCAGGCGAATGGTTCAGGCTCGATCTGAATGATTGTCGTGATGTCATCCAGTGCACAGAGGCAGGGC 240
     P  T  E  D  E  A  D  A  F  T  K  E  L  C  R  D  K  G  A  G  E  W  F  R  L  D  L  N  D  C  R  D  V  I  Q  C  T  E  A  G

241  TTCAGGCGCTGAGATGTCCTCACGGTCTGGCTTTCAACCTGGAATTGCAGACCTGTGACTGGAAGGACAACGTCAAGAACTGCAACCAGAAGGAGAAGAAGAGGGTCGTCAAGCCCCTGC 360
     L  Q  A  L  R  C  P  H  G  L  A  F  N  L  E  L  Q  T  C  D  W  K  D  N  V  K  N  C  N  Q  K  E  K  K  R  V  V  K  P  L

361  TCAACACCGTCGAGCCTCTTTGTCAGGAGAACCTGCTGGCCTGCGGCGATGGAACGTGCATTGATCGAACCCTGTTTTGCGATGGCAAATTTGACTGTGCCGATGAGTCCGACGAAAACA 480
     L  N  T  V  E  P  L  C  Q  E  N  L  L  A  C  G  D  G  T  C  I  D  R  T  L  F  C  D  G  K  F  D  C  A  D  E  S  D  E  N

481  CCTGTGATATCAAGAGTGATCCCAACAGCGCACCCATTTGCAACCCGGATGAGTGCCGCCTCCCCGACTGTTACTGCTTTAATAACGCCAACGAGGTCCCCGACAACATGAACCCTACCA 600
     T  C  D  I  K  S  D  P  N  S  A  P  I  C  N  P  D  E  C  R  L  P  D  C  Y  C  F  N  N  A  N  E  V  P  D  N  M  N  P  T

601  ATGTACCCCAAATGATCACCATAACATTCGACGATGCTGTCAACATTGAAAACATCGACCTTTACAATATCATTTTCGATAGCCGCTTCAACCCTAACCAGTGCTCCATCAAGTCGACCT 720
     N  V  P  Q  M  I  T  I  T  F  D  D  A  V  N  I  E  N  I  D  L  Y  N  I  I  F  D  S  R  F  N  P  N  Q  C  S  I  K  S  T

721  TCTTCGTCTCCCACAAATACACAAACTACTCCGCTGTGCAGGATTTGCATCGCCTTGGTCACGAAATTGCCATCCACTCAATCAGCCACAGCAACAATGAAACATTCTGGACAAAGGCTT 840
     F  F  V  S  H  K  Y  T  N  Y  S  A  V  Q  D  L  H  R  L  G  H  E  I  A  I  H  S  I  S  H  S  N  N  E  T  F  W  T  K  A

841  CCCCCGATGAATGGGAACGTGAGATGGCAGGTGGTCGTGTGATTGTAGAAAGATTTGCCAACATCACCGACTCTTCTGTGATTGGTGTGAGATCTCCCTACCTACGTGTGGGTGGAAACA 960
     S  P  D  E  W  E  R  E  M  A  G  G  R  V  I  V  E  R  F  A  N  I  T  D  S  S  V  I  G  V  R  S  P  Y  L  R  V  G  G  N

961  ACCAATTCGGCATGATGGAGCAGAATGCCTTCCTCTACGACTCCACCATGACTGCCCCACTGCAGAACCCCCCACTCTGGCCTTACACCCTTTATTACCGCATGCCACACGCTTGCCACG 1080
     N  Q  F  G  M  M  E  Q  N  A  F  L  Y  D  S  T  M  T  A  P  L  Q  N  P  P  L  W  P  Y  T  L  Y  Y  R  M  P  H  A  C  H

1081 GCAACCTCCAGAATTGCCCCACCCGTTCCTTCGCGGTCTGGGAAATGGTCATGAACGAGATGGACCGTCGTGAGGAACCAACCATTGAAGAAGATTTGCCTGGATGTGCTATGGTGGATT 1200
     G  N  L  Q  N  C  P  T  R  S  F  A  V  W  E  M  V  M  N  E  M  D  R  R  E  E  P  T  I  E  E  D  L  P  G  C  A  M  V  D

1201 CTTGCTTCTCCAACAAGCCAACAGCTGATCAGTTCTATAAATTCCTCGTAAACAACTTTGACCGTCATTACCTCACCAACCGTGCCCCAATGGGTCTCTACTTCCATTCTGCCTTCCTCA 1320
     S  C  F  S  N  K  P  T  A  D  Q  F  Y  K  F  L  V  N  N  F  D  R  H  Y  L  T  N  R  A  P  M  G  L  Y  F  H  S  A  F  L

1321 AAAATGATCCAGAAATATTGGACGCCTTCCTCTTCTGGCTGGATGAGACCTTGGCAAACAACCCTGATGTTTACTTCGTCACCATGACTCAAGTCATTCAATGGATCGAGGACCCACAGC 1440
     K  N  D  P  E  I  L  D  A  F  L  F  W  L  D  E  T  L  A  N  N  P  D  V  Y  F  V  T  M  T  Q  V  I  Q  W  M  Q  D  P  Q

1441 CCGTCAGCAACCTCAAGAACTACGAGCCCTGGAAGGAGAAGTGCAACGTAGCTGGACCTCCTTTCTGCTACGGTGGCACCAACTGTGAACTCAACACTGATGAACTTCCCGGTGAGACCC 1560
     P  V  S  N  L  K  N  Y  E  P  W  K  E  K  C  N  V  A  G  P  P  F  C  Y  G  G  T  N  C  E  L  N  T  D  E  L  P  G  E  T

1561 TTCGCCTGGCCACCTGCATGCGATGCCCCAACAGGTATCCCTGGTTATTGGATCCTTTGGGAGAGGGTTACTTCTAAGAGCACTGCTGCTGCATTAATGCTGTCGCCCTGTCCCCTGGTT 1680
     L  R  L  A  T  C  M  R  C  P  N  R  Y  P  W  L  L  D  P  L  G  E  G  Y  F  *

1681 GAGTCAGGCCCCCTCCTCTCCCGTGGGGACAGTTGTGCTCATCTGTAAATATATTGTTTTATAATTTGTACATGATCGTGTCCAGGCATGTGTAGCCTCATTCCTAACTAAGCAGTGGTT 1800
1801 GGCTGGTCAGGGGTGCGGCGGCAACACCACCATCATCACTGTCGCTAAGGCCAAGAAATCAGGTTTGCTACAATGAACTCTGTGCAACGGTCTCCAGAGTTGATGCTGCTGTTGCTGATG 1920
1921 CTGCTGCCTTTCCTGTCAGCCTCTATGACAGGTCGGAGCTCATATTGACAAACTATTCCAAATCCCCTGGTAACCCCTTAAATCACTGTCGGGGATGAGCTGGGGCTGCTCTCTTCCAAA 2040
2041 CCTTGCGGATCCCAAAACTTCCCCCTTTCAACTCTTATCTCATCTATGGGAATACCGCCGGGGTCCCAATTATATTTATTAGACCTGGAGGGGCCCTCATCACCTTTTTTCTCTAATATC 2160
2161 CTCCACCCCCTTAAAAATTACACAAAAACATCACCACCAACCAGGTGGGGGCCATGTGACAGTGCCCCTACCACCTGAGCACATTTTTGAGATTTAACCTAGCTGTGATGATCGTTTAGA 2280
2281 ATTTTAGCGTCACTTGTTTTTACCTTCCATATTATGTTTAAATGTTACCCAAATAAAAAAACATTATTCCTAATATTATCTCATTATCCTTAGAAATAATATACTTCTACATAAAGAAAA 2400
2401 AATATAGATGCCCTGTGCATGGAAAAAAATATCTTTCATTACCCTACCTCCAACTATTACACACGAAACTCATACCAACGAACTCTCAATCTTGAATATAAAAACGCAAAAAAACAGACG 2520
2521 TACAGGCATGAGAACCACAGTACGAGTATTTATTACATAATGATGAATATGATGAATTATTAACAAATAGTACAACTTCAAAAACTATTAGATAAGAGATCGATAACAAGCTTACAATAA 2640
2641 TAGTACAAAAACTGACCAAAGAAGAATATGAATTCCAGAAATGCAATGACAGAAAAAAAAACTCCAGCAACTACTTTACATGAAAAAACCTTGAAACAATTTTGCGACCACATCCATAAT 2760
2761 TAAACTATCAAGAAATCTACTGGTTCCATAACCCATTCACTTGAAGATATGTAGATTCTAGACCTAACTATAAATTTCTAATATGCATGCTAATACATTAGAAGCACGGGTCTATATACG 2880
2881 CTTAAAG 2887
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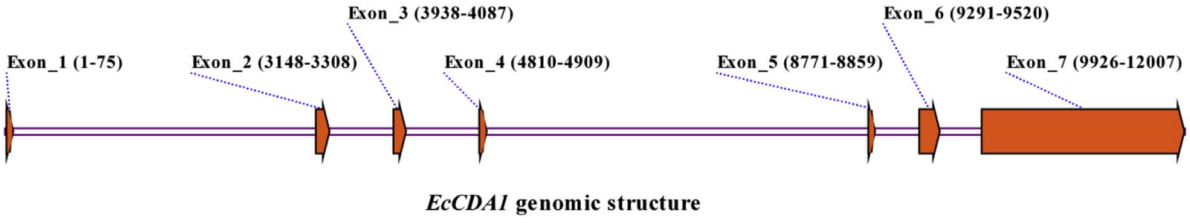
(A)



(B)

Fig. 1. (A) The nucleotide sequence and deduced amino acid sequence of EcCDA1. The predicted signal peptide is underlined in red. Chitin-binding peritrophin-A domain (ChtBD2) is underlined in black, low-density lipoprotein receptor class A domain (LDLa) is underlined in pink, and the Polysacc_deac_1 domain is underlined in blue. The N-glycosylation sites are circled in red. (B) The genomic structure of *EcCDA1*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)