Journal of Economic Entomology, 110(4), 2017, 1460–1464
doi: 10.1093/jee/tox126
Advance Access Publication Date: 28 April 2017
Research article

OXFORD

Biological and Microbial Control

The Role of p38 MAPK, JNK, and ERK in Antibacterial Responses of *Chilo suppressalis* (Lepidoptera: Crambidae)

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Subject Editor: Denny Bruck

Received 15 January 2017; Editorial decision 6 April 2017

Abstract

The mitogen-activated protein kinases (MAPKs) are conserved signal transduction pathways and broadly responsible for bacterial infection from yeast to mammals, and virus, fungi, and bacteria, specifically *Bacillus thuringiensis*, to insects. But little is known about the MAPK pathways in antibacterial responses in *Chilo suppressalis* (Walker), an important lepidopteran pest of rice. In this study, we used the bacteria of *Bacillus thuringiensis*, *Escherichia coli*, and *Staphyloccocus aureus* to infect *C. suppressalis* larvae, and the responses of MAPK pathways were analyzed. The results showed that *E. coli* infection induced the up-regulated expression of *Csp38* and *CsERK1* at 24 h postinfection (pi). Meanwhile, injection of *B. thuringiensis* and *S. aureus* resulted in strong activation of *Cs*JNK phosphorylation at 3 h pi. These results suggest that MAPK signaling pathways play important functional roles in antibacterial responses in *C. suppressalis* larvae.

Key words: p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, extracellular signal-regulated kinase, *Chilo suppressalis*, antibacterial responses

[illegible]

Table 1. Specific primers used in the experiments

(5′-3′)	
EF-1-F	GAACCCCCA ACAGCGAA CC
EF-1	C CCG GCCAACCAGAAA AGG
P38-F	CA ACGCGG GAG GCAA
P38	GCAAAC G CGA CGC GAA
JNK-F	GGCAGC ACGA AC GGCA
JNK	AC CCCGA G C GCG
ERK1-F	GCC GCC A A CGGCAAC
ERK1	CCGG GGAAGGG GAGG C
ERK2-F	C GCG C G ACGGGAG G C
ERK2	CGAACAG A GCCACCAGAAG

Materials and Methods

Insect Rearing

Chilo suppressalis D C , , 2012 4 . (. 2012); 28 ± 1 °C, >80% , 16:8 (D) . .

Real-Time Quantitative PCR

A *S. aureus*, *B. thuringiensis*, *E. coli* . B B 37 °C 180 . B (10,000 , 4 °C) 5 B ( ) . B B 1.0, 2 µ B . F 0, 3, 6, 12, 24 . A D A D A (; , D , C) CB (*C. suppressalis* -1 (*EF-1*) (. 2011, . 2016). C : 95 °C 30 , 40 95 °C 10 59 °C 30 C B E (, D , C) B D 2 55 95 °C C C , D A 1 µ A (D A .), C : $E = (10^{-1/\text{slope}} - 1) \times 100$ (2001, . 2004; 2).

Western Blot Assays

C. suppressalis (300 , 5 EG A, 17 7.5 , 1) 0, 3, 6, 12, 24 . B (B 1976) 5 , 10 µ 8% D AGE DF) (B , C , A). F 38, - , -/ 1:1,000 (C , D , A); 38, -/ , - G G ( )- 1:5,000 EC (F F , A) .

Data Analysis

$2^{-\Delta\Delta C_T}$ (2001). A A (, C ,).

Results and Discussion

Effect of Bacteria on *C. suppressalis* p38 MAPK Pathway Activation

(. 2003 , ; F D 2007; G C 2008; . 2012; . 2012). E 38 A (B. 2002, C . 2010). C. *suppressalis* . A F . 1A, *E. coli* C*sp38* 12 24 B ( ). C*sp38* 2.6- 24 *S. aureus* - 38 38 38 12. *B. thuringiensis* (F . 2A). 38 *B. thuringiensis* (. 2004, C . 2010, . 2011); 38 A *S. aureus* *E. coli*, (. 2009, . 2013).

Table 2. Primer specifications for optimized qRT-PCR amplification of *C. suppressalis*

	Eff	R^2	
CsEF1	99.8%	0.992	−3.326
Csp38	100.9%	0.994	−3.300
CsJNK	103.9%	0.995	−3.235
CsERK1	99.9%	0.992	−3.325
CsERK2	103.5%	0.980	−3.242

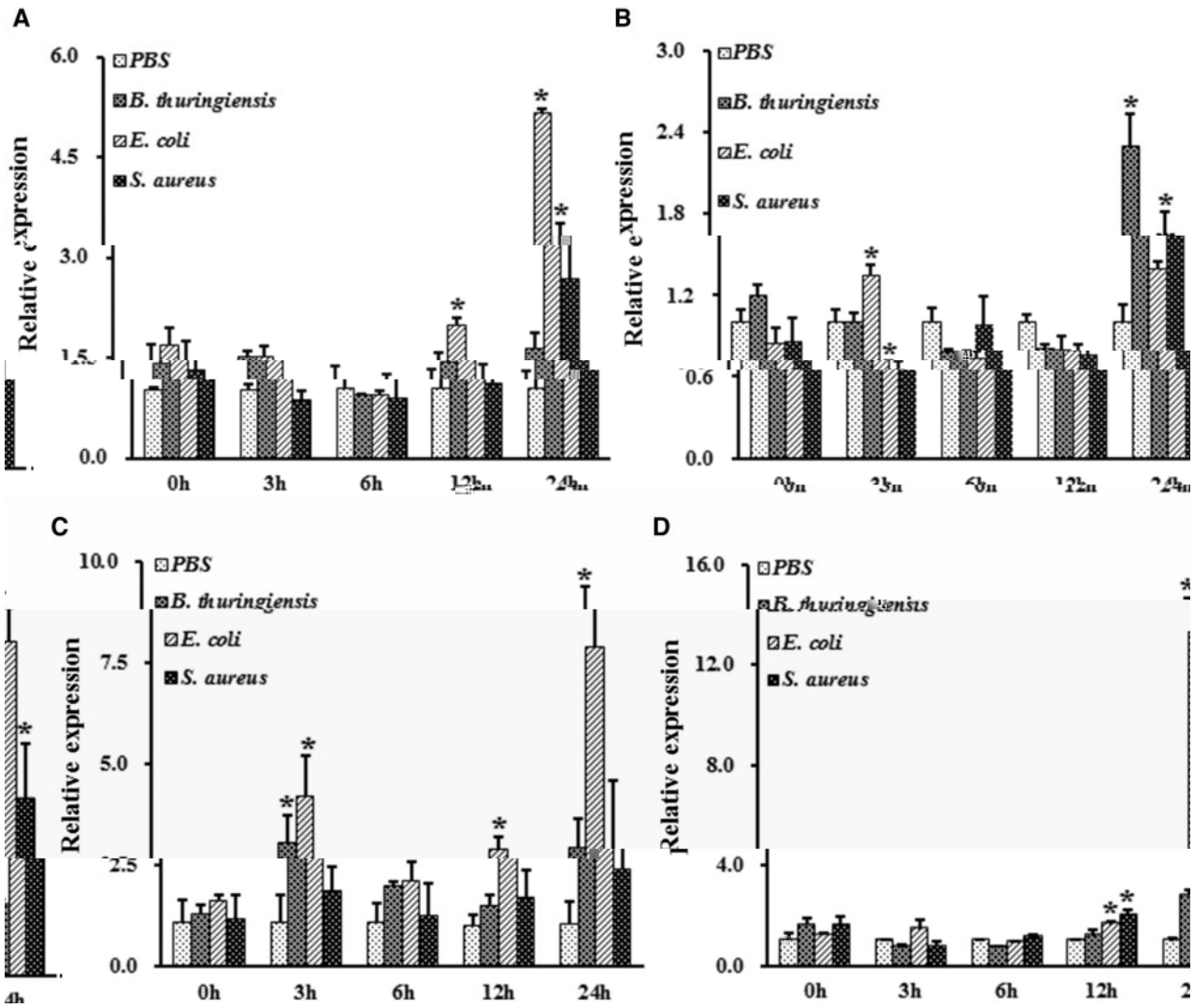

Fig. 1. Effects of bacterial treatment on *C. suppressalis* MAPKs. (**A**) Relative estimates of *Csp38* transcripts from qRT-PCR of *C. suppressalis* larvae treated with bacteria for 0, 3, 6, 12, and 24 h. (**B**) Relative estimates of *CsJNK* transcripts from qRT-PCR of *C. suppressalis* larvae treated with bacteria for 0, 3, 6, 12, and 24 h. (**C**) Relative estimates of *CsERK1* transcripts from qRT-PCR of *C. suppressalis* larvae treated with bacteria for 0, 3, 6, 12, and 24 h. (**D**) Relative estimates of *CsERK2* transcripts from qRT-PCR of *C. suppressalis* larvae treated with bacteria for 0, 3, 6, 12, and 24 h. Relative amounts of genes transcript were normalized to the expression of *EF1*. Each symbol and vertical bar represents the mean ± SE ($n = 3$). Asterisks indicate significant differences ($P < 0.05$; ANOVA).

Responses of *C. suppressalis* JNK Pathway to Bacteria Challenge

F *CsJNK* 24 *B. thuringiensis* (2.3-) *S. aureus* (1.6-f) *CsJNK* *E. coli* 3 (F . 1B). / *C. suppressalis*, / / / 3 *B. thuringiensis*; 3 24 *S. aureus* (F . 2B). / *S. aureus* *B. thuringiensis*. / . A / (1996, . 2007).

Analysis of *C. suppressalis* ERK1/2 Pathways in Response to *S. aureus*, *B. thuringiensis*, and *E. coli* Immune Stimulations

C E (F D. 2007). C *CsERK1* *CsERK2* *CsERK1* 3 (4.2-f), 12 (2.8-f), 24 (7.9-f) *E. coli* (F . 1C), 3 *B. thuringiensis* . *CsERK2* *E.coli* *S. aureus* 12 24 (F . 1D) F .

Bradford, M. M. 1976. A [illegible] . A B [illegible]. 72: 248–254.

Cancino-Rodezno, A., C. Alexander, R. Villasenor, S. Pacheco, H. Porta, Y. Pauchet, M. Soberon, S. S. Gill, and A. Bravo. 2010. [illegible] 38 [illegible] *Bacillus thuringiensis*. [illegible] B [illegible] B [illegible]. 40: 58–63.

Chen, J. M., C. C. Xie, L. L. Tian, L. X. Hong, X. R. Wu, and J. H. Han. 2010. [illegible] 38 [illegible] *Drosophila* [illegible] A. 107: 20774–20779.

Chouvenc, T., N. Y. Su, and A. Robert. 2009