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꿀벌에 대한 dsRNA의 급성섭식독성 평가

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Acute Oral Toxicity of dsRNA to Honey Bee, *Apis mellifera*

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Received: 18 October 2017/ Revised: 17 November 2017/ Accepted: 22 November 2017

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Abstract

BACKGROUND: RNA interference (RNAi) eliminates or decreases gene expression by disrupting the target mRNA or by interfering with translation. Recently, RNAi technique was applied to generate new crop traits which provide protection against pests. To establish the environmental risk assessment protocol of RNAi LMO in lab scale, we developed dsRNA expression system using *E. coli* and tested acute oral toxicity assay to honey.

METHOD AND RESULTS: The dsRNA expression vector, L4440, was chosen and cloned 240 bp of Snf7 and GFP gene fragment. To develop the maximum dsRNA induction condition in *E. coli*, we tested induction time, temperature and IPTG concentration in media. To estimate the risk assessment of dsRNA to honey bee, it has been selected and cultured with dsRNA supplement for 48 hours according to OECD guideline. As a result, the optimum condition of dsRNA induction was 37°C, 4 hours and 0.4 mM IPTG concentration and the difference between Snf7 and GFP dsRNA molecules from *E. coli* was not significant in survival and behavior to honey bee. Furthermore, blast search results indicated that effective match of predicted dsRNA fragments were not existed in honey bee genome.

CONCLUSION: In this study, we developed and tested the acute oral toxicity of dsRNA using *E. coli* expression system to honey bee.

Key words: Acute oral toxicity, *Apis mellifera*, dsRNA, Living modified organisms

서론

가 가
 (Living Modified, LM) . International
 Service for the Acquisition of Agribiotech Application
 (ISAAA) 2016 LM
 28 179 (ha)
 가
 39 LM
 LMO가
 LM
 RNAi LMO가 가
 가 가
 LMO

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가 LMO Snf7 V-ATPase GFP dsRNA
 RNA (RNA interference, RNAi) *Caenorhabditis* *in vitro* transcription
elegans (Fire et al., 1998) HT115 (DE3) dsRNA
 가 가 Snf7 GFP dsRNA
 가 LMO RNAi 가 dsRNA 가
 mRNA dsRNA dsRNA가 dsRNA 가
 RNAi *Aedes aegypti*,
Harmonia axyridis, *Acyrtosiphon pisum*, *Epiphyas*
postvittana (Niimi et al., 2005,
 Turner et al., 2006, Jaubert-Possamai et al., 2007, Coy
 et al., 2012). RNAi *Hyalophora*
cecropia, *Spodoptera litura*, *Plutella xylostella*,
Spodoptera frugiperda, *Choristoneura fumiferana*
 (Bettencourt et al., 2002; Rajagopal et al.,
 2002, Bautista et al., 2009, Rodriguez-Cabrera et al.,
 2010, Quan et al., 2013), *Spodoptera litura*
Bemisia tabaci
 RNAi 가 (Jeon et al.,
 2014, Kim et al., 2015).
 RNAi LMO
 가 (WCR, *Diabrotica*
virgifera virgifera Leconte)
 Snf7 mRNA dsRNA
 (Bolognesi
 et al., 2012, Ramaseshadri et al., 2013, Ko i et al., 2014).
 Snf7 ESCRT-III endosomal-
 autophagic
 (Henne et al., 2011, Wegner et al., 2011).
 가 가
 가 (Henry et al.,
 2012). 가 OECD
 semi-filed
 가
 가
 가
 가
 DvSnf7 mRNA
 dsRNA 가
 (Tan et al., 2015, Vélez et al., 2016).

재료 및 방법

dsRNA 발현 시스템 구축

dsRNA L4440 vector HT115
 (DE3) L4440 vector
 T7 promoter가 IPTG
 (Isopropyl β-D-thiogalactopyranoside)
 RNA가 dsRNA vector, HT115 (DE3)
 RNaseIII가 dsRNA
 (Timmons et al., 2001). L4440
 vector 240bp Snf7 GFP
 Bachman(2013) Snf7 (주)
 GFP
 mGFP PCR N-
 BamH I C- Xho I
 L4440 Snf7 BamH I /Xho
 I L4440 GFP BamH I /Xho I
 plasmid
 plasmid HT115 (DE3)
 colony T7 promoter primer
 PCR plasmid

dsRNA 발현 조건 확립 및 정제

Snf7 GFP dsRNA
 (22°C, 30°C, 37°C), IPTG (0.1 mM, 0.4
 mM, 1 mM) (2, 4)
 4°C, 4,000 rpm 10
 200 µl
 TE (Tris-EDTA) pellet Lysozyme
 (400 µg/ml) DNaseI (1 unit/ml) RNeasy mini kit
 (Qiagen, Germany) total RNA
 RNA (NanoDrop ND-2000,
 Thermo Scientific, USA) -80°C

dsRNA 발현 확인

dsRNA 10 µg total

RNA 1.5% agarose gel 150 V, 20
 ChemiDoc™ XRS+ System(Bio-Rad, USA), 48
 band dsRNA
 1 µg total RNA
 ReverTra Ace-α(TOYOBO, Japan)
 primer cDNA cDNA
 10², 10³, 10⁴ 10⁴
 cDNA 1 µl PCR (Quantitative
 Realtime PCR, qRT-PCR) Power SYBR Green
 PCR Master Mix (ThermoFisher Scientific, USA)
 StepOnePlus Real-Time PCR system(Applied
 Biosystem, USA) qRT-PCR
 primer GFP Snf7
E.coli 16SrRNA reference
 (Clifford *et al.*, 2012).
 (one-way ANOVA)

시험물질 처리농도
 가
 10~100 Snf7 dsRNA가
 LMO Snf7 RNA
 , 가
 10~100
 1 µg/g Snf7 dsRNA
 GFP dsRNA가 total RNA 50% 1:1

꿀벌 준비
 4
 (15 cm, 5 cm)
 10
 25%
 Snf7 dsRNA
 GFP dsRNA total RNA(2 µg/ml) 1 ml 50%
 1 ml
 stock solution 0.2 ml
 2 4
 25%
 (assay control)
 GFP dsRNA
 (SATO, Japan)

꿀벌 사육 및 치사율 확인
 dsRNA 가
 24.5~25.5°C, 61~66%

꿀벌 Genome Blast
 Snf7 dsRNA genome dsRNA
 가
 bioinformatic tool tool
 Ensembl Metazoa (<http://metazoa.ensembl.org/index.html>) Blast Snf7 240 bp
 genome

결과 및 고찰

dsRNA 최적 발현 조건 확립
 L4440 Snf7 L4440 GFP plasmid가
 HT115(DE3) plasmid
 T7 promoter primer PCR (Fig. 1).
 vector 223 bp 가
 , Snf7 GFP가 L4440 Snf7 L4440
 GFP 381 bp 가
 (Fig. 1).

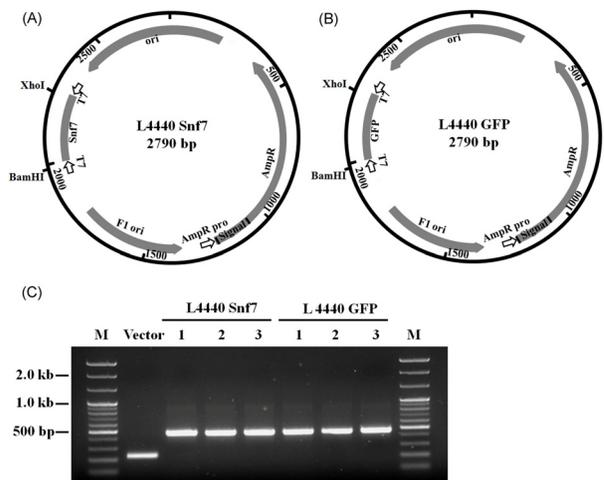


Fig. 1. Diagrams of dsRNA expression plasmid DNA and diagnostic PCR result of transformed HT115 (DE3). (A-B) The dsRNA expression plasmids L4440 with Snf7 (A) and GFP (B) gene fragment. (C) Agarose gel electrophoresis of T7 promoter primer PCR of L4440 empty vector, L4440 Snf7 and L4440 GFP plasmid transformed into *E. coli* HT115. Vector represented L4440 empty vector, lane 2-4 indicated L4440 Snf7 transformed individual *E. coli* cell line and lane 5-7 indicated L4440 GFP transformed individual *E. coli* cell line. M represented 100 bp size marker.

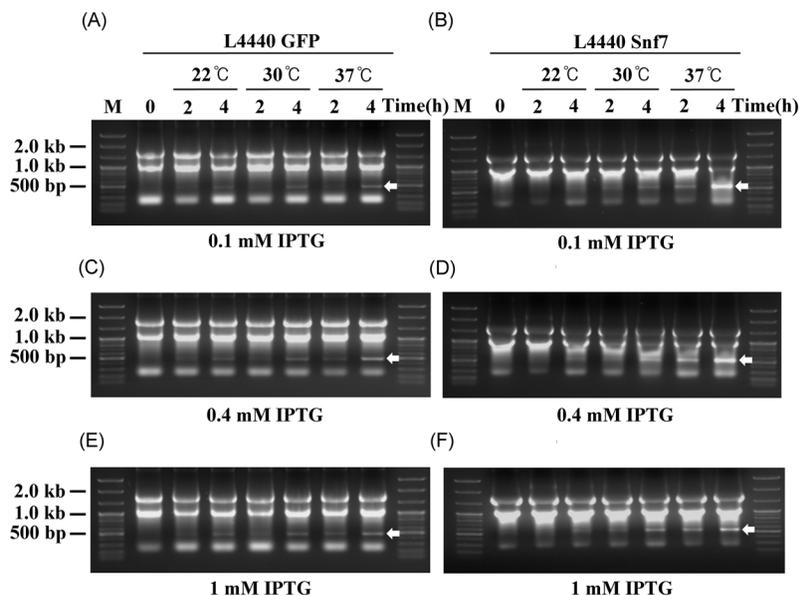


Fig. 3. Optimal induction condition of Snf7 dsRNA and GFP dsRNA. The dsRNA induction test in various IPTG concentrations, induction time and temperature. L4440 GFP and L4440 Snf7 transformed HT115 (DE3) with different IPTG concentration (A-B, 0.1 mM; C-D, 0.4 mM; E-F, 1 mM), induction time(2, 4 hour) and incubation temperature (22 , 30 , 37). Arrow indicated dsRNA band.

Snf7 GFP 37°C, 0.4 mM
 IPTG Snf7 GFP 가 dsRNA가 (25%), GFP dsRNA
 (Fig. 3). (1 µg/ml), Snf7 dsRNA (1 µg/ml) 48
 dsRNA
 dsRNA (Table 2). dsRNA
 dsRNA
 dsRNA total
 RNA 가 (Table 3). dsRNA가 가 mRNA ,
 Snf7 dsRNA GFP dsRNA가 48 21~22 nt dsRNA

Table 2. Number of dead honey bees after dsRNA exposure

Nominal concentration (µg a.i./bee)	Number of Honey bee tested	Cumulative number of dead Honey bee			
		1 hour	4 hour	24 hour	48 hour
Assay Control	60	0	0	0	0
GFP	60	0	0	0	0
Snf7	60	0	0	0	0

Table 3. Symptoms of general intoxication of acute oral toxicity to adult honey bees with dsRNA treatment

Nominal concentration (µg a.i./bee)	Symptoms of general intoxication			
	1 hour	4 hour	24 hour	48 hour
Assay Control	N (60)	N (60)	N (60)	N (60)
GFP	N (60)	N (60)	N (60)	N (60)
Snf7	N (60)	N (60)	N (60)	N (60)

(): Number of honey bee
 * Abbreviation of observable symptoms of intoxication
 N: Normal

Table 4. Blast results of honeybee genome to Snf7 sequence

Genomic Location	Overlapping Gene	Orientation	Length (mer)	Score	E-value	Identity (%)
15:2240755-2240782	GB54443	Forward	28	20	0.046	92.9
6:996087-996105		Forward	19	19	0.18	100.0
8:9822750-9822768	GB54818	Forward	19	19	0.18	100.0
GroupUn98:8202-8224		Reverse	23	19	0.18	95.7
7:1592493-1592514		Forward	22	18	0.72	95.5
2:12443904-12443921		Reverse	18	18	0.72	100.0
GroupUn10:63736-63757		Reverse	22	18	0.72	95.5
GroupUn106:41095-41116		Reverse	22	18	0.72	95.5
15:3706933-3706954	GB49490	Reverse	22	18	0.72	95.5
4:2993224-2993245		Forward	22	18	0.72	95.5
10:11029753-11029770		Forward	18	18	0.72	100.0
5:14182702-14182719		Reverse	18	18	0.72	100.0
11:1290363-1290379	GB43935	Reverse	17	17	2.8	100.0
11:7439654-7439670	GB47256	Forward	17	17	2.8	100.0
GroupUn121:12940-12956	GB45916	Forward	17	17	2.8	100.0
GroupUn1713:377-393		Reverse	17	17	2.8	100.0
GroupUn1624:9202-9218		Reverse	17	17	2.8	100.0
7:4249356-4249372	GB49246	Forward	17	17	2.8	100.0
2:2666985-2667001		Forward	17	17	2.8	100.0
1:6855091-6855107	GB40737	Reverse	17	17	2.8	100.0
1:22868151-22868167		Forward	17	17	2.8	100.0
GroupUn4315:178-194		Reverse	17	17	2.8	100.0
13:6204662-6204678		Reverse	17	17	2.8	100.0
GroupUn299:21884-21900		Reverse	17	17	2.8	100.0
6:17651796-17651816	GB55706	Reverse	21	17	2.8	95.2
GroupUn3912:3027-3043		Reverse	17	17	2.8	100.0
GroupUn337:40489-40505		Forward	17	17	2.8	100.0
9:5938018-5938034		Reverse	17	17	2.8	100.0
GroupUn4008:5044-5060		Reverse	17	17	2.8	100.0
12:1624966-1624982	GB40196	Reverse	17	17	2.8	100.0
GroupUn3:266997-267013		Reverse	17	17	2.8	100.0
14:7055042-7055058		Reverse	17	17	2.8	100.0
GroupUn2563:826-842		Reverse	17	17	2.8	100.0
8:174565-174589	GB47875	Reverse	25	17	2.8	92.0
8:5608614-5608630		Forward	17	17	2.8	100.0
4:1062331-1062347	GB54885	Forward	17	17	2.8	100.0
4:9252465-9252481		Reverse	17	17	2.8	100.0
GroupUn477:2503-2519		Reverse	17	17	2.8	100.0
GroupUn3426:203-219		Forward	17	17	2.8	100.0
5:6470785-6470805		Forward	21	17	2.8	95.2
5:6697631-6697647		Forward	17	17	2.8	100.0
5:7735920-7735936	CALX	Forward	17	17	2.8	100.0

(Elbashir *et al.*, 2000).
 Snf7
 Bioinformatic tool
 dsRNA
 (Table 4). Snf7
 가
 240 bp Snf7
 dsRNA
 , (17~19 nt)
 가
 HT115 (DE3) Snf7 dsRNA GFP
 dsRNA
 ,
 가 dsRNA
 가 dsRNA
 가

적 요

RNAi LMO
 LMO
 가가
 dsRNA , ()
 가
 . L4440 vector Snf7 GFP
 plasmid HT115 (DE3)
 , IPTG
 37°C, 0.4 mM IPTG, 4
 가 dsRNA가
 가 가
 dsRNA
 Snf7 dsRNA GFP
 dsRNA 가
 가
 가 dsRNA

Notes

The author declare no conflict of interest.

Acknowledgement

This study was supported by the National Institute of Ecology (NIE), Republic of Korea.

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