

辣椒疫霉病无毒基因 RXLR128001 克隆与蛋白表达纯化

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摘要: SD33 1 RXLR128001 BLAST and SignalP4.1 Server *Escherichia coli* RXLR128001

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Clone of Nontoxic Gene RXLR128001 in *Phytophthora capsici* and Expression and Purification of Effector Protein

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Abstract: In this study, we cloned a nontoxic gene RXLR128001 from *Phytophthora capsici* and then analyzed the sequence structure of it based on the BLAST and SignalP4.1 Server software. At the same time, we expressed and purified RXLR128001 gene in *Escherichia coli* and determined the optimal conditions that could induce RXLR128001 gene to be effectively expressed. Finally, we prepared the high pure protein of RXLR128001 gene to be beneficial to the further study on the crystal cultivation and optimization of it.

Keywords: *Phytophthora capsici*; effector protein; gene clone; expression and purification of protein

(*Phytophthora capsici*) [1,2]
[3]
20 [4] [5,6]
effectors [7]
[7]
[8] N-
RXLR-dEER motif 100 400
[9-11] 300 RXLR RXLR
1 RXLR

1 材料与方法

1.1 材料

SD33

1.2 辣椒疫霉 RxLR 效应分子基因克隆

1.2.1 辣椒疫霉效应蛋白分子 RXLR128001 界定与引物设计

JGI (<http://genome.jgi.doe.gov/>) 1 RXLR128001 RXLR128001
RxLR128001-F: 5`-ATGCGCCTCCTTTATTTGGC-3` RXLR128001-R:
5`-CTACACACTGTTGAGTTTCG-3`

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1.2.2 RXLR128001 基因克隆

Escherichia coli DH5 JM109

pGEM-T Easy Vector

Promega

Sangon

DNA

RXLR128001 PCR 95 5 min 94 30 s 55 30 s 72 2 min 30 72
 5 min DNA 1) 100 µg/mL Amp LB
 2~3 h 2) -80 *E. coli* DH5 JM109
 1.5 mL Ep 10 µL 50 µL
 30 min 3 42 90 s 2 min 4) 500 µL LB
 37 200 r/min 45 min 5) 1 min 8000~10000 r/min 100 µL LB
 100 µL 37 12~16 h 6) LB
 37 200 r/min 12~16 h OD=0.5 PCR

1.3 RXLR128001 基因原核表达与蛋白纯化

1.3.1 原核表达载体构建

pET-28a

5' 3'

BamHI

EcoRI

RXLR128001-F:

5'-CGCGGATCCCTCTCCACGCCTACGGCACCTG-3';RXLR128001-R:5'-CCGGAATTCTTAGCCGG

ACACCACACCCAC-3'

RxLR128001

PCR

PCR

RXLR128001

PCR

[12]

[12]

[12]

PCR

1.3.2 转化宿主蛋白表达

BL21(DE3)

Kana

1) LB 5 5 mL LB 50 mg/L Kana 15 mL
 37 OD 0.6~0.8
 2) 500 µL 15 1:1(V/V) -80
 3) 500 µL
 4) 5 µL IPTG 1 mM/L 37 200 r/min 4 h
 5) 500 µL 12000 r/min 2 min
 6) 10 µL 5× 100 5 min , 12000 r/min 5 min;
 7)15 SDS-PAGE

1.3.3 蛋白表达条件优化

1 5 100 mL LB 100 mg/mL Kana 250 mL 1~5
 RXLR128001-28a 1 mL 37 200 r/min
 2 OD600 0.6~0.8 1~5 IPTG
 0.2 mM/L 0.4 mM/L 0.6 mM/L 0.8 mM/L 1.0 mM/L 4
 5000 r/min 6 min 10 mL 4
 4 15000 r/min 30min 1~5 10 µL 5× 100
 5 min , 12000 r/min 5 min

3)15 SDS-PAGE

1.3.4 融合蛋白大量表达

37 OD600=0.6~0.8 1:100 1 L LB 50 mg/L Kana
 37 OD600=0.6~0.8 16 IPTG
 20 h 5000 r/min 6 min

1.4 融合蛋白的纯化

1.4.1 亲和层析纯化 1) 1 L 50 mL 20 mM Tris-HCl 150 mM
 NaCl pH 8.5 4 (600 W 6 s 6 s 100) 4 14000 r/min

30 min 0.22 μm

2) Ni-NTA 4

3 20 mM/L Tris-HCl 150 mM/L NaCl 20 mM/L pH 8.5

Ni-NTA 50~1000 mM (20 mM/L Tris-HCl 150 mM/L NaCl pH 8.5)

SDS-PAGE

1.4.2 离子交换纯化 1) 10 KD 1 mL

20 mM Tris-HCl 50 mM/L NaCl pH 8.5

2) 4 12000 rpm 15 min

3) ResourcesQ AKTA 20 mM Tris-HCl 50 mM/L NaCl

pH8.5 5

4) 20 mM Tris-HCl 2 M/L NaCl pH8.5 20 mM Tris-HCl 50 mM/L NaCl pH 8.5

5) SDS-PAGE

2 结果与分析

2.1 辣椒疫霉 RxLR128001 基因克隆与基因序列结构分析

RXLR128001 BLAST RXLR128001 SD33 Genbank

(Genbank no KT726224), SignalP4.1 Server RXLR128001

RXLR128001 1

RXLR128001 75.8 kDa 21

ORF 2016 bp 671

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1000 1040 1080 1060 1070 1080
GAGAAATTCAGCAAGAAAGATCCGAAAGCAGTCCGAGAGATCAATAGAGATGTGG
E K S T T K K I A R Q P G A D D L E M W
1090 1100 1110 1120 1130 1140
GTGAACAGTGGACAACTCGCGAGCAATGTACAACTTTTGAACCTGCTCCCGGAGA
V N S G Q S V D D V Y K L L N L P S R R
1150 1160 1170 1180 1190 1200
GATCTATGGTCAGCTTGGTAATCAGAAAGCTGTCAGACCTGGTGTGAGCTTATGAA
D L E V D P F E N Q K L F D T W E T P F N
1210 1220 1230 1240 1250 1260
GCCTGACGCTCAAGACTCCAGAGAAACTTCTGCTATAATTCGGAGTGGGACATAGT
A V S V K T P E K T S A I F S K L A F S
1270 1280 1290 1300 1310 1320
TTCGAGACAGCAATGCGAAATTCCTGGAGCAGCAATAAGTCTCCGAGCAAGAA
F E D R P W M Q I L E A A N K F S S M E
1330 1340 1350 1360 1370 1380
AAGCGCGCCCAATTCGAGTGGAGAAAGCCCAAGGATTTCTCCCAAGGATATCA
K A A T R I Q L E R A Q S I F S T G V S
1390 1400 1410 1420 1430 1440
CCCGTAAGGCTTAAAGTGGTGGCTTCGATAATGGGGATTCCTTCAGTAGT
P R K A F K W V A I D N V G D S V L S S
1450 1460 1470 1480 1490 1500
CCTCTGTGAAGAGTGGTGGCTTACGTGGAGATTCACAGAGAAATCCAGGCAAG
P L K K W M L Y V E D F N K K N P G K
1510 1520 1530 1540 1550 1560
GAGGAATCTGGTCTTGGCACTACGTTAACTATCAAACTCCGAGACAGATGAC
E E S W F L P L R W N Y Q N I G R E T D
1570 1580 1590 1600 1610 1620
ANGGGAAGAAAGCCAGTACAGTGAACCTGGCCACCTGGCGAGAGAGAAACAAG
K A M R D P S T V K L A Q L V Q K E R M
1630 1640 1650 1660 1670 1680
AAGATAGCTAGAAAGTGGAAATATCTCCAAATATGGGCTTCCCTGGATGGCACTC
K E W L E R W K Y S P N M A F R E I H L
1690 1700 1710 1720 1730 1740
AACAAAGCAGGAGAAAGTGTFTCCGGCCAGTATGGGTGGTGGTCAAGTATG
N K A G E K V F S A P N F E L W V K Y L
1750 1760 1770 1780 1790 1800
GATGACTGGAAACAAAGATACTCCAGCAAGAGAGACTATGATCAACGGGTTCCGGGT
D D W N Q A V P S K K E T M I D G P K G
1810 1820 1830 1840 1850 1860
AATFACCAATGACTGGATCTAGTGGCAATGCTGGCCAGAGGAGAAAGCCCGAGCACG
N Y H D L D L V F M L A A E K A P S E T
1870 1880 1890 1900 1910 1920
ANGAAGTGGGTCAGAAATGAGGATGGGCTGGTGGATGAGTGGGTCGAAAGAAA
K K L A S E L K D A L V D K N V A E K K
1930 1940 1950 1960 1970 1980
ACACTGGTACTCAATCTGGCTCAAGGGATCTCACTCTCCGAGGATATGCTGGAG
T L A V K S W L R G I S S N D D M I E
1990 2000 2010
CGATTCAGCCGGAACATCAAGTGTGTAG
R F T A K L N S V *
661

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图 1 辣椒疫霉菌效应蛋白分子 RXLR128001 基因氨基酸序列
Fig.1 Amino-acid sequence of *Phytophthora capsici* effector gene RXLR128001

阴影部分为信号肽氨基酸序列,*为终止密码子

Amino acids within shady indicate the signal peptide, * is terminal code

2.2 RXLR128001 表达与纯化

2.2.1 RXLR128001 重组质粒构建与鉴定 PCR 1.0%

DNA 2016 bp 2

RXLR128001-28a PCR RXLR128001-28a

2.2.2 RXLR128001 蛋白表达 pET28a(+)-RXLR128001 BL21 IPTG

15% SDS-PAGE 3 RXLR128001

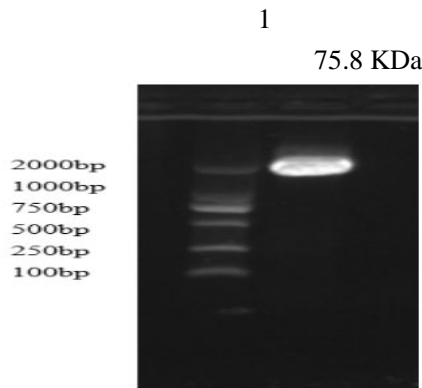


图 2 RXLR128001 基因 PCR 扩增结果
Fig.2 PCR amplification of RXLR128001

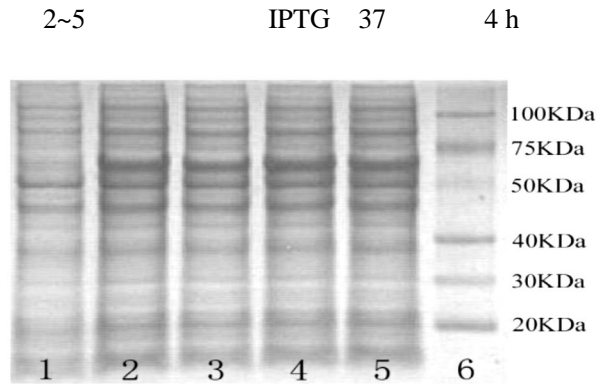


图 3 RXLR128001 蛋白在不同条件下的诱导表达

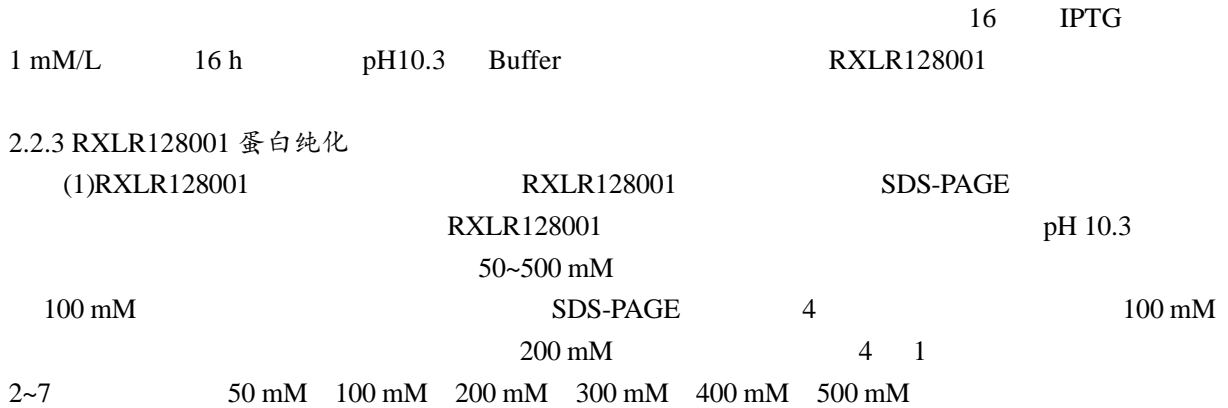


图 4 RXLR128001 蛋白亲和层析纯化

Fig.4 Purification of RXLR128001 protein with affinity chromatography

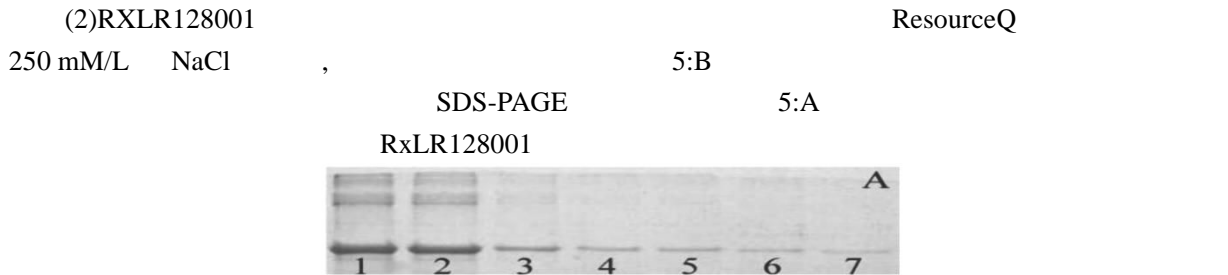


图 5 A: RXLR128001 蛋白离子交换层析纯化; B: RXLR128001 蛋白 ResourceQ 柱纯化峰值, * 号所示

Fig.5 A: Purification of RXLR128001 protein with ion exchange chromatography B: Purification of RXLR128001 protein with ResourceQ column, The peak value was showed by *

3 结论与讨论

JGI (<http://genome.jgi-psf.org/>) 1 RXLR,
 128001 RXLR128001
 SD33 RXLR128001 ORF
 2016 bp 671 75.8 kDa
 RXLR 100~400 [9-11]
 RXLR128001 1 21 [11]
 RXLR128001
 RXLR128001
 RXLR128001
 RXLR128001
 4 5

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