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辣椒疫霉病无毒基因 RXLR128001 克隆与蛋白表达纯化

王晓梅*,张钟文,杨 楠
, 130118

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

关键词: ; ; ;
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Clone of Nontoxic Gene RXLR128001 in *Phytophthora capsici* and Expression and Purification of Effector Protein

WANG Xiao-mei*, ZHANG Zhong-wen, YANG Nan
College of Agronomy/Jilin Agricultural University, Changchun 130118, *China*

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 effective 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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作者简介: (1971-), , , . E-mail:wxm820@126.com
*通讯作者:Author for correspondence. E-mail:wxm820@126.com

1.2.2 RXLR128001 基因克隆 pGEM-T Easy Vector Promega *Escherichia coli* DH5α JM109 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 10 mL LB 50 mg/L Kana 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 , SD33 RXLR128001 BLAST RXLR128001 Genbank (Genbank no KT726224), SignalP4.1 Server RXLR128001 RXLR128001 1

RXLR128001 ORF 2016 bp 671 75.8 kDa 21

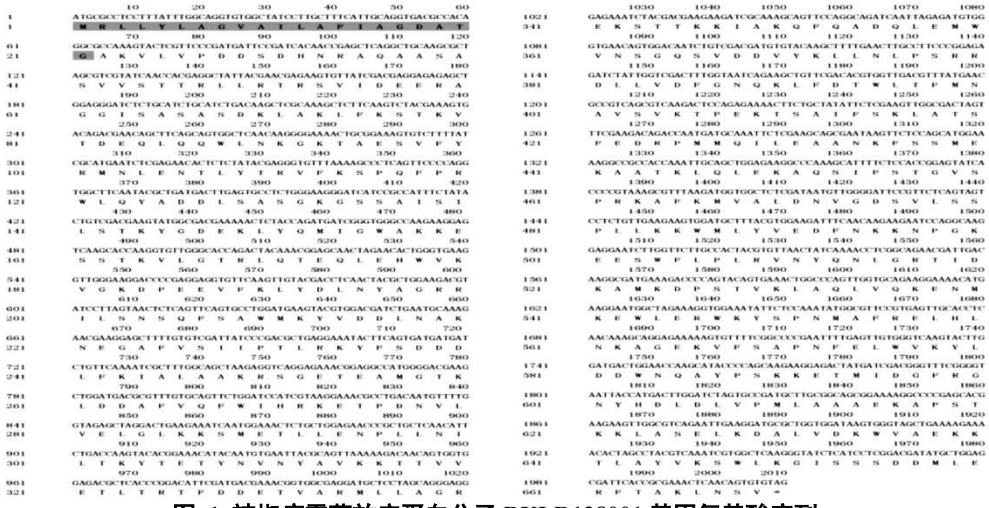

图 1 辣椒疫霉菌效应蛋白分子 RXLR128001 基因氨基酸序列

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

1 2~5 IPTG 37 4 h

75.8 KDa

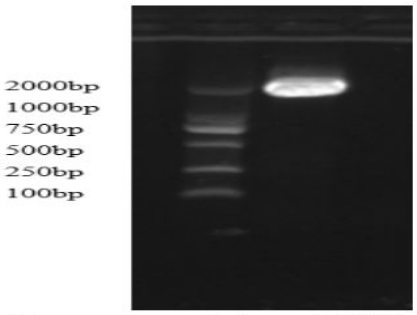

图 2 RXLR128001 基因 PCR 扩增结果

Fig.2 PCR amplification of RXLR128001

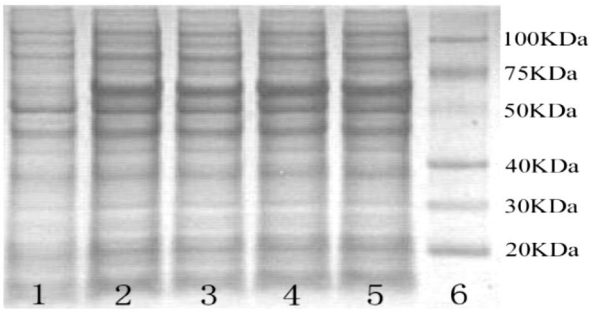

图 3 RXLR128001 蛋白在不同条件下的诱导表达

Fig.3 The expression of RXLR128001 protein under different induced conditions

16 IPTG

1 mM/L 16 h pH10.3 Buffer RXLR128001

2.2.3 RXLR128001 蛋白纯化

(1)RXLR128001 RXLR128001 SDS-PAGE

RXLR128001 pH 10.3

50~500 mM

100 mM SDS-PAGE 4 100 mM

200 mM 4 1

2~7 50 mM 100 mM 200 mM 300 mM 400 mM 500 mM

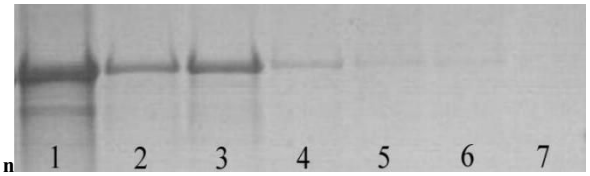

图 4 RXLR128001 蛋白亲和层析纯化

Fig.4 Purification of RXLR128001 protein with affinity chromatography

(2)RXLR128001 ResourceQ

250 mM/L NaCl , 5:B

SDS-PAGE 5:A

RxLR128001

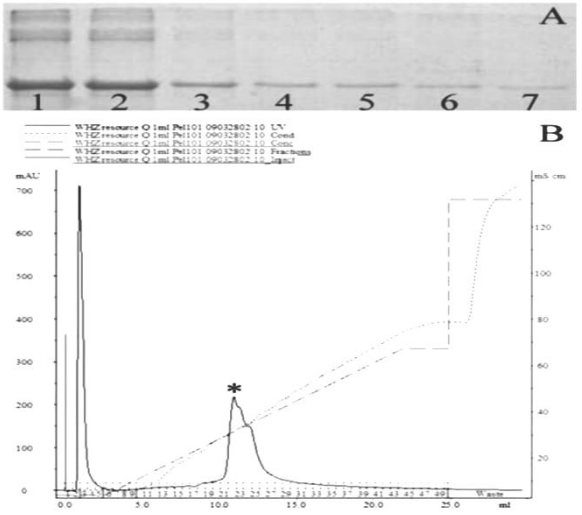

图 5 A: RXLR128001 蛋白离子交换层析纯化；B: RXLR128001 蛋白 ResourceQ 柱纯化峰值,＊号所示

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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