BbJEN1

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通讯作者。

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球孢白僵菌羧基转运蛋白基因 J. ( H, ( ! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( )! ( }
1.2  
3'-Full RACE Core Set RNA PCR Kit
AMV P Enzyme TaKaRa P GFP™ PCR DNA and Gel Band Purification Kit Ready-To-Go™ DNA Labelling Beads dCTP Amersham Biosciences Hybond N+ Phamarcia pGEM Easy-T Promega

1.3 RNA P DNA EST
Sma I Sea I Dra I EcoRV 4

1.4 YADE AJ273226 EST

1.5 3' RACE
Bjen1-RT1 5'-AGGTAGATATCTCCGACA-3' 3'-Full RACE Core Set

1.6 RT-PCR
RNA cDNA Bjen1-RT1 Bjen1-RT2 RNA PCR Kit AMV Enzyme RT-PCR Bjen1-RT1 1.5 Bjen1-RT2 5'-TCGTGAGCTCGATTGGAAT-3'

1.7 DNA Est
DNA Gel Band Purification Kit GFP™ PCR DNA and Gel Band Purification Kit Hybond N+ pGEM Easy-T

1.8 Southern

2

2.1 JEN1 GenBank AY187631

Bjen1-RT1 5'-JEN1

GBbJEN1

Saccharomyces cerevisiae JEN1

77% 66% 30%
2.2 GBbJEN1

YADERGBbJEN1 977bp

GenBank AY187634 TATA

CAATGMaJEN1

Mig1 Mig2 Mig1

GBbJEN1

STREŚ CCCCT

pH

2.3 Southern

Dra I Eco R I/Pst I DNA 32 P
dCTP GbJEN1 DNA

GBbJEN1 DNA


1 2

Fig. 1 Southern blot analysis of Beauveria bassiana genomic DNA hybridized with 32 P labeled GBbJEN1

A. Agrose gel electrophoresis of B. bassiana genomic total DNA digested with Dra I and Eco R I/Pst I.[B. Genomic Southern blot hybridization with GBbJEN1 fragment as probe M. Marker XM] MBI. 1. Dra I

2. Eco R I/Pst I.

2.4 BbJEN1

RT-PCR BbJEN1

1 2

Fig. 2 Analysis of BbJEN1 expression by RT-PCR

1. Marker XM MBI 2 – 5. RT-PCR using RNA which was extracted from the cultures containing cicada cuticle lane 2 cicada cuticle lane 3 cockroach cuticle lane 4 and cockroach cuticle and glucose 2% lane 5 respectively.
Cloning and Characterization of a Carboxylic Transport Protein JEN1 and Its Upstream Sequence from Beauveria bassiana

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Abstract The degenerate primers were designed based on an EST of Metarhizium anisopliae which was highly homologous with a DNA tagging from a T-DNA insert mutant of Beauveria bassiana. And homologous DNA fragment of the EST and it’s extending sequence were amplified from B. bassiana using YADE method. The sequence analysis showed that the amplified DNA fragment was a partial fragment of gene encoding carboxylic transport protein JEN1 because the putative amino acid sequence was similar to carboxylic transport protein JEN1 from Neurospora crassa. Thereafter the whole DNA sequence of GbJEN1 encoding JEN1 from B. bassiana was obtained by extending upstream and downstream sequence of the amplified fragment using YADE method. The cDNA of JEN1 designated BbJEN1 was cloned from B. bassiana by 3’RACE according to the sequence of GbJEN1 which is 1656bp long and encoded a protein of 514 amino acid with Mr = 55975.37 Da and PI = 9.32. The amino acid sequence of the gene showed 77% 66% and 30% similarity to MAJEN1 of M. anisopliae JEN1 of Neurospora crassa and JEN1 of Saccharomyces cerevisiae respectively. Sequence analysis indicated that GbJEN1 contained two introns. Southern analysis indicated that GbJEN1 was present as a single copy in B. bassiana. The result of RT-PCR showed that transcription of BbJEN1 was induced by the cuticle of cockroach or cicada and repressed by glucose. A 977bp upstream sequence of GbJEN1 was amplified using YADE method which contain several putative binding domains of glucose repressor and one stress response element STRE.

Key words: Beauveria bassiana  Carboxylic transport protein