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The Function of Major Sperm Proteins (MSPs) in reproduction of pine wood nematode

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Abstract

BxMSP1, BxMSP2 and BxMSP3 of *Bursaphelenchus xylophilus* were cloned in this study. The senior structure of these proteins was rich of β sheets, which was highly conserved in MSP members of the nematode species. In situ hybridization showed that these genes were specifically expressed in the seminal vesicle tissue of the male adults. The reproduction ability of *B. xylophilus* decreased when the nematodes were soaked by the dsRNA of BxMSP1 and BxMSP2. qPCR analysis showed lower transcript abundance of the targeted mRNAs when the nematodes were soaked by the dsRNA of the MSPs. These results indicated that BxMSP1 and BxMSP2 were required for reproduction of *B. xylophilus*.

1 Introduction

The major sperm protein (MSP) is a nematode specific protein. MSP has first been identified in *Caenorhabditis elegans*. It is the most abundant protein present in nematode sperm, MSP is the key player in the motility machinery of nematodes that propels the crawling movement of nematode sperm in *C. elegans*. But the function of MSP in the plant nematode is still very limited known. In this paper, three MSPs were cloned form *B. xylophilus* and gene function were identified by RNAi method.

2 Materials and methods

BxMSP1, BxMSP2 and BxMSP3 were cloned by the methods of transcriptomic sequencing and rapid amplification of cDNA ends (RACE). In situ hybridization was used to locate the gene expressed tissue site. Nematodes were treated 48h by the dsRNA of BxMSP1, BxMSP2 and BxMSP3 respectively. Then these nematodes were fed on the *Botrytis cinea* to evaluate the reproduction ability. qPCR was used to detect the gene expressed level.

3 Results

3.1 Gene cloning of BxMSP1, BxMSP2 and BxMSP3

Genomic sequence analysis indicated that BxMSP1, BxMSP2 and BxMSP3 contained a intron respectively. Mobile-Sperm domain was contained in these MSPs. These proteins was rich of β sheets, which was highly conserved in MSP members of the nematode species.

3.2 Tissue expression site of MSPs

In situ hybridization showed that three MSPs were specifically expressed in the seminal vesicle tissue of the male adults. There is no hybridization signal in the females and larvas.

3.3 RNAi of MSPs

dsRNA of BxMSP1 and BxMSP2 significantly suppressed the reproduction ability of *B. xylophilus*, and significantly decreased the yield and the hatching rate of eggs. But dsRNA of BxMSP3 had no significantly effect on the nematode reproduction.

3.4 qPCR of gene expressed level of MSPs

qPCR indicated that dsRNA of BxMSP1 and BxMSP2 significantly decreased the expression level of BxMSP1 and BxMSP2. But the dsRNA of BxMSP3 had no significantly effect on the expression level of BxMSP3. These results indicated BxMSP1 and BxMSP2 had important roles of regulating the reproduction of *B. xylophilus*.

4 DISCUSSION

The pine wood nematode is a disastrous pathogen of the pine forests in East Asia and European. But because of limited understanding of its pathogenic mechanism, there is no efficient measures to control this nematode. In this study, BxMSP1 and BxMSP2 were required for reproduction of *B. xylophilus*. But how these genes regulate the pine wood nematode is still unknown. The interactional proteins and regulation network of BxMSP1 and BxMSP2 in the reproduction process need to be illuminated in the future. These work will help us to understand the molecular mechanism of nematode sperm development and reproduction. It is useful for screening the potential target gene for control this nematode.

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