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家蝇抗真菌肽-1的cDNA克隆及其编码蛋白序列的生物信息学分析（英文）

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cDNA Cloning and Sequence Analysis of Musca domestica Antifungal Peptide-1 (MAF-1)

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摘要 目的 克隆家蝇抗真菌肽-1(MAF-1）的cDNA序列并对其编码的蛋白序列进行生物信息学分析。 方法 根据MAF-1的N端30个氨基酸序列设计简并引物，采用RACE法和巢式PCR技术克隆MAF-1的3′端和5′端cDNA序列，获得MAF-1的cDNA序列和氨基酸序列。根据所得MAF-1成熟肽部分的cDNA序列设计引物进行RT-PCR，对序列进行验证并运用生物信息学软件进行分析。 结果 MAF-1的3′端cDNA序列长度为568 bp，开放阅读框长度为441 bp，编码蛋白序列共147个氨基酸，3′端非编码区127 bp。经NCBI中Blast比对未找到同源序列，提示该基因为一全新序列，登录到GenBank，获得登录号HM178948。该基因编码的147个氨基酸加上MAF-1的N端未用来设计引物的9个氨基酸，全长共156个氨基酸。由5′ RACE获得139 bp cDNA序列，分析所得MAF-1成熟肽的氨基酸序列与前述一致。RT-PCR结果证实了RACE所得MAF-1序列的正确性。根据生物信息学分析方法对所推导的MAF-1蛋白全长序列进行分析，其理论相对分子质量和等电点均与其实际检测值相近。利用ExPASy的各种分析工具对MAF-1分析得知，该蛋白具有信号肽，富含α螺旋，有3个α螺旋区，亚细胞定位分析其主要分布于细胞核内。利用PredictProtein分析发现，MAF?鄄1序列中有2个蛋白激酶C磷酸化位点、1个N端酰基化位点，并预测MAF-1为非球形蛋白。最后，利用ExPASy中的3D-pssm(Phyre Version0.2)模建了MAF-1的三维空间结构图。 结论 成功克隆了家蝇抗真菌肽-1（MAF-1）的cDNA序列，获知其编码的氨基酸序列和生物信息学信息。

关键词： 家蝇 抗真菌肽 RACE cDNA 生物信息学

Abstract： Objective To clone the cDNA sequence of Musca domestica antifungal peptide-1 (MAF-1) and analyze the amino acid sequence of MAF-1 by bioinformatics method. Methods Based on the primer designed according to the N-terminal amino acid sequence of MAF-1, the cDNA and amino sequence of MAF-1 were obtained by the methods of RACE and NestPCR. The accuracy of the experiment was confirmed by RT-PCR. The characteristic of the sequence was analyzed by bioinformatics software. Results The length of the cDNA sequence of MAF-1 was 568 bp by 3′ RACE, including an open reading frame (ORF) of 441 bp length and 3′ UTR of 127 bp. It was a novel sequence with the submission number of HM178948 in GenBank since none homology was found when compared with other sequences by Blast. Added with the 9 amino acids that were not used to design primer, the whole sequence of MAF-1 was 156 amino acids conferred from its cDNA. 139 bp cDNA sequence was obtained by 5′ RACE and the result was consistent to 3′ RACE. The result of RT-PCR showed the cDNA of MAF-1 mature peptide was accurate. The bioinformatics analysis deduced that the theoretic molecular weight and isoelectric point of the whole protein sequence of MAF-1 gene were similar to those detected. The ExPASy illustrated that the MAF-1 gene had a signal peptide. There were abundant α-helix in it, the domain located between the 128 and 153 amino acid residuals. Subcellular analysis showed MAF-1 was almost in the nucleus. PredictProtein found two protein kinase C phosphalation sites and one N-myri-stoylation site, and predicted that it was not a globular protein. In the end, the three dimension image of MAF-1 was set up with 3D-pssm of ExPASy. Conclusion The cDNA sequence and the amino acid sequence of MAF-1 have been obtained and analyzed successfully.

Keywords： *Musca domestica* Antifungal peptide RACE cDNA Bioinformatics

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