

论著

赖型钩端螺旋体溶血素HlyX基因的克隆、表达及其细胞毒效应

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摘要 目的: 克隆、表达HlyX基因并研究其细胞毒性效应。方法: 从赖型钩端螺旋体017株全基因组中用PCR方法扩增出目的基因, 双酶切构建重组质粒, 转化E.coliJM109, 诱导表达HlyX; 将表达的目的蛋白免疫新西兰大白兔, 制备多克隆抗体, Western blotting鉴定其免疫原性; 将目的蛋白纯化、复性后作用于ECV304细胞, 通过检测ECV304细胞乳酸脱氢酶(LDH)和一氧化氮(NO)的释放量研究目的蛋白的细胞毒性效应。结果: 扩增出1 179 bp的目的基因HlyX, 重组质粒经双酶切、PCR鉴定, 测序结果表明重组质粒构建成功。经IPTG诱导表达的融合蛋白约64 kD, 主要以包涵体的形式表达, 经免疫动物得到多克隆抗体, ELISA检测效价达1 : [KG-*2] 64 000; Western blotting鉴定在目的蛋白的位置处有特异性阳性条带。经过HlyX融合蛋白作用的ECV304细胞LDH和NO释放量明显高于对照组(P<0.01)。结论: HlyX能在大肠杆菌中表达, 表达的目的蛋白对细胞有毒性效应。

关键词 [钩端螺旋体属](#) [溶血素类](#) [细胞毒性](#); [基因,HlyX](#)

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Cloning and expression of Leptospira strain 017 hemolysin HlyX gene and its cytotoxicity

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Abstract

AIM: To clone HlyX gene and observe its expression and cytotoxicity. METHODS: The HlyX gene was amplified from Leptospira strain 017 genome by the polymerase chain reaction (PCR) and through enzyme digestion, and cloned into pET32a(+), then transformed into E.coliJM109. After induced with IPTG, the target protein was immunized to New Zealand white rabbit. Western blotting was used to identify the immunogenicity of the expressed protein. The purified and renatured protein was acted on ECV304 cells to detect its cytotoxicity by examining the release of LDH and NO from the cells. RESULTS: The full length of the HlyX gene about 1 179 bp was obtained by PCR. The recombinant plasmid was identified by enzyme digestion, PCR and DNA sequencing. After induced with IPTG, the expressed protein existed in the form of inclusion bodies about 64 kD, which was consistent with the expected size of the fused protein. After immunity, the titre of the multiclonal antibody reached 1 : [KG-*2] 64 000 by ELISA. Western blotting analysis found a positive band specifically in the target protein position. The release of the LDH and NO in the ECV304 cells treated with HlyX fusion protein showed significant increase compared with the control group (P<0.01). CONCLUSION: HlyX gene is expressed successfully in E.coli JM109, and the expressed products shows cytotoxicity.

Key words [Leptospira](#) [Hemolysins](#) [Cytotoxicity](#) [Genes](#) [HlyX](#)

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