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研究报告

猪IFNα基因在毕赤酵母中的高效分泌表达High Level Secretion Expression of PoIFNα in Pichia pastoris

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摘要

巴斯德毕赤酵母载体质粒pPICZαA含有强启动子PAOX1和α-MF信号肽序列,构建猪IFNα基因的重组质粒pPICZαA-IFNα，并转入E.coli JM109中，得到转猪IFNα基因工程菌，经酶切鉴定克隆到载体pPICZαA上的外源基因即为猪IFNα基因。通过电击将经Sac Ⅰ酶切后线性化的pPICZαA-IFNα质粒转化到巴斯德毕赤酵母KM71中。SDS-PAGE和Western blot鉴定表达产物的结果表明，分泌于胞外的猪IFNα蛋白分子量比猪IFNα理论值分子量稍大，估计是糖基化的原因。表达的蛋白可发生正确的抗原-抗体反应，表达量为 0.45 mg/mL。将蛋白表达上清经细胞毒性实验检测表达产物的抗病毒活性为2.1×104 IU/mL。Abstract: The porcine alpha interferon gene was inserted into the Pichia pastoris expression vector of pPICZαA which contains AOXⅠpromoter and α-factor signal sequence.The recombinant plasmid was transformed into host cell E.coli JM109 and then was extracted for analysis of restriction enzymes.It was confirmed that heterogeneous gene spliced into vector pPICZαA was IFNα gene. The recombinant plasmid of pPICZαA-IFNα was linearnized by Sac Ⅰ and transformed into KM71 by electroporation. SDS-PAGE and Western blot analysis showed that IFNα product was observed in the supernants with a little larger molecular weight size than the natural IFNα.The rIFN gene has the same antigenicity as natural one.The expressed rIFN accumulated up to about 0.45mg/mL.The cytokine activity of the supernants was vertified by WISH/VSV system,which is about 2.1×104IU/mL.

关键词 猪IFNα 巴斯德毕赤酵母 基因重组 分泌表达 Key words Po IFNα Pichia pastoris gene recombination secretory expression

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Abstract

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