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研究报告

青鱼生长激素的重组表达及其多克隆抗体的制备

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摘要 以含有的青鱼生长激素编码区cDNA的重组质粒pbcGHc为模板，高保真PCR扩增青鱼生长激素（GH）成熟肽cDNA序列，定向插入原核表达载体pET-28a，构建青鱼GH原核表达质粒pET-bcGH。将pET-bcGH转化大肠杆菌BL21(DE3)，IPTG诱导青鱼GH基因在大肠杆菌中的融合表达，SDS-PAGE凝胶电泳结果显示一条23 kDa的诱导表达重组青鱼GH带。以草鱼GH多克隆抗体为一抗，Western Blot证明，该重组青鱼GH具有免疫学活性。将经过亲和层析、透析纯化后的重组青鱼GH作为抗原，采用改进的方法对家兔进行皮下免疫注射，获得青鱼GH多克隆抗血清。以该多抗为一抗，Western Blot 可以检测出4 ng的抗原量；并且在青鱼垂体组织抽提液中和血清中检测到一种能与该抗血清作用的大小为21 kDa的蛋白质。这些结果表明本研究得到的青鱼GH多克隆抗血清具有较好的免疫特性。

关键词 青鱼；生长激素；重组表达；多克隆抗体

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In Vitro Expression and Antibody Preparation of Black Carp (Mylopharyngodon piceus) GH

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Abstract

The cDNA fragment encoding the mature polypeptide of growth hormone (GH) for the black carp (Mylopharyngodon piceus) was PCR amplified and subcloned into pET-28a. The recombinant expression plasmid pET-bcGH was transformed into E.coli BL21(DE3) and fusion polypeptide containing a 6xHis-tag at the N-terminus was expressed after IPTG induction. The fusion protein band of 23 kDa or so showed immunoreactivity to the polyclonal antibody aganist grass carp GH. The recombinant GH for black carp was purified by affinity chromatography and dialysis. Using the fusion protein as an antigen, through the modified immunization method, the polyclonal antiserum to black carp GH was obtained. Immunochemistry results showed that the antiserum could detect the antigen as low as 4ng. The protein of 21 kDa in black carp pituitary protein extracts and blood serum could be detected by western blot analysis in which polyclonal antiserum to black carp GH was used as the primary antibody. All these results showed that the polyclonal antiserum against black carp GH was not only effective but also highly specific.

Key words black carp growth hormone; recombinant expression; polyclonal antibody

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