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日本血吸虫两种酪氨酸酶的克隆、表达和转录特异性分析

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Cloning, Expression and Transcription Specificity Analysis of Two Tyrosinases from Schistosoma japonicum

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摘要 参考文献 相关文章

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摘要 目的 克隆、表达日本血吸虫2种酪氨酸酶SjTYR1和SjTYR2的全长基因，并研究这2种基因在日本血吸虫不同性别和不同发育阶段的转录特异性。 方法 设计特异性引物，从日本血吸虫成虫cDNA文库中扩增出SjTYR1和SjTYR2的全长片段，并克隆入原核表达载体pSJ2，验证正确的重组质粒转化大肠埃希菌（E. coli）Rosetta Gami细胞，使用异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达，并用组氨酸标签对表达产物进行亲和层析纯化。将纯化后获得的重组蛋白SjTYR1和SjTYR2分别免疫新西兰白兔制备免疫兔血清。分别以重组蛋白免疫兔血清和日本血吸虫感染兔血清为一抗，用蛋白质印迹（Western blotting）分析重组蛋白的免疫原性和免疫反应性；以重组蛋白免疫兔血清为一抗，用Western blotting观察其与日本血吸虫虫体蛋白的反应情况。制备感染后14、16、18、20、22、24、26和28 d雌虫和雄虫的总RNA，利用RT-PCR分析SjTYR1和SjTYR2基因在日本血吸虫不同性别及不同发育时间的转录水平的差异。 结果 构建了重组原核表达载体SjTYR1/pSJ2和SjTYR2/pSJ2，诱导后获得包涵体形式表达的重组蛋白，相对分子质量（Mr）分别为55 000和56 800，与预测的融合蛋白相对分子质量相符。纯化后的重组蛋白SjTYR1可被日本血吸虫感染兔血清识别，而SjTYR2则不能。rSjTYR1免疫兔血清与虫体蛋白反应后，可见一条约Mr 100 000的条带，rSjTYR2免疫兔血清不与虫体蛋白反应。在雄虫体内，2种酪氨酸酶均不转录；在雌虫体内，2种酪氨酸酶在感染后24 d内不转录，自24 d开始出现激增，之后逐渐提高，感染后28 d达到相对最大值。 结论 构建了SjTYR1和SjTYR2的重组质粒并表达，2种重组蛋白均具有免疫原性和免疫反应性。SjTYR1和SjTYR2在血吸虫雌虫中特异表达，且在感染终宿主后24～28 d转录水平升高。

关键词： 日本血吸虫 酪氨酸酶 克隆 表达 转录特异性

Abstract： Objective To clone and express the recombinant proteins based on the whole open reading frame of two tyrosinases（tyrosinase 1 and tyrosinase 2） from Schistosoma japonicum, and study the transcription specificity of the two tyrosinases in different sex and developmental stages of S. japonicum. Methods The full-length of SjTYR1 and SjTYR2 were amplified with specific primers and subcloned into pSJ2. The recombinant plasmids were transformed into E. coli Rosetta Gami strains and induced with IPTG for expression. The recombinant proteins were purified by Ni-NTA agarose. The recombinant proteins SjTYR1 and SjTYR2 were used to produce the specific antibodies by immunizing the rabbits. The immunogenicity of the recombinant proteins SjTYR1 and SjTYR2 were detected by Western blotting using sera of recombinant proteins-immunized rabbits and S. japonicum-infected rabbit serum as the primary antibody, respectively. The reactivity of sera from recombinant proteins-immunized rabbits was analyzed by Western blotting against the native protein of S. japonicum worm. Total RNA was extracted from 14, 16, 18, 20, 22, 24, 26, and 28-day male and female worms. Transcription levels of the two tyrosinases in different sex and different stage were determined via RT-PCR method. Results The expression vector of SjTYR1/pSJ2 and SjTYR2/pSJ2 were constructed and the recombinant proteins SjTYR1 and SjTYR2 were expressed in inclusion body in E. coli（about Mr 55 000 and Mr 56 800）. The sera of S. japonicum-infected rabbits reacted positively with the purified recombinant protein SjTYR1, but not with recombinant protein SjTYR2. The native protein of S. japonicum worm could be recognized by sera of rSjTYR1-immunized rabbits （Mr 100 000）, but not by sera of rSjTYR2-immunized rabbits. Transcription levels of the two tyrosinases in male worms were nearly zero. In female worms, the transcription levels of the two tyrosinases increased sharply from the 24th day post-infection and reached maximum on the 28th day. Conclusion The recombinant proteins of SjTYR1 and SjTYR2 show immunogenicity and immunoreactivity. SjTYR1 and SjTYR2 are both expressed specifically in female worms