A B C D α β γ δ  

1 2  *  2  2  2  2  2  2

1.2.1 pBV/cpa408  1:50  Amp + LB  30°C  3 - 5h

1.2.2  3.0mL pH8.0 TE  80%  80%  pH7.4 PBS 200cm²

1.2.3  cpa408  1.0 mg/kg  18g  8  18g  1/2

1.2.4 IgG  ELISA
攻毒实验:取静止厌氧培养周的"型产气荚膜梭菌菌株浓缩培养上清,分不同剂量腔注射昆明小鼠,筛选确定最小致死剂量。以腹腔注射次免疫周后昆明小鼠,观察免疫保护效果。

结果
诱导表达蛋白的纯化分析显示,诱导表达菌体超声破碎上清经饱和硫酸铵沉淀后得到了浓缩(图4),沉淀物经透析后,进一步以凝胶过滤层析方法有效地纯化到了目的蛋白(图7)。经薄层凝胶扫描分析,纯化到的蛋白纯度达以上(图7)。图4饱和硫酸铵沉淀后上清及沉淀鉴定

用制备好的表达目的蛋白免疫昆明小鼠后,以倍的"型产气荚膜梭菌浓缩培养上清腹腔攻击,被免疫小鼠获得了的保护。文献报道,[毒素中第、位上的组氨酸对其具有活性至关重要,位或位组氨酸残基被其它氨基酸残基取代,即可丧失全部的溶血性和致死性。文献报道,[毒素中第位上的氨基酸被其它氨基酸残基取代,就可丧失!毒素的溶血活性。本研究所克隆的基因去掉了在!毒素中起关键作用的位氨基酸残基,从理论上讲,该克隆基因的表达产物就可丧失!毒素本身的溶血性和致死性。昆明小鼠因表达产物主动免疫保护实验结果证明,!基因表达产物无!毒素本身的致死性且具有良好的免疫原性,其免疫昆明小鼠后产生的抗体效价值在第周时就达,到第周时达。腹腔攻击后,被免疫小鼠获的保护,揭示所产的抗体具有良好的免疫保护作用,说明!基因可作为保护性抗原的候选基因,其表达产物为保护性抗原。
Preparation of Alpha-toxin's Protective Antigen of Clostridium perfringens Type A and Research for Its Primary Immunological Protective Function

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Abstract Induced by 42℃ the recombinant engineering bacterial pBV/cpa408 was highly expressed. After having been pelletted by 80% NH4SO4 and dialysised the expressed protein was isolated and purified by the gel filtration chromatography. Then according to an amount of 1.0mg/kg the Kunming Mice body weighted 18g were immuned with the purified protein by intraperitoneal inoculation. One week after the first enhanced immunization the Kunming Mice were attacked with an amount of 1.0MLD alpha-toxin in which the eight mice immuned all survive and the control group all died. During the period of immunization the titre of the mouse’s serum antibody was measured by ELISA. One week after the first immunization the titre of the mice’s serum antibody was 1:800 but that of one week after the first enhanced immunization reached to 1:6400.

Key words alpha-toxin protective antigen preparation immunological protective function