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鉴定痢疾杆菌毒力基因的SW480细胞模型构建 Construction of SW480 Cell Model Identifying Shigella Virulent Genes

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摘要

信号标签诱变技术（STM）是一种在体内高通量筛选病原体毒力基因的新方法，在应用时的一个先决条件是要建立合适的体内筛选系统。为将该技术应用于福氏痢疾杆菌，我们使用三个福氏痢疾杆菌菌株进行了预试验：通过同源重组构建而成的带有氯霉素抗性且aroA和virG基因失活的突变株RC426；因在侵袭质粒上自发缺失3个基因座(ipaBCDA, invA 和 virG)的另一减毒突变株T32，其曾被用作福氏痢疾杆菌的口服疫苗；还有具侵袭宿主细胞能力的野生性菌株2457T。将RC426、T32和2457T混合后侵袭结肠细胞系SW480，不同时间回收经侵袭后细胞裂解液中的菌体并统计。结果显示在侵袭12h内回收到减毒突变株的量与野生有毒株存在显著性差异，表明SW480 细胞系可用于痢疾杆菌的STM研究。Abstract: Signature-tagged mutagenesis (STM) is a novel technology with high throughput screening ability to identify virulent genes of pathogen in vivo. An appropriate animal or cell line model is one of prerequisites by exploiting this technique. In order to apply STM to Shigella flexneri, RC426 was constructed as an attenuated mutant with chloramphenicol resistance and aroA and virG genes inactivated by homologous recombination; Another attenuated strain T32 was used as an oral S. flexneri 2a vaccine due to a spontaneous deletion in three loci (ipaBCDA, invA and virG) on the virulence plasmid. The wild type strain 2457T had the invasion ability into host cells. The three strains, RC426, T32 and 2457T, were mixed together to invade colon cancer cell line SW480, and the distinct strains were recovered and counted from cell lysates of invaded SW480 in different time. The results showed that there were statistically significant differences between the amounts of two attenuated strains recovered and that of virulent strain within 12h invasion, indicating SW480 was a suitable cell model for applying STM to screen virulent genes of Shigella flexneri.

关键词 SW480 细胞模型 痢疾杆菌 信号标签诱变技术 构建 Key words SW480 Cell model Shigella spp. Signature-tagged mutagenesis Construction

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