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大肠杆菌噬菌体 T4 基因42和43 DNA片段无性繁殖系的鉴定和分析

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摘要　含泡嘧啶的T4DNA 用核酸内切酶Eco RI、SalI、Hind III切割，特别是用Eco RI不完全切割，所得片段以质粒pBR 322作载体，在大肠杆菌E.coli KH802菌株中进行了无性繁殖。5，000个无性繁殖系经标记获求地作遗传鉴定，筛选基因42和43的无性繁殖系，并对其所带T4DNA片段的图位和有关基因的功能表达进行了初步分析。试验证明，10个无性繁殖系带有基因43片段，其中CC-20还带有全部imm基因及基因42；基因43内B22和B263突变位点之间有一个EcoRI酶切位点，基因42或imm内也有一个酶切位点，无性繁殖系CC-20的N55突变位点表现为野生型，这一突变点的回复突变率为10-6-10-10；CC-20的无细胞发酵过滤液对amN55和amN122亦有互补作用，表明过滤液中有功能性的基因42产物脱氧胞嘧淀羟甲基化酶的存在；从杂种质粒DNA长度及已知pBR322的长度推算，CC-20的T4DNA片段约为6kb，肯定能包括基因42全部。CC-20所带的基因imm亦明显的表现抗T4野生型和无意义突变体的侵染功能，这将可能为发酵工业中噬菌体的防治开辟新的途径。

关键词

分类号

Identification and Analysis of Clones Carrying Bacteriophage T4 Gene 42 and 43 D NA Fragments

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Abstract

Cytidine containing T4 DNA was cleaved by endonucleases EcoRI,SalI,and HindIII,especially by partial digestion of EcoRI,and DNA fragments were cloned in Escherichia coli KH 802,using the plasmid pBR322 as vector.About 5,000 clones were identified by marker rescue spot test to select clones carrying gene 42 and 43 fragments,whose mapping position and relevant gene expression were analysed.Ten clones were identified to carry gene 43 fragments,among which CC-20 also carries complete gene 42 and immunity gene (imm).Two EcoRI cleavage sites were identified,one between amber sites B22 and B263 in gene 43,the other in gene 42 or imm.The amber site N55 in clone CC-20 apparently reverted to wild,since it complemented the amber mutant N55,with the production of wild T4.The rate of this reversion was proved to be 10-6—10-10.The fact that the cell free filtrate of the liquid culture of CC-20 specifically complemented amN55 and amN122 suggested the existence of the functional enzyme,deoxycytidylate hydroxymethylase,the product of gene 42,in the filtrate.The estimated length (～6kb) of the T4 DNA fragment in CC-20 by subtraction of the known length of pBR322 from the measured contour length of the hybrid plasmid,together with the production of the functional enzyme provided the evidence that gene 42 in CC-20 was complete.The immunity function in CC-20 was clearly shown by its invariable resistance against infection by wild T4 as well as its amber mutants.This provided a new approach for phage control by plasmid carrying immunity gene in industrial fermentation.

Key words

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