遗传学报　1984 11 (5): 339-347　ISSN: 0379-4172　CN: 11-5450/R

枯草芽孢杆菌中的分子克隆和单体杂种质粒转化的研究

陈乃用，郭三堆，印小明

中国科学院微生物研究所，北京

收稿日期　修回日期　网络版发布日期　接受日期

摘要　枯草芽孢杆菌感受态细胞的质粒转化与质粒的分子构型有关。用琼脂糖凝胶电泳法分离纯化 的pUB110质粒DNA单体是很少或没有转化活性的。在pUB110质粒DNA上插入一段受体菌染色体 DNA，这种杂种质粒的单体可以转化感受态细胞，但受体菌必须是重组型的。转化效率和插 入片段的大小有关，片段愈大转化活性愈高，片段小于0.6kb，就和pUB110 DNA单体一样， 几乎没有转化活性。在pUB110质粒DNA上插入一段解淀粉芽孢杆菌染色体DNA，这种杂种质粒 的单体转化效率都很低。本文还讨论了用鸟枪法在枯草芽孢杆菌中进行分子克隆的一些问题。

关键词

分类号

Molecular Cloning and Monomeric Hybrid Plasmid Transformation in Bacillus subtilis

Chen Naiyong, Guo Sandui, Yin Xiaoming

Institute of Microbiology,Academia Sinica,Beijing

Abstract

The transformability of plasmid DNA in B.subtilis competent cell depends on its molecular structure.Monomeric plasmid DNA of pUB110 isolated from agarose gel el ectrophoresis is found to be inactive in transformation,but both monomeric plasm id DNA and unfractionated plasmid DNA preparations could transform B.subtilis pr otoplasts.

When an enough length of DNA fragment homologous to the recipient chromosoma l DNA was inserted into the plasmid molecule,the monomeric form of such hybrid p lasmieds became active in transformation,provided the recipient cells were recom bination proficient.

A series of hybrid plasmids consisting of pUB110 with an insert of B.subtili s BR151 chromosomal DNA were constructed.The specific activity of the monomeric hybrid plasmids increased with the size (~1.2—3.5 kb) of the inserted chromosom al DNA fragments,but when the inserted fragment was smaller than 0.8kb,the trans forming activity markedly decreased,and when the cloned fragment was smaller tha n 0.6 kb activity markedly decreased,and when the cloned fragment was smaller th an 0.6 kb there was nearly no activity.

We also constructed a series of hybrid plasmids consisting pUB110 with an in sert of B.amyloliquefaciens chromosomal DNA of (~0.5—4.6 kb).The transforming a ctivity of all these hybrid plasmids so constructed was very low.

In shotgun cloning experiment,homologous chromosomal DNA fragments were used as donor DNA,pUB110 as cloning vector.Plasmid DNA's were extracted from 387 Neo R clones transformed.Fifty nine plasmids,which were larger than pUB110 as judged by agarose gel electrophoresis,represented 15.2% of transformants and small fra gments were preferentially cloned.The cloned fragments of 72.9% of the hybrid pl asmids were smaller than 0.6 kb .The main problem in shotgun cloning in competen t B.subtilis seems to be the difficulty in cloning large fragments of DNA and th e requirement for oligomeric DNA in plasmid transformation.

Key words

DOI:

扩展功能

本文信息

Supporting info

PDF(982KB)

[HTML全文](0KB)

参考文献

服务与反馈

把本文推荐给朋友

加入我的书架

加入引用管理器

复制索引

Email Alert

文章反馈

浏览反馈信息

相关信息

本刊中 无 相关文章

本文作者相关文章

· 陈乃用

· 郭三堆

· 印小明

通讯作者