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STABILITY OF RECOMBINANT *ESCHERICHIA COLI* IN CONTINUOUS CULTURE

Introduction

A wide spectrum of different theoretical and practical problems can be solved using plasmids. This explains the fact that the regulation of plasmid replication and of the expression of plasmid-coded genes has attracted a great deal of attention. Instability of plasmids during the cultivation of the recipient cells has been observed by several authors (Jones et al., 1980; Aiba and Koizumi, 1984; Dwivedi et al., 1982 etc.) The most popular model explaining instability of plasmids during the cultivation is the segregation model (Imanaka and Aiba, 1981), further developed by several authors (Ollis, 1982; Ollis and Chang, 1982; Seo and Bailey, 1985). Random segregation of plasmid DNA between daughter cells during the cell division and slower growth rate of plasmid-containing cells in comparison with the plasmid-free cells are regarded as reasons of plasmid instability in this model. Data presented in this paper are not explainable within the limits of the segregation model but indicate that the disappearance of the plasmid markers is connected with the regulation of the expression of plasmid-coded genes.

Materials and methods

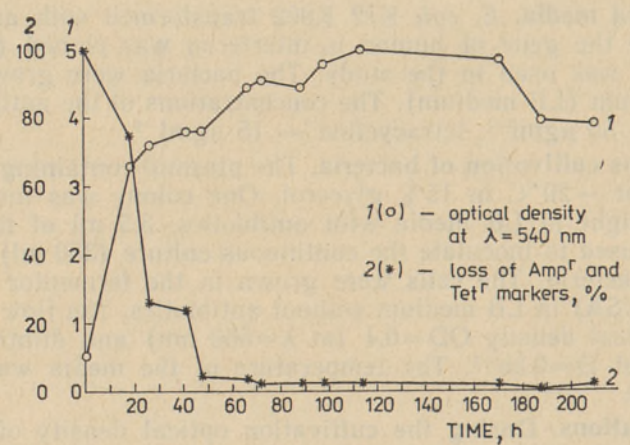
Strain and media. *E. coli* K12 K802 transformed with an expression vector where the gene of human α_3-interferon was cloned (Овчинников et al., 1982) was used in the study. The bacteria were grown in Luria-Bertani medium (LB medium). The concentrations of the antibiotics were: ampicillin — 50 $\mu g ml^{-1}$, tetracycline — 15 $\mu g ml^{-1}$.

Continuous cultivation of bacteria. The plasmid-containing culture was maintained at −20°C in 15% glycerol. One colony was inoculated and grown overnight in LB media with antibiotics. 3.5 ml of the overnight culture was used to inoculate the continuous culture (350 ml) of plasmid-containing bacteria. The cells were grown in the fermentor C-30 (New-Brunswick, USA) in LB medium without antibiotics. The flow was switched in at optical density OD = 0.4 (at $\lambda = 560$ nm) and dilution rate was maintained at $D = 0.5 h^{-1}$. The temperature of the media was 37 C° and $pH = 7.0 \pm 0.2$.

Determinations. During the cultivation optical density of the culture was determined and the outplatings of the culture on the LB solid media with and without antibiotics were regularly carried out. The method of Holmes and Quigley (1981) was used to isolate plasmid DNA. Restriction analysis and electrophoresis of DNA on agarose were carried out as described by Maniatis et al. (1982).

Results and discussion

The growth curve of bacterial culture and relative amount of Amp^r and Tet^r cells are shown in Fig. 1. The number of antibiotic-resistant cells declines rapidly from initial 100% to final 2% during the 70 h of cultivation. According to the optical density data, steady state of the growth was not achieved during this period. The remaining 2% resistance was maintained during the next 100 h of cultivation. The same pattern was observed in all the experiments carried out. Electrophoresis of DNA on agarose from resistant and nonresistant colonies showed the presence of plasmids in all the investigated colonies with approximately the same copy number. No large-scale changes of plasmid structure were observed using the restriction mapping. The data obtained demonstrated that the resistance to antibiotics disappeared quickly after the selective pressure was removed. Fast decrease of the markers observed is in good agreement with the segregation model of Imanaka and Aiba. However, the observed basal 2% resistance to the antibiotics which did not change during 100 h of cultivation is not in agreement with the segregation model. In conformity with the latter, the result will be invariably the complete disappearance of the cells resistant to the antibiotics according to the exponential law in case $p>0$ and $\Delta\mu>0$. It should be added that almost similar behaviour of plasmid-dependent markers, which was observed by us, was also observed in the case of TOL-plasmid during continuous cultivation of *Pseudomonas putida* PPK1 (Keshavarz et al., 1984). In the latter case the final 1% catabolic activity was maintained during about 500 h of cultivation, but the loss of markers was concomitant with the loss of plasmid DNA. Our data indicate that the instability of recombinant cultures may be caused not only by elimination of plasmids or due to the occurrence of mutations in them but may also be the result of the mechanisms on protein level. For example, plasmid-coded proteins are mostly foreign to the cells, therefore the bacterial proteases may also cause the loss of plasmid markers. In the case of heat-shock proteases degradation of recombinant proteins has already been shown (Goff and Goldberg, 1985).



Growth of recombinant *Escherichia coli* K12 K802 in continuous culture on Luria-Bertani medium without antibiotics. At the moment time = 0, flow (dilution rate $D=0.5h^{-1}$) was started. The inoculum was grown under antibiotic pressure.

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REKOMBINANTSE *ESCHERICHIA COLI* GENEETILINE STABIILSUS PIDEVAL KULTIVEERIMISEL

On uuritud rekombinantse bakteri *E. coli* K12 K802 kasvu mitteselektiivsel rikkal (Luria-Bertani) söötmel ning analüüsitud eksperimendis täheldatud ekspressioonivektori (plasmiidi pBR 322 tuletis kloneeritud inimese α_3 interferooni geeniga) poolt kodeeritud markerite ebastabiilsuse ja nn. jääkresistentsuse võimalikke põhjusi. Katse tulemusi on seletatud vastavate matemaatiliste mudelite abil.

Катрин ТОМСОН, Райво ВИЛУ

ИЗУЧЕНИЕ ГЕНЕТИЧЕСКОЙ СТАБИЛЬНОСТИ РЕКОМБИНАТНОГО ШТАММА *ESCHERICHIA COLI* ПРИ НЕПРЕРЫВНОМ КУЛЬТИВИРОВАНИИ

Изучен рост рекомбинантного штамма *E. coli* в неселективных условиях в богатой (Luria—Bertani) среде. Показано, что основная часть клеток быстро (50 поколений) теряет маркеры, кодируемые генами вектора экспрессии (дериват плазмиды pBR 322, содержащий ген человеческого интерферона), однако после этого остается т. н. остаточная резистентность к антибиотикам (3—5% клеток), которая сохраняется в течение 300—500 поколений. Обсуждаются возможные клеточные механизмы обнаруженных эффектов. Экспериментальные данные сопоставлены с результатами математического моделирования.