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COTRANSFORMATION OF LINEAR CHROMOSOMAL DNA AND PLASMID DNA IN *ESCHERICHIA COLI*

H.E.N. BERGMANS, D.H. KOOIJMAN and W.P.M. HOEKSTRA

Department of Molecular Cell Biology, State University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

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1. Introduction

Transformation of Ca^{2+}-heat shocked *Escherichia coli* cells with plasmid DNA has become an indispensable technique in the study of plasmids and in genetic engineering. The process of induction of competence by Ca^{2+}-heat shock is still poorly understood [1,2], but it has been argued [3,4] that only a small proportion of the shocked cells is effectively rendered competent in the process. This has been deduced from the fact that in transformation with two different plasmids as donor DNA, the observed frequency of double transformants is higher than would be expected from the observed transformation frequencies of each of the plasmids separately.

This phenomenon has been used for construction by transformation of strains carrying a plasmid for which direct selection is not possible [3]. Direct screening for transformants that have received the non-selectable plasmid is usually not feasible due to low transformation frequencies (about 10^{-3} plasmid transformants per viable cell). When transformation is performed with excess DNA of the non-selectable plasmid in the presence of non-saturating concentrations of a selectable one, selection for the latter yields a population of transformants with a high proportion of clones that have also received the non-selectable plasmid.

We wondered whether the same phenomenon would occur in transformation of *E. coli* with linear chromosomal DNA together with circular plasmid DNA. If so, it would imply that plasmid DNA and chromosomal DNA are preferentially taken up by the same subpopulation of competent cells. When applied, the method would have the advantage that at no time do two, possibly interfering, plasmids have to be present in the same cell. The practical drawback that only *recB recC sbcA/B* strains are transformable with chromosomal DNA [5,6] can be circumvented since we have shown recently [7] that transformation of Rec^+ strains with chromosomal DNA is possible, albeit at low frequencies.

2. Materials and Methods

2.1. Bacterial strains

All strains are *E. coli* K12 derivatives. AM1268 is a Rec^+Sbc^+ derivative of the transformable strain AM1095 (*recB21 recC22 Sbc15 leu*) [8].

2.2. DNA isolation

Chromosomal DNA was isolated from a prototrophic strain according to the method of Cosloy and Oishi [6]. A slightly modified cleared lysate method [9] was used to isolate pBR322 and ColE1 DNA. The quick screening method of Birnboim and Doly [10] was used to check plasmid content of individual transformant colonies. Agarose slab gel electrophoresis was performed on 0.6% agarose gels [11].

2.3. Transformation procedures

The procedure is a modification of one previously reported [7]. AM1268 was grown in phosphate-buffered minimal salts medium [12] till the absorbance

reached a reading of 30 in a Klett photometer (660 nm filter). The culture was chilled and all subsequent operations were at 0°C unless otherwise indicated. Cells were collected by centrifugation, washed with 10 mM NaCl and resuspended in 0.05 vol. 20 mM HEPES (*N*-2-hydroxyethyl-*N'*-ethane sulfonic acid) buffer pH 6.0. 0.3 ml of this suspension was mixed carefully with DNA and with $CaCl_2$ and $MgCl_2$ to a final concentration of 30 mM and 26 mM, respectively, in a final volume of 0.5 ml. After 10 min incubation at 0°C the mixture was heated at 42°C (6 min), chilled to 0°C and further incubated at 0°C during 30 min. The total mixture was then diluted 1 : 10 into a minimal salts medium buffered with $1.2 \cdot 10^{-1}$ M morpholinopropane sulfonic acid (MOPS) buffer pH 7.0, containing all growth requirements and 10^{-4} M phosphate, and incubated at 37°C under aeration during 90 min, to allow expression of plasmid functions.

Leu^+ transformants were selected on solid minimal salts medium, buffered with $1.2 \cdot 10^{-1}$ M Tris–HCl pH 7.0, supplemented with all growth requirements minus leucine, and 10^{-4} M phosphate. Selection for antibiotic resistance was performed on complete medium containing 80 μg/ml penicillin (Gist-Brocades, Delft, The Netherlands) or 20 μg/ml tetracycline (Sigma Co.).

Colicin production of single colonies was tested after UV (254 nm) irradiation (30 J/m^2) of the colonies after 6 h of growth at 37°C on complete medium. After another 14 h of growth, the cells were killed by chloroform vapour and 5 ml of an early logarithmic culture of AM1268 in soft agar was spread on the plates. Zones of inhibited growth could be seen around the colicin producing colonies after another 8 h incubation at 37°C.

3. Results

In initial experiments the cotransformation frequency of a selectable plasmid (pBR322, carrying Ap^r and Tc^r determinants) and linear chromosomal DNA was studied. Primary selection was made for transformants that had received a chromosomal marker, thus mimicking the situation in which no direct selection for plasmid transformants is feasible. The selectable properties of the plasmid then allow an easy secondary screening of plasmid transformants.

TABLE 1

Comparison of the frequency of plasmid transformants among the total population of viable cells and among transformants for a chromosomal marker (Leu^+).
AM1268 (Rec^+ *leu*) was transformed with 3.2 μg/ml prototrophic chromosomal DNA together with plasmid DNA in the concentrations indicated.

Plasmid	DNA conc. (μg/ml)	Frequencies	
		Plasmid transformants viable cells	Plasmid transformants Leu^+ transformants
pBR322	27.5	$5.5 \cdot 10^{-4}$	$9.0 \cdot 10^{-2}$ (8/89)
pBR322	55.0	$1.1 \cdot 10^{-3}$	$1.0 \cdot 10^{-1}$ (12/120)
ColE1	54.2	$2.6 \cdot 10^{-4}$	$5.3 \cdot 10^{-2}$ (5/95)

AM1268 (Rec^+ *leu*) was transformed with pBR322 DNA at high concentrations (Table 1) well above the saturating concentration (cf. Fig. 1). Chromosomal DNA was added at a concentration of 3.2 μg/ml, which in our procedure is not saturating (Fig. 1), but still yields a reasonable number of transformants. The frequency of plasmid transformants in the total population of viable cells was assessed by plating on complete medium with or without penicillin. Leu^+ transformants, selected on Tris-based minimal medium minus leucine, were screened for the presence of pBR322 by streaking for single colonies on complete medium with penicillin. Some transformants yielded large numbers of penicillin-resistant colonies, which

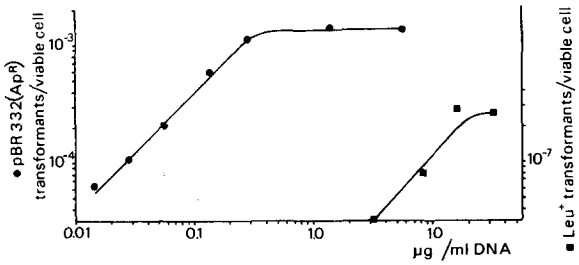

Fig. 1. Frequency of transformants of AM1268 (Rec^+ *leu*) transformed with plasmid (pBR322) DNA (selection Ap^r) or chromosomal DNA (selection Leu^+).

upon further testing turned out to be Leu^+ and simultaneously resistant against penicillin and tetracycline. Most of the other transformants yielded a few penicillin-resistant colonies; these were however Leu^-. Apparently in those cases some background (Leu^-) colonies that had only been transformed with plasmid DNA were picked up together with the Leu^+ colonies.

Some colonies of the first and second type were inoculated directly from the medium selective for chromosomal transformants onto plates of the same composition. Cells collected from these cultures were tested for plasmid content by the method of Birnboim and Doly [10]. Colonies of the first type contained a plasmid with the same mobility as pBR322 in agarose slab gel electrophoresis, the second type contained no plasmid. This shows that it is possible to identify plasmid transformants without direct selection for plasmid properties. Table 1 summarizes the results of these experiments.

There is a difference of two orders of magnitude between the frequency of plasmid transformants among chromosomal transformants compared to their frequency in the total cell population.

A similar experiment was performed with the non-selectable plasmid ColE1. Table 1 summarizes the conditions of this experiment and the results. In this case plasmid transformants were screened by testing colicin production after UV-induction of single colonies on complete medium. About 12 000 colonies from the total cell population, as well as streaks for single colonies from the Leu^+ transformants were tested in this way. Of 5 Leu^+ transformants all single colonies produced colicin, the other Leu^+ transformants did not show any colicin activity. Again a difference of two orders of magnitude was observed between the frequency of plasmid transformants among Leu^+ transformants and among the total cell population.

Plasmid isolation [10] was performed on cultures of the 5 colicin-producing Leu^+ transformants and on cultures of some of the non-producing Leu^+ transformants. Colicin producing transformants contained a plasmid with the same mobility as ColE1 in agarose slab gel electrophoresis. The non-producing colonies contained no detectable plasmid.

4. Discussion

The induction of competence in *E. coli* by heat shock and divalent cations is poorly understood. Recently an interesting model has been presented [1], in which it is assumed that induction of competence is equivalent with the generation of membrane disturbances (viz. "holes" or "pores") by Ca^{2+}, through which a Ca^{2+}-DNA complex can enter the cell. Uptake of linear chromosomal DNA would not be expected to be fundamentally different from uptake of circular plasmid DNA, although a supercoiled molecule could need a larger "hole" for entrance than a linear molecule entering in a linear fashion.

The cells that are effectively rendered competent are only a minor fraction of the viable cells in the population: from the transformation frequency of plasmid DNA at saturating DNA concentrations a maximal level of competence of 0.1% can be inferred, while we have argued [2] that for *recB recC sbcB* strains transformed with linear chromosomal DNA at most 1% of the cells are competent. The results presented here show that the same cells that are transformed by chromosomal DNA are preferentially transformed by plasmid DNA. Clearly there is at least an overlap between the cells competent for transformation with either kind of DNA. This preferential transformability must originate in the ability to take up DNA successfully, as the processes after uptake will be different for either type of DNA: plasmid DNA should settle as autonomously replicating DNA, while chromosomal DNA should integrate into the chromosome by recombination.

Our experiments show that double transformation can be used effectively to minimize the screening needed to find a transformant with plasmid DNA carrying no readily selectable properties. The fast screening methods available make the screening of at least 100 potential transformant colonies per day feasible. In our experiments this would have been sufficient to identify at least 5 transformed strains.

Our method of double transformation has some advantages over the method with two plasmids [3]. In the latter method the selectable and non-selectable plasmids have to co-exist for a large number of generations in the same cell. If the two plasmids are not compatible the non-selective plasmid might be lost during this period from a substantial part of the

cells in the transformant colony. Moreover special precautions have to be taken to remove the selectable plasmid out of the double transformant after the primary selection. Neither of these problems apply to our method. Our method has the disadvantage that the recipient strain should be a sufficiently transformable Rec^+ strain, with some auxotrophic marker that reverts at a sufficiently low frequency to allow transformation. Alternatively a *recB recC sbcA/B* strain may be used, although these strains cannot harbour all plasmids stably (e.g. ColE1, which is incompatible with *sbcB* [13]).

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