

# Evidence for Uptake of Plasmid DNA into Intact Plants (*Lemna perpusilla*) Proved by an *E. coli* Transformation Assay

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DNA Uptake, *Lemna perpusilla*, Plasmids

The water plant *Lemna perpusilla* has been incubated with the *E. coli* plasmids pMB9 and pBR325, respectively. Uptake of plasmids has been shown by subsequent transformation of *E. coli* cells to tetracycline resistance after treatment with *Lemna* DNA from plasmid-incubated plants. In 7 out of 15 assays we found stable transformants. From the transformation rate an amount of  $10^{-4}$  to  $10^{-6}$   $\mu$ g plasmid DNA per 10  $\mu$ g of plant DNA can be calculated.

## Introduction

*E. coli* plasmids can be used as vectors for the transfer of eucaryotic or procaryotic genes into higher plant cells or intact plants (for review see [1]). The aim of this investigation was to show uptake of plasmids into an intact plant. As recipient we used the water plant *Lemna perpusilla* cultivated under sterile conditions. The plasmids pMB9 or pBR325 conferring the tetracycline or the tetracycline, ampicillin and chloramphenicol resistance markers were applied to the intact plants. Whereas previous studies on DNA uptake by plant cells often included the analysis of the DNA preparation by caesium chloride gradient centrifugation [2–4] we tried as a more sensitive method for detection of plasmid DNA the bioassay of *E. coli* transformation. The plasmids of the resulting transformants were analyzed after a miniscreen procedure to see whether a rearrangement of plasmid occurred during the passage through the plant.

## Materials and Methods

### Recipient plants

*Lemna perpusilla*, strain 6746, was cultivated under sterile conditions in the medium described by

Pirson and Seidel [5] at 27 °C and continuous light of 3000 lux.

### Plasmids and Bacteria

*E. coli* plasmids pMB9 and pBR325 were harvested from *E. coli* K-12, HB101, kindly given by Prof. A. Pühler, University of Bielefeld, and by Dr. R. B. Meagher, University of Athens, Georgia. *E. coli* K-12, RRI [6] was used for the transformation assays.

### Isolation of plant DNA and plasmids

DNA of *Lemna perpusilla* was isolated after homogenization in a glass homogenizer following a slightly modified procedure described by Hemleben *et al.* [3]. The purified and reprecipitated DNA was used in the transformation assay.

Plasmid DNA was isolated from *E. coli* by ethidium bromide-caesium chloride gradient centrifugation [7] after amplification with chloramphenicol (200  $\mu$ g/ml for pMB9) or with spectinomycin (300  $\mu$ g/ml for pBR325).

### Incubation of *Lemna* with plasmid DNA

Ca. 200 exponentially growing *Lemna* plants were transferred to 10–20 ml 0.01 M KCl-0.001 M sodium citrate buffer pH 6.7 containing sterile prepared plasmid DNA (50–70  $\mu$ g/ml) and streptomycin (10  $\mu$ g/ml). The incubation was carried out at 27 °C or 6 °C. The lower temperature improves the stability of the plasmids in the medium. The plants were in contact with the medium with the root-like organs and the lower surface of the leaves. At the end of the incubation time (5 min as control or 20–22 h) the plants were washed three times with distilled water, treated with DNase I in 0.002 M  $MgCl_2$  (20  $\mu$ g/ml, 15 min, 37 °C) and then with proteinase K (100  $\mu$ g/ml, 30 min, 37 °C) before isolation of the DNA.

### Transformation of *E. coli*

Competent *E. coli* cells were obtained by the  $CaCl_2$  method [8]. 5–30  $\mu$ g *Lemna* DNA from plasmid-incubated plants or pure plasmid DNA (0.0003–3  $\mu$ g) were added to 200  $\mu$ l of competent *E. coli* K-12, RRI cells.

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Table I. Incubation of *Lemna perpusilla* with the *E. coli* plasmids pMB9 (exp. 1–3) and pBR325 (exp. 4–7) and subsequent transformation of competent *E. coli* cells to tetracycline resistance with the DNA isolated from the plasmid-incubated plants or pure plasmid as control.

| Plasmid concentration [ $\mu\text{g/ml}$ ] | Incubation conditions | <i>Lemna</i> DNA in the transformation assay [ $\mu\text{g}$ ] | Resulting transformants [ml] | Transformation rate/ $\mu\text{g}$ <i>Lemna</i> DNA | Transformation rate/ $\mu\text{g}$ pure plasmid | Calculated $\mu\text{g}$ of plasmid DNA/10 $\mu\text{g}$ <i>Lemna</i> DNA |
|--------------------------------------------|-----------------------|----------------------------------------------------------------|------------------------------|-----------------------------------------------------|-------------------------------------------------|---------------------------------------------------------------------------|
| 1 a) 57                                    | 5 min/27 °C           | 10                                                             | 0                            | 0                                                   | $4.5 \times 10^{-4}$                            | 0                                                                         |
| 1 b) 57                                    | 20 h/27 °C            | 10                                                             | 11.4                         | $3.0 \times 10^{-9}$                                | $4.5 \times 10^{-4}$                            | $8.9 \times 10^{-5}$                                                      |
| 2) 65                                      | 20 h/27 °C            | 5                                                              | 5.0                          | $1.6 \times 10^{-8}$                                | $1.7 \times 10^{-3}$                            | $2.3 \times 10^{-4}$                                                      |
| 3 a) 75                                    | 5 min/27 °C           | 30                                                             | 0                            | 0                                                   | $8.6 \times 10^{-5}$                            | 0                                                                         |
| 3 b) 75                                    | 20 h/27 °C            | 30                                                             | 8.6                          | $2.7 \times 10^{-10}$                               | $8.6 \times 10^{-5}$                            | $3.1 \times 10^{-5}$                                                      |
| 4) 70                                      | 22 h/27 °C            | 18                                                             | 5.0                          | $6.5 \times 10^{-11}$                               | $6.7 \times 10^{-6}$                            | $1.7 \times 10^{-4}$                                                      |
| 5 a) 57                                    | 22 h/27 °C            | 20                                                             | 6.7                          | $3.8 \times 10^{-9}$                                | $1.9 \times 10^{-3}$                            | $2.2 \times 10^{-5}$                                                      |
| 5 b) 57                                    | 22 h/6 °C             | 20                                                             | 33.3                         | $1.9 \times 10^{-8}$                                | $1.9 \times 10^{-3}$                            | $9.6 \times 10^{-5}$                                                      |
| 6 a) 57                                    | 22 h/27 °C            | 18                                                             | 5.0                          | $6.6 \times 10^{-10}$                               | $5.5 \times 10^{-3}$                            | $1.6 \times 10^{-5}$                                                      |
| 6 b) 57                                    | 22 h/6 °C             | 24                                                             | 13.3                         | $1.3 \times 10^{-9}$                                | $5.5 \times 10^{-3}$                            | $3.0 \times 10^{-5}$                                                      |
| 7) 73                                      | 22 h/6 °C             | 20                                                             | 1.8                          | $2.1 \times 10^{-10}$                               | $5.5 \times 10^{-3}$                            | $4.5 \times 10^{-6}$                                                      |

### Miniscreen

Plasmids present in the transformed bacteria were isolated as described by Birnboim and Doyle [9], treated with the restriction endonuclease *Hind*III and analyzed by gel electrophoresis on 1% agarose gels.

### Results and Discussion

*Lemna* plants were incubated with pMB9 or pBR325 plasmid DNA under various conditions for 0 to 22 h at 6 or 27 °C, respectively. After the incubation the plants were thoroughly washed, treated with DNase I twice to remove the plasmids from the plant surface and subsequently with proteinase K and then the plant DNA was isolated. These *Lemna* DNA preparations were used for transformation of *E. coli* cells to tetracycline resistance. Table I shows the results. Out of 15 incubation assays 7 *Lemna* DNA preparations from plasmid-incubated plants transformed *E. coli* with a transformation rate of  $10^{-8}$ – $10^{-10}$ / $\mu\text{g}$  DNA. These transformants were not due to contamination of the plants or of the competent *E. coli* cells. Both possibilities were tested by plating incubation medium on nutrient broth agar plates and competent cells on tetracycline-containing plates. DNA from *Lemna* plants treated only for 5 min with plasmid DNA never resulted in transformed *E. coli* cells.

The amount of plasmid DNA present in *Lemna* DNA preparations could be determined by compar-

ing the transformation rates with those obtained after treatment of competent *E. coli* cells with pure plasmid DNA (Table I). These rates are linear to the amount of plasmid between 3 to 0.0003  $\mu\text{g}$  plasmid DNA applied to *E. coli* cells. Mixing plant DNA and plasmids *in vitro* (100:1) this only slightly lowered the transformation rate. Therefore it is possible to calculate the portion of plasmid DNA taken up by the plants. Per 10  $\mu\text{g}$  of *Lemna* DNA  $10^{-4}$  to  $10^{-6}$   $\mu\text{g}$  plasmid DNA are estimated. Regaining ca. 0.5  $\mu\text{g}$  DNA per *Lemna* plant this means that about 100–900 intact plasmid molecules are taken up by one *Lemna* plant. Using intact plants as recipients one could argue that the plasmids do not actually enter the plant cells or even the cell nucleus but stay in the intracellular spaces. One argument against this possibility is that in several experiments we did not get any transformants.

The transformants obtained after treatment of *E. coli* with DNA from plasmid incubated plants were analyzed in more detail after a miniscreen with the restriction endonuclease *Hind*III which cuts the normal pMB9 and pBR325 plasmids only once giving rise to one band on agarose gels. Some of these *Lemna*-plasmid transformants contained in addition a new band with a molecular weight of ca.  $3 \times 10^5$ , whereas most of these appeared unchanged. This phenomenon is under further investigation and might be an indication that some kind of rearrangement of the plasmids occur in the plant cells.

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