Optimal Conditions for Transformation of *Azotobacter vinelandii*

WILLIAM J. PAGE* AND MARGARET VON TIGERSTROM

*Department of Microbiology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9*

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Optimal transformation of *Azotobacter vinelandii* OP required a 20-min incubation of the competent cells with deoxyribonucleic acid at 30°C in buffer (pH 6.0 to 8.0) containing 8 mM magnesium sulfate. Nitrogen-fixing transformants of nitrogen fixation-deficient recipients could be plated immediately on selective medium, but transformants acquiring rifampin and streptomycin resistance required preincubation in nonselective medium. The three phenotypes achieved an approximately equal and stable frequency after 17 h (six generations) of growth in nonselective medium.

Recently a liquid medium for the production of competent *Azotobacter vinelandii* was described (12). This induction procedure utilizes an iron-limited Burk medium containing ammonium acetate and glucose (OFe+N medium) and provides a major improvement in the *Azotobacter* transformation procedure. Formerly, a combined competence induction and transformation medium (plate assay) was the only procedure for genetic exchange in this organism (11). The plate assay was used to map nitrogenase-deficient (Nif') lesions (1) and to perform intergeneric transformation of *A. vinelandii* with *Rhizobium* spp. DNA (2, 10). In these studies the transformant cells and the transformation frequencies were examined, but the mechanisms of DNA binding, uptake, and recombination could not be studied by the plate assay, which required a solid support (11).

*A. vinelandii* OP strain UW1, a previously described (1), nitrogenase-deficient (Nif'), rifampin- and streptomycin-sensitive strain (obtained from W. Brill, University of Wisconsin, Madison), was used as a recipient in the present study. Donor DNA was prepared from *A. vinelandii* ATCC 12837 strains 113 (Nif' Rif') and 114 (Nif' Str') by lysis in 15 mM saline citrate buffer (pH 7.0) containing 0.05% sodium dodecyl sulfate as described previously (11). Prototrophic (Nif') cells were maintained or selected on Burk nitrogen-free medium (pH 7.2) containing 1% glucose (11). Burk buffer refers to Burk medium without glucose. The Nif' strains were grown on Burk medium containing 1.1 g of ammonium acetate per liter (BBGN medium).

The Nif transformation frequency was 3.95 × 10⁻³ when recipient cells were induced to competence in the OFe+N liquid competence medium and were assayed by the plate method.

The same competent cells also could be transformed in a liquid assay containing Burk buffer. The frequency of transformation was dependent on the magnesium concentration in the assay (Fig. 1). The Nif transformation frequencies were comparable in the plate and the liquid assays when the magnesium concentration of the Burk buffer was increased from the normal 0.81 mM to 8.5 mM.

Increasing the magnesium concentration to 40 mM did not further increase the transformation frequency. The results confirm the previous observation that magnesium was essential for the transformation of *A. vinelandii* (11). There was no transformation in the liquid assay without the addition of magnesium or DNA, or when DNase was added at zero time. Unlike other genera that require magnesium for transformation (4, 7, 8, 13, 15), calcium would not substitute for magnesium in the transformation assay. Whether magnesium is required for the binding of DNA to the competent cell or for uptake of the transforming DNA remains to be elucidated.

The crude lysate DNA was purified by precipitating 1 volume of crude DNA with 3 volumes of 95% ethanol at 4°C. The precipitated DNA was wound onto a sterile glass rod, dried, and redissolved in sterile 10 mM saline (pH 7.0), and the DNA concentration was estimated by the Burton procedure (3). When equal amounts of purified or crude DNA were used in the liquid assay, the resultant Nif transformation frequencies were 6.16 × 10⁻³ and 5.76 × 10⁻³, respectively. It was proposed previously that crude lysate DNA was the best donor for the transformation of *A. vinelandii* and that purification of the DNA was very detrimental to the transformation frequency (11). The earlier erroneous conclusion was probably due to the inability of
Transformation was more sensitive to increased temperature, showing 96% inhibition at 7°C above the optimum compared to 96% inhibition at 13°C below the optimum. There was no transformation at 4°C, and the frequencies at 42 and 46°C were $3.82 \times 10^{-6}$ and $1.17 \times 10^{-6}$, respectively. The viability of the recipient cells in the assays was not affected from 4 to 42°C. At 46°C, however, there was a 98% decrease in viability. Because of the disproportionate loss of transformation versus viability at 37 and 42°C, it was suspected that a temperature-sensitive event was involved in transformation. This was proven to be the case by preincubating competent strain UW1 cells at 17, 25, 30, 37, and 42°C for 30 min, and then incubating for 20 min at 30°C with DNA present. The frequencies of Nif transformants from the assays preincubated at 17, 25, and 30°C were identical to the 30°C value reported in Fig. 3. Upon preincubation at 37 and 42°C, however, the Nif transformation frequencies were $6.53 \times 10^{-5}$ and $1.57 \times 10^{-6}$, respectively. Identification of this temperature-sensitive "competence factor" is in progress (unpublished data).

The Nif marker proved to be a useful transformation indicator because cells could be plated
directly from the assay onto selective medium. There apparently was enough carry-over of 
NH₄⁺ in the recipient cell pools to permit phenotypic expression of Nif on the nitrogen-free 
selective medium. As shown in Fig. 4, however, Rif and Str resistance was first expressed after 
3 h of incubation in nonselective medium, which coincided with the onset of cell division. As the 
culture grew (mean generation = 1.8 h) in the nonselective medium, the apparent frequencies of 
the antibiotic resistances increased with time while the frequency of Nif decreased with time. 
The frequency of all three markers stabilized after approximately six generations or after 11 h 
of exponential growth. Because there was a 6-h lag before the onset of exponential growth, the 
total incubation required before marker frequency stabilization was approximately 17 h. 
This marker frequency stabilization period could have been caused by a lag in (i) DNA uptake, 
(ii) DNA integration, (iii) nuclear segregation, (iv) cell growth and division, (v) product synthe-

FIG. 3. The effect of assay temperature on transformation frequency. Competent strain UW1 cells 
were preincubated in Burk buffer containing 8 mM MgSO₄ (final assay concn) for 10 min at the appro-
riate temperature, then transformed with strain 113 crude DNA for 20 min at 30°C. Transformation fre-
cuency was estimated as described in Fig. 1.

FIG. 4. The time required for phenotype frequency stabilization. Competent strain UW1 cells were 
transformed with strain 113 or strain 114 crude DNA for 20 min at 30°C. After the addition of DNase, 0.2 
ml of the assay tube contents was added to a 10-ml Erlenmeyer flask containing 4.8 ml of BBGN me-
dium, and this was incubated at 30°C in a water bath rotary shaker (New Brunswick Scientific Co., New 
Brunswick, N.J.). Selective media for Rif and Str transformants consisted of the BBGN medium con-
taining 20 μg of rifampin per ml or 20 μg of streptomycin per ml, respectively. At time intervals, the transformation frequencies of the Nif marker (●, strain 113 DNA; ○, strain 114 DNA), Str marker 
(Δ), and Rif marker (■) were estimated as described in Fig. 1. The viable count of the BBGN cultures was 
also determined (dotted line).

lag before cell division in the nonselective me-
dium probably affected the timing of the first 
appearance of the Rif and Str transformants. 
The initial frequencies of these two markers also 
may be determined by the turnover rate of their 
respective products and by the number of cell 
divisions required to yield a phenotypically re-
sistant cell. It has been estimated that A. vine-
landii may contain 10 to 50 genome equivalents 
per cell (H. L. Sadoff, B. Shimeki, and S. Ellis, 
H99, p. 151, 1977). This would require a long 
period of nuclear segregation before the produc-
tion of genotypically homogeneous progeny with 
a stable phenotype frequency during cell divi-

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LITERATURE CITED


