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Transformation of the Archaebacterium *Halobacterium volcanii* with Genomic DNA

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We describe optimization of a transformation system for the halophilic archaebacterium *Halobacterium volcanii*. Transformation of spheroplasts in the presence of polyethylene glycol permits the uptake and expression of high-molecular-weight linear fragments of genomic DNA as well as plasmid or bacteriophage DNA. Transformations can be performed with either fresh or frozen cell preparations. Auxotrophic mutants were transformed to prototrophy with genomic DNA from wild-type cells with efficiencies of 5×10^4/μg of DNA and frequencies of 8×10^{-5} per regenerated spheroplast. The overall efficiency of transformation with genomic DNA implies that genetic recombination is an efficient process in *H. volcanii*.

The recent development of broadly useful genetic tools for studying the halobacteria promises to yield new insights into the molecular biology of the archaebacterial kingdom. A mating system (13) and techniques for protoplast fusion (16) have been described for *Halobacterium volcanii*, while bacteriophage DNA transfection (3, 5) and plasmid transformation (3, 9) have been developed for both *H. volcanii* and *Halobacterium halobium*. Most recently, selectable shuttle vectors which can be maintained in either *H. volcanii* or *Escherichia coli* have been constructed (W. L. Lam and W. F. Doolittle, Proc. Natl. Acad. Sci. USA, in press).

Long-standing interest in bacteriorhodopsin, the membrane-bound light-driven proton pump of *H. halobium*, suggests that these new genetic techniques will find considerable application with the purple membrane-producing halophiles. As a model organism for general genetic studies of halophilic archaebacteria, however, *H. volcanii* perhaps shows even greater promise. The ability of *H. volcanii* to grow on simple, defined media has enabled Mevarech and co-workers to generate a variety of auxotrophic mutants (13, 17). In addition, Charlebois and co-workers have made substantial progress in preparing a physical map of the *H. volcanii* genome by ordering a library of overlapping cosmid clones (2).

With eubacteria, transformation with linear fragments of chromosomal DNA has long been a basic technique for genetic mapping and an invaluable tool for strain construction. Here we describe transformation of *H. volcanii* auxotrophs to prototrophy with wild-type chromosomal DNA and establish conditions under which transformation, even of previously frozen cells, occurs at high efficiency.

MATERIALS AND METHODS

Strains, phage, media, and growth conditions. WFD11, the strain of *H. volcanii* (synonymous with *Haloferax volcanii*) used here as wild type, is a derivative of strain DS2 which has been cured of the plasmid pHV2 (3). Auxotrophic *H. volcanii* strains (17) were from M. Mevarech. *H. halobium* R_1 (20) and its phage ϕH1 (19) were from W. Zillig.

The *H. volcanii* minimal medium of Kauri and Kushner (T. Kauri, R. Wallace, and D. J. Kushner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, I52, p. 189; D. J. Kushner, personal communication) contained, per liter, 125 g of NaCl, 50 g of $MgCl_2 \cdot 6H_2O$, 5 g of K_2SO_4, and 0.2 g of $CaCl_2 \cdot 2H_2O$ in addition to the following, which were added from separate sterile stocks: 5 ml of 1 M NH_4Cl, 5 ml of 10% glycerol, 45 ml of 10% sodium succinate, 2 ml of 0.5 M K_2HPO_4 (pH 7), 1 ml of trace elements solution, 1 ml of 0.8-mg/ml thiamine hydrochloride, and 1 ml of 0.1-mg/ml biotin. The trace element solution contained, per 100 ml, 47 mg of $MnCl_2 \cdot 4H_2O$, 44 mg of $ZnSO_4 \cdot 7H_2O$, 5 mg of $CuSO_4 \cdot 5H_2O$, and 42 mg of $FeSO_4 \cdot 7H_2O$. Minimal plates contained 15 g of agar (Difco Laboratories, Detroit, Mich.) per liter in addition to the constituents of minimal medium. Complex medium contained 3 g of yeast extract (Difco) and 5 g of tryptone (Difco) per liter in addition to the constituents of minimal medium.

Regeneration overlay medium for spheroplasts, medium preparation procedures, and growth conditions were as previously described (6).

Amino acid auxotrophs were supplemented as required by adding 100 μl of a 10-mg/ml solution of the amino acid to 3-ml portions of regeneration overlay medium. Adenine auxotrophs were similarly supplemented by 300 μl of 2-mg/ml solution of adenine hemisulfate.

H. halobium R_1 for the indicator lawn was grown in a complex medium containing, per liter, 250 g of NaCl, 20 g of $MgSO_4 \cdot 7H_2O$, 3 g of trisodium citrate, 2 g of KCl, 0.2 g of $CaCl_2 \cdot 2H_2O$, 3 g of yeast extract, and 5 g of tryptone. Support plates for phage DNA transfections contained 15 g of agar per liter in addition to the constituents of the *H. halobium* medium. For phage DNA transfections, 3-ml portions of regeneration overlay medium were supplemented with 0.3 ml of a solution containing 3 g of yeast extract and 5 g of tryptone per 100 ml.

Preparation of DNA. High-molecular-weight genomic DNA was prepared as follows. Cultures (50 ml each) of *H. volcanii* were grown to stationary phase, cells were pelleted and suspended in 10 ml of 1 M NaCl–20 mM Tris hydrochloride (pH 8.0), and 10 ml of 100 mM EDTA–0.2% sodium dodecyl sulfate was then added. The resulting lysate was overlaid with 40 ml of 95% ethanol, and DNA was spooled onto a glass rod. The DNA was dissolved in 5 ml of TE (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA) plus 500 μg of RNase A (Worthington Diagnostics, Freehold, N.J.) by gently rocking the tube at 37°C. After several hours, proteinase K (Boehringer Mannheim Canada, Dorval, Quebec, Canada) was added to 0.1 mg/ml, and mixing was continued

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overnight. The DNA was extracted twice with 5 ml of water-saturated phenol (neutralized with 1 M Tris hydrochloride [pH 8.0]) by gently rocking the tube for several hours. A 2-ml portion of the aqueous phase was then precipitated with 1 ml of 7.5 M ammonium acetate and 6 ml of 95% ethanol. The DNA was pelleted by centrifugation, and the pellet was washed first with 70% ethanol and then with 95% ethanol and finally dissolved in 2 ml of TE by gently rocking it overnight.

Size-fractionated genomic DNA was prepared as follows. Samples of genomic DNA were passed through a French pressure cell at 69, 207, 414, 827, or 1,379 bars (1 bar = ~100 kPa). These sheared DNAs were pooled with untreated genomic DNA. The pooled DNA was fractionated by rate-zonal ultracentrifugation on a 13-ml, 10 to 40% sucrose gradient (in 1 M NaCl–5 mM EDTA–20 mM Tris hydrochloride [pH 7.5]) run for 14 h at 35,000 rpm and 20°C in an SW40 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The gradient was unloaded as 0.25-ml fractions. Samples from fractions were used directly for transformation without further treatment.

The sizes of genomic DNA shear products were determined by comparison with *Hin*dIII-digested phage lambda DNA and multimers of phage lambda DNA (Promega Biotec, Madison, Wis.) following contour-clamped homogeneous electric-field electrophoresis (4) in 1% agarose for 12 h at 10°C with a 5-s switching time.

Phage ϕH DNA was prepared as described by Schnabel et al. (19).

DNA concentrations were determined by comparing scanning densitometer traces of genomic DNA with lambda DNA standards on photographic negatives of ethidium bromide-stained agarose gels or by measuring the A_{260} of DNA solutions.

Transformation solutions. Spheroplasting solution contained 0.8 M NaCl, 27 mM KCl, 50 mM Tris hydrochloride (pH 8.2), and 15% sucrose. Spheroplast regeneration salt solution for dilutions and washes contained 3.4 M NaCl, 175 mM $MgSO_4$, 34 mM KCl, 5 mM $CaCl_2$, 50 mM Tris hydrochloride (pH 7.2), and 15% sucrose. Polyethylene glycol (PEG) solution contained 6 g of PEG 600 (PEG with a molecular weight of approximately 600) for every 4 ml of spheroplasting solution. PEG 600 was purified as previously described (6).

Transformation procedure. Unless otherwise noted, all steps were performed at room temperature. Cells grown to densities of 0.8 to 1.6 A_{550} (0.6×10^9 to 1.2×10^9 viable cells per ml) were pelleted in a clinical centrifuge and gently suspended in a 1/10 volume of spheroplasting solution by pumping up and down with a pipettor. A portion (0.1 ml) of 0.5 M EDTA (pH 8.0) was added per milliliter of concentrated cells and immediately mixed by gently swirling the tube. Typically, 0.22-ml portions of spheroplasts were distributed to the bottoms of 15-ml polypropylene tubes (Falcon 2059; Becton Dickinson and Co., Lincoln Park, N.J.). DNA (usually 10 μl in spheroplasting solution) was added to the spheroplast suspension and immediately mixed by gently swirling the tube. An equal volume (230 μl) of PEG solution was then added to the lip of the horizontally held tube, the tube was capped, and spheroplasts and PEG solution were mixed by rocking the tube gently until schlieren lines were no longer visible. Spheroplast regeneration salts solution was then added to bring the total volume to 10 ml and mixed well by inversion.

If few transformants were expected, all or most of the transformation mixture was plated following pelleting and gentle suspension of the spheroplasts in 1 ml of spheroplast dilution solution. When larger numbers of transformants were expected, pelleting of the spheroplasts was omitted and dilutions were prepared directly as required. Spheroplasts were plated by adding 0.1 to 1.0 ml of an appropriate dilution of the transformation mixture to 3 ml of molten (60°C) regeneration overlay medium and pouring the mixture onto support plates. Plates were supplemented, as described above, depending on selection requirements. For transfections with ϕH DNA, 0.2 ml of a fresh late-log-phase *H. halobium* R_1 culture was added to overlay medium to serve as an indicator lawn (strain R_1 has been shown to lack a restriction enzyme present in the wild-type strain NRC817 [18] and in our hands appeared to be lacking any restriction system). When the overlay had set, the plates were inverted, wrapped to prevent desiccation, and incubated at 37 to 42°C. Regeneration efficiency was monitored by plating 10^{-6} of the transformation mixture without any selection.

Further practical suggestions for performing this transformation procedure have been described elsewhere (6). In particular, maximum efficiency and reproducibility depended on avoiding cell lysis (which may be caused by rough handling of spheroplasts or contamination by traces of detergent) and ensuring that the final PEG concentration was correct.

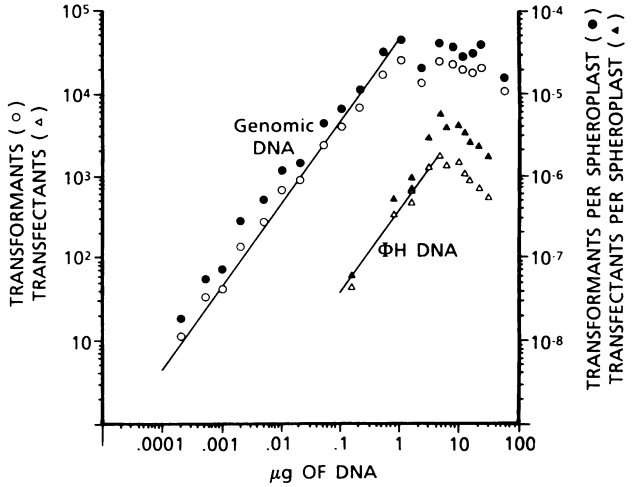

FIG. 1. Dependence of total transformants and transformation frequency on input DNA. For transformation of WR102 (*ade-2*) with WR211 (*ser-2*) genomic DNA, total Ade^+ colonies (○) and transformation frequency as Ade^+ colonies per regenerated spheroplast (●) are shown. For transfection of WFD11 with ϕH DNA, total plaques (△) and transfection frequency as plaques per regenerated spheroplast (▲) are shown.

RESULTS

We have defined transformation efficiency as the number of transformants produced per microgram of DNA, transformation frequency as the number of transformants per regenerated spheroplast, and regeneration efficiency as the number of regenerated spheroplasts relative to the viable cell count of the initial culture.

Dependence of DNA concentration. Transformation with genomic DNA and transfection with phage ϕH DNA both depended on DNA concentration in a linear fashion (Fig. 1). For transformation of strain WR102 (*ade-2*) to prototrophy with unfractionated WR211 (*ser-2*; from M. Mevarech [17])

TABLE 1. Transformation of *H. volcanii* auxotrophs with WFD11 (wild-type) genomic DNA[a]

Strain[b]	Genotype	Selection	No. of colonies with:	
			No DNA	WFD11 DNA
WR102	*ade-2*	Ade^+	6, 0	2,504, 2,204
WR203	*ser-1*	Ser^+	0, 0	436, 432
WR217-1	*orn-1 tmp-5*	Orn^+	72, 50	1,110, 803
WR218	*ade-21 tmp-6*	Ade^+	2, 3	231, 339
WR256	*his-1 arg-1*	His^+	2, 4	363, 301
		Arg^+	50, 64	1,076, 1,181
		His^+ Arg^+	0, 0	0, 1
WR268	*ade-18 arg-2*	Ade^+	0, 0	336, 357
		Arg^+	2, 2	291, 228
		Ade^+ Arg^+	0, 0	0, 0

[a] Samples (~2×10^9 viable cells) were transformed with 30 ng of DNA. Transformations were performed in duplicate with cells which had been stored frozen (6), except that fresh cells were used in the case of strain WR217-1.

[b] All strains were obtained from M. Mevarech, Tel Aviv University. Strains have been previously described (17) except for WR256.

DNA (range, 10 to 250 kilobase pairs [kbp]), the slope of the transformation efficiency corresponded to 5×10^4 transformants per μg of DNA, and the slope of transformation frequency corresponded to 8×10^{-5} transformants per regenerated spheroplast per μg of DNA. Transformation efficiency and frequency plateaued around 1.0 μg of DNA per transformation. Each transformation contained 1.2×10^9 cells of strain WR102, so there were about 0.2 genome equivalents per cell per μg of transforming DNA. (We have estimated a genome size of 3.8×10^6 bp, based on sizing by contour-clamped homogeneous electric-field gel electrophoresis of *Bam*HI digestion products.) Regeneration efficiencies in this experiment averaged 51%.

The slope of the transfection efficiency for ϕH DNA (linear 59-kbp molecule [19]) corresponded to 400 plaques per μg of DNA. The decline in efficiency above 5 μg appeared to correlate with PEG precipitation of transfecting ϕH DNA. DNA precipitation was just visible at 1.5 μg of input DNA and became more pronounced as the DNA concentration increased. In contrast, the plateau observed with genomic DNA (at 1.0 μg of DNA) did not appear to correlate with DNA precipitation (just visible at 10 μg of input DNA). PEG precipitation at a particular concentration of DNA depends on DNA size and other factors (11), which may explain why genomic DNA was less susceptible to precipitation than was ϕH DNA.

Transformation of various auxotrophic mutants. Table 1 presents the results of transformations of a number of different auxotrophic mutants with 30 ng of genomic DNA (range, 10 to 100 kbp) from the wild-type strain WFD11. Most of these transformations were performed with frozen cell preparations as previously described (6).

Even though the ethyl methanesulfonate-induced mutations in these strains (13, 17) are expected to be single-base changes (14), we found reversion rates to be low (10^{-9} to 10^{-7}). Most colonies seen when cells (which had not been spheroplasted) were plated under selective conditions were slow-growing pseudorevertants easily distinguished from wild-type or true revertants. An exception to this was the *arg-2* allele of strain WR256, which gave revertants uniformly indistinguishable from true Arg^+ colonies. In control transformations without added DNA, the background for most of these strains was even further reduced, presumably because pseudorevertants were unable to regenerate under selective conditions. The *orn-1* allele of WR217-1, however, gave a relatively high frequency of slow-growing revertants even after mock transformation.

Transformation efficiencies spanned a range of 0.9×10^4 transformants per μg of input DNA for the *ade-21* allele of strain WR218 and the *arg-2* allele of WR268 to 8×10^4 for the *ade-2* allele of WR102. When double auxotrophs WR256 and WR268 were selected for simultaneous transformation of both of their auxotrophies, only background levels of colonies were seen, as would be expected if these markers were unlinked.

Transformation of auxotrophs with genomic DNA lacking the complementing wild-type allele produced only background numbers of revertants. In experiments with unfractionated genomic DNA, strains WR256 (*his-1 arg-1*) and WR220 (*ade-18 tmp-8*; from M. Mevarech [17]) gave normal background transformation levels when transformed with their own DNA and selected for His^+ and Ade^+, respectively. When each strain was transformed with DNA from the other, selection of WR256 for His^+ and WR220 for Ade^+ produced numbers of transformants comparable to the numbers observed following transformation with wild-type WFD11 DNA.

Optimization of conditions for transformation. Several parameters were varied experimentally in order to approach maximum efficiency in this system, but the optimum PEG molecular weight of 600 and the optimum final PEG concentration of 30% previously determined for *H. halobium* (5) were assumed to hold for the *H. volcanii* system.

(i) Chelation of divalent cations. Even though *H. volcanii* tended to convert from its normal cupped, disk shape to a spherical form when cells were suspended in spheroplasting solution (which does not contain divalent cations), no transformants were seen (a >1,000-fold reduction from standard conditions) unless cells were also treated with EDTA.

(ii) NaCl concentration. The optimum NaCl concentration in spheroplasting solution was 0.8 M (Fig. 2). Transformation frequencies fell to less than 50% of maximum levels below 0.4 M or above 1.2 M. The efficiency of regeneration was unaffected by variation in the NaCl concentration of the spheroplasting solution (Fig. 2). The data in Fig. 2 were obtained by using wild-type WFD11 DNA to transform strain WR256 to His^+, but similar results were obtained by varying NaCl concentration with ϕH DNA transfection of WFD11.

(iii) pH. The maximum transformation efficiency was obtained at a spheroplasting solution pH of 8.2. Tris, which has been shown to stimulate transformation in some eubacterial systems (8), did not produce any special advantage compared with the dipolar ionic buffer TAPS [*N*-tris(hydroxymethyl)methylaminopropane sulfonic acid, sodium salt] at 50 mM concentrations. The pH of the spheroplating solution did not have any appreciable effect on regeneration efficiency between pHs 7.0 and 9.8 (above pH 8.8 these results were based on buffering with the dipolar ionic buffer CHES [cyclohexylaminoethane sulfonic acid, sodium salt]).

(iv) Phase of growth and cell density. Transformations were performed on comparable numbers of viable cells (~2×10^9) from various phases of growth. Transformation efficiency varied little between a culture density of 0.45 A_{550} (the lowest density tried) and 2.0 A_{550}. Stationary-phase cultures with a density of 3 A_{550} gave poor results, apparently because of cell lysis and the accompanying coprecipitation of genomic and transforming DNA upon addition of PEG.

The numbers of cells per transformation was varied between 4×10^8 and 1×10^{10} in our standard procedure with

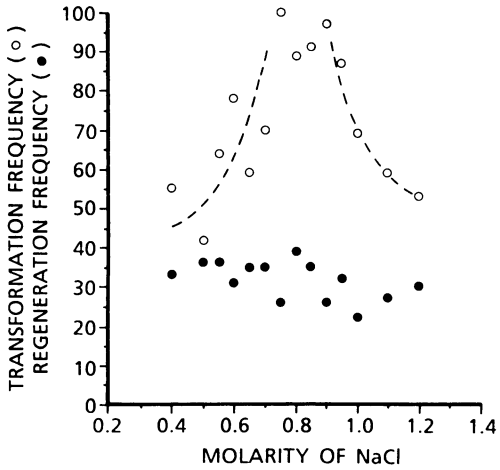

FIG. 2. Effect of NaCl concentration on transformation. Samples of WR256 (*his-1 arg-1*) suspended in spheroplasting solutions of various NaCl concentrations were transformed with 30 ng of size-fractioned WFD11 (wild-type) genomic DNA of 43 kbp average size. Spheroplasting solutions with correspondingly varied NaCl concentrations were used to make up the PEG solutions. Transformations were plated with selection for His^+ phenotype, yielding between 500 and 1,200 His^+ colonies. Total transformants were normalized for minor fluctuations in spheroplast regeneration to give transformation frequency (transformants per spheroplast) (○). Data are presented as percentages of the concentration which resulted in the highest frequency (900 transformants per 30 ng of input DNA and 1.1×10^9 regenerated spheroplasts resulted in 8×10^{-7} transformants per spheroplast at the 0.75 M NaCl concentration). Spheroplast regeneration (●) is represented as a percentage of initial viable culture count.

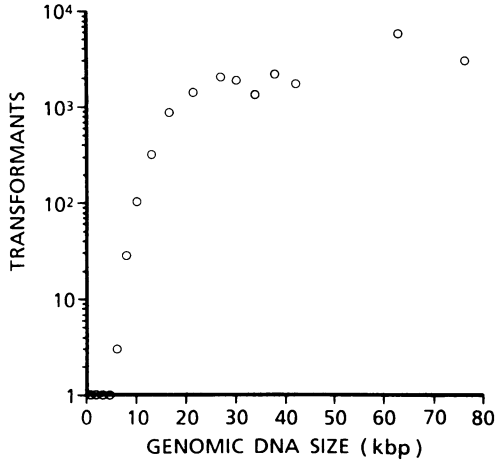

FIG. 3. Effect of DNA fragment size on transformation. Samples (1.5×10^9 cells) of WR102 (*ade-2*) were transformed with size-fractionated WFD11 (wild-type) genomic DNA and selected for the Ade^+ phenotype. Data were normalized to 30 ng of input DNA and 5×10^8 regenerated spheroplasts.

30 ng of input genomic DNA. Optimal transformation efficiency was obtained when cultures were suspended in spheroplasting solution at a concentration of 10^9 cells per 200 μl. A marked decrease in efficiency was seen with $\geq 5 \times 10^9$ cells per 200 μl.

(v) Lengths of incubations. Cultures remained useful for transformation for at least 24 h following removal from usual growth conditions to room temperature. When varied between 1 and 20 min, the length of DNA incubation had no effect on transformation. Similarly, varying the length of PEG incubation between 1 and 20 min had no effect on transformation or regeneration efficiencies. After transformation of auxotrophs, a period of free expression (overnight incubation of spheroplasts in complex medium) before plating produced no higher efficiencies than did plating with selection immediately following transformation.

(vi) Spheroplast regeneration. Regeneration efficiencies for fresh cells varied between 15 and 60% of the viable count of the initial culture. Generally higher regeneration efficiencies were seen by using cells from cultures with densities below 1.5 A_{550}. Regeneration efficiencies for stored frozen cells were 50 to 100% of those obtained with fresh cells. Although regeneration efficiencies varied between batches of cells, the transformation frequency (transformants per spheroplast) for a particular allele remained reasonably constant from culture to culture and between fresh and frozen cell preparations. The use of 1 to 4% bovine serum albumin in spheroplasting solutions has been reported to improve regeneration for *Halobacterium cutirubrum* and *Halobacterium salinarium* spheroplasts (10). In our hands, the inclusion of bovine serum albumin in spheroplasting solutions had no effect on regeneration or transformation of *H. volcanii*. Bovine serum albumin in this concentration range did, however, improve pelleting of spheroplasts following the post-PEG-treatment wash.

Effect of DNA size. Transformation efficiency was demonstrated to depend on DNA size. WFD11 (wild-type) genomic DNA was size fractionated by rate-zonal sucrose gradient ultracentrifugation. Fractions spanned a range from 1 to 75 kbp and exhibited fairly sharp upper size limits on contour-clamped homogeneous electric-field gels. WR102 was transformed with about 30 ng of DNA for fractions above 6 kbp, selecting for an Ade^+ phenotype. Transformations with fractions between 6 and 1 kbp contained increasing amounts of DNA with decreasing size (reflecting a bias in the concentrations of various molecular weight fragments in the pooled sheared DNA applied to the fractionating gradient), with the 1-kbp fraction containing about 1.5 μg of DNA. Even so, fractions of 5 kbp and less produced only background levels of Ade^+ colonies. Results were adjusted for minor variations in spheroplast regeneration and exact amounts of input DNA to give the results shown in Fig. 3. Transformation efficiencies of fractions above 25 kbp were comparable to those of unfractionated high-molecular-weight genomic DNA. Below 25 kbp, transformation efficiency declined sharply to less than one Ade^+ colony per 30 ng of DNA at 5 kbp or below. Because of the possibility of trailing of higher-molecular-weight DNAs in the sucrose gradient, the decline in transformation efficiency below 25 kbp may be more precipitous than our results indicate.

DISCUSSION

Using high-molecular-weight genomic DNA and ϕH DNA, we have characterized a PEG-mediated spheroplast transformation system for *H. volcanii*. This transformation system was modified from methods which we previously developed for *H. halobium* (5). Since *H. volcanii* and *H. halobium* are not closely related (12, 21), we expect that similar procedures will work for other species of halophilic archaebacteria.

Like *H. halobium* transfection (5), *H. volcanii* transformation depended on the chelation of divalent cations by EDTA. The primary difference between the two systems

was the optimal concentration of NaCl in the spheroplasting solutions: 0.8 M for *H. volcanii* and 2.0 M for *H. halobium*, which in either case is about one-half the NaCl concentration found in optimal growth media. In many respects, such as phase of culture growth, length of incubation with DNA, and length of incubation with PEG, *H. volcanii* appeared to have less-stringent requirements for transformation than did *H. halobium* (5). The technique of storing frozen cells for transformation (6) greatly facilitated our experiments.

We believe that the efficiencies of uptake and expression of exogenous DNAs for *H. halobium* and *H. volcanii* are comparable, but comparisons are indirect. ϕH DNA used for transfection of *H. volcanii* must be propagated in *H. halobium* because *H. volcanii* is outside the host range of ϕH. Consequently, ϕH DNA is restricted by a factor of about 10^{-4} following uptake by *H. volcanii* (2, 6), and it is not possible to make a direct comparison of phage DNA transfection efficiencies in the two species. Genomic DNA carries modifications and is not restricted upon uptake by *H. volcanii*, but data for transformation of *H. halobium* with genomic DNA are unavailable.

We have measured the efficiency of transforming an Ade^- mutant (strain WR102) to prototrophy with size-fractionated DNA and observed a decline in efficiency by 3 orders of magnitude between 25 and 6 kbp (Fig. 3). In general, the relationship between DNA size and transformation for *H. volcanii* is typical of results obtained with both naturally and artificially transformed eubacterial species (1, 7, 15).

Various factors such as membrane transit, nuclease degradation, recombination, and the size and nature of the mutational lesion could affect the dependence of transformation efficiency on DNA size. We cannot separate these factors in this set of experiments, but taken as a lower limit, the data suggest that recombination can occur at high frequency in *H. volcanii*. For example, we have seen efficiencies of 7×10^4 transformants per μg with randomly sheared genomic DNA whose size (28 kbp) corresponds to about 0.75% of the genome (data from the experiment are summarized in Fig. 3). Thus, a pure fragment of similar size with a complementing sequence optimally positioned might be expected to produce 9×10^6 (or more) transformants per μg. This compares quite favorably with previously reported efficiencies for plasmid transformation of *H. volcanii* (5×10^7 to 1×10^8 per μg [Lam and Doolittle, in press]) or phage DNA transfection of *H. halobium* (5×10^6 to 2×10^7 per μg [5]).

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