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Polyethylene Glycol-Dependent Transfection of *Acholeplasma laidlawii* with Mycoplasma Virus L2 DNA

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Phenol-extracted DNA from mycoplasma virus L2 was able to transfect *Acholeplasma laidlawii* in the presence of polyethylene glycol. Transfection was sensitive to DNase and was most efficient with 36% (wt/vol) polyethylene glycol 8000 and cells in logarithmic growth. Virus production by the transfected cells was similar to that of the cells infected by intact virus. L2 DNA transfected *A. laidlawii* with a single-hit dose-response curve, reaching saturation at high DNA concentrations. Optimum transfection frequencies were about 10^{-7} transfectants per L2 DNA molecule and 10^{-4} transfectants per CFU. When DNA was present in saturating amounts, the number of transfectants increased linearly with the number of CFU present in the transfection mixture, suggesting that DNA uptake does not occur by a mechanism involving cell fusion. The cleavage of the superhelical mycoplasma virus L2 genome with restriction endonucleases that cleave the DNA molecule once reduced the transfection frequency. Host cell modification and restriction of transfecting L2 DNA were similar to those for infecting L2 virions.

Mycoplasma is the general name for a group of procaryotes that do not have cell walls; each cell is bounded only by a lipoprotein membrane (reviewed in references 23 and 30). These are the smallest known free-living cells, with genomes 20 to 40% those of typical eubacteria (30), and arose by degenerative evolution as a subline of the gram-positive eubacteria (35).

The small genome sizes of these organisms would appear to make them amenable to genetic studies; unfortunately, few reports of systems for genetic exchange in the mycoplasmas have been published. Folsome (7) reported that *Acholeplasma laidlawii* was capable of binding high-molecular-weight double- or single-stranded DNA in a DNase-resistant form, but transformation could not be detected. However, the transformation of cation-treated, tetracycline-sensitive *Mycoplasma hominis* and *Mycoplasma salivarium* cells to tetracycline resistance by DNA extracted from tetracycline-resistant *M. hominis* cells has been described by Furness and Cerone (8).

An alternate system for genetic exchange in mycoplasmas is transfection, the uptake and expression of exogenous viral nucleic acid. Three viruses infecting *A. laidlawii* have been described elsewhere (reviewed in reference 21). Mycoplasma virus L1 is a bullet-shaped virion containing circular, single-stranded DNA of 4.5 kilobase pairs or 1.5×10^6 daltons. Mycoplasma virus L2 is an enveloped, quasispherical virion containing circular, superhelical, double-stranded DNA of 11.8 kilobase pairs or 7.8×10^6 daltons. Mycoplasma virus L3 is a T7-like virion containing linear, double-stranded DNA of about 26×10^6 daltons. We are interested in having a transfection system for L2 virus, since such a system is needed for genetic studies of this unique temperate, enveloped, budding phage.

Liss and Maniloff (18) demonstrated that *A. laidlawii* cells in the late-logarithmic growth phase could be infected by DNA isolated from L1 mycoplasma viruses simply by mixing cells and viruses. We found that this protocol does not work with L2 viral DNA and, therefore, investigated other methods for getting exogenous DNA into membrane-bounded cells. Recently, an efficient system for the introduction of plasmids into *Streptomyces* protoplasts was developed (2). The method involves the uptake of plasmid DNA in the presence of polyethylene glycol (PEG) and the subsequent regeneration of the bacterial cell wall. This system of plasmid transformation has been extended to a number of *Bacillus* species (3, 12, 24, 33) and has been used to transform *Bacillus subtilis* protoplasts for chromosomal markers (16). PEG-mediated transfection of protoplasts with bactriophage DNA has been reported in *Streptomyces* sp. (15) and in *Streptococcus* sp. (9). In *B. subtilis* L-

forms, PEG has been shown not only to induce transfection with phage DNAs but also to cause the uptake of phage particles (34).

Since mycoplasmas lack cell walls, like bacterial protoplasts, we investigated whether the uptake of DNA by mycoplasmas could be induced in the presence of PEG. We report here the development of a PEG-mediated system for the uptake and expression of genetic material in mycoplasmas, using *A. laidlawii* cells and mycoplasma virus L2 DNA.

MATERIALS AND METHODS

Cells, virus, and medium. *A. laidlawii* JA1 (19) and K2 (13) were used in these studies. Mycoplasma virus L2 (10) was grown on strain K2, unless indicated otherwise. The cells were assayed as CFU, the virus was assayed as PFU, and transfected cells were assayed as infectious centers (on lawns of K2 unless otherwise indicated). Tryptose broth and agar plates were used for culturing *A. laidlawii* cells and L2 as described previously (17).

Buffers and PEG solution. T buffer was 0.01 M Tris, pH 8.0. TE buffer was T buffer with 0.001 M EDTA. TES buffer was TE buffer with 0.1 M NaCl. TS buffer was T buffer with 0.1 M NaCl.

PEG solution was 40% (wt/vol) PEG (PEG 8000; Fisher Scientific Co., Rochester, N.Y.) in 0.5 M sucrose–0.01 M Tris, pH 6.5.

Purification of L2 virus. An early-log-phase culture of *A. laidlawii* was infected with L2 at a multiplicity of infection of about 1. After overnight incubation at 37°C, the cells were removed by centrifugation (10 min at 9,000 rpm at 4°C in a Beckman JA-10 rotor), and the virus was pelleted (30 min at 25,000 rpm at 10°C in a Beckman SW28 rotor). The virus pellet was suspended in TS buffer and purified by sedimentation (3 h at 25,000 rpm at 10°C in a Beckman SW28 rotor) through linear 15 to 30% sucrose gradients. The fractions were collected, and those containing L2 were pooled, concentrated by centrifugation, and suspended in TS buffer.

Isolation of L2 DNA. The infection of cells by L2 was performed as described above. The isolation of viral DNA by phenol extraction of PEG-precipitated virus was performed essentially as previously described (27), except that the virus-containing supernatant was not filtered. DNAs had a 260 nm/280 nm absorbance ratio greater than 1.8. The DNA concentration was estimated by the absorbance at 260 nm, where 1 absorbance unit corresponds to 50 μg of DNA per ml (4).

Transfection. *A. laidlawii* cultures were grown to an optical density of 0.1 at 610 nm, measured with a spectrophotometer (model 240; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The cells were pelleted (10 min at 6,000 rpm at room temperature in a Sorvall SS-34 rotor) and then suspended in the original volume of fresh media. Fifty microliters of DNA in TE buffer was added to 250 μl of the suspended cells, and the solution was mixed. Two milliliters of PEG solution was added, and the mixture was blended again. After 2 min, the mixture was diluted with 10 ml of T buffer and blended, and the cells were pelleted (12 min at 12,000 rpm as described above). The cell pellet was suspended in 1 ml of tryptose broth and immediately assayed for infectious centers.

The number of cells per transfection mixture was determined by plating samples of suspended cells (before PEG treatment) for CFU. It was found that PEG treatment did not affect cell number as measured by the CFU assay (data not shown).

Isolation of single-cell clones. Single-cell clones were isolated by plating a liquid culture of strain K2, picking individual colonies with a sterile inoculating loop, and streaking to allow the isolation of single colonies. A second streak was performed to yield the clones used in transfection.

Enzymes. DNase I, RNase, and pronase (Calbiochem-Behring, San Diego, Calif.) were dissolved in buffers as previously described (18). RNase was heated to 100°C for 10 min to destroy residual DNase activity. L2 DNA and purified L2 virus were treated with 100 μg each of DNase and RNase and 500 μg of pronase per ml of 37°C for 1 h.

Restriction endonucleases *Bst*EII, *Cfo*I, and *Sst*I (Bethesda Research Laboratories, Gaithersburg, Md.) were used as recommended by the manufacturer. A portion of each reaction mixture was run on agarose gels (27) to ensure that the DNA had been digested completely.

RESULTS

Effect of PEG concentration. The effect of PEG concentration on the transfection frequency of strain K2 by L2 DNA is shown in Fig. 1. The optimal number of transfected cells was obtained with 36% (wt/vol) PEG. No transfectants were observed in the absence of PEG.

Transfection and cell growth phase. To determine the effect of the cell growth phase on the transfection frequency, we removed the cells from a K2 culture at various times during growth and performed transfection. Cells in all phases of growth could be transfected by L2 DNA (Fig. 2), but optimal transfection was obtained with cells in the logarithmic growth phase, particularly with those in late-logarithmic growth.

Enzymatic treatment of DNA and virus. The infectivity of L2 DNA and virus after treatment with various enzymes was investigated. Table 1 shows that the infectivity of L2 DNA, but not that of L2 virions, was destroyed after treatment with DNase. These results confirm that the infectious centers observed in PEG-mediated transfection experiments were the result of transfection by viral DNA.

Table 1 also shows that the treatment of L2 virus with pronase substantially reduced its infectivity. This result is consistent with the finding that a number of viral proteins are apparently located on the surface of the viral envelope (11; S. Cadden and J. Maniloff, unpublished data).

Virus production by transfected cells. To examine the production of virus by transfected cells, we measured viral one-step growth in cells transfected by L2 DNA (Fig. 3). Virus produc-

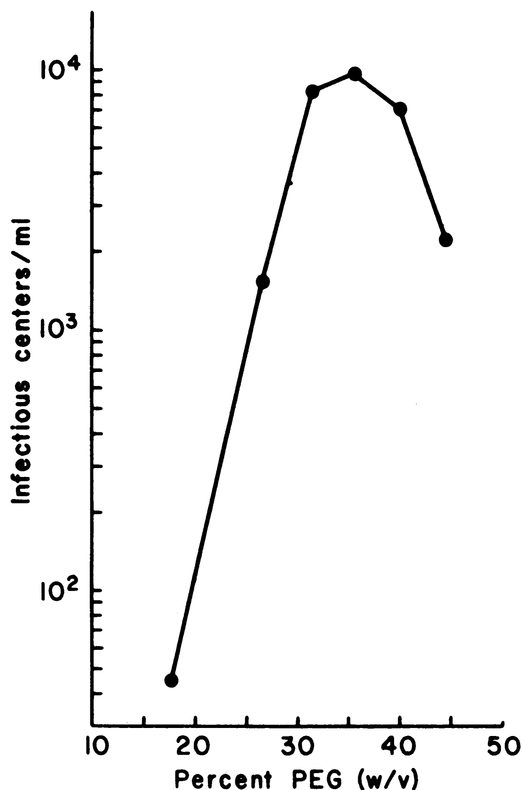

FIG. 1. Effect of PEG concentration on number of transfectants. The transfection mixture contained 1 μg of DNA (50 μl), 2.6 × 10^7 CFU (250 μl) of strain K2, and various concentrations of PEG solutions (2 ml), giving the final indicated PEG concentrations. The cells were pelleted, suspended in 1 ml of tryptose broth, and assayed for infectious centers.

tion by transfected cells was similar to that of infected cells. At 100 min after transfection, the one-step growth curve was in the rise period, and each transfected cell had already released approximately 50 progeny L2. Progeny virus release lasted 4 to 6 h, and at 7 h after transfection, each transfected cell had produced approximately 550 progeny viruses (Fig. 3). This is about the yield observed for cells infected by L2 virus (29).

L2 DNA dose response. The effect of the DNA concentration on transfection was examined by measuring the number of transfected cells as a function of the amount of L2 DNA. The dose-response curve for L2 transfection (Fig. 4) was linear, with a slope of 1.07, indicating that single L2 DNA molecules give rise to transfectants. In addition, DNA saturation could be achieved in this system, and in the experiment shown, saturation was produced with 4 μg of L2 DNA (about 10^7 molecules of L2 DNA per transfectant). However, we noticed that the amount of DNA required to produce saturation varied from one DNA preparation to the next. It has previously been shown that most of the L2 DNA isolated by phenol extraction of PEG-precipitated virus is in the superhelical form (27). However, agarose gel electrophoresis of DNA purified in this way shows that relaxed (nicked) circles, and sometimes linear molecules, are present in the preparations. These probably arise from superhelical forms during the handling of the DNA in the purification process. Since different preparations of L2 DNA have different relative amounts of nicked circular, linear, and superhelical DNA molecules, the observation that different DNA preparations saturate the transfection system at different amounts of DNA could be explained if the nicked circular and linear forms of L2 molecules are less efficient in transfection and complete with superhelical molecules.

Transfection with linear L2 DNA molecules. To test the above hypothesis, we examined the transfection efficiency of L2 DNA preparations which had been treated with restriction endonucleases. The restriction endonucleases *Bst*EII and *Cfo*I each have been shown to cleave the L2 DNA at single sites (6). *Sst*I also cleaves L2 DNA once, and the site of cleavage maps very

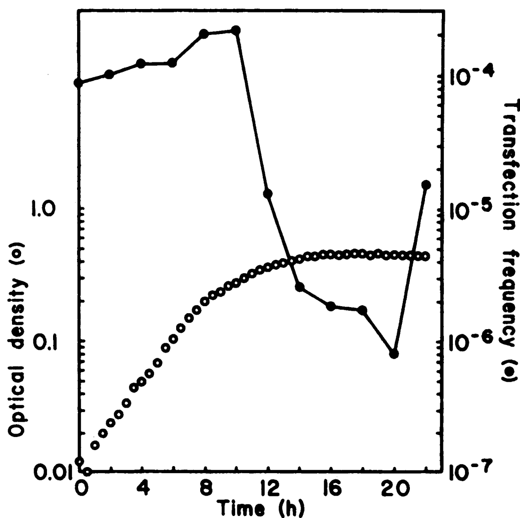

FIG. 2. Transfection and cell growth phase. At various times, cell samples were removed from a growing culture of strain K2, the optical density at 610 nm was measured, and the sample was then diluted to 0.01 optical density units. Ten milliliters of the diluted cells was centrifuged, and the cells were suspended in 1 ml of fresh medium. Transfection was carried out by adding 12 μg of L2 DNA (50 μl) to 250 μl of the suspended cell sample. The remaining cell sample was plated to assay CFU. Transfection frequency is expressed as the number of infectious centers per CFU in the transfection mixture.

TABLE 1. Effect of enzymes on infectivity of L2 DNA and virions[a]

Treatment	Infectious centers/reaction mixture	
	L2 DNA	L2 virus
DNase	0	2.6×10^{11}
RNase	2.6×10^4	3.0×10^{11}
Pronase	2.7×10^4	5.5×10^6

[a] DNA and purified virus were treated with DNase, RNase, and pronase as described in the text. After treatment, 5 μg of treated DNA (50 μl) was phenol and chloroform extracted before being added to 9.9×10^7 CFU (250 μl) of strain K2 in transfection experiments. The virus was assayed directly for PFU after enzymatic treatment.

near the single *Cfo*I site (J. Nowak, T. Sladek, and J. Maniloff, unpublished data). Table 2 shows that the linearization of L2 DNA reduced the number of transfected cells to less than 10% of the number obtained by transfection with untreated DNA. Although only linear L2 DNA molecules were observed by agarose gel electrophoresis in these nuclease-treated preparations, the presence of some uncut and nicked circular L2 DNA molecules cannot be ruled out.

To determine whether linear L2 DNA molecules compete for transfection with superhelical molecules, we performed transfection with a constant, saturating amount of superhelical L2 DNA and increasing amounts of linear L2 DNA (Table 3). These data show that linear L2 DNA can efficiently compete with superhelical molecules in transfection, since the number of infectious centers decreased as increasing amounts of linear DNA were added to a constant amount of superhelical DNA. Therefore, differences in the amounts of DNA needed to produce saturation in various DNA preparations must be due to differences in the amounts of linear (and possibly of open circular) molecules in these preparations, which compete with superhelical molecules in transfection. The ability of linear DNA to compete with superhelical molecules suggests that individual cells are able to take up only limited amounts of DNA.

Transfection and cell number. The data in Table 3 show that, as the quality of the mycoplasma virus L2 DNA preparation decreased (i.e., as the relative amounts of open circular and linear molecules in a preparation increased), the number of transfectants obtained at a saturating amount of DNA decreased. Nevertheless, in transfection experiments with DNA preparations of many different qualities, we have not found transfection frequencies greater than about 10^{-4} transfectants per CFU. This low efficiency might be due to transfection's involving a complex mechanism, such as a requirement of cell fusion for DNA uptake. In this case, the relationship between the number of transfectants and the cell number would not be linear. Therefore, transfection was carried out with a constant (saturating) amount of L2 DNA and increasing cell numbers (Fig. 5). As the number of cells per transfection mixture was increased, the number of transfectants increased linearly (slope = 0.99), indicating that cooperative effects between cells are not involved in L2 transfection.

Transfection of isolated clones. The low transfection frequency of 10^{-4} could be explained by two genetic models. The first model postulates two subpopulations of cells, with only the smaller subpopulation (of the order of 1 per 10^4 cells) being competent for transfection. The second model postulates that all cells are equally competent, but with the same low frequency of about 10^{-4}. These two models can be distinguished by determining the transfection frequencies of clones of K2 cells. The first model would predict that, since competent cells are so rare, it

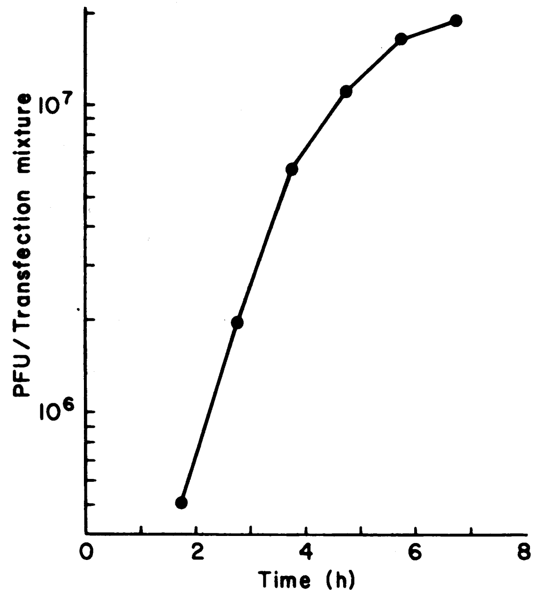

FIG. 3. One-step virus growth in cells transfected with L2 DNA. Transfection was performed by adding 10 μg (50 μl) of L2 DNA to 1.5×10^8 CFU (250 μl) of strain K2. After transfection, the cells were diluted 10^4-fold in tryptose broth. At the indicated times after transfection, the samples were centrifuged to remove cells, and the supernatant was assayed for PFU to measure progeny virus production. The data are shown as the total number of PFU in the original transfection mixture, which contained 3.7×10^4 infectious centers (transfected cells) in this experiment. Zero time in this figure is the time at which the PEG solution was added to induce transfection.

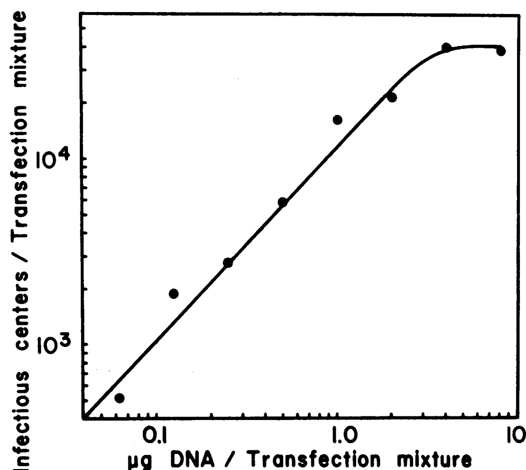

FIG. 4. L2 DNA transfection (dose-response) curve. Transfection was performed by adding various amounts of L2 DNA (50 µl) to 6.4×10^8 CFU (250 µl) of strain K2. The slope of the linear part of the curve is 1.07.

would be improbable to randomly pick a competent K2 clone and most clones would not be competent. The second model predicts that all clones have the same low (10^{-4}) transfection frequency as the initial K2 population.

Three clones were isolated and found to have the same transfection frequency of 1.9 ($\pm$0.2) $\times$ 10^{-4} infectious centers per CFU. Hence, the second model applies: all cells in the population have the same low transfection frequency. This experiment shows that, with regard to transfection, every cell is genetically the same. However, these data provide no information on possible physiological differences between cells which might account for the observed transfection frequency by either of the two models described above.

DNA binding in transfection. DNA binding by *A. laidlawii* is a slow process, and at 37°C the amount of DNA bound increases approximately 10-fold over a period of 30 to 40 min (7). Since, in the system described here, transfection is initiated by the addition of PEG immediately after the DNA is added to the cells, we tested whether or not allowing more time for DNA binding before the addition of PEG would increase the number of transfectants. Figure 6 shows that increasing the time for DNA binding caused a decrease, rather than an increase, in the number of transfectants. Hence, transfection is not improved by using longer binding times. The observed decrease is probably due to the inactivation of L2 DNA by mycoplasma nucleases (28).

Host cell modification and restriction. This transfection system has allowed us to investigate an aspect of host modification and restriction of L2 virus. *A. laidlawii* JA1 and K2 both restrict and modify mycoplasma virus L2 (20; Table 4). Strain K2 contains methylated adenine and cytosine (6), which presumably is the basis of modification in these cells. However, strain JA1 contains no detectable modified bases (6), and the mechanism of modification in these cells is unknown. It is possible that modification in JA1 involves an alteration of the viral envelope rather than of the DNA. This possibility was investigated by comparing the restriction frequencies of infections due to L2 virions with those due to L2 DNA. Table 4 shows similar restrictions by K2 of L2 virions propagated on JA1 cells and of DNA from those virions and by JA1 of L2 virions propagated on K2 cells and of DNA from these virions. Therefore, the possibility that JA1 modification is due to a viral membrane effect can be eliminated. JA1 somehow modifies L2 DNA so that it is restricted by K2.

DISCUSSION

The most important result of this work is the demonstration that the PEG-mediated method of genetic exchange used for gram-positive protoplasts can also be applied to the mycoplasma *A. laidlawii*. In the system described here, the transfection of *A. laidlawii* with L2 DNA occurred in all phases of cell growth; however, transfection was most efficient when exponentially growing cells were used. Hence, some physiological component to the transfection of mycoplasmas that varies with cell growth must be present.

Once L2 viral DNA gains entry into the cells, the viral growth curve is essentially the same as that observed in cells infected with intact virus (29). This indicates that DNA uptake occurs

TABLE 2. Effect of a single restriction endonuclease cleavage on transfection activity of L2 DNA[a]

Treatment	Infectious centers/ transfection mixture (% of maximum)
None	2.5×10^4 (100)
*Bst*EII	1.5×10^3 (6.2)
*Sst*I	1.4×10^2 (0.6)
*Cfo*I	6.5×10^1 (0.3)

[a] The enzymatic treatment of DNA was performed as indicated in the text. Restriction endonuclease-digested DNA was used directly in transfection experiments. The transfection mixture contained 3 µg of L2 DNA (50 µl), 1.3×10^8 CFU (250 µl) of strain K2, and 2 ml of PEG solution, giving a final concentration of 36% PEG.

TABLE 3. Transfection using a constant saturating amount of superhelical L2 DNA and various amounts of linear L2 DNA[a]

Amt of linear L2 DNA (μg)	Infectious centers/ transfection mixture ($\times 10^4$)
0	4.0 (100)[b]
0.5	3.0 (75)
1.1	2.6 (65)
5.3	2.2 (55)

[a] L2 DNA was treated with *CfoI* as described in the text. The reaction mixture was then heated to 65°C for 1 h to inactivate the enzyme. The indicated amounts of cleaved DNA were added to 12 μg of untreated L2 DNA (sufficient to saturate the dose-response curve), and transfection was performed by the addition of the DNA (50 μl of total volume) to 1.4×10^8 CFU (250 μl) of strain K2. Before transfection, a portion of each mixed DNA sample was run on an agarose gel to confirm that there was no residual *CfoI* activity which would have converted superhelical L2 DNA to the linear form.

[b] The percentage of the maximum is given in parentheses.

rapidly after PEG addition. The latent period of one-step growth after PEG-dependent L2 transfection is the same as (or perhaps slightly shorter than) that for L2 infection, and the virus yield for transfection is similar to that for infection (Fig. 3). This is the reverse of the situation with other phages—where the latent period for transfection is significantly longer than that for infection, and the virus yield for transfection is less than for infection—e.g., mycoplasma virus L1 transfection of *A. laidlawii* (22), *B. subtilis* phage transfection of naturally competent cells (32), and PEG-mediated transfection of *Streptomyces lividans* 66 protoplasts (15) and *B. subtilis* L-forms (34).

The DNA transfection dose-response curve is linear, with a slope of 1 (Fig. 4), which means that transfection is due to single L2 DNA molecules. This is in agreement with the results for *B. subtilis*, where it was found that plasmid DNA molecules were not damaged during PEG-dependent uptake into protoplasts (5). However, other transfection systems have dose-response curves with slopes greater than 1, indicative of several (perhaps damaged) DNA molecules having to interact to produce an infection (32).

The DNA transfection dose-response curve (Fig. 4) shows that the efficiency of L2 transfection is about 1 transfectant per 10^7 L2 DNA molecules. This is low, but within the range reported for other phage transfection systems (1, 22).

At saturating L2 DNA concentrations, the optimal transfection frequencies were about 10^{-4} transfectants per CFU (Fig. 2). This contrasts markedly with the frequencies of PEG-mediated plasmid transformation in *Streptomyces* sp. and *B. subtilis* where up to 20% (2) and 80% (3), respectively, of protoplasts could be transformed. However, PEG-mediated plasmid transformation in other *Bacillus* species (12, 25) and PEG-mediated transfection in *Streptococcus* sp. (9) are several orders of magnitude less efficient than this. The transfection of *Streptomyces* protoplasts by bacteriophage DNA is also inefficient, and Suarez and Chater (31) have presented evidence for the presence of a minor subpopulation of protoplasts that are competent for transfection in this system. It is possible that a similar subpopulation of cells exists in *A. laidlawii* transfection. However, since all *A. laidlawii* clones retain the low transfection frequency (Table 4), a genetically determined competent subpopulation of cells can be ruled out. The possibility that there is a physiologically determined competent subpopulation cannot be eliminated.

The optimal concentration of PEG for the efficient transformation and transfection of *Streptomyces* protoplasts is 20% (14). For *B. subtilis* transformation, concentrations of from 20 to 30% PEG have been used (14). Higher concentrations of PEG in these organisms promote protoplast fusion, and therefore, it has been suggested that protoplast transformation does not involve a protoplast fusion mechanism (14). The *A. laidlawii* transfection system described here has an optimum PEG concentration of 36%, a PEG concentration which promotes

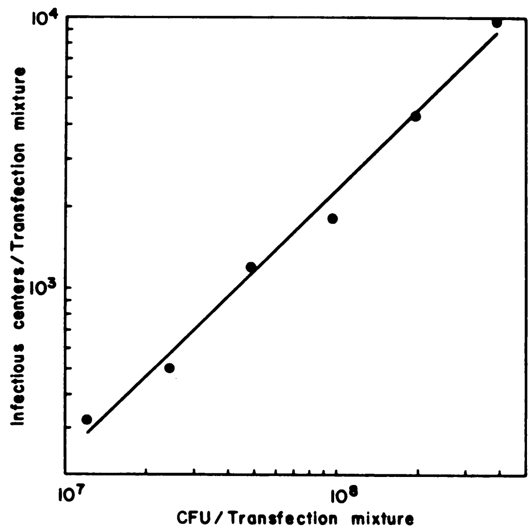

FIG. 5. Transfection and cell number. Transfection was performed by the addition of 10 μg of L2 DNA (50 μl) to the indicated numbers of CFU (in 250 μl) of strain K2. The slope of the curve is 0.99.

fusion in both *Streptomyces* and *Bacillus* spp. However, this system does not involve cell fusion, since the cell concentration-transfection (dose-response) curve (Fig. 5) has a slope of 1, ruling out a multicell model for DNA uptake.

Table 2 shows that the treatment of the L2 genome with restriction endonucleases that cleave the DNA molecule once decreased the number of transfectants to less than 10% of the number obtained with untreated DNA. Possible explanations for this are that the cleavage of the circular genome destroys the biological activity of the DNA or that linear DNA is more sensitive to cellular nucleases than are circular molecules. The trivial explanation, that a small number of molecules escaped cleavage by the enzymes, cannot be ruled out even though no uncleaved forms were visible when the treated DNA was analyzed by agarose gel electrophoresis. Linear molecules compete efficiently with superhelical DNA in this transfection system (Table 3).

The data in Table 4 show that the modification and restriction of L2 DNA do occur in transfection. The values shown are similar to those for modification and restriction in infection with intact virus (6, 20, 26). It is concluded that the modification and restriction that occur in infection with L2 virus are mediated at the DNA level. The data do not exclude a component of the modification and restriction system which could act at the level of the viral and cellular membrane. However, because of the similar magnitude of the restriction values for transfection and infection, any membrane interaction contribution to modification/restriction must be minor if it does occur. It is interesting that modification and restriction occur with L2 DNA from virus grown on strain JA1, since no biochemical basis for the modification of this DNA has been found (6).

TABLE 4. Comparison of restriction frequencies of L2 virions and L2 DNAs[a]

Infection with:	Relative PFU on strain:	
	JA1	K2
L2 · JA1 virions	1.0	0.007
L2 · JA1 DNA	1.0	0.011
L2 · K2 virions	0.016	1.0
L2 · K2 DNA	0.048	1.0

[a] L2 · JA1 virions were propagated on strain JA1 cells, and L2 · JA1 DNA was isolated from these virions. L2 · K2 virions were propagated on strain K2 cells, and L2 · K2 DNA was isolated from these virions. Transfection was carried out by the addition of 5 μg of DNA (50 μl) to either 9.1×10^7 CFU of JA1 or 8.2×10^7 CFU (250 μl) of strain K2. For each infection, the data are normalized to the original host: the titer of L2 · JA1 virions on JA1 was 3.7×10^{10} PFU/ml, that of L2 · JA1 DNA on JA1 cells was 6.7×10^3 infectious centers per ml, that of L2 · K2 virions on K2 cells was 7.0×10^{10} PFU/ml, and that of L2 · K2 DNA on K2 cells was 1.2×10^4 infectious centers per ml.

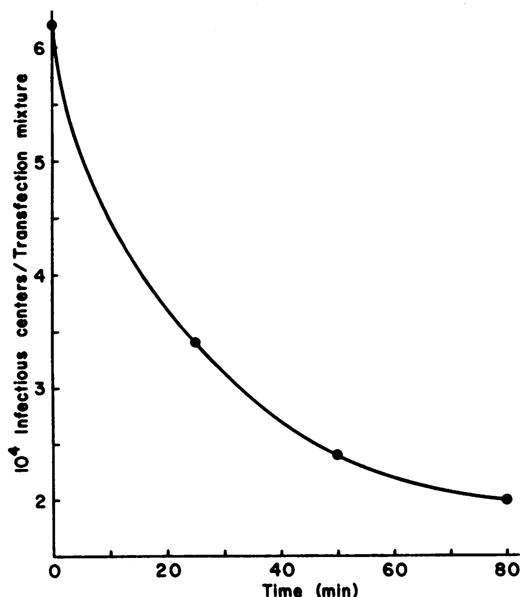

FIG. 6. DNA binding and transfection. L2 DNA (19 μg in 50 μl) was incubated with 1.8×10^8 CFU (250 μl) of strain K2 at 37°C for the indicated times before transfection was initiated by the addition of 2 ml of PEG solution.

In conclusion, the data in this paper show that the PEG method of genetic exchange can be applied to *A. laidlawii* to cause transfection with L2 DNA. We have already used the transfection technique to isolate conditional lethal mutants of L2 after the treatment of viral DNA with mutagens (T. Sladek and J. Maniloff, unpublished data). This technique can be applied to develop systems of genetic exchange in other species of mycoplasmas, a group of organisms that, as a whole, have not been studied genetically, and to introduce macromolecules other than DNA into these cells (36). The inefficiency of transfection in the *A. laidlawii* system, as in some other systems of PEG-mediated transformation and transfection, is perplexing and because of the simplicity of the *A. laidlawii* system (lack of the necessity of removing and regenerating cell walls), the system described here may be a good model for ascertaining why only a small proportion of cells are transfected and for studying the mechanism of action of PEG.

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