Transformation of bacteria by electroporation

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The introduction of DNA into bacteria by transformation is an essential step in the construction of recombinant strains. Recently, electroporation, or electropermeabilization, in which a brief high voltage electric discharge is used to render cells permeable to DNA, has revolutionized the transformation of bacteria. The technique is fast, simple, reproducible, frequently gives very high transformation frequency and appears to be applicable to a wide range of bacterial types previously thought untransformable. The technique can also offer advantages for transformable bacteria such as *Escherichia coli*.

Both the biotechnology industry and academic molecular biologists depend heavily on recombinant DNA technology. The power to clone genes and introduce them into novel hosts is derived from three technical capabilities: (1) the discovery and introduction into the marketplace of restriction endonucleases that cleave DNA at specific sites; (2) the commercial availability of DNA ligases with which recombinant DNA molecules can be constructed *in vitro*; and (3) the development of techniques with which recombinant DNA molecules can be introduced into target organisms by transformation. The first two technologies are available for application to all research and development in molecular biology.

However, there is a need to apply recombinant DNA technology to a range of new and different bacteria, possibly with novel and exciting properties and potential. Researchers will require a transformation system for each new bacterium they wish to manipulate genetically. We were interested in transforming the lactic acid bacteria with which we work. Although they are of economic significance, until recently many strains simply could not be transformed[1–3].

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Transformation: natural and induced

Bacterial transformation was first described by Griffith[4] in 1928, years before DNA was recognized as the genetic material. Griffith observed that mixing killed cells of 'S' (smooth colonial morphology) pathogenic pneumococci with live cells of a non-pathogenic 'R' (rough colonial morphology) strain could cause a permanent 'transformation' of the rough strain to the virulent S cell type. It was subsequently recognized that the transforming principle was DNA which was taken up by the R-type cells which were naturally competent (i.e. they could take up DNA and thus acquire new genetic traits)[5].

Members of at least 15 genera of bacteria are naturally competent; current knowledge of the natural DNA uptake process has been reviewed[5,6]. Few natural transformation systems have been described. It is not known if this is because the appropriate conditions for transformation have yet to be found, or because only a limited number of bacterial species are naturally competent. What is known is that specific genes determining competence must be present and expressed for natural transformation to occur. Even when a natural transformation system exists, its use to engineer bacteria can have drawbacks[3].

Without natural DNA uptake, researchers must seek alternative means of getting DNA into target cells in order to perform genetic manipulation (for review see Ref. 3). In some cases the task can be simple; e.g. the induction of 'pseudocompetence' in *Escherichia coli* by pretreatment of cells with Ca^{2+} or other divalent metal ions[7,8]. In other cases, extreme measures must be taken. One approach, used first for the transformation of certain streptomycetes[9] and bacilli[10], is to remove the bacterial cell wall, which is thought to present a barrier to DNA, with lytic enzymes. Transforming DNA can then be introduced into the resulting protoplasts by the action of a fusant such as polyethylene glycol. Walls are induced to re-form on protoplasts by growing them on regeneration media. Such methods tend to take time to develop and perform; usually they are restricted in scope to the specific strain for which they were developed.

Until recently, a researcher seeking to transform a novel bacterial strain was forced into either a needle-in-the-haystack hunt for a natural transformation system or the painful (and not always successful) labor of inducing competence artificially or establishing a protoplast transformation system. Recently, the situation has changed radically. This review will focus on a new and exciting technique, electrotransformation (transformation by electroporation) of bacteria, which could greatly expand the number of strains and species that can be transformed, and which can also be used to increase the efficiency (i.e. transformants per μg DNA) and frequency (i.e. proportion of cells transformed) of transformation of strains for which transformation systems are available.

What is electroporation?

A number of physical and biological changes take place in cells that are exposed to electric fields. These changes were first investigated by Neumann and Rosenheck[11] in 1972 and then by Zimmermann *et al.* in 1973 in the course of studies on the effect of electric pulses on cell membranes[12]. At low electric field strengths the membranes of the cells become polarized (Fig. 1). When the changes in electric-field-induced membrane potential reach a critical

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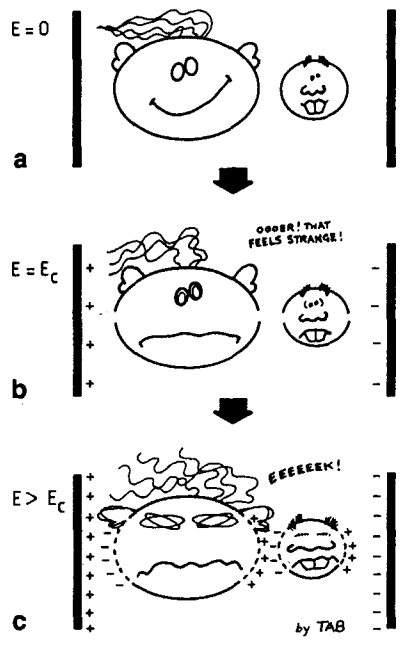
value in the range 200–400 mV, areas of reversible local disorganization and transient breakdown occur[13–15], making the membrane permeable to molecules and macromolecules. Both molecular influx and efflux have been observed[13,14]. Cells in this state (Fig. 1, second panel) may be thought of as truly 'electropermeabilized'. There is evidence, however, that the permeabilization to small molecules requires a lower threshold voltage than is required for permeabilization to macromolecules (J. Teissie *et al.* unpublished). There is evidence that the lipid matrix can be considered as the locus of permeabilization[15] and it has been postulated that lipid–protein junctions may be sites for electropermeabilization.

Because 'pore' formation in eukaryotic cells was postulated to explain effects observed during their exposure to electrical fields of critical strenth[16], the process of introducing macromolecules such as transforming DNA has come to be known as electroporation. It would probably be more proper to refer to the process as 'electrotransformation' or 'transformation by electropermeabilization'; a shift towards this more accurate terminology may be afoot.

Whatever the nature of the membrane disturbance, it seems that DNA can enter bacteria at specific transient loci in the membrane and bring about transformation. Other events are also associated with exposure of cells to electric fields. For example, net ATP synthesis occurs in *Escherichia coli* cells subjected to brief high intensity electric pulses by the establishment of an electrochemical gradient that drives formation of a chemical bond by the respiratory chain[17]. Another technique, electrofusion, depends on the induction of membrane depolarization by electric fields[18,19]. In electrofusion heterologous cells can be fused by a process of intercellular, rather than intracellular, membrane 'healing'. As pointed out by Zimmermann[18], electrical permeabilization had great potential in the introduction of molecules into cells. It should be noted that electrofusion has been used to fuse bacterial protoplasts[20,21].

Above a critical voltage, the cell

Fig. 1



Electropermeabilization of cells. (Reprinted from Ref. 18 with permission.)

membrane is irreversibly damaged by electric fields: the state of disorganization of the membrane (Fig. 1, third panel) may become too great to repair or reverse. This or excessive loss of cellular contents may cause cell death. Thus electropermeabilization of cells will occur above a lower specific critical threshold voltage and below a critical lethal voltage. In practice it has been observed that both of these critical parameters are genus, species and strain dependent.

Electroporation was successfully used to introduce DNA first into mouse myeloma cells[22] and subsequently into mouse lymphocytes[23], *Bacillus cereus* protoplasts[24], yeasts[25] and plant protoplasts[26]. Electroporation is now established as a technique for eukaryote cell biologists. For a number of reasons the prokaryotes presented a greater challenge that has only recently been overcome.

Apparatus used for electroporation of bacteria

The electroporation of eukaryotic cells can be accomplished with millisecond pulses of a few hundred to a few thousand volts per cm in amplitude. These conditions impose a potential gradient of ~1 V across cells of 10 μm diameter (or more), although the gradient is not uniform across the cell surface. At first glance, imposing a 1 V gradient across a microbial cell would seem to be a simple task. However, to create a potential gradient of 1 V across the ~1 μm diameter of a hypothetical bacterial cell, a field strength of 10 000 V cm^{-1} should be generated[27] for periods as long as 10 ms. Such high voltages usually make the power supplies used for bacterial electroporation more complex and costly than those used for higher cells. Both US and European manufacturers offer units ranging in price from $1000 to as much as $10 000; the most successful supply sells for about $2500 in the US.

The various machines differ in their effectiveness and in the features they offer, yet most are similar in basic design. Commonly a circuit charges a capacitor which is then discharged through a cell suspension which rests in a sample chamber between anode and cathode. Controls to vary the voltage and the duration of the pulse simultaneously should be provided. Power supplies producing square or sinusoidal waves are also available. However, exponentially decaying pulses work well and they are most often used.

Several sample cell designs have emerged independently. Some manufacturers provide individually wrapped, sterile disposable cuvettes; other designs employ reusable chambers with steel or platinum electrodes that can be cleaned and sterilized between each use. Most designers attempt to reduce the spacing between the electrodes to 0.05–0.4 cm. As the gap between the electrodes is made smaller, the field strength in V cm^{-1} increases for a given power supply voltage setting and the voltage requirements placed on power supply design are reduced.

The technique is easy to perform (Fig. 2). Often, the electroporation process itself takes only a few minutes for a dozen experiments.

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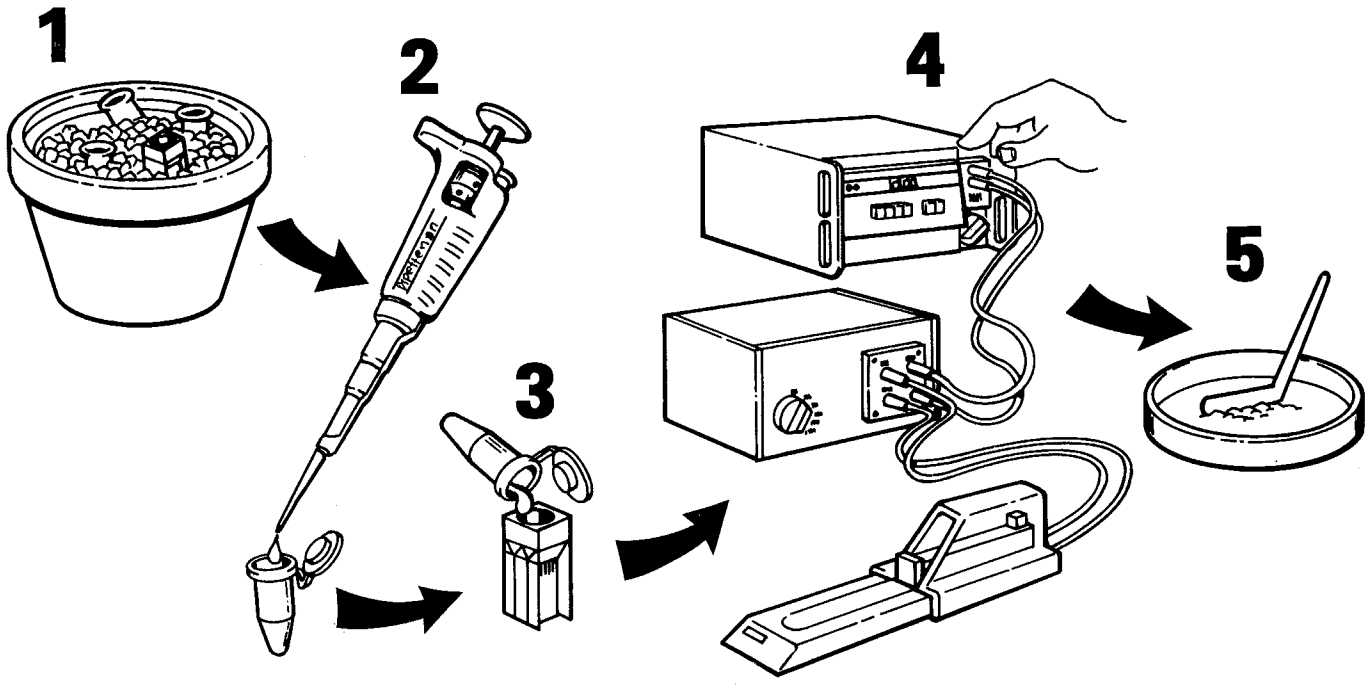
The first application of electroporation to bacteria *(Bacillus cereus)*[24]

Fig. 2



Apparatus used for electroporation of bacteria. Cells are cultured, harvested, washed free of media components and resuspended in water, or a low conductivity buffer. The resuspension solution might contain an osmotic stabilizer, most often sucrose. An aliquot of cells is placed on ice and chilled for ten minutes (1). DNA is added (2) and the cells are immediately placed in a chilled sample cuvette (3) and positioned in a shielded sample holder where the high voltage pulse is delivered (4). With the apparatus depicted here, the voltage and capacitance are set on the primary power supply. The auxiliary resistance network interposed between the supply and the sample cell allows the time constant to be varied independently of the voltage. Immediately after the pulse is applied, the cell suspension is removed from the cuvette and diluted into growth medium. In some cases, transformed cells may be plated directly on selective media (5), while in other cases it is necessary to incubate the cells for one to four hours in growth medium prior to plating to obtain good transformation efficiency or frequency.

did not offer any particular advantage over other methods since protoplasts had to be prepared and regenerated. The transformation of whole bacterial cells by electroporation would require DNA molecules to permeate the intact wall. Conventional wisdom argued against this possibility. The lack of power supplies capable of delivering the sustained high voltage pulses theoretically required for electroporation of bacteria may also have deterred researchers.

However, in 1985, Harlander presented the first preliminary data on the successful electroporation of whole cells of *Lactococcus lactis*[28] at the Streptococcal Genetics meeting in Miami, USA. At that time, one of us (BC) collaborated with Harlander to apply electrofusion and electroporation to lactic acid bacteria. Neither technique could be extended to the bacteria with which we worked. The promising results with *L. lactis* were inconsistent and difficult to repeat; but the promise was there and Harlander's results inspired others to pursue the possibility of bacterial electrotransformation. The inappropriate design and unsatisfactory performance of the power supplies then available probably hindered the early attempts.

In the following year, Bill Dower at Bio-Rad Laboratories obtained efficient high frequency transformation of *E. coli*. Already a manufacturer of an electroporation supply useful for eukaryotic cells, Bio-Rad claimed that year that efficiencies of 10^6 transformants per μg of DNA could be achieved through electrotransformation of whole untreated *E. coli* cells. Upon reading the Bio-Rad publicity, we and numerous other investigators showed a renewed interest in electroporation of whole cells. The advertised findings were subsequently published[29].

A method for the electroporation of protoplasts of *Streptomyces lividans* appeared in 1987 (Ref. 30). At about the same time, two of us (BC and JF) reported[31] high frequency transformation of untreated cells of the Gram-positive bacterium, *Lactobacillus casei*. A similar protocol was successful with *Streptococcus thermophilus*[32] and *L. lactis* (AM *et al.*, unpublished). Word spread quickly that untreated bacterial cells could be transformed by electroporation. The barrier presented by the formidable bacterial cell wall had seemingly vanished.

Table 1 presents a list of bacteria that have been electroporated. A large number of workers have very quickly helped to establish electroporation as a method with broad applicability. New reports are

appearing at such a rapid rate that the list presented in Table 1 is probably incomplete. Transforming a particular bacterium by this technique is no longer in itself remarkable. Papers have appeared that describe the testing of every strain in the authors' culture collection, but these reveal little about the mechanisms of, and barriers to, electroporation. However, as will be seen, the conditions required for efficient electroporation of a selected strain may be quite specific and much research into the underlying mechanisms of electrotransformation remains to be done.

Table 1

Bacterial strains that can be transformed by electroporation.

Organism	Ref.
Bacillus anthracis	G. Dunny (pers. commun.)
Bacillus cereus	33, 34
Bacillus subtilis	B. Beliveau and J. Trevors (pers. commun.), B. Karan-Tamir and M. Zukowski (pers. commun.), J. F. Viret (pers. commun.)
Bacillus licheniformis	B. Karan-Tamir and M. Zukowski (pers. commun.)
Bacillus thuringiensis	G. Dunny (pers. commun.)
Bordetella pertussis	J. Miller (pers. commun.)
Brevibacterium lactofermentum	J. Flickinger (unpublished), S. Bonnassi and M. Sicard (pers. commun.)
Campylobacter jejuni	35
Campylobacter coli	35
Clostridium perfringens	36
Corynebacterium glutamicum	H. Wolff and E. Neumann (unpublished)
Cyanobacteria spp.	A. Grossman (pers. commun.)
Enterococcus faecalis	33, 34, 37
Enterococcus faecium	M. Solioz and A. Mercenier (unpublished)
Erwinia stewartii	D. Coplin (pers. commun.)
Erwinia carotovora subsp. *carotovora*	M. Mount (pers. commun.)
Erwinia herbicola	M. Mount (pers. commun.)
Escherichia coli	28, 29, 37–41
Hemophilus pleuropneumoniae	G. Lalond *et al.* (unpublished)
Klebsiella pneumoniae	V. Piantanida and F. Bayliss (pers. commun.)
Listeria monocytogenes	D. Hoffman and F. Bayliss (pers. commun.)
Lactobacillus spp.	31, 33, 34, 39
Leuconostoc dextranicum	34
Leuconostoc cremoris	W. de Vos (pers. commun.)
Mycobacterium leprae	W. Jacobs (pers. commun.)
Mycobacterium smegmatis	B. Giquel-Sanzay (pers. commun.)
Myxococcus xanthus	J. Rodriguez *et al.* (pers. commun.)
Pediococcus acidilactici	34
Propionibacterium jensenii	34
Pseudomonas putida	37
Rhizobium meliloti	W. Dower *et al.* (pers. commun.)
Salmonella typhimurium	39, 40
Streptococcus spp. (including *lactococcus*)	28, 32–34, 37, 39, 42, 43
Staphylococcus aureus	33, 34, M. Sicard and A. Mercenier

What are the critical factors?

While the phenomenon of electrotransformation of bacteria is relatively new, it may be useful to examine some of the factors and parameters that contribute to successful introduction of DNA into a bacterial strain that is not naturally competent. Since most investigators have used power supplies that deliver exponentially decaying pulses for electrotransformation, the discussion will be largely restricted to results obtained with such systems. Some of the observations, however, will probably hold true for all systems. For example, transformation efficiencies of greater than 10^6 transformants per μg of DNA are obtainable using a square-wave generator (J. Teissié *et al.*, pers. commun.).

In attempting to establish an electrotransformation system for an 'untransformable' bacterium one usually starts by following the manufacturers' suggested protocols for bacterial electropermeabilization. The effects of altering buffers, voltages and time constants are explored. This frequently gives positive results; but how does one proceed if no transformants are observed, or if the frequencies or efficiencies are unacceptably low?

The most obvious parameters controlling electropermeation are the voltage applied and the time constant of the pulse. Typically, field strength values fall in the range of 6–12 kV cm^{-1}, but each bacterium displays its own unique optimum voltage. For example, some strains of *Lactobacillus bulgaricus* are extremely sensitive to electric fields – 50% of the population fails to survive 2.5 kV cm^{-1}; optimal transformation occurs at ~2 kV cm^{-1} (B. Boizet and BC, unpublished). In contrast, some strains of *S. thermophilus* are best transformed at voltages above 10 kV cm^{-1} (AM *et al.*, unpublished). *E. coli* cells can withstand 12 kV cm^{-1} with almost no cell death; they are also maximally transformable at this voltage. It can be assumed that the critical voltage varies with membrane composition, cell diameter and cell-wall characteristics.

The percentage of the population that survives the electric discharge decreases as the voltage increases.

Usually some loss of viable cells occurs at the voltage found most effective for transformation; however, the extent of this loss varies so widely that there is no simple rule of thumb governing how much cell death is optimal. Cell death during electroporation may result from irreversible membrane damage and the loss of intracellular components including plasmid DNA[41].

The electroporation medium can play a large role in determining efficiency. Concentrated sucrose is commonly added to the electroporation medium; sucrose is frequently used to stabilize protoplasts although there is no evidence that it acts in this way in electroporation media. Growth of electroporated cells on regeneration media or osmotically stabilized media neither increases the recovery of viable cells nor improves the transformation frequency. In some cases (e.g. *E. coli*), cells previously frozen in 15% glycerol or in sucrose-containing electroporation buffer can be electroporated at the same efficiencies and frequencies as freshly prepared cells. The transformation frequency observed with previously frozen cells of *L. casei* is a half to a third of that observed for freshly cultured cells (BC and JF, unpublished); most of this effect is probably due to loss of viable cells on freezing, although membrane damage cannot be discounted. The ability to use frozen cells affords the investigator an 'off-the-shelf' supply of cells and assures batch to batch uniformity.

A series of voltages must be evaluated to determine the optimum for each strain. Fortunately, this is not difficult experimentally. The choice of time constant is restricted by power supply capacity as well as by the conductivity of the electroporation medium. Decay times of the discharge of 2.5 to 10 msec duration are frequently the most effective. Changes in buffer ions, pH, conductivity, dielectric constant and buffer concentration may effect the time constant as well as the transformation frequency. A variety of electroporation buffers should be evaluated.

Attempts to increase frequency by repetitively pulsing the cuvette are not particularly effective. Cell survival decreases, while the number of transformants remains relatively constant. Some of this effect may be attributed to joule heating; a single high voltage pulse can cause a 15–25°C rise in temperature. Since chilled cells seem to be preferable for electroporation, re-cooling the cells between pulses has also been attempted, but with little effect on frequency[37].

While chilling cells on ice prior to electroporation appears virtually obligatory, it has been found that preincubation of cells with DNA is not necessary. It has been reported that DNA does not bind to the bacterial cells prior to entry[38,41]. Furthermore, preincubation can lead to decreased frequencies if cell-associated nucleases are present[31] (unpublished). Incubation of cells on ice after electroporation, thought to be necessary for eukaryotic cells, is not required for bacteria. In fact, unlike eukaryotic cells, bacterial cells do not appear to be permeable to macromolecules for a significant period of time after electro-shock (BM, JF and AM, unpublished). The addition of DNase one minute after application of the electric pulse does not lower the transformation frequency (J. Teissié *et al.*, unpublished).

Is there an upper size limit for the DNA used for electroporation? This is not yet clear. In the few reports that did consider the size of the DNA, high molecular weight plasmid and phage DNA entered cells as efficiently as small plasmids. A cosmid library prepared in *E. coli* has been transferred to *Salmonella typhimurium* by high frequency electrotransformation (K. Sanderson, pers. commun.).

Is electroporation applicable to every bacterium, and if so, does it work equally well for all bacteria? The answer to both questions is clearly no. The efficiency of transformation for the organisms listed in Table 1 varied from 10^{10} transformants per μg DNA for *E. coli*[38,41] to a single transformant of *Staphylococcus aureus*[34]. Total failures occur as well. The basis for these differences is complex and not completely understood. Failure may stem from choice of DNA, host restriction barriers, the non-replication of plasmids or the non-expression of a marker gene; it is usually difficult to determine the exact cause. The best choice of transforming DNA for a given strain is an indigenous plasmid or phage isolated from an isogenic strain of the same bacterium. For a complete discussion of these types of problem see Ref. 3.

Physical barriers surrounding the cell might also affect frequencies. *Klebsiella pneumoniae* strains that transform poorly by the classical $CaCl_2$ method[7,8] (perhaps because they are surrounded by capsular polysaccharide) can be transformed efficiently by electroporation (V. Piantanida and F. Bayliss, pers. commun.). *Salmonella typhimurium* strains vary greatly in the efficiency by which they can be transformed; mutants deficient in lipopolysaccharide[44], and in particular *gal*E mutants, are often superior transformers when compared with wild type strains. When transformed by electropermeation, most strains behave in a more nearly equivalent manner (K. Sanderson, pers. commun.).

The factors most likely to affect 'electrotransformability' of a bacterium are the membrane composition and the wall thickness, density and structure. The differences between the membranes of various bacteria are (arguably) smaller than the differences between their walls. The wide range of voltages available for electroporation gives one confidence that both a threshold and a critical voltage will be defined for the membrane of almost any bacterium. It is a matter of physics that the imposed electric field will disrupt the membrane structure at the correct voltage. Evidence points to the wall as being at least partly responsible for failures or low frequencies. Gram-positive bacteria have denser and thicker cell walls than Gram-negative bacteria, and, in general, Gram-negative strains electroporate more efficiently.

E. coli transformation frequency increases in a linear manner as the ratio of transforming DNA molecules to cells is raised over a ten thousand-fold range[38]. At the highest DNA

concentrations virtually 100% of the cells that survive are transformed. When the same experiment is performed with *L. casei,* an equivalent linear response is observed at low DNA concentrations; however, saturation is observed when about 1% of the surviving cells are transformed (BC and JF, unpublished). By manipulating growth conditions (see below) the value can be raised to 3–10% transformation of surviving cells before saturation occurs. One possible interpretation of these results is that only a subpopulation of the *L. casei* cells are able to be transformed. We refer to this subpopulation as 'electro-competent'.

Although we believe it derives from cell-to-cell differences in the density or continuity of the cell wall, with some cells presenting a reduced barrier to DNA entry, there is no direct experimental evidence for or understanding of the basis for low electro-competence. No strong correlations have been found between transformation and cell diameter, shape and morphology. Spherical cells with small diameters should require greater field strengths to electroporate than larger rod-shaped cells; the near spherical streptococci generally require higher voltages and give lower frequencies than rod-shaped lactobacilli. Although the evidence is fragmentary, the nature of the cell wall seems to impose the greatest limitation on electrotransformation of bacteria.

To improve frequencies researchers can attempt to determine empirically conditions that increase the proportion of cells in this subpopulation. Growing Gram-positive bacteria in media containing L-threonine or L-lysine[45] weakens the cell wall cross-links and leads to improvements in electroporation frequencies. Growing cells in high concentrations of glycine (>1%) can have a similar effect (G. Dunny, pers. commun.). Treating Gram-positive cells with lytic enzymes to remove or loosen wall structure[46] (and, in the extreme, to prepare protoplasts[30,37]) may also improve transformability. However, attempts to improve the frequency of transformation of *S. thermophilus* in this way have not met with success (AM *et al.,* unpublished).

Fig. 3

The hazards of electroporation.

The use of cell wall synthesis inhibitors, alteration of the composition of growth medium conditions and age of culture may have dramatic but poorly defined effects on efficiency. Optimization of a variety of parameters and conditions can lead to 100–1000-fold improvements in frequency (G. Dunny, pers. commun.).

Unfortunately, there are still some strains that fail to transform and others defy attempts to improve upon the low frequency at which they are transformed by electroporation. Perhaps, when electroporation is better understood, a mechanism for removal of the remaining barriers will become evident.

The future of electroporation

Electroporation is fast becoming the method of choice for the transformation of bacterial cells but it is not universally applicable. It has other drawbacks as well. Dangerous high voltages are required. Occasional arcing through bacterial suspensions can explode sample cuvettes and create bacterial aerosols (Fig. 3). These kinds of technical problem require improvements in both power supply and sample cell design and performance.

In the last year, two very different and apparently significant new developments in electroporation have appeared. One is an extension of present technology: a resistor network was added to an existing power supply to limit current (and thus reduce the potential for arcing) and allow the time constant to be varied[38]. In addition, a new cuvette design reduced the electrode gap to 2 mm (thereby effectively doubling the effective electrical field strength) and permitted volumes as low as 40 μl to be electroporated. This combination improved the transformation of *E. coli* to near theoretical maximum values: 10^9–10^{10} transformants per μg DNA with 80% of the surviving population recovered as transformants. In our hands, the same apparatus and cuvettes improved the efficiency and frequency of transformation of *L. casei* approximately 100-fold (JF and BC, unpublished). These innovations do not always have the same impact: the frequency of transformation observed for *S. thermophilus* is unaffected by the

new equipment (AM *et al.*, unpublished). This is consistent with the hypothesis that only a subfraction of the cells is electro-competent; when all of that population is transformed, higher frequencies cannot be achieved without enhancing the percentage of electro-competent cells.

The second innovation centered around a novel power supply and sample chamber design[41]. The power supply delivers a falling sinusoidal waveform pulse. Using this apparatus, high efficiencies and frequencies of transformation were obtained with *E. coli*.

These, and future, developments will lead to increased acceptance of electroporation as a method. It seems safe to conclude that most Gram-negative and many Gram-positive bacteria can best be transformed by electroporation. There will be exceptions, but as the underlying reasons for failure are determined, the scope of electroporation will grow. It is amazing how quickly researchers have cast off skepticism about the impenetrable barrier presented by the bacterial cell wall and embraced the electroporation technology.

We are now at the stage of investigating why some strains are recalcitrant to electrotransformation. It is a challenging scientific and technical problem. We may learn much about bacterial physiology and structure in the process. At the moment, the bacterial cell wall can still apparently present a formidable barrier to DNA entry.

Although we heralded the electrotransformation method for bacteria as a significant advance, we have emphasized short-comings more than successes. This is because progress will only come when we understand our failures. The reader should note the formidable list of organisms that can be transformed by this method (Table 1). A balanced appraisal justifies the conclusion that electroporation is now the fastest and simplest method for transforming bacteria. It offers the greatest chance of success at transforming a currently untransformable bacterium. This will prompt many to attempt to develop new transformation systems. Compared with the cost of a fruitless search for a transformation system, electroporation is relatively inexpensive. Even an occasional rapid, but inexpensive, failure can be accepted; few methods are truly universal. The high frequencies and efficiencies, and its technical ease, make it an attractive alternative for organisms for which transformation systems already exist. Future developments will only improve the technology.

References

1 Chassy, B. M. (1985) *Trends Biotechnol.* 3, 273–275
2 Chassy, B. M. (1987) *FEMS Microbiol. Rev.* 46, 297–312
3 Mercenier, A. and Chassy, B. M. (1988) *Biochimie* 70, 503–517
4 Griffith, F. (1928) *J. Hyg.* 27, 113–159
5 Stewart, G. J. and Carlson, C. A. (1985) *Annu. Rev. Microbiol.* 40, 211–235
6 Saunders, J. R., Dougherty, A. and Humphreys, G. O. (1984) *Methods Microbiol.* 17, 61–95
7 Mandel, M. and Higa, A. (1970) *J. Mol. Biol.* 53, 159–162
8 Hanahan, D. (1983) *J. Mol. Biol.* 166, 557–580
9 Bibb, M. J., Ward, J. M. and Hopwood, D. A. (1978) *Nature* 274, 398–400
10 Chang, S. and Cohen, S. N. (1979) *Mol. Gen. Genet.* 168, 111–115
11 Neumann, E. and Rosenheck, K. (1972) *J. Membr. Biol.* 10, 279–290
12 Zimmermann, U., Schultz, J. and Pilwat, G. (1973) *Biophys. J.* 13, 1005–1013
13 Knight, D. E. (1981) *Tech. Cell. Physiol.* P113, 1–20
14 Knight, D. E. and Scrutton, M. C. (1986) *Biochem. J.* 234, 497–506
15 Teissié, J. and Tsong, T. Y. (1981) *Biochemistry* 20, 1548–1554
16 Sowers, A. E. and Lieber, M. R. (1986) *FEBS Lett.* 205, 179–184
17 Teissié, J. (1986) *Biochemistry* 25, 368–373
18 Zimmermann, U. (1983) *Trends Biotechnol.* 1, 149–155
19 Zimmermann, U. and Vienken, J. (1982) *Membr. Biol.* 67, 165–182
20 Shivarova, N. *et al.* (1983) *Bioelectrochem. Bioenerg.* 11, 181–185
21 Reed, W. M. (1987) *J. Gen. Appl. Microbiol.* 33, 287–294
22 Neumann, E., Schaefer-Ridder, M., Wang, Y. and Hofschneider, P. H. (1982) *EMBO J.* 1, 841–845
23 Potter, H., Weir, F. and Leder, P. (1984) *Proc. Natl Acad. Sci. USA* 81, 7161–7165
24 Shivarova, N., Förster, W., Jacob, H-E. and Grigorova, R. (1983) *Z. Allg. Mikrobiol.* 23, 595–599
25 Hashimoto, H., Morikawa, H., Yamada, Y. and Kimura, A. (1985) *Appl. Microbiol. Biotechnol.* 21, 336–339
26 Fromm, M., Taylor, L. P. and Walbot, V. (1985) *Proc. Natl Acad. Sci. USA* 82, 5824–5828
27 Bernhardt, J. and Pauly H. (1973) *Biophysik* 10, 89–98
28 Harlander, S. K. (1986) in *Streptococcal Genetics* (Ferretti, J. J. and Curtiss, R. C., eds), pp. 229–233, ASM Publications, Washington DC
29 Dower, W. J. (1987) *Mol. Biol. Rep.* (Bio-Rad Laboratories) 1, 5
30 MacNeil, D. J. (1987) *FEMS Microbiol. Lett.* 42, 239–244
31 Chassy, B. M. and Flickinger, J. L. (1987) *FEMS Microbiol. Lett.* 44, 173–177
32 Somkuti, G. A. and Steinberg, D. H. (1987) *Proceedings of the 4th European Congress on Biotechnology* Vol. 1 (Neijssel, O. M., van der Meer, R. R. and Luyben, K. C. A. M., eds), p. 412, Elsevier
33 Muriana, P. M., Luchansky, J. B. and Klaenhammer, T. R. (1988) *American Society of Microbiology 88th Annual Meeting, Miami,* Abstract H-64, p. 155
34 Luchansky, J. B., Muriana, P. M. and Klaenhammer, T. R. *Mol. Microbiol.* (in press)
35 Miller, J., Dower, W. and Tompkins, L. S. (1988) *Proc. Natl Acad. Sci. USA* 85, 856–860
36 Allen, S. and Blaschek, H. (1988) *American Society of Microbiology 88th Annual Meeting, Miami,* Abstract H-57, p. 154
37 Fiedler, S. and Wirth, R. (1988) *Anal. Biochem.* 170, 38–44
38 Dower, W. J., Miller, J. F. and Ragsdale, C. W. (1988) *Nucleic Acids Res.* 16, 6127–6145
39 Dahlman, D. and Harlander, S. (1988) *American Society of Microbiology 88th Annual Meeting, Miami,* Abstract H-63, p. 155
40 Taketo, A. (1988) *Biochim. Biophys. Acta* 949, 318–324
41 Calvin, N. M. and Hanawalt, P. C. (1988) *J. Bacteriol.* 170, 2796–2801
42 van der Lelie, D., van der Vossen, J. M. B. M. and Venema, G. (1988) *Appl. Environ. Microbiol.* 54, 865–871
43 Somkuti, G. A. and Steinberg, D. H. (1988) *Biochimie* 70, 579–585
44 MacLachan, P. R. and Sanderson, K. (1985) *J. Bacteriol.* 161, 442–445
45 Chassy, B. M. and Giuffrida, A. (1980) *Appl. Environ. Microbiol.* 39, 153–158
46 Powell, I. B., Achen, M. G., Hillier, A. J. and Davidson, B. E. (1988) *Appl. Environ. Microbiol.* 54, 655–660