Biochimica et Biophysica Acta 819 (1985) 105–113
Elsevier

BBA 72732

Fusion of bacterial spheroplasts by electric fields

Hans-Jürgen Ruthe and Julius Adler

Departments of Biochemistry and Genetics, University of Wisconsin-Madison, Madison, WI, 53706 (U.S.A.)

(Received March 20th, 1985)

Key words: Membrane fusion; Electrofusion; Dielectrophoresis; Spheroplast; Cell fusion; (*E. coli, S. typhimurium*)

Spheroplasts of *Escherichia coli* or *Salmonella typhimurium* were found to fuse in an electric field. We employed the fusion method developed by Zimmermann and Scheurich (1981): Close membrane contact between cells is established by dielectrophoresis (formation of chains of cells by an a.c. field), then membrane fusion is induced by the application of short pulses of direct current. Under optimum conditions the fusion yield was routinely 90%. Fusable spheroplasts were obtained by first growing filamentous bacteria in the presence of cephalexin, then converting these to spheroplasts by the use of lysozyme. The fusion products were viable and regenerated to the regular bacterial form. Fusion of genetically different spheroplasts resulted in strains of bacteria possessing a combination of genetic markers. Fusion could not be achieved with spheroplasts obtained by growing the cells in the presence of penicillin or by using lysozyme on bacteria of usual size.

Introduction

Cell fusion has proven to be a valuable tool in genetic engineering. Fusion by chemical means has led to new strains of bacteria [1,2], hybrid mammalian cell lines [3,4] and hybrid plants [5,6]. Cell fusion was introduced as early as 1909 by Küster [7], but even today standard chemical fusion techniques exhibit limitations like low selectivity for wanted fusion products, poor control of the fusion process, considerably varying fusion conditions, and low viability due to the chemical fusogens.

To avoid the shortcomings of chemical methods an electrical fusion technique has been developed by Zimmerman and co-workers (see Refs. 8, 9, for reviews). This method was originally used to fuse plant protoplasts [10,11]. Cells are brought into tight membrane contact in an alternating electric field of high frequency [12,13] and then they are fused by short direct current pulses of high intensity [14]. Chemical fusogens are not involved, the number and types of cells to be fused can be preselected [15], the fusion is rapid and synchronous, and cell fusion can be observed under the microscope to identify and withdraw individual fusion products [8].

Whereas the fusion of plant protoplasts [16] or mammalian cells [17] is well established in Zimmermann's method, and protoplasts of Gram-positive bacteria (*Bacillus thurigensis*) have been fused after agglutination with poly(ethylene glycol) and subsequent application of electric field pulses [18], the electrofusion of spheroplasts of Gram-negative bacteria has not been demonstrated.

We report here how spheroplasts of *Escherichia coli* or *Salmonella typhimurium* can be fused effectively employing Zimmermann's method. We show that the fusion products are viable and capable of reverting to the bacterial form.

Materials and Methods

Chemicals and media. Sucrose (Reagent, A.C.S.) came from the MCB Manufacturing Chemists, Inc. Lysozyme, deoxyribonuclease I, cephalexin,

kanamycin sulfate, and tetracycline hydrochloride were purchased from Sigma Chemical Co.

Luria-Bertani (LB) medium contained 1% Difco Bacto Tryptone, 0.5% Difco Bacto yeast extract, and 1% NaCl; it was autoclaved for sterility. Reversion medium was 23% (w/v) sucrose/2% (w/v) Difco Bacto Antibiotic Medium 3/20 mM $MgCl_2$/0.1 mM $CaCl_2$; this solution was filtered for sterility. Selection plates for *Escherichia coli* were made from Luria-Bertani medium plus 1.3% Difco Bacto Agar, kanamycin (50 μg/ml) and tetracycline (14 μg/ml). Selection plates for *Salmonella typhimurium* were made from Vogel-Bonner medium [19]. They contained 0.2% citric acid as a sole carbon source; kanamycin (80 μg/ml) was added.

Cell fusion apparatus. The alignment field (alternating current) and the fusion pulses (square waves of direct current) were generated by a Zimmermann Cell Fusion System, purchased from the GCA Corporation, Chicago. To avoid contamination a closed fusion chamber was constructed by use of a 200 μm open fusion chamber (N. Z1200) from the GCA Corporation (see Fig. 1).

Bacterial strains. E. coli AW405 [20], *E. coli* DH1 [21], and *S. typhimurium* TH32 [22] have been described previously.

Plasmids. The plasmids RSF 1010 Tn5 [23] carrying kanamycin resistance and pR Z 341 [23] carrying tetracycline resistance were used to transform *E. coli* DH1.

Growth of bacteria and preparation of giant spheroplasts. Cells were grown in Luria-Bertani medium by shaking at 35°C overnight to stationary phase. Then they were diluted 1 : 100 in Luria-Bertani medium and grown at 35°C with shaking to an A_{590} of 0.5.

Three ml of these cells were diluted to 30 ml in Luria-Bertani medium. To grow long filamentous cells, cephalexin (60 μg/ml) was added [24,25] and the culture was grown with shaking at 42°C for 2 h [26]. Cells were harvested by centrifugation at 1500 × *g* at 4°C. The pellet was washed once in 1 ml of 0.8 M sucrose solution and suspended in 2.5 ml of the same solution.

Spheroplasts from filamentous cells were formed [27] by adding the reagents listed at their final concentrations in the following order [28,29]: Tris buffer pH 7.8 (50 mM), lysozyme (100 μg/ml for *E. coli*, 200 μg/ml for *S. typhimurium*), DNAase (50 μg/ml), and EDTA (4 mM) to a final volume of 2.8 ml. (The final sucrose concentration was 0.7 M.) After 6 min at room temperature spheroplast formation had initiated. 1 ml of a solution of 20 mM $MgCl_2$/0.7 M sucrose was then added (locally high concentrations of $MgCl_2$ should be prevented) to stabilize the spheroplasts and to activate the DNAase. After 4 more minutes of incubation at room temperature spheroplast formation was completed.

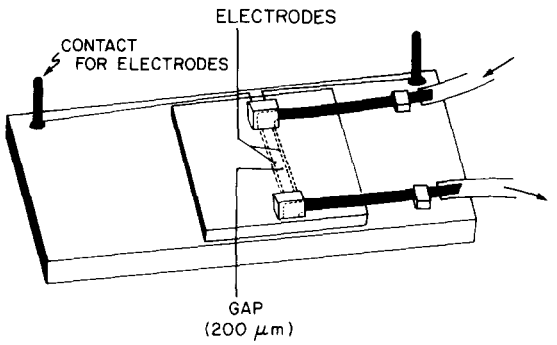

Fig. 1. Diagram of a closed fusion chamber. As a cover slip a thin (0.5 mm) piece of plastic was glued with silicone rubber over the 200 μm gap of an open fusion chamber (No. Z1200 from the GCA Corporation). Two openings were connected to two thin metal pipes (injection needles, 0.75 mm outer diameter, drawn in black) which fitted to polyethylene tubing and served as inlet or outlet for the fusion medium. The dimensions of the chamber are 2.5 × 7.6 cm, the gap between the electrodes (200 μm) is not drawn to scale.

This cell suspension (1.5 ml) was layered over 7 ml of a solution of 0.8 M sucrose/0.5 mM $MgCl_2$ and centrifuged at 1000 × *g* in a swinging bucket rotor for 5 min at 4°C to transfer the spheroplasts gently into a low ionic medium and to separate them from slower sedimenting debris of cells which lysed during either filament or spheroplast formation. After centrifugation the supernatant was removed with a Pasteur pipette to avoid any ionic contamination of the sedimented spheroplasts. Spheroplasts were then resuspended in the desired fusion medium (usually 0.8 M sucrose/1 mM $MgCl_2$), to $3 \cdot 10^7$ cells/ml (determined in a Petroff-Hausser Bacteria Counter) and stored on ice. If viability of the fusion products was desired, storage on ice did not exceed 60 min.

Cell fusion procedure. 25 μl of the final cell

suspension (see previous section) were placed on an open fusion chamber and covered with a cover slip. If sterile conditions were desired, cells were injected into a closed fusion chamber (Fig. 1) with a syringe. The alignment field and the fusion pulses were then applied. After cell fusion was observed under the microscope, the alignment field was gradually (5 s) turned off. The fused cells were then replaced with unfused ones by injecting new cell suspension. This was repeated until 20 μl of cell suspension had passed through the fusion chamber.

Quantitation of fusion yield. The number of aligned cells in a given 'pearl chain' (usually 5 to 12 cells) was determined before fusion pulses were applied. After the fusion process the number of unfused cells in the pearl chain was counted, and the percentage of fused cells (fusion yield) was then calculated. This was repeated three times to determine the fusion yield (%) reported in the figures. In the case of cell lysis during the fusion process experiments were not scored.

Reversion of spheroplasts. After the fusion process the concentration of $MgCl_2$ in the 20 μl of fusion medium was increased by addition of 20 μl 30 mM $MgCl_2$/0.8 M sucrose. Reversion medium was then added stepwise, to a final volume of 4 ml. (Abrupt changes of media should be prevented.) Cells were incubated at 37°C with gentle shaking (50 rpm) in 125 ml Erlenmeyer flasks.

Transformation. Transformation of *E. coli* by plasmid DNA was essentially done as described elsewhere [30].

Chemical measurements. DNA content was determined by the method of Dische [31], and protein content was determined by the method of Lowry et al. [32].

Results

Fusion of bacterial spheroplasts

In Zimmermann's fusion method cells are first collected on electrodes by a high frequency (approx. 100–1000 kHz) a.c. field (alignment field). In this process the cells form 'pearl chains' along the field lines. This phenomenon (dielectrophoresis), first discovered by Muth [12] and described in detail by Pohl [13], occurs in media of low ionic strength that contain emulsified fat droplets or cells. Cells subjected to the electric field turn into dipoles due to a new distribution of their electrical charges. Opposite poles of different cells then attract each other and tight membrane contacts are established in a few seconds. The fusion process is then initiated by one or more high intensity d.c. pulses (fusion pulses), which induce a reversible membrane breakdown in the contact zone between the cells. The membrane breakdown triggers pore formation between cells and finally results in membrane fusion [10,11]. Fusion pulses of too high intensity cause cell lysis [9].

In the present work we could align normal cells of *Escherichia coli* or *Salmonella typhimurium*, but fusion was not possible, even at 25 kV/cm, presumably because of the rigidity of the peptidoglycan layer of the bacterial cell envelope. In order to obtain cells with the peptidoglycan layer removed (spheroplasts), cells were grown in the presence of penicillin, an inhibitor of peptidoglycan synthesis,

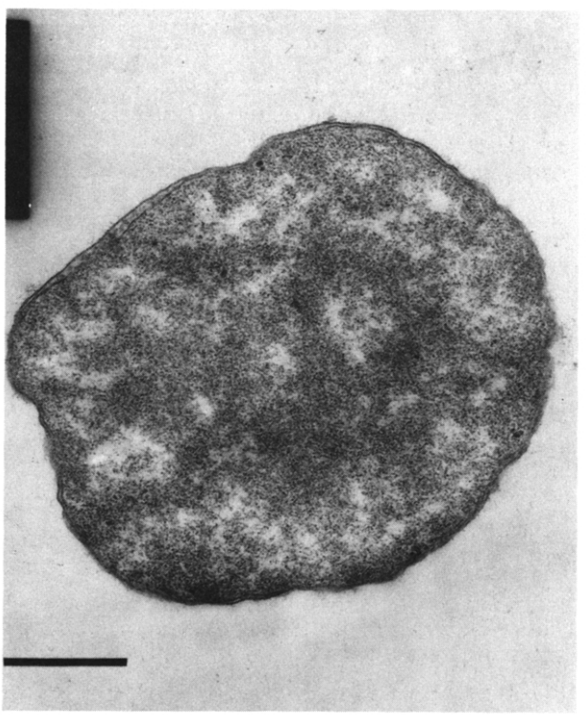

Fig. 2. Thin-section electron microscopy of a spheroplast prepared from filamentous *E. coli* AW405. The spheroplast is enveloped by a double layer of membranes. The protoplasm is homogenous and not septated. Such spheroplasts were used for fusion. The bar in the lower left represents 1 μm.

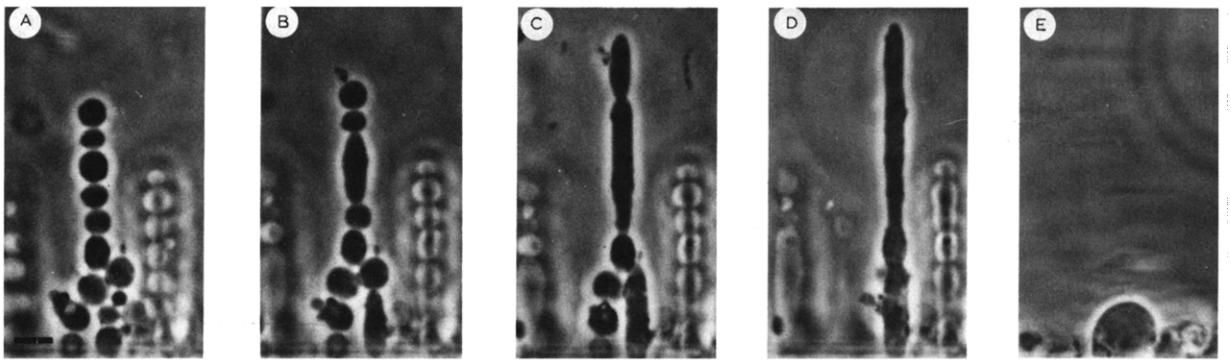

Fig. 3. Fusion of *E. coli* AW405 spheroplasts. (A) 'Pearl chain' formation of cells in an a.c. field (1.0 kV/cm, 1.0 MHz). (B) Partial fusion after application of two d.c. pulses (1.5 and 2.5 kV/cm, 15 μs). (C) Application of a third d.c. pulse (4.0 kV/cm, 15 μs). (D) Complete fusion after two more pulses (4.0 kV/cm, 15 μs). (E) 10 min after disconnecting the a.c. field. Time between pulses: 5 s. The process of membrane fusion after each pulse was completed in less than one second. Fusion medium: 0.8 M sucrose/1 mM $MgCl_2$. The bar in the lower left of (A) represents 10 μm. The same magnification was used for all figures.

for usually 2–4 h [33]. After that cells were spherical and highly sensitive to an osmotic shock. In subsequent fusion experiments though, spheroplasts of various sizes (2–10 μm in diameter) were not fusable. Neither was fusion possible with spheroplasts (0.5–1.0 μm in diameter) obtained from bacteria treated with Tris/EDTA/lysozyme to hydrolyze the peptidoglycan layer [28,29].

Finally we used a procedure for making spheroplasts that was successful for fusion, perhaps because these have more membrane than is needed to house their cytoplasm and this gives them a flexibility that may be necessary for excellent membrane contact in the electric field. Bacteria were first grown to long filamentous cells, 50 to 150 μm long, in the presence of cephalexin, a β-lactam antibiotic [24,25]. Shorter filaments (8 to 40 μm) were not very useful. Then these filaments were treated with Tris/EDTA/lysozyme to form giant non-septated spheroplasts with an average diameter of 7 μm (Fig. 2). Electrofusion of these giant spheroplasts could then be obtained easily (Fig. 3).

Efficiency of fusion and survival of cells were increased by determining optimum fusion conditions. A suitable fusion medium contained 0.8 M sucrose (Fig. 4) and 1 mM $MgCl_2$ (Table I). Mg^{2+} was not absolutely required for membrane fusion, but cell lysis due to intense fusion pulses was largely prevented by addition of Mg^{2+}; this allowed application of more fusion pulses of higher

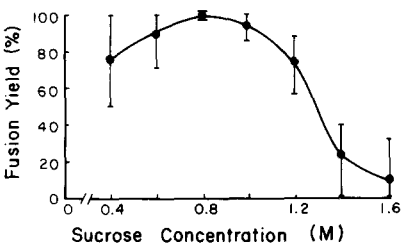

Fig. 4. Effect of sucrose concentration on the fusion yield. Spheroplasts of *E. coli* AW405 were subjected to 5 d.c. pulses (4.0 kV/cm, 15 μs). A.c. field: 1.0 kV/cm, 1.0 MHz. The fusion medium was supplemented with 1 mg MgCl. Cell lysis occurred at sucrose concentrations of less than 0.4 M. The mean values of three experiments are given (●); bars indicate range of fusion yield obtained.

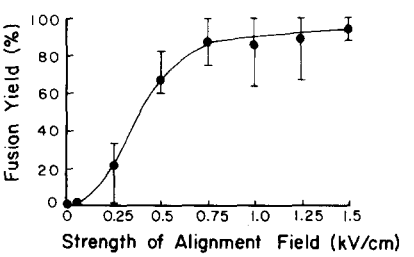

Fig. 5. Dependence of the fusion yield on the strength of the a.c. field. Frequency of the field was 1.0 MHz. Results were obtained after application of five fusion pulses (4.0 kV/cm, 15 μs) on E. coli AW405 spheroplasts. The fusion medium contained 0.8 M sucrose and 1.0 mM $MgCl_2$. The mean values of three experiments are given (●); bars indicate range of fusion yield obtained. Cell lysis started at forces of more than 1.5 kV/cm.

TABLE I

EFFECT OF $MgCl_2$ CONCENTRATION ON FUSION YIELD AND CELL STABILITY

Spheroplasts of *E. coli* AW405 were fused in media of 0.8 M sucrose and various concentrations of $MgCl_2$. Cells were subjected to 10 d.c. pulses of 4.0 kV/cm, 15 μs (except the first pulse was 1.5 kV/cm, the second pulse 2.5 kV/cm). A.C. field: 1.0 kV.cm, 1.0 MHz. Three experiments were performed for each concentration. The mean values are given. The standard deviation ranged from ±10% for fusion yield above 60% to ±35% for fusion yield of less than 60%.

Number of pulses	Fusion yield (%); $MgCl_2$ (mM)						
	0	0.1	0.5	1	1.5	2	2.5
1	0–5	0	0	0	0	0	[a]
2	80	58	37	46	26	25	
3	Lysis	77	60	77	66	66	
4		82	93	77	76	80	
5		Lysis	Lysis	86	80	80	
6				94	88	80	
7				94	88	80	
10				Lysis	88	80	

[a] 'Pearl chain' formation was inhibited by turbulence.

TABLE II

EFFECT OF $CaCl_2$ CONCENTRATION ON FUSION YIELD AND CELL STABILITY

E. coli AW405 cells were fused in media of 0.8 M sucrose and various concentrations of $CaCl_2$. ($MgCl_2$ was not present.) Cells were subjected to 10 fusion pulse of 4.0 kV/cm, 15 μs (except the first pulse was 1.5 kV/cm, the second pulse was 2.5 kV/cm). A.C. field: 1.0 kV/cm, 1.0 MHz. Three experiments were performed for each concentration. The mean values are given. The standard deviation ranged from ±5% for fusion yield above 60% to ±30% for fusion yield of less than 60%.

Number of pulses	Fusion yield (%); $CaCl_2$ (mM)						
	0	0.1	0.5	1	1.5	2	2.5
1	0–5	0–1	0	0	0	0	[a]
2	77	41	34	13	11	8	
3	Lysis	67	58	49	48	23	
4		Lysis	75	62	48	27	
5			Lysis	62	50	45	
6				62	60	45	
7				62	60	45	
10				62	60	45	

[a] 'Pearl chain' formation was inhibited by turbulence.

intensity, and consequently a higher fusion yield resulted (Table I). Addition of Ca^{2+}, recommended for other cell systems [8], was not as useful as Mg^{2+} (Table II) and rather inhibited fusion. Inhibition of fusion by Ca^{2+} has been noticed also by others [34]. Addition of pronase, recommended by other workers [35], was not found helpful here (data not shown). The strength of the a.c. field, used to align spheroplasts into pearl chains, was found to be sufficient at 1 kV/cm (Fig.5) and 1 MHz (Table III). The d.c. field, used then to fuse aligned spheroplasts, was usually administered as five pulses of 4 kV/cm (Fig. 6, top) at a duration of 15 μs (Fig. 6, bottom). Sometimes the first of these five pulses resulted in lysis of one or more spheroplasts. This could be avoided by an initial application of two pulses of lower intensity (1.5–2.5 kV/cm).

The size of the fusion products could be selected by altering the cell density in the fusion medium. A higher cell density resulted in longer pearl chains and larger fusion products. Usually a cell density of $3 \cdot 10^7$ cells/ml was used to obtain fusion of typically 4–6 spheroplasts. Fusion products were stable in the fusion medium for at least 10 h.

TABLE III

EFFECT OF ALTERNATING CURRENT FREQUENCY

The strength of the a.c. field was 0.75 kV/cm. Five d.c. pulses (4.0 kV/cm, 15 μs) were administered. The fusion medium was: 0.8 M sucrose, 1 mM $MgCl_2$. The mean values from three experiments are given. The standard deviation ranged from ±3% to ±17%.

	Frequency of alternating current (kHz)						
	10	50	100	500	800	1000	1300
Fusion yield (%)	[a]	[b]	73 [c]	83	85	93	93

[a] At 10 kHz quantitative analysis was impaired by turbulences in the medium. Spontaneous cell fusion without application of direct current pulses occurred. These fusion products subsequently lysed.

[b] At 50 kHz turbulences and cell rotation impaired quantitative analysis.

[c] At 100 kHz alignment was disturbed by cell rotation. Pearl chains were short (2–5 cells).

Reversion of (unfused) giant spheroplasts

The ratio of DNA/protein in filaments of an average length of 100 μm was determined to be 33 ng DNA/μg protein, and in untreated cells (length 2 to 4 μm) it was about the same, 36 ng DNA/μg protein (both at an $A_{590} = 0.9$). It can be concluded that about 30 bacterial genomes are present in such a long filament and thus also in a resulting giant spheroplast.

Spheroplasts of *E. coli* or *S. typhimurium* with diameters of about 7 μm largely reverted to the bacterial form in 8 h (*S. typhimurium*) or 10 h (*E. coli*) of shaking at 37°C in reversion medium; after overnight incubation only 1 to 2% unreverted spheroplasts remained. Extensive lysis of the spheroplasts is unlikely, because spheroplasts incubated in this medium in the presence of an inhibitor of protein synthesis, 80 μg/ml kanamycin, stayed stable during incubation overnight. We estimate that more than 90% of the spheroplasts reverted to the bacterial form.

Several steps during the reversion process were observed (see Fig. 7): (1) Spheroplasts grow one or more buds from their surfaces. (2) With progressive growth of these buds, the spheroplasts convert into long rod-like cells with a size of 30–70 times 1.2 μm. (3) These rods divide into 20–30 small sections in the size of normal bacteria. We assume that each of these sections is a viable bacterium. Similar processes have been reported for the reversion of spheroplasts obtained from nonfilamentous bacteria [36,37].

Reversion of fusion products

To demonstrate the reversion and viability of fusion products, a mixture of cells with two different genetic markers was fused, the fusion products were released from the fusion chamber by flushing with 0.8 M sucrose, and individuals expressing both markers were selected.

For these experiments *E. coli* DH1 was chosen because its properties, F^-, λ^-, should decrease the chance of spontaneous exchange of genetic material. *E. coli* DH1 was transformed by nonselftransmissible plasmids carrying either tetracycline- or kanamycin-resistance. Giant spheroplasts of the two transformed strains were mixed and fused electrically (as described above); the predominant fusion product containing 4–6

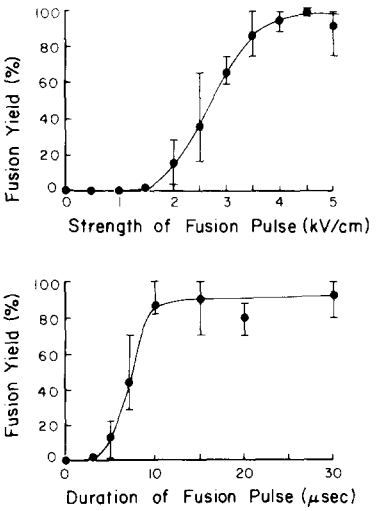

Fig. 6. Effect of strength and duration of fusion pulses on fusion yield. Upper presentation: Five fusion pulses of given strength and a duration of 15 μs were administered. Lower presentation: Five fusion pulses of given duration and a voltage of 4.0 kV/cm were administered. All experiments were carried out on *E. coli* AW405 spheroplasts. The a.c. field was 1.0 kV/cm, 1.0 MHz. The fusion medium contained 0.8 M sucrose and 1.0 mM $MgCl_2$. The mean values of three experiments are given (●); bars represent range of fusion yield obtained. Cells started to lyse at pulse strengths of 6.0 kV/cm (top curve) or pulse durations of 40 μs (bottom curve), respectively.

spheroplasts. The fusion products were then allowed to regenerate in reversion medium. This medium did not contain antibiotics. After completition of the reversion process (10 h) the reversion medium was diluted 1 : 1 with Luria-Bertani medium; tetracycline (12 μg/ml) and kanamycin (40 μg/ml) were added at the same time. Cells were then incubated for another 20–24 h. Finally cells were streaked out for single colonies on selective plates. 5–200 colonies of *E. coli* now resistant to both tetracycline and kanamycin could be obtained on plates having both antibiotics. Three such experiments were carried out and genetic markers for *E. coli* DH1 (F^-, λ^-, thi-1, recA1, supE44) were found for all doubly-resistant colonies tested. For controls, spheroplasts of both strains (one carrying tetracycline resistance, the other kanamycin resistance) were not fused but were mixed and incubated in reversion medium as described above. In this case regenerated bacteria formed no colonies on plates containing both antibiotics.

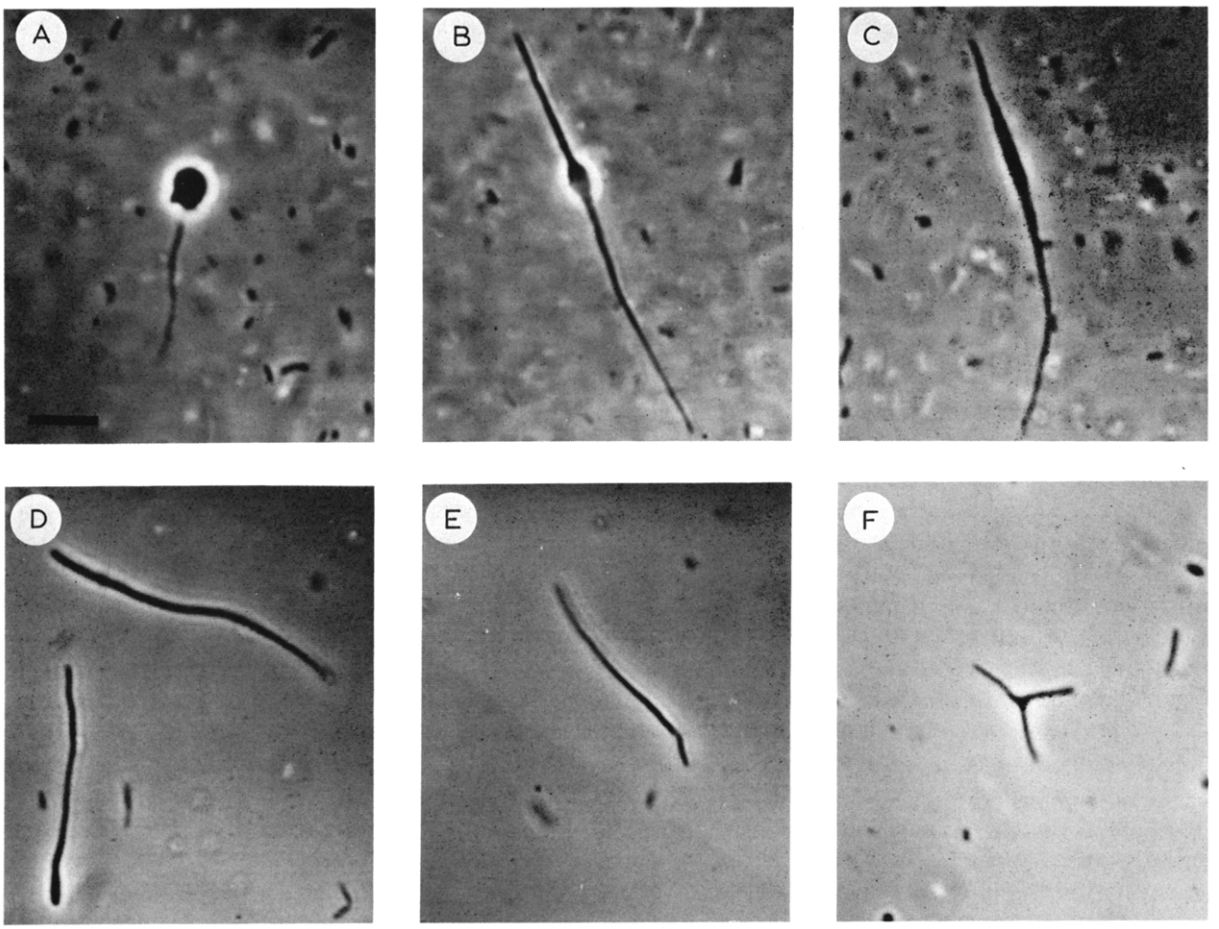

Fig. 7. Morphological aspects of the reversion of giant spheroplasts. Filaments of *E. coli* AW405 (100–150 μm long), grown in the presence of cephalexin, were treated with Tris/EDTA/lysozyme. The resulting spheroplasts were incubated in reversion medium for 8 h (see Materials and Methods). After that time most spheroplasts had reverted to the bacterial form, but typical stages of the reversion process were still present. (A) Buds grow out from the surface of a spheroplast. (One bud already gained considerable length.) (B–D) With progressive growth of these buds, spheroplasts elongate to rod-like cells of 30–70 μm in length. (E) Long rods divide into sections in the size of normal bacteria, as seen in the bottom of that rod. (F) Sometimes spheroplasts emerge into 'Y' shaped forms. The bar in the lower left of (A) represents 10 μm. The same magnification was used for all figures.

Spheroplasts of S. typhimurium fused with E. coli

S. typhimurium TH32 (highly motile) can grow on citrate as sole carbon source and is sensitive to kanamycin, while *E. coli* DH1 (not motile) can not utilize citrate, but when transformed with the kanamycin resistance plasmid, can grow in the presence of kanamycin. Giant spheroplasts from the two kinds of bacteria were mixed and fused electrically. The reversion to bacteria was done as described in the previous two sections. Approximately a hundred colonies which grew on citrate as sole carbon source in the presence of 80 μg/ml kanamycin were obtained in each of 7 experiments. Ten colonies were tested for motility. They all exhibited motility characteristics of *S. typhimurium* TH32.

Discussion

We found that the fusion method of Zimmermann and co-workers can be applied to giant spheroplasts of *Escherichia coli* or *Salmonella typhimurium* prepared from long filamentous forms produced by growth in the presence of cephalexin.

The fusion process is rapid, and highly effective. The size of the fusion products can be controlled well. The fusion products are viable and regenerate to the bacterial form.

In our hands Zimmermann's method was not suitable for fusing penicillin- or lysozyme-induced spheroplasts of nonfilamentous bacteria.

Fusable spheroplasts elongate when the alignment field is applied, whereas nonfusable spheroplasts retain their spherical shape in the same field. An excess of membrane results from the conversion of long filamentous bacteria to spheroplasts as less membrane is needed to enclose a sphere than to enclose a rod. Consequently these spheroplasts must be relatively flexible, a quality that allows them to flatten out and to provide greater areas of membrane contact during pearl chain formation in the alignment field. Plant protoplasts also require large regions of membrane contact for optimum fusion as shown by others [9].

Exact setting of electronic parameters, such as strength of the alignment field or duration of the fusion pulse is not extremely critical for the fusion process. Most of the parameters can be altered over almost an order of magnitude without loss of fusion. These properties might become of importance if fusion of bacteria to other cell types is desired.

The envelope of spheroplasts of Gram-negative bacteria consists of a cytoplasmic (inner) membrane and an outer membrane (see Fig. 1). The outer membrane of Gram-negative bacteria contains mainly lipopolysaccharide. Electrofusion is not inhibited by this structure. It is not necessary to have protoplasts, which are lacking an outer membrane and possess the cytoplasmic membrane only.

Some workers complain about intractable sticking of their fusion products to the electrodes [16]. In our experiments fusion products could easily be released from the fusion chamber by flushing with a sucrose solution.

Spheroplasts exposed to an a.c. field of relative low frequency (10 kHz) aligned to some extent to form short pearl chains. A moderate field strength of 0.75 kV/cm, well tolerated at a frequency of 1 MHz, now resulted in membrane breakdown leading to membrane fusion and finally to cell lysis (see Table III). This phenomenon can be explained by theoretical considerations, which imply that the electric potential superimposed over the membrane by an external alternating field varies inversely with the field's frequency [8,38]. When the breakdown potential of the membrane is reached, in the alignment field, fusion can occur without application of d.c. pulses. Electrostimulated fusion may open additional possibilities for gene transfer between different bacteria or between bacteria and eucaryotic cells; this may lead to the construction of cells with new, desired properties.

Acknowledgements

We are indebted to Michael Lentz for technical assistance and for building a closed fusion chamber, and to Steven Limbach for doing the electron microscopy. We thank Jerry Yin for plasmid DNA; the *E. coli* Genetic Stock Center, Yale University, New Haven, Connecticut, for *E. coli* DH1; and James Hudson (previous GCA Corporation) for helpful discussion. H.-J. Ruthe thanks the Deutsche Forschungsgemeinschaft for granting a scholarship for study abroad.

References

1 Fodor, K. and Alföldi, L. (1976) Proc. Natl. Acad. Sci. USA 73, 2147–2150
2 Gabor, M.H. and Hotchkiss, R.D. (1979) J. Bacteriol. 137, 1346–1353
3 Köhler, G. and Milstein, C. (1975) Nature 256, 495–497
4 Croce, C.M., Linnenbach, A., Hall, W., Steplewski, Z. and Koprowski, H. (1980) Nature 288, 488–489
5 Carlson, P.S., Smith, H.H. and Dearing, R.D. (1972) Proc. Natl. Acad. Sci. USA 69, 2292–2294
6 Melchers, G. and Labib, G. (1974) Mol. Gen. Genet. 135, 277–294
7 Küster, E. (1909) Ber. Dtsch. Bot. Ges. 27, 589–598
8 Zimmermann, U. (1982) Biochim. Biophys. Acta 694, 227–277
9 Zimmermann, U. and Vienken, J. (1982) J. Membrane Biol. 67, 165–182
10 Zimmermann, U., Scheurich, P., Pilwat, G. and Benz, R. (1981) Angew. Chem. 93, 332–351; Angew. Chem. Int. Edn. Engl. 20, 325–344
11 Zimmermann, U. and Scheurich, P. (1981) Planta 151, 26–32
12 Muth, E. (1927) Kolloid Z. 41, 97–102
13 Pohl, H.A. (1978) Dielectrophoresis, Cambridge University Press, Cambridge
14 Senda, M., Takeda, J., Abe, S. and Nakamura, T. (1979) Plant Cell Physiol. 20, 1441–1443

15 Vienken, J. and Zimmermann, U. (1982) FEBS Lett. 137, 11–13
16 Bates, G.W., Gaynor, J.J. and Shekhawat, N.S. (1983) Plant Physiol. 72, 1110–1113
17 Pilwat, G., Richter, H-P. and Zimmermann, U. (1981) FEBS Lett. 133, 169–174
18 Shivarova, N., Grigorova, R., Förster, W., Jacob, H.-E. and Berg, H. (1983) Bioelectrochem. Bioenerg. 11, 181–185
19 Vogel, H.J. and Bonner, D.M. (1956) J. Biol. Chem. 218, 97–106
20 Mesibov, R. and Adler, J. (1972) J. Bacteriol. 112, 315–326
21 Hanahan, D. (1983) J. Mol. Biol. 166, 557–580
22 Hong, J.-S. and Kaback, H.R. (1972) Proc. Natl. Acad. Sci. USA 69, 3336–3340
23 Johnson, R.C. and Reznikoff, W.S. (1983) Nature 304, 280–282
24 Greenwood, D. and OGrady, F. (1973) J. Infect. Dis. 128, 211–222
25 Rolinson, G.N. (1980) J. Gen. Microbiol. 120, 3217–323
26 Ishihara, A., Segall, J.E., Block, S.M. and Berg, H.C. (1983) J. Bacteriol. 155, 228–237
27 Onitsuka, M.O., Rikihisa, Y. and Maruyama, H.B. (1979) J. Bacteriol. 138, 567–574
28 Repaske, R. (1958) Biochim. Biophys. Acta 30, 225–232
29 DePamphilis, M.L. and Adler, J. (1971) J. Bacteriol. 105, 376–383
30 Gubler, U. and Hoffman, B.J. (1983) Gene 25, 263–269
31 Dische, Z. (1930) Mikrochemie 8, 4–32
32 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
33 Kaback, H.R. (1971) Methods Enzymol. 22, 99–120
34 Chapel, M., Teissie, Y. and Alibert, G. (1984) FEBS Lett. 173, 331–336
35 Zimmermann, U., Pilwat, G. and Richter, H.-P. (1981) Naturwissenschaften 68, 577–578
36 Hirokawa, H. (1962) J. Bacteriol. 84, 1161–1168
37 Udou, T., Ogawa, M. and Mizuguchi, Y. (1982) J. Bacteriol. 151, 1035–1039
38 Bernhardt, J. and Pauly, H. (1973) Biophysik 10, 89–98