ORIENTATION OF *SCHIZOSACCHAROMYCES POMBE* NONLIVING CELLS UNDER ALTERNATING UNIFORM AND NONUNIFORM ELECTRIC FIELDS

F. J. IGLESIAS,* M. C. LÓPEZ,‡ C. SANTAMARÍA§, AND A. DOMÍNGUEZ‡
*Departamento de Física Fundamental, Facultad de Ciencias, ‡Departamento de Microbiología, Facultad de Biología, Universidad de Salamanca, and §Departamento de Física, Universidad del País Vasco, Bilbao, Spain

ABSTRACT When nonliving cells of *Schizosaccharomyces pombe* were subjected to the action of alternating uniform and nonuniform electric fields, two types of orientation were produced. The first one, with its longest axis parallel to the field lines, is similar to that obtained with living cells. The second, perpendicular to the direction of the field, is produced for relatively high frequencies and low conductivities; this probably takes place when the conductivities of the external and internal media (cell cytoplasm) become equal. A mixed cell population is produced in a discrete interval of the parameters used. Our results provide direct evidence that cell alignment does not depend on the physiological state of the cells.

INTRODUCTION

Alternating current, field-induced force effects have been described for many years. However, their detailed understanding in general, and the field-induced effect in yeasts, have not been well defined. Several authors have reported that living, nonspherical cells can orient in several directions in an alternating electric field (reviewed by Zimmerman [1]). Teixeira-Pinto et al. (2) observed that a unicellular organism like *Euglena gracilis* and asymmetric cytoplasmic inclusions in immobilized cells of *Paramecium* and *Ameba* can have parallel orientation with the field lines at lower frequencies or perpendicular orientation at higher frequencies. They also observed that the frequency ranges associated with the migration vary from species to species. Griffin and Stowell (3) extended the results described above and showed that the turnover frequencies could be changed by varying the external conductivity of the medium.

Schwarz et al. (4) and Saito et al. (5) proposed that a nonspherical particle, suspended in a medium of different dielectic properties, tends to be oriented by a sufficiently intense alternating homogeneous electric field. They also showed that the direction of stable orientation is determined by the geometry of the particle, the dielectic properties of the cell, and the frequency of the field.

In previous works we analyzed the dielectrophoretic behavior of yeast cells with different shapes and their response to several physical and biological parameters (Iglesias et al. [6]; Lopez et al. [7]). We have also shown that some of the properties of yeast cells are similar to those of macromolecular suspensions, while others depend on the metabolic state of the cells (7, 8).

The present work attempts to compare the behavior of living and nonliving cells of *S. pombe* with regard to the orientation phenomena under uniform and nonuniform electric fields.

MATERIALS AND METHODS

Schizosaccharomyces pombe (*S. pombe*) 972 h^- was kindly supplied by P. Nurse, University of Sussex. The culture was propagated in YED (yeast extract 1%, glucose 2%) in Ehrlenmeyer flasks at 250 rpm and 24°C. The cells were killed by autoclaving at 121°C, under ultraviolet (UV) light, permeabilized with mixtures of toluene-ethanol (9), treated with cetrimide (7) sodium azide, (2, 4) dinitrophenol, or sodium cyanide at the concentrations indicated in each experiment. In all cases the permeability of the cells was determined by the methylene blue vital staining technique of Arnold (10). Viability was also assayed by plating cell suspensions onto solid YED and incubating at 24°C for 48 h.

The orientation of living and nonliving *S. pombe* cells in a uniform electric field was studied in a setup consisting of a microscope slide onto which two electrodes 0.75 mm apart had been painted with silver, as described by Teixeira-Pinto et al. (2) (see Fig. 3). Dielectrophoresis was carried out in a pin-pin type electrode arrangement. The platinum wire diameter was 65 μm and 1 mm apart. The pin type tips were rounded (approximately spherical). The electrodes were mounted directly above the surface of a glass microscope slide and the rounded ends were placed about 0,1 mm above the surface of the slide.

For each assay, aliquots of the cells were collected, centrifuged, washed three times with deionized water, and resuspended to the desired cell concentration. A small drop (50 μl) of the suspension to be examined was placed between the electrodes.

The alternating current (AC) voltage was supplied by a function generator (Model 2000; Krohn-Hite Corp., Avon, MA) amplified by a single stage amplifier for the frequency range 0.2–8 MHz. The frequency of the field supplied to the electrodes was monitored by a Hewlett-

Please address reprint requests to Dr. Domínguez.

Packard Universal Counter (model 5314A. Hewlett-Packard Co., Palo Alto, CA) and an oscilloscope. Voltage was determined by a Hewlett-Packard (400E) voltmeter and an oscilloscope. The conductivity of the suspension was determined with a Crison 522 (C.R. Mares, S.A., Barcelona, Spain) conductimeter and adjusted by addition of 0.1 M KCl.

RESULTS

To test for the orientation of *S. pombe*, living and nonliving cells were exposed to an alternating electric field. Fig. 1 *a* shows the dielectrophoretic yield of living cells, and Fig. 1 *b* the low yield of dead ones with only a few anchored to the electrodes. Similar results were obtained with yeasts differing in shape or in cell wall composition (6). This and all the following experiments were conducted at voltages in which the radiofrequency field did not affect cell viability, as could be detected by measuring the number of cells forming colonies after the dielectrophoretic pulse (all the cells remained viable; see Table I). Furthermore, under the conditions chosen (collection time, voltage, conductivity, and cell concentration) there were no stirring problems and the mean size of the cell chains formed on the electrodes was that which gave the minimal experimental error. Neither were there any interferences between the chains formed on both electrodes.

Our next step was to analyze the behavior of nonliving cells under uniform and nonuniform electric fields. Cells of *S. pombe* treated by autoclaving at 121°C or permeabilized with toluene-ethanol produced an almost identical response in both types of fields (Figs. 2 and 3), which differs from that of living cells. Although both types of treatment provide rapid and complete permeabilization of the cells, probably by equalizing the external conductivity of the medium and the internal conductivity of cell (cell cytoplasm), they differ in some aspects. In the first (autoclaving at 121°C), all enzymatic activities are destroyed and a partial solubilization of some of the cell-wall components (mainly galactomannan; A. Domínguez, unpublished work) probably occurs. With the second, toluene-ethanol permeabilization, the cells maintain their integrity as could be demonstrated by electron microscopy (11); pore size probably permits only small molecules (10,000–15,000 mol. wt.) to escape from the cell and with this treatment several different enzymatic activities could be assayed (9).

With both treatments a transition took place from the parallel orientation of the cells to the field lines to a perpendicular (Fig 2). This transition was dependent on the electric frequency of the field and on the conductivity of the medium. When the value of the conductivity increased, higher frequencies of the field were necessary for the transition. In our experimental conditions, a mixed orientation of nonliving cells also occurred between the ranges shown in Fig. 2. The range of frequencies assayed from 5,000 kHz to 8 MHz are smaller than those previously described by *Euglena*, although no data concerning the dielectrophoretic behavior of this microorganism were reported (2, 3).

Fig. 3 shows the orientation pattern of *S. pombe* nonliving cells for nonuniform and uniform electric fields (parallel Fig. 3 *a* and *d*; mixed, *b* and *e*, and perpendicular *c* and *f*). In the two last pictures, the stockade arrangement of the cells is evident. Our results suggest that in the orientation phenomena detected, neither the integrity of the cell wall nor that of the cytoplasmic content are essential (see Discussion). When the same experiments were carried out with nonliving cells of *S. cerevisiae*, the results due to the sphericity of this yeast are less clear than those reported for *S. pombe*. However, a perpendicular orientation to the

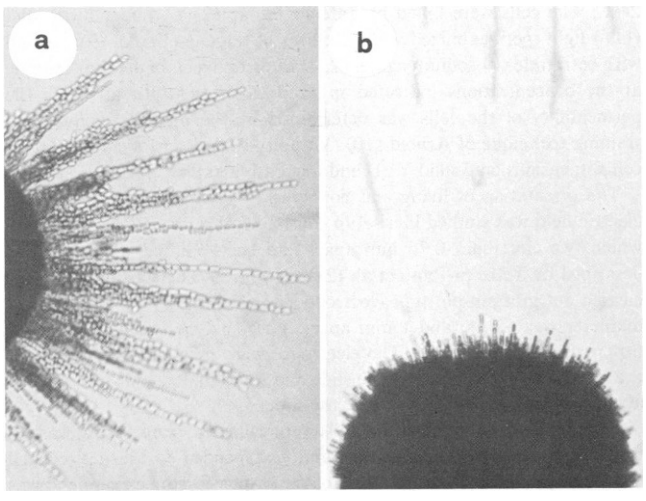

FIGURE 1 Living (*a*) and nonliving (*b*) cells of *S. pombe* collected in a nonuniform alternating current field. The electrodes are of the pin-pin type. Collection time was 2 min and frequency was 1 MHz. rms. voltage was 20 V and electric conductivity was 2×10^{-3} $(\Omega m)^{-1}$.

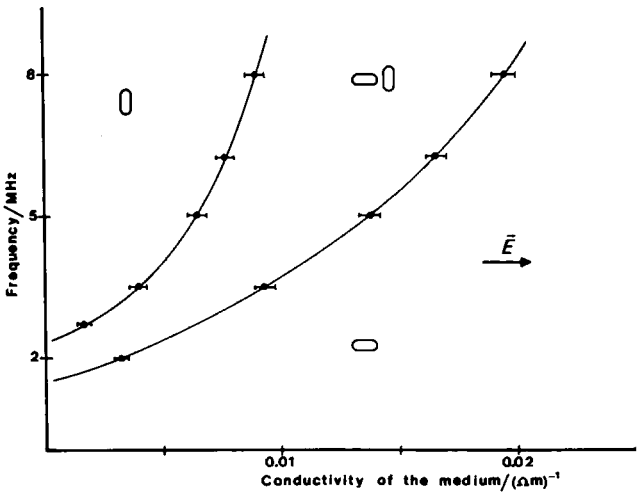

FIGURE 2 Orientation of nonliving cells of *S. pombe* in an alternating field as a function of frequency and of the conductivity of the medium. The values of each intersection point were calculated from a series of 10 micrographs by counting 100 cells in each, assuming that the cells were either in parallel or perpendicular to the field lines when 90 ± 5% of them adopted one or the other disposition.

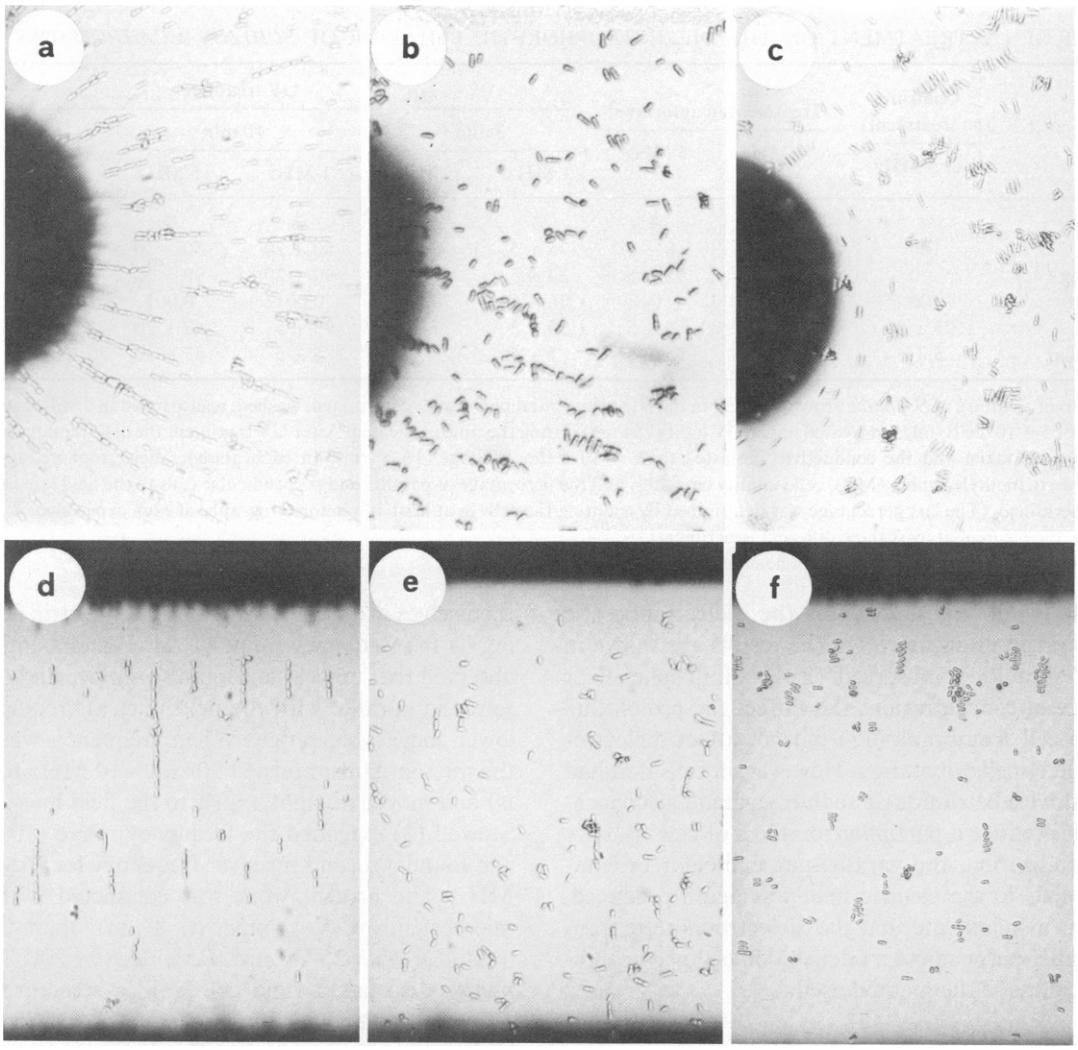

FIGURE 3 Sequential micrographs of the orientation of *S. pombe* nonliving cells under nonuniform (*a–c*) and uniform (*d–f*) electric fields as a function of the conductivity of the medium. Conductivity (*a, d* parallel), 2×10^{-2}; (*b, e* mixed), 8×10^{-3} and (*c, f* perpendicular), 2×10^{-3} $(\Omega m)^{-1}$. Frequency 4 MHz.

field lines was also seen with budded cells (permeabilized by autoclaving or by toluene-ethanol; data not shown).

To determine whether the change in orientation was due to the equalizing in conductivities between the external medium and the interior of the cell (cell cytoplasm), the same parameters, dielectrophoretic yield, cell viability, and cell orientation, were determined in aliquots of *S. pombe* cultures treated with UV light or with chemical reagents. The working hypothesis consisted of using one of these treatments to isolate dead cells (unable to form colonies on culture dishes) still retaining a practically intact cell membrane and thereby preventing the free diffusion of molecules. Table I shows the results of the UV treatment (for details on the effect of UV light on yeast cells see reference 12). A good correlation was observed between the dielectrophoretic yield, methylene blue staining, cell viability, and cell orientation, similar to that obtained with the permeabilizing or autoclaving treatments, except for UV treatment of 10 min. For this irradiation time (and for UV irradiation times of 7, 8, 9, 11, and 12 min, data not shown) a larger degree of uncertainly, up to 20%, and a discrepancy between some of the parameters assayed, were observed. Up to the present, the only possible explanation would be that not all the cells unable to form colonies are permeable to the methylene blue stain; it is also possible that there could be different degrees of permeabilization; i.e., if all the cells permeabilized at this irradiation time (10 min UV) displayed the same degree of permeabilization, the dielectrophoretic yield would be similar to that obtained by the autoclaving and 30 min UV treatment (8 and 12%, respectively). The same rationale could be applied to the cell orientation data (parallel-perpendicular).

We attempted to confirm the previous results by treatment of the cells with several chemical compounds able to inhibit cell metabolism (13), or with detergents that dam-

TABLE I

EFFECT OF UV TREATMENT ON THE DIELECTROPHORETIC BEHAVIOR OF *SCHIZOSACCAHROMYCES POMBE*

	Control (no treatment)	Treatment in autoclave*		UV treatment					
				3 min		10 min		30 min	
	1 or 5 MHz	1 MHz	5 MHz	1 MHz	5 MHz	1 MHz	5 MHz	1 MHz	5 MHz
	%	%	%	%	%	%	%	%	%
DEPY	100	8 ± 5	8 ± 5	75 ± 5	75 ± 5	50 ± 20	50 ± 20	12 ± 5	12 ± 5
MB staining	0	95 ± 5	95 ± 5	32 ± 5	32 ± 5	90 ± 20	90 ± 20	99 ± 5	99 ± 5
Cell viability	100	0	0	76 ± 5	76 ± 5	0,001	0,001	0	0
Parallel	95 ± 5	95 ± 5	5 ± 5	95 ± 5	77 ± 5	95 ± 5	43 ± 20	95 ± 5	0
Perpendicular	5 ± 5	5 ± 5	95 ± 5	5 ± 5	23 ± 5	5 ± 5	57 ± 20	5 ± 5	95 ± 5

Aliquots of cells of a culture of *S. pombe* grown on YED in the late exponential phase were centrifuged, washed, resuspended in distilled sterile water at a concentration of 5×10^6 cells/ml, and treated under UV light (254 nm) during the times indicated. After UV treatment the cells remained for 0.5 h in the dark; aliquots were taken and the conductivity adjusted to 2×10^{-3} Ωm. Voltage, 10 V rms. In each aliquot, dielectrophoretic yield (DEPY), permeabilization to methylene blue (MB), cell viability on plates, and the percentage of parallel and perpendicular cells to the field line forces at 1 and 5 MHz were determined. (The last percentage was determined by counting the cells in at least 10 photomicrographs of each experiment.)

The results are the mean of at least three different experiments.

*The same results were obtained with the toluene-ethanol treatment.

age the cell membrane and affect the dielectrophoretic yield as described previously (7). The results are shown in Table II. As could be observed (2, 4), dinitrophenol or sodium azide at concentrations that affect the proton flux through the cell membrane (13) did not affect dielectrophoretic yield or cell orientation. However, in cells that had been treated with cetrimide or sodium cyanide at concentrations that produce a partial or total loss of cell viability and permeabilization, the parallel-perpendicular orientation of the cells to the electric field was again produced. These results also indicate that the dielectrophoretic transitions (parallel-perpendicular) depend on cell permeabilization, regardless of the method used.

DISCUSSION

Nonspherical or asymmetric cytoplasmic inclusions in immobilized cells of microorganisms are able to orient themselves in an alternating or pulse electric field, depending on the frequency range (1, 2). Teixeira-Pinto et al. (2) observed that unicellular organisms move and orient themselves in parallel with the field lines at frequencies in the lower megacycle range. When frequency was increased, the microorganism turned 90° (at ~10 MHz for *E. gracilis*) and moved at right angles to the field lines. Griffin and Stowell (3) extended the frequency range (10–200 MHz) and found a second turnover frequency for *Euglena* at 100 MHz. The present work was conducted with a smaller microorganism, *S. pombe,* (6–8 μm), than the previous ones (*Euglena* 25–30 μm, *Paramecium* 200–300 μm). *S. pombe* also has a rigid cell wall, a structure lacking in *Euglena* or *Paramecium,* and a lower polarizability than both these microorganisms. The dielectrophoretic behavior of *S. pombe* has been extensively studied, and the lower frequencies used, from 500 kHz to 8 MHz, were selected

TABLE II

EFFECT OF TREATMENT WITH TOXIC REAGENTS AND DETERGENTS ON THE DIELECTROPHORETIC BEHAVIOR OF *SCHIZOSACCHAROMYCES POMBE*

	Control (no treatment)	2,4 DNF (5 mM*)		Na_3N‡ (25 mM)		Cetrimide (8×10^{-3} mM)		CN^- (600 mM)	
	1 or 5 MHz	1 MHz	5 MHz	1 MHz	5 MHz	1 MHz	5 MHz	1 MHz	5 MHz
	%	%	%	%	%	%	%	%	%
DEPY	100	95 ± 5	96 ± 5	96 ± 5	95 ± 5	38 ± 5	38 ± 5	5 ± 5	5 ± 5
MB	0	1 ± 5	1 ± 5	1 ± 5	1 ± 5	57 ± 5	57 ± 5	98 ± 5	98 ± 5
Cell viability	100	99 ± 5	99 ± 5	99 ± 5	99 ± 5	40 ± 5	40 ± 5	0	0
Parallel	95 ± 5	95 ± 5	95 ± 5	95 ± 5	95 ± 5	95 ± 5	36 ± 5	95 ± 5	5 ± 5
Perpendicular	5 ± 5	5 ± 5	5 ± 5	5 ± 5	5 ± 5	5 ± 5	63 ± 5	5 ± 5	95 ± 5

Aliquots of cells of a culture of *S. pombe* grown on YED in the late experimental phase were centrifuged, washed, resuspended in distilled sterile water at a concentration of 5×10^6 cells/ml, and treated with the reagents over 1 h. The cells were centrifuged, washed, and resuspended at a conductivity of 2×10^{-3} mho/m. Voltage, 10 V rms. In each sample, dielectrophoretic yield (DEPY), permeabilization to methylene blue (MB), cell viability on plates, and percentage of parallel and perpendicular cells to the field line forces at 1 and 5 MHz were determined. (The last percentage was determined by counting the cells in at least 10 photomicrographs of each experiment.)

The results are the mean of at least three different experiments.

*The same results were obtained with 2,4 DNF (2,5 mM).

‡The same results were obtained with Na_3N (5 mM).

because *S. pombe* shows a maximum dielectrophoretic yield in this frequency range (6). Our experimental results differ in several aspects from those reported previously. One of these is that we could not find changes in the orientation of living cells of *S. pombe*; however, this is probably because our experimental setup cannot produce higher frequencies than 8 MHz and a transition from the parallel to the perpendicular orientation to the field line forces could reasonably be expected at higher frequencies.

Saito et al. (5) proposed that in general there can be only one stable direction of equilibrium, and that the change in the stable direction with frequency can occur either as a gradual change or as a sudden jump of 90°. The same authors also stated that for ellipsoidal particles of symmetrical composition, with or without a shell and with parameters of biological interest, only the latter (sudden jump) can occur.

Our results do not show the existence of orientation patterns other than the parallel perpendicular ones for dead cells of *S. pombe*. However, in the frequency and electrical conductivity ranges in Fig. 2, cells with both orientations are seen to coexist. Note that in this work the cells were killed by several different procedures (autoclaving at 121°C, UV, irradiation, toxic agents, and detergents) and in all cases the same results were obtained both in uniform and nonuniform electric fields, which indicates that neither the killing procedure nor the type of field are relevant parameters in our experimental conditions. Accordingly, the discrepancy observed could be due to heterogeneities in our working conditions that we have been hitherto unable to detect, or to the fact that up to now, the theoretical models elaborated (5, 14), are not based on sufficient experimental evidence although they do interpret some of the dielectrophoretic phenomena correctly. (We have shown that particle size, considered as nonbiological, is the main parameter affecting the dielectrophoretic field of yeast cells and fungal spores; López et al., (15). In this sense we feel that the contribution of the metabolic status of the cells (6), the effect of different cations in the suspension medium (7), or of the cell wall (15) have not been sufficiently evaluated. It should also be mentioned that the mixed orientation with nonliving cells of *S. pombe* occurs only at a small interval of frequency and conductivity, and that previous work with *Euglena* has shown that the transition from the parallel to the perpendicular form passes from 10 to 18 MHz without intermediate measures, it being possible that a mixed orientation could take place in this interval (3). In agreement with our results, Griffin pointed to the existence of mixed orientation in human and avian erythrocytes according to the glucose concentration employed (16).

Pohl (14) stated that changes in orientation only occur in living cells, and suggested that these changes are due to the polarizability of the lipid layers of the cell membrane and to the ions bound to its surface. At low frequencies, ionic double layer polarization will dominate and cause cells to line up with the field. On the contrary, at high frequencies the double layer response will begin to fail, and membrane capacitance charging, maximized by having the cells aligned at right angles to the field, is favored. Our results, however, show that *S. pombe* dead cells can change their orientation under uniform and nonuniform electric fields.

Such findings are probably due to two factors; one of them is the equalizing of the conductivity of the external medium and that of the cell's interior (cell cytoplasm), as may be deduced from the data presented in Table I. The table shows that for intermediate UV irradiation times (~10 min) all the cells have lost their ability to grow and reproduce (cell viability 0.001%), whereas not all cells are completely permeabilized, as may be inferred from the data referring to the methylene blue staining and dielectrophoretic yield. Thus part of the cells, the nonpermeabilized or incompletely permeabilized ones, cannot carry out the change in orientation. This hypothesis is supported by the experiments described in Table II in which it is shown that reagents at concentrations able to inhibit cell metabolism, but which do not induce changes in cell viability nor in cell permeability, do not cause alterations in cell orientation. In contrast, a detergent, cetrimide, at concentratinos that will permeabilize part of the cells, or sodium cyanide at concentrations sufficient to induce losses in cell viability and total permeabilization, do produce the parallel-perpendicular transition in the cells.

The second fact able to account for the parallel-perpendicular transition of *S. pombe* dead cells is the existence of a very rigid cell wall composed mainly of galactomannan, α-glucan and β-glucan (17). In those cells lacking a wall, when the plasma membrane is disorganized (thereby allowing the passive diffusion of metabolites), changes take place that induce alterations in the intracellular structures and in the shape itself of the cell. In *S. pombe,* however, alterations in the permeability of the plasma membrane and even in the components of the cell wall (galactomannan) allow the cells to retain their shape. It is probably this, the maintenance of cell shape by the β-glucan of the cell wall, which permits the changes in orientation described in *S. pombe* dead cells. Accordingly, our results show that reorientation phenomena also occur in nonliving cells and thus a reevaluation of the previous hypothesis to explain this fact would seem to be in order.

We wish to thank N. Skinner for revising the English version of the manuscript. M.C. Lopez is a recipient of a fellowship from FIS (Fondo de Investigaciones Sanitarias).

Received for publication 8 March 1985.

REFERENCES

1. Zimmerman, U. 1982. Electric field-mediated fusion and related phenomena. *Biochim. Biophys. Acta.* 694:227–277.
2. Teixeira-Pinto, A. A., L. L. Nejelelski., J. L. Cutler, and J. H. Heller. 1960. The behaviour of unicellular organisms in an electromagnetic field. *Exp. Cell Res.* 20:548–564.

3. Griffin, J. L., and R. E. Stowell. 1966. Orientation of Euglena by radiofrequency fields. *Exp. Cell Res.* 44:684–688.
4. Schwarz, G., M. Saito, and H. P. Schwan. 1965. On the orientation of nonspherical particles in an alternating electrical field. *J. Chem. Phys.* 43:3562–3569.
5. Saito, M., H. P. Shwan, and G. Schwarz. 1966. Response of nonspherical biological particles to alternating electric fields. *Biophys. J.* 6:313–327.
6. Iglesias, F. J., M. C. López, C. Santamaria, and A. Domínguez. 1984. Dielectrophoretic properties of yeast cells dividing by budding and by transversal fission. *Biochim. Biophys. Acta.* 804:221–229.
7. López, M. C., F. J. Iglesias, C. Santamaria, and A. Dominguez. 1984. Dielectrophoretic behaviour of *Saccharomyces cerevisiae*: effect of cations and detergents. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 24:149–152.
8. Santamaria, C., F. J. Iglesias, and A. Dominguez. 1985. Dielectrophoretic deposition in suspensions of macromolecules: polyvinylchloride and Sephadex G-50. *J. Colloid. Interface Sci.* 103:508–515.
9. Dominguez, A., M. V. Elorza, J. R. Villanueva, and R. Sentandreu. 1980. Regulation of chitin synthase activity in *Saccharomyces cerevisiae*: effect of the inhibition of cell division and of synthesis of RNA and protein. *Curr. Microbiol.* 3:263–266.
10. Arnold, W.N. 1972. The structure of the yeast cell wall. Solubilization of a market enzyme, β-fructofuranoside, by the autolytic enzyme system. *J. Biol. Chem.* 247:1161–1169.
11. Elorza, M. V., G. Larriba., J. R. Villanueva, and R. Sentandreau. 1977. Budding in *Saccharomyces cerevisiae*: formation of the cross-wall. *Trans. Br. Mycol. Soc.* 69:451–457.
12. Haynes, R. H., and B. A. Kunz. 1981. DNA repair and mutagenesis in yeast. *In* The Molecular Biology of the Yeast Saccharomyces. I. Life Cycle and Inheritance. J.N. Strathern, F. W. Jones, and J. R. Broach, editors. Cold Spring Harbor, New York. 371–414.
13. De la Peña, P., F. Barros, S. Gascón, S. Ramos, and P. S. Lazo. 1982. The electrochemical proton gradient of Saccharomyces. The role of potassium. *Eur. J. Biochem.* 123:447–453.
14. Pohl, H. A. 1978. Dielectrophoresis. Cambridge University Press, London.
15. López, M. C., F. J. Iglesias, C. Santamaría, and A. Domínguez. 1985. Dielectrophoretic behavior of yeast cells: Effect of growth sources and cell wall and a comparison with fungal spores. *J. Bacteriol.* 162:790–793.
16. Griffin, J. L. 1970. Orientation of human and avian erythrocytes in radio-frequency fields. *Exp. Cell Res.* 61;113–120.
17. Bush, D. A., M. Horisberger, I. Horman, and P. Wursh. 1974. The wall structure of *Schizosaccharomyces pombe. J. Gen. Microbiol.* 81:199–206.