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Practical Evaluation of Continuous Particle Electrophoretic Separation of Bacterial Species

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NOTE

Practical Evaluation of Continuous Particle Electrophoretic Separation of Bacterial Species

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Summary

Experiments were conducted in which the continuous particle electrophoresis apparatus was utilized for the separation of five bacterial species and an ascosporogenous yeast. The separated bands observed when bacteria or bacteria-yeast binary mixtures were electrophoresed were not composed of pure cultures. This was confirmed by classical cultural techniques. The reasons for the poor resolution of microorganisms into pure cultures using this instrument are discussed.

INTRODUCTION

In 1966, Strickler and Gafford (*1*) reported that *Chromobacterium violaceum*, *Bacillus globigii*, *Escherichia coli*, and *Serratia marcescens* could be separated from each other by continuous particle electrophoresis (CPE). They assumed that resolution of a bacterial mixture into discrete bands could be equated with the separation of individual organisms into pure cultures. No attempt was made to validate the cultural purity of their separations by morphological examination or by classical cultural techniques. Because of the desirability of a simple and rapid technique for the absolute resolution of a mixed population into component species, the present study was initiated.

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MATERIALS AND METHODS

A full description of the continuous particle electrophoresis apparatus has been given elsewhere (*2*, *3*).

Five bacterial species and an ascosporogenous yeast were used as the test organisms for this study: *Sarcina lutea*, *Staphylococcus aureus*, *Bacillus megaterium*, *E. coli*, *S. marcescens*, and *Saccharomyces cerevisiae*. The bacteria were subcultured from reserve slants to 100 ml of Trypticase Soy Broth (T-Soy), grown at 35°C and harvested by centrifugation during the late logarithmic phase of growth. *S. cerevisiae* was grown on the same medium at 26°C and harvested in a similar manner. The cells were then washed twice with 10^{-3} *M* sodium veronal buffer, pH 8.6, and adjusted to a concentration of O.D. 50. Mixtures of two organisms were obtained for the separation experiments by mixing together equal volumes of the O.D. 50 suspensions. (All reagents were Difco except the sodium veronal buffer, which was Beckman.)

The apparatus was turned on 30 min prior to each trial to allow for temperature equilibration of both the buffer and the apparatus. A stable zero voltage reference band was established by adjusting the curtain flow and sample injection flow rates, which are interdependent (for any given curtain flow rate there is only one satisfactory rate of sample injection). Contents of the reference band were collected in several adjacent collection tubes. A 3-mm loopful from each tube was streaked to appropriate media to determine the effect of passage through the cell on the viability of the organisms. Typical operational parameters were as follows: Curtain buffer temperature 7°C, electrode rinse buffer temperature 10°C, curtain flow rate 9 ml/min, sample injection flow rate 20 μl/min, and applied voltage gradient 50 V/cm.

A potential was applied after the zero reference band was established. After banding occurred, the contents of the curtain were collected in tubes. (The entire buffer curtain on the apparatus is 48 mm wide; each collecting tube can collect only about a 1-mm slice of that curtain. Therefore, a single band more than 1 mm wide must be collected in several adjacent tubes.) The contents of the tubes and the buffer from clear areas devoid of any visible particulates were streaked on blood agar and tryptone–glucose–yeast extract agar (S.P.C.) and the streaked plates were incubated at 35°C for 48 hr. S.P.C. plates streaked with *S. marcescens* or *S. cerevisiae* were incu-

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TABLE 1

Band Positions and Operational Parameters in Separation of Microorganisms by Continuous Particle Electrophoresis

Organisms	Band characteristics: Position (mm) Ref. V_0	Band characteristics: Position (mm) Voltage applied	Band characteristics: No.	Band characteristics: Description	Operational parameters: Buffer temperature (°C) Curtain	Operational parameters: Buffer temperature (°C) Electrode rinse	Operational parameters: Curtain flow rate (ml/min)	Operational parameters: Sample injection rate (μl/min)
S. lutea and *B. megaterium*	19	A: 29–32 B: 34–35	2	A: Concn[a] B: Weak	8	14	6.5	30
E. coli and *S. aureus*	21	A: 31–34 B: 38–39	2	A: Weak B: Concn	4	6	10	30
E. coli and *S. marcescens*	21	27–32	1	Left to right decreasing opacity gradient	6.5	10	7.5	20
S. marcescens and *B. megaterium*	17	30–33	1	Weak	10	14	7.5	30
E. coli and *B. megaterium*	14	A: 28–30 B: 31–32	2	A: Concn B: Weak	8	13	10	30
E. coli and *S. cerevisiae*	17	A: 29–31 B: 32–38	2	A: Concn B: Weak	6	10	9.5	30

[a] Concentrated.

bated at room temperature (22–25°C) to stimulate pigment production for the former and to provide an optimal growth temperature for the latter. Morphologically distinct colonies on these agars were gram stained and their relative numbers noted.

RESULTS AND DISCUSSION

The operational parameters and band positions for the six typical separation experiments performed are given in Table 1. The correlative cultural-analytical data for the microbiological content of individual bands appearing within a single separation experiment are given in Table 2.

TABLE 2

Microbiological Content of Resolved Bands

Organisms	Blood[a]	S.P.C.[b]
S. lutea and *B. megaterium*	Band A: Equal proportions of both organisms Band B: Slightly higher numbers of *B. megaterium*	—
E. coli and *S. aureus*	Band A: Equal proportions of both organisms Band B: Equal proportions of both organisms	—
E. coli and *S. marcescens*	—	Left half—band (concn[c]): High numbers, *S. marcescens;* low numbers, *E. coli* Right half—band (weak): High numbers, *E. coli;* low numbers *S. marcescens*
S. marcescens and *B. megaterium*	—	One band: Equal proportions of both organisms
E. coli and *B. megaterium*	—	Band A: Equal proportions of both organisms Band B: Slightly higher numbers of *E. coli*
E. coli and *S. cerevisiae*	—	Band A: Equal proportions of both organisms Band B: Equal proportions of both organisms

[a] 5% Defibrinated sheep blood in trypticase soy agar base.
[b] Tryptone–glucose–yeast extract agar.
[c] Concentrated.

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The selection of organisms for study was based on two considerations: first, the possibility of electrophoretic separation; and second, the ease with which they could be identified culturally or morphologically. The separation of two organisms is dependent on a difference in their zeta potentials. Unfortunately, the zeta potential for any particular organism could not be known in advance. Therefore, it was assumed that if the chosen organisms differed greatly in cellular morphology, their surface charges would also differ greatly and their ease of separation would be enhanced correspondingly. All of the six organisms used appeared to possess these physical attributes. Furthermore, differences in gram staining, colonial morphology, pigmentation, and biochemical characteristics made these organisms especially easy to identify. The results show that under an applied voltage, bacterial or bacterial–yeast mixtures do resolve into separate bands. Unfortunately, the proportions of mixed microorganisms in each band are similar or not substantially different from the composition of the zero reference band. This is true even when the microorganisms are as different from each other as *S. cerevisiae* and *E. coli.* As seen in the viewing window of the CPE apparatus, *E. coli* and *S. marcescens* did not separate at all, but formed a single broad band with a left-to-right decreasing opacity gradient. Surprisingly, cultural analysis of the collection tube contents showed that the left-hand part of the band was composed predominantly of *S. marcescens* while the right-hand portion was predominantly *E. coli.* That was the best separation achieved in any of the experiments.

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The question of the significance of the banding which appears on electrophoresis still remains. Before the application of a potential, the zero voltage reference stream exists as a band approximately 1 mm wide. When a potential is applied, the bacterial stream deviates towards the cathode; obviously these organisms possess an electrical charge. Furthermore, for any single test organism, the deviated band widens and exhibits an internal concentration gradient. This suggests that individual organisms within a pure culture possess varying zeta potentials, possibly because of the heterogenicity of age groups within the culture.

When a pure culture of *E. coli* was subjected to electrophoresis, the band width of the migrating stream was narrower than that seen for corresponding cultures of *S. aureus* or *B. megaterium.* It is very likely that this occurs because *E. coli* cultures are composed of discrete units, probably of similar electrical charge, whereas cultures of *S.*

aureus and *B. megaterium* form variable clumps or chains which upset the charge distribution pattern. Hence, for these two organisms, a broad band width might be expected. This obstacle can be overcome only if some method can be devised to prevent clumping or chain formation in bacteria. Improved methods of sample preparation may contribute to a more successful application of CPE in the separation of bacterial mixtures.

Another problem still remains. In order to obtain clearly visible bands of bacteria in the viewing window of the apparatus, the injected sample must be very concentrated (approximately O.D. 50). Unless a method of band detection appreciably more sensitive than visual observation can be developed, this limitation would severely handicap practical utilization of the CPE for bacterial separations even if there were no other complications.

Under presumably favorable conditions, it was not possible to obtain a good resolution of a binary mixture of microorganisms into pure cultures. It is concluded that use of continuous particle electrophoresis at its present "state of the art" is not a practical method for separating bacteria.

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