APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1990, p. 2870–2875
0099-2240/90/092870-06$02.00/0

Vol. 56, No. 9

Single-Cell Entrapment and Microcolony Development within Uniform Microspheres Amenable to Flow Cytometry

RAPHAEL NIR, RAPHAEL LAMED, LEON GUETA, AND ELI SAHAR*

Department of Biotechnology, Tel-Aviv University, Tel-Aviv, Israel 69978

Received 22 February 1990/Accepted 19 June 1990

A method is presented for encapsulating single microbial cells in small spheres suitable for analysis and sorting by flow cytometry. The entrapped cells are able to multiply and form colonies contained within their respective microspheres. The system is based on ejecting the cells suspended in a gellable liquid through an orifice vibrating at ultrasonic frequencies, thus shearing the cell-containing jet into uniform droplets. When low-melting-temperature agarose was used, the droplets could be gelled into solid spheres during flight by appropriately directed cooling air streams. This gelling was accompanied by significant dehydration, resulting in a twofold decrease in bead diameter and a corresponding increase in agarose concentration. Nevertheless, the microbeads obtained were highly uniform and had diameters which could be precisely controlled in the range of 10 to 40 μm. A variety of bacterial and yeast species were entrapped in agarose beads by using this system. In all cases the cells were able to develop into microcolonies containing as many as several hundred cells. This system enables one to apply the powerful method of flow cytometry to the analysis and sorting of whole microbial colonies. Potential applications of this technology in various areas of microbiology are considered.

Flow cytometry has proved to be an extremely powerful technique in the analysis and sorting of mammalian cells (8). The application of flow cytometry to the study of microorganisms has been relatively limited for several reasons, including the small dimensions of common microbial cells, leading to smaller optical signals; filamentous morphology in many important species; and inability to examine secreted products, which often are the desired entity. We have previously suggested (12) that such drawbacks may be overcome if the cells were entrapped within small spheres (termed microbeads [MBs]) in a way which allows the single cells to develop into a microcolony confined within the MB. In addition to offering amplification of signals, such constructs may allow the flow-cytometric analysis of products secreted by the cells, provided these products are retained within the sphere. Recently it has been shown that entrapping cells in gel microdroplets may be useful for viable counting of microbial cells (14).

Although many methods have been described for immobilization of cells and cellular components for a variety of purposes (2, 5, 6), the particles obtained have usually had diameters in the range of 0.1 mm to several millimeters or were highly variable in size and thus not suitable for flow-cytometric analysis.

In this communication we describe a method to entrap single cells within spheres of homogeneous size in the range of 10 to 40 μm, suitable for flow-cytometric analysis. The conditions of the entrapment preserve the viability of the cells and allow their growth within the MB to form microcolonies.

MATERIALS AND METHODS

Microbial strains, media, and growth conditions. The strains used in this study were *Escherichia coli* 346, B, O2, JM101, and BE280; *Acinetobacter calcoaceticus* A2 and RAG1; *Pseudomonas aeruginosa* YS7 and PAKS-1; *Myxococcus xanthus* ER1500, ER1010, and DK5057; *Candida pseudotropicalis* IP-513; *Saccharomyces cerevisiae* 352-1C; *Streptomyces peucetius* ATCC 29050, and *Streptomyces lividans* 1326. All strains were obtained from scientists at Tel-Aviv University.

MSM medium contained (per liter of distilled water) 22.2 g of K_2HPO_4, 7.26 g of KH_2PO_4, 4.0 g of $(NH_4)_2SO_4$, and 0.2 g of $MgSO_4 \cdot 7H_2O$. The medium was adjusted to pH 7.0 with KOH and was autoclaved at 121°C for 20 min. The carbon source, unless otherwise indicated, was glucose (1%, wt/vol). YL medium contained (per liter) 3.0 g of KH_2PO_4, 1.0 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 2.0 g of yeast extract, and 20.0 g of lactose (autoclaved separately). Brain heart infusion (BHI) broth contained 37 g of brain heart infusion per liter (Difco Laboratories, Detroit, Mich.). CT medium contained (per liter) 10.0 g of Casitone (Difco) and 2.0 g of $MgSO_4 \cdot 7H_2O$. YPD medium contained (per liter) 20.0 g of glucose, 20.0 g of Bacto-Peptone (Difco), and 10.0 g of yeast extract (Difco). LB medium contained (per liter) 10.0 g of tryptone (Difco), 5.0 g of yeast extract, and 5.0 g of NaCl. YP medium contained (per liter) 3.0 g of yeast extract, 5.0 g of Bacto-Peptone, and 0.5 g of $Ca(NO_3)_2 \cdot 4H_2O$. It was adjusted to pH 6.4 with NaOH. All media were solidified, when required, by addition of 20 g of Bacto-Agar (Difco) per liter.

All growth experiments were performed with 100-ml flasks containing 20 ml of medium. The flasks were incubated at 30°C with shaking at 180 rpm in a forced-air shaker incubator (New Brunswick Scientific Co., Inc., Edison, N.J.).

E. coli 346, B, ESS, O2, BE280, and JM101 and *P. aeruginosa*YS7 and PAKS-1 were grown in MSM or LB medium; *A. calcoaceticus* RAG1 was grown in MSM medium containing 1% ethanol; *A. calcoaceticus* A2 was grown in brain heart infusion medium; *M. xanthus* strains were grown in CT medium; *Streptomyces* strains were grown in YP medium; *C. pseudotropicalis* IP-513 was grown in YL medium; *S. cerevisiae* 352-1C was grown in YPD medium.

* Corresponding author.

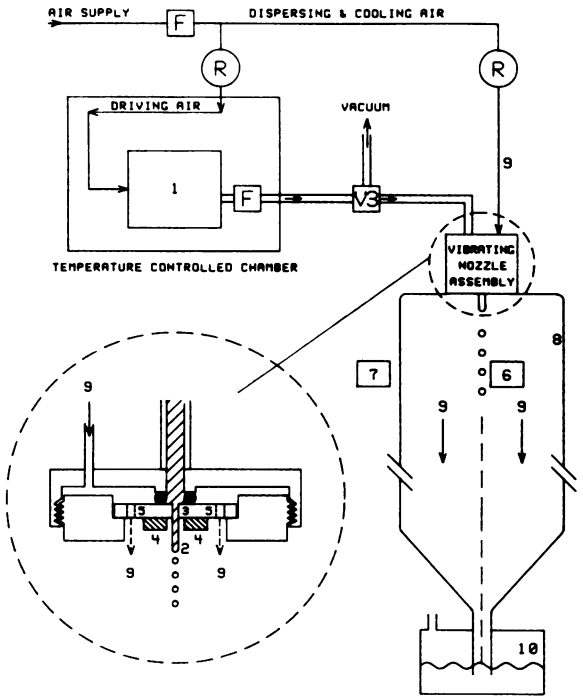

FIG. 1. Schematic description of the MB preparation device. Abbreviations: F, filter; R, pressure regulator; V3, three-way valve; 1, reservoir containing the microbial suspension in gelling medium; 2, liquid jet; 3, orifice; 4, piezoelectric crystal; 5, base plate; 6, light-emitting diode; 7, viewing microscope; 8, guiding cylinder; 9, dispersing and cooling air; 10, collection vessel.

Preparation of cells for entrapment in MBs. Cells were grown in the appropriate medium until the mid-log phase, harvested, washed, and suspended to approximately 10^9 cells per ml in MSM medium, unless otherwise indicated. *M. xanthus* cells were suspended in 10 mM MOPS (morpholinepropanesulfonic acid) buffer, and yeast cells were suspended in KMN buffer [3.0 g of KH_2PO_4 per liter, 1.0 g of $(NH_4)_2SO_4$ per liter, 0.1 g of $MgSO_4 \cdot 7H_20$ per liter]. The cells were then diluted to the desired concentration in molten agarose at 39°C. The agarose solution was prepared by dissolving type VII low-melting-point agarose (Sigma Chemical Co., St. Louis, Mo.) in the appropriate medium (MSM or MOPS buffer for bacteria; KMN buffer for yeasts) to a final concentration of 0.6% (wt/vol) and sterilizing it by autoclaving (121°C for 20 min).

MB preparation. MBs were prepared by using a vibrating-orifice system developed in our laboratory; this system is depicted schematically in Fig. 1. In this system the cells, suspended in a gellable or polymerizable medium such as agar and contained in a temperature-controlled reservoir (labeled 1 in Fig. 1) maintained at 39°C, were driven by sterile air pressure through an orifice (labeled 3). The air supply was sterilized by passage through a submicrometer filter (M-623; Motor Guard Corp.). Before reaching the orifice the cell suspension was filtered by a nylon mesh filter (F) (pore size, 6 to 8 μm; Swiss Silk Bolting Cloth Mfg. Co., Zürich, Switzerland). The continuous liquid jet (labeled 2) exiting the orifice was broken up into droplets by the vibrations of a ring-shaped piezoelectric crystal (Vernitron, Bedford, Ohio) (labeled 4) mounted on a base plate (labeled 5) which contained the interchangeable orifice. These narrow orifices (diameter, 10 to 25 μm) were created by using optical pinholes drilled in thin steel plates (Ealing Corp., South Natick, Mass.). The piezoelectric crystal was driven by a variable-frequency oscillator (70 V, 1 A), which also drove a light-emitting diode array (labeled 6) that allowed, by viewing the stroboscopic illumination through a ×10 to ×40 variable magnification microscope (labeled 7) (model VMT; Olympus, Tokyo, Japan), to monitor droplet formation. The droplets were dispersed (to prevent aggregation) and cooled by streams of air (labeled 9), emanating from a series of 12 0.5-mm-diameter holes located concentrically around the orifice. The air stream also served to guide the droplets through the cylindrical path (labeled 8) (made of transparent Plexiglas, 75 cm in length and 13 cm in diameter), facilitating their collection in a vessel (labeled 10) containing an appropriate liquid (usually MSM buffer). The vibrating-orifice assembly was heated by a heating coil wound around its housing, and the temperature was controlled by a temperature sensor embedded in the housing. The droplets were solidified into MBs by various means, depending on the medium used for their construction. When alginate was used (0.2% sodium alginate from *Laminaria hyperborea*; BDH, Poole, England), polymerization was achieved by collecting the droplets in a 2.0% solution of $CaCl_2$. When temperature-dependent gelling media such as agar or agarose were used, gelling was achieved by cooling the droplets in flight by the air streams (labeled 9) before the droplets were collected in the liquid medium.

Fluorescent MBs were produced by coupling the dye dichlorotriazinyl-aminofluorescein (DTAF; Research Organics Inc., Cleveland, Ohio) to the agarose (before the bead preparation). The DTAF solution (1 mg/ml of isopropanol) was added at 100-fold dilution to 0.6% molten agarose solution at pH 9.0 followed by incubation for 1 h at 39°C.

Determination of the number of cells entrapped in MBs. Samples were removed from the MB suspension and stained with acridine orange (Molecular Probes, Inc., Eugene, Oreg.) at a final concentration of 25 μg/ml. The stained MBs were observed microscopically under epifluorescent illumination, and the number of cells per bead was counted directly. Staining was not necessary for yeast cells, and the number of cells per bead was determined by using phase-contrast illumination. At least 200 beads were counted for each sample.

Development of microcolonies and determination of cell viability in the MBs. The suspension of MBs containing the entrapped cells was incubated in the appropriate medium as described for free cells. To determine viability, we removed samples at intervals and observed them microscopically under phase illumination to monitor the development of colonies within the MBs and to count them. The colonies were usually visible within a few hours. The viability of cells in the MBs was determined by dividing the average number of colonies that eventually developed per bead by the average number of cells per bead initially entrapped in the MBs. At least 500 beads were counted to obtain these averages. In some cases cells were fluorescently stained with 25 μg of acridine orange per ml to facilitate the counting of single cells.

Flow cytometry. MB suspensions were analyzed by using an unmodified flow cytometer (Cytofluorograf model 50H; Ortho Diagnostic Systems). Narrow-angle forward light scatter and fluorescence were measured for each particle, using 200 mW of light emitted by an argon ion laser at a wavelength of 488 nm. At least 10,000 particles were analyzed for each histogram.

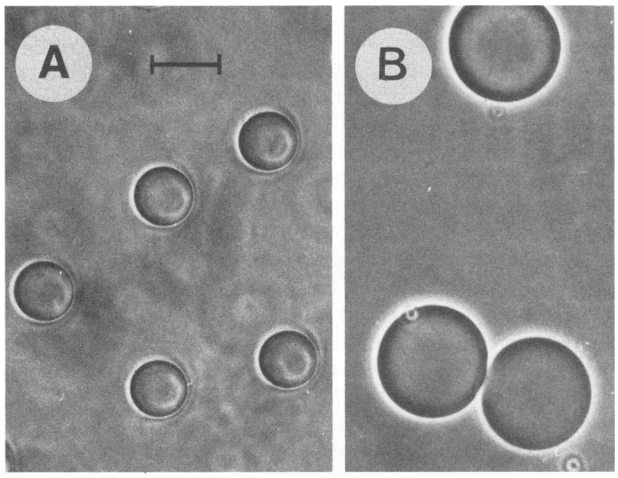

FIG. 2. Phase micrographs of MBs obtained with two different orifice diameters (D): (A) D = 10 μm; (B) D = 20 μm. MBs were prepared from 0.6% agarose. Experimental conditions were adjusted in each case to obtain MBs with the highest degree of homogeneity in bead size. Bar, 20 μm.

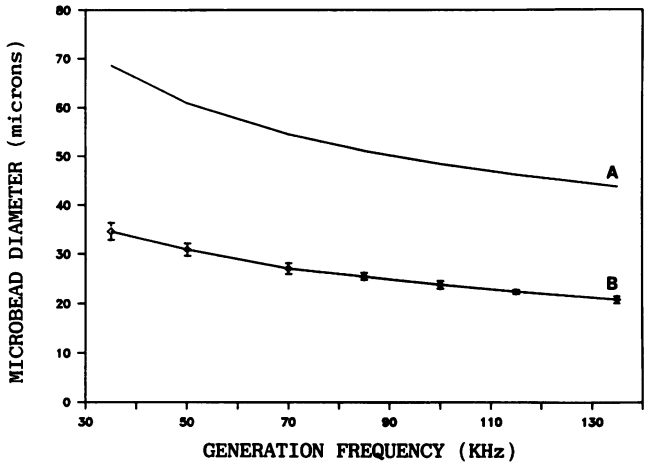

FIG. 3. Diameter of MBs as a function of the vibration frequency. Constant fluid flow rates were maintained. The theoretical values (curve A) were calculated from equation 1. The experimental values (curve B) were measured from photographs of the MBs and calibrated by comparison with a known graticule photographed under identical conditions. Each point on the graph represents the mean value ± standard deviation for 20 to 80 beads.

RESULTS

Preparation of MBs and their characteristics. The entrapment of cells within MBs of homogeneous, controllable size was achieved by using a system based on ejection of the cell suspension through a vibrating orifice, as described in detail in Materials and Methods. Low-melting-point agarose (type VII; Sigma) was found to be a suitable medium for microbial entrapment by the methods described here. All results described below were obtained with a 0.6% solution of this medium.

The size of the MBs produced by this system, as well as their homogeneity, depended on a complex interplay of the fluid velocity, the vibration frequency, and the orifice diameter, as well as on the gelling medium and the conditions under which it was solidified. Figure 2 illustrates the results obtained with orifice diameters of 10 μm (Fig. 2A) and 20 μm (Fig. 2B) under optimal conditions when fluorescently labeled agarose was used as the MB-forming medium. Flow-cytometric analysis (results not shown) revealed that in both cases, more than 95% of the MBs comprise one homogeneous group with a coefficient of variation of 1.7% in the fluorescence signal and 2.0% in the light scatter signal. Most of the remaining 5% are MBs that resulted from the merging of two droplets before solidifying, as can be deduced from the fact that their average integrated fluorescence is approximately twice that of the MBs contained in the first group.

The diameter D (in centimeters), of the droplets generated is a function of the rate, Q (in milliliters per hour), of fluid flow through the orifice and the vibration frequency, f (in Hertz), according to the following equation:

$$D = (Q/600\pi f)^{1/3} \quad (1)$$

Therefore, varying Q or f offered a measure of control over the size of the MBs when using a constant-orifice diameter. Figure 3 depicts typical results obtained with agarose MBs by varying the vibration frequency under constant fluid flow rate. The average MB diameter was measured microscopically for each preparation by using a calibrated graticule (Fig. 3, curve B). The MB diameter obtained was considerably smaller than the value expected from equation 1 (Fig. 3, curve A). Despite this discrepancy, a linear correlation was found (Fig. 4) between the theoretical droplet volume as calculated from equation 1 and both the average MB fluorescence and forward light scatter values (which are a measure of bead mass) as measured by flow cytometry.

To determine whether this discrepancy in size was due to contraction during the agarose gelling process or whether it was due to the disintegration of each droplet into several smaller droplets, we prepared MBs from agarose in which small fluorescent latex particles were suspended at a precisely measured concentration, C. If the decrease in MB size was due to contraction, the average number, N_m, of latex particles measured per MB would be identical to N_c, the value calculated from the measured value of C and the initial

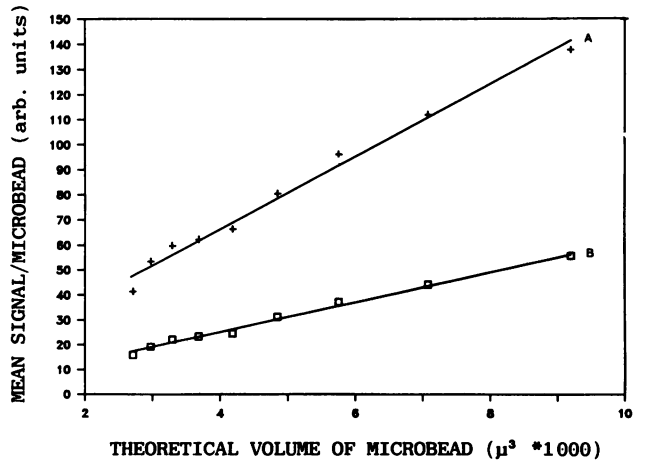

FIG. 4. Light scatter and fluorescence of MBs as a function of their initial volume at the orifice. The average light scatter (+) and fluorescence (□) values were measured by flow cytometry. The theoretical volume of the MB was calculated from equation 1. Different-sized MB populations were prepared by using fluorescently labeled agarose and varying the generation frequency. The solid lines were calculated by linear regression.

TABLE 1. Comparison of theoretical and observed numbers of latex particles per MB as a function of the vibration frequency

Vibration frequency (kHz)	No. of latex particles per bead	
	Theoretical (N_c)	Measured (N_m)
135	0.53	0.55
115	0.62	0.57
100	0.71	0.62
85	0.84	0.78
70	1.01	0.90
50	1.42	1.06
35	2.03	1.63

droplet volume obtained from equation 1. In contrast, if the droplets disintegrate before MBs are formed, N_m would be smaller than N_c. The results of these experiments (Table 1) show that there was good agreement between the values of N_m and N_c. Therefore, the agarose MBs indeed contracted during solidification. The precise degree of shrinkage (Fig. 3) was independent of the MB generation frequency and was equal to a decrease in MB volume by a factor of 8.3, as calculated from the microscopically measured average MB diameter. Such a contraction should be accompanied by a corresponding increase in the average concentration of agarose within the MB.

To verify this conclusion, the carbohydrate content of an MB suspension containing 1.5×10^6 MBs was determined by the phenol-sulfuric acid total-sugar assay (4), calibrated by the 6-mg/ml agarose solution used for the MB preparation. The average agarose concentration in the MBs was 49 mg/ml, confirming the high degree of compaction each MB underwent after its initial formation at the orifice and in excellent agreement with the value obtained, as described above, by direct measurement of the MB diameter.

MB preparation could be performed under aseptic conditions. Generation rates of up to 5×10^8 beads per h were easily achieved. The agarose MBs could be stored for many weeks without any sign of deterioration and were completely stable even under the high shear forces experienced during flow cytometric analysis (results not shown).

Cell entrapment and colony development in MBs. We entrapped microbial cells in the MBs by suspending them in the agarose in its melted state (at 39°C). The distribution of the number of cells per bead was expected to follow the Poisson distribution:

$$P(m,n) = e^{-m}m^n/n! \quad (2)$$

where m is the mean initial concentration of cells and $n = 0,1,2,3....$

To prove the applicability of the Poisson equation to the actual distribution of cells entrapped in the MBs, we analyzed 13 different preparations in which cells of various species (*S. cerevisiae*, *C. pseudotropicalis* and *E. coli*), as well as fluorescent latex particles (diameter, 1.55 μm), were entrapped in the MBs under different conditions. The distribution of the number of cells per bead, as counted microscopically, was in excellent agreement with the theoretical value calculated from equation 2 (Fig. 5). Therefore, for example, by choosing the initial cell concentration so that each MB contained on average 0.1 cell, 9% of the beads contained a single cell and 0.47% contained two or more cells. In this case more than 95% of the colonies that developed in the MBs originated from a single cell.

When MBs containing cells were suspended in appropriate growth media, the cells were able to divide within the MB and to form a microcolony which, if allowed sufficient time and sufficient nutrients, grew to fill the entire MB. Figure 6 illustrates the types of colonies obtained for *E. coli* (Fig. 6A), *S. cerevisiae* (Fig. 6B), and the gliding myxobacterium *M. xanthus* (Fig. 6C). A colony that grew to completely fill an MB contained several hundred microbial cells, as estimated from approximate counts performed on the cell population obtained by rupturing single MBs while observing them under the microscope. The local cell density within the MB was calculated to be as high as 10^{11} cells per ml.

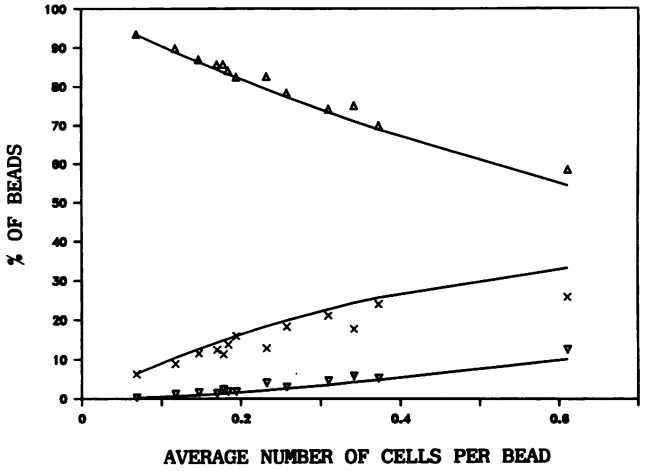

FIG. 5. Distribution of cell number per bead as a function of the average cell concentration. Continuous lines were calculated from the Poisson distribution. The discrete symbols represent the experimental values measured by using different preparations of MBs containing cells: △, empty beads; × one cell per bead; ▽, two cells per bead.

Table 2 presents a list of strains encapsulated and grown in this system to date. In all cases studied, cells were able to grow and form colonies that were confined within the MBs. The cell viability, as determined by the percentage of entrapped cells that developed into microcolonies, depended on the strain used and ranged between 30% (for bacteria) and 90% (for yeasts). Preliminary evidence indicates that the growth rate of cells within the MB, as measured by direct counting, was comparable to their growth rate in suspension (results not shown).

DISCUSSION

The results presented here demonstrate that single microbial cells can be entrapped in small, uniform semisolid spheres in a way that preserves their viability and allows their development into microcolonies entrapped within the spheres.

The vibrating-orifice technique was selected for the entrapment process, since it had been previously shown that it was capable of producing uniform, small liquid droplets in large numbers (1). This method has also been used recently for the generation of macroscopic alginate or carrageenan beads in which cells were immobilized for use as a catalyst in packed columns (13).

The medium composing the matrix of the MB should conform to several restrictions depending on the method used for their formation and the desired application. Induction of flow of the polymeric solution through a narrow orifice and the ultrasonic shearing of the liquid jet into

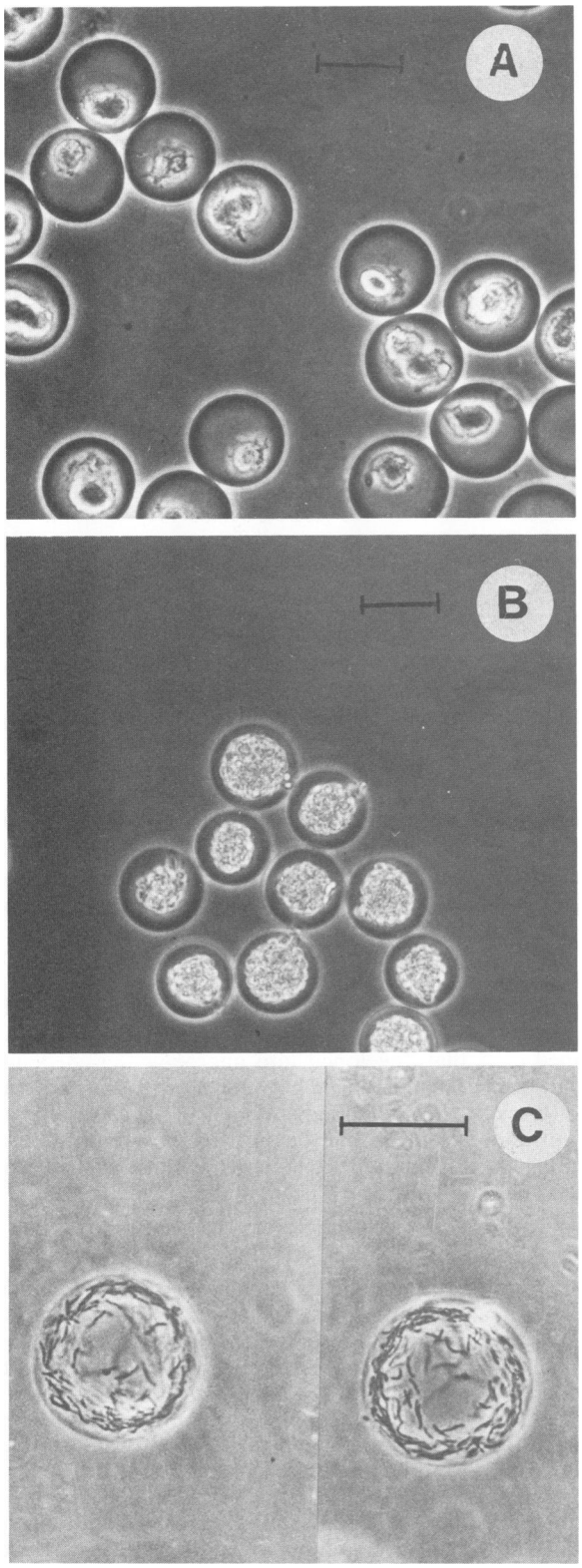

FIG. 6. Phase-contrast microscopy of microcolonies of different species in MBs. (A) *E. coli*; (B) *S. cerevisiae*; (C) *M. xanthus*. For panels A and B, microcolonies contained within MBs were sorted by flow cytometry directly onto glass slides to enrich the relative population of occupied beads. Bar, 20 μm.

TABLE 2. Microorganisms grown in MBs and their viability

Strain	Viability (%)
E. coli	
B	36.8
346	ND[a]
ESS	ND
O2	ND
BE-280	ND
JM101(pUC19)	ND
A. calcoaceticus	
A2	39.8
RAG-1	ND
P. aeruginosa	
YS-7	36.6
PAKS-1	30.4
M. xanthus	
ER1500	ND
ER1010	ND
DK5057	ND
S. peucetius	ND
S. lividans	ND
C. pseudotropicalis IP-513	56.0
S. cerevisiae 352-1C	91.0

[a] ND, Not done.

uniform droplets dictates a medium of relatively low viscosity. For cell proliferation in the MBs, a medium which can entrap the microbial cells and allow their growth is needed; preferably a gelling or polymerizable material which is favorable for the cells in both its liquid and polymerized or gelled forms. Agarose and agar were both suitable for our purpose. Agarose of low gelling temperature was compatible with high cell viability, since it remains liquid down to 30°C at the concentrations used. Its relatively low viscosity permitted free flow through a 10-μm nozzle and allowed shearing of the liquid jet into droplets at sonic frequencies as high as 200 kHz. Alternatively, selection of a high-melting-point agarose yielded beads which were suitable for the growth of thermophilic bacteria at temperatures as high as 70°C (results not shown). An additional advantage of agarose, in the context of this study, is the fact that it is used as a gelling agent in the food industry (10). Its use should therefore pose no safety problems even for encapsulating cells used to produce food-grade chemicals.

The methods previously used to entrap cells in agarose (7, 9) were usually based on emulsification of the aqueous agarose solution in a hydrophobic medium; this resulted in highly nonuniform beads, the majority of which had diameters larger than 0.1 mm. The method described here yielded highly uniform beads with coefficients of variation of bead diameter as low as 1.7% and bead diameters as small as 10 μm. The bead size could be precisely controlled by varying the vibration frequency or the fluid flow rate, as well as by choosing the appropriate orifice diameters.

The solid beads obtained were considerably smaller than the initial liquid droplet. Although the kinetics and dynamics of this contraction have not been studied in detail, it indicates that the droplets underwent a dehydration process during flight before final solidification. The contraction in bead diameter was approximately twofold, irrespective of the initial droplet size. As a result, this method allows one to produce dense beads with final agarose concentrations in the range of 5%, a concentration which would have hindered the free flow of the melted agarose solution and demanded

considerably higher shear forces to break the jet into droplets. It is somewhat surprising that despite this high degree of contraction, the resulting beads were still highly uniform in size. One may speculate that the degree of dehydration of each droplet is determined not by its individual trajectory but rather by the final agarose concentration reached.

The distribution of cells within the MBs was in excellent agreement with that predicted by the Poisson equation. Therefore, the distribution of cells in the beads could be precisely predicted and controlled. This may be especially important when this system is used for mutant selection, when it is desirable that the fraction of beads initially occupied by a single cell is at a maximum.

The system described here may have several applications. Most notably, it allows us, for the first time, to apply the power of flow cytometry to the analysis of microbial colonies rather than to single cells. We have recently shown that this may be used to study cellular behavior and cell-cell interactions during the development of a colony (11). The amplification resulting from the simultaneous observation of many cells of the same clone may allow the measurement of signals which are too weak to measure from a single cell. In addition, the flow-cytometric measurement of extracellular secreted products, the selection of desired mutants in cases in which no growth-selective conditions are available, and the analysis of filamentous microorganisms may become possible. Finally, the small size of the beads described here would result in a decrease in the time needed for diffusion into or out of the MB. The extreme homogeneity in bead size ensures that each cell is subject to conditions more nearly identical than is possible with other entrapment procedures. The application of this procedure to the immobilization of a variety of other biological entities, such as mammalian cells, enzymes, and biological insecticides, awaits further investigation.

ACKNOWLEDGMENTS

This work was supported in part by grants 86/101 and 84/87 from The Israel Ministry of Industry and Commerce. R.N. is the recipient of a fellowship from the the Israel National Council for Research and Development.

The expert technical assistance of Nahum Lavie is gratefully acknowledged. We are also grateful to Yossef Shabtai for many helpful discussions.

LITERATURE CITED

1. **Berglund, R. N., and B. Y. H. Liu.** 1973. Generation of monodisperse aerosol standards. Environ. Sci. Technol. **7**:147–153.
2. **Chibata, I., T. Tosa, and T. Sato.** 1986. Methods of cell immobilization, p. 217–229. *In* A. L. Demain and N. A. Solomon (ed.), Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, D.C.
3. **Cook, P. R.** 1984. A general method for preparing intact nuclear DNA. EMBO J. **3**:1837–1842.
4. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
5. **Jackson, D. A., J. Yuan, and P. R. Cook.** 1988. A gentle method for preparing cyto- and nucleo-skeletons and associated chromatin. J. Cell Sci. **90**:365–378.
6. **Klein, J., and F. Wagner.** 1983. Methods for the immobilization of microbial cells. Appl. Biochem. Bioeng. **4**:11–51.
7. **Mosbach, K., S. Birnbaum, K. Hardy, J. Davies, and L. Bulow.** 1983. Formation of proinsulin by immobilized *Bacillus subtilis*. Nature (London) **302**:543–545.
8. **Muirhead, K. A., P. K. Horan, and G. Poste.** 1985. Flow cytometry; present and future. Biotechnology **3**:337–356.
9. **Nilsson, K., S. Birnbaum, S. Flygare, L. Linse, U. Schroder, U. Jeppsson, P. O. Larsson, K. Mosbach, and P. Brodelius.** 1983. A general method for the immobilization of cells with preserved viability. Eur. J. Appl. Microbiol. Biotechnol. **17**:319–326.
10. **Roels, J. A., and H. A. C. Thyssen.** 1987. Impact of biotechnology on the food industries, p. 21–35. *In* D. Knorr (ed.), Food biotechnology. Marcel Dekker, Inc., New York.
11. **Rosenbluh, A., R. Nir, E. Sahar, and E. Rosenberg.** 1989. Cell-density-dependent lysis and sporulation of *Myxococcus xanthus* in agarose microbeads. J. Bacteriol. **171**:4923–4929.
12. **Sahar, E., R. Nir, Y. Yisraeli, Y. Shabtai, and R. Lamed.** 1987. A system for microbial strain improvement by product measurement and flow sorting of microcolonies. Cytometry Suppl. **1**:93.
13. **Scott, C. D.** 1987. Techniques for producing monodispersed biocatalyst beads for use in columnar bioreactors. Ann. NY Acad. Sci. **501**:487–493.
14. **Weaver, J. C., G. B. Williams, A. Klibanov, and A. L. Demain.** 1988. Gel microdroplets: rapid detection and enumeration of individual microorganisms by their metabolic activity. Biotechnology **6**:1084–1089.