APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1996, p. 4180–4185 Vol. 62, No. 11
0099-2240/96/$04.00+0

Autoradiographic Method for Isolation of Diverse Microbial Species with Unique Catabolic Traits

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Received 8 May 1996/Accepted 3 September 1996

A novel autoradiographic method for isolation of bacteria with unique catabolic traits was developed to overcome many of the limitations of traditional selective enrichment techniques. The method consists of five steps. (i) An environmental sample is directly plated (without enrichment) on a microporous filter atop a solid medium that allows cultivation of diverse kinds of microorganisms. (ii) Once colonies form, two replicas of the filter are prepared and the colonies are regrown. (iii) The replica filters are starved 24 to 72 h to deplete intracellular carbon reserves and then (iv) placed on $Na_2{}^{35}SO_4$-containing solid media with and without a test compound. (v) Following an incubation period, the replica filters are exposed to film in order to identify colonies that incorporate more ^{35}S into cell biomass in the presence of the test compound than in its absence, providing presumptive evidence for metabolism of the compound. The colonies identified in this manner can be recovered from the master filter. To demonstrate this technique, bacteria capable of degrading benzoate were isolated from a single soil slurry by traditional enrichment as well as by autoradiography. From the enrichment culture, a single isolate able to degrade benzoate was obtained. In contrast, 18 distinct strains were obtained by purifying 19 putative benzoate-degrading colonies identified by autoradiography. Each of the 18 strains was able to completely transform the substrate, as determined by high-performance liquid chromatography analyses. The doubling times of a subset of the isolates grown in benzoate medium ranged from 1.4 to 17.1 h, whereas the doubling time of the isolate obtained by enrichment was 2.0 h. These data demonstrate that the method described here can be used to obtain a collection of diverse organisms able to metabolize a specific compound.

Selective enrichment culture techniques have traditionally been used to isolate bacteria in studies of microbial ecology and physiology. These techniques typically involve incubation of mixed populations (from soil or activated sludge, for example) in a medium designed to foster the growth of only those microorganisms exhibiting a particular phenotype. Serial transfers of the enrichment culture to fresh medium are continued until the microbial culture appears to consist mainly of one or very few types of microorganisms exhibiting the desired properties. Undesired organisms are eliminated from the culture through competitive exclusion or dilution. Although selective enrichment techniques have historically been used with great success, there are two major limitations to these techniques which hamper their effectiveness in the study and exploitation of the metabolic diversity and ecology of microorganisms.

First, the diversity of isolates within a sample and their frequencies in the environment are difficult to assess by the use of enrichment culture techniques. Batch cultures are competitive environments which typically select organisms with rapid growth rates. Thus, organisms with inherently slower growth rates or growth rates which are lower under the conditions used are excluded. Organisms with nutritional requirements are often overlooked as well since minimal media are frequently used for selective enrichments in an effort to limit or prevent the growth of undesired organisms. Thus, the diversity of organisms obtained from a single enrichment culture is low.

Second, enrichment procedures make it difficult to rapidly obtain organisms able to degrade recalcitrant substrates. Reports of isolation times from several months to a year are not uncommon (1, 12, 19, 24). With enrichment cultures, the length of time required to purify bacteria is determined primarily by bacterial growth rates. Substrates which are metabolized inefficiently, toxic at high concentrations, or slightly soluble can substantially increase the time required for organisms of interest to become established as dominant members of a culture.

Because of the limitations described above, we developed a simple method that does not rely on the use of selective enrichment techniques for screening and isolating microorganisms with particular phenotypes. The method involves cultivation of bacteria on filters atop a nonselective agar medium that fosters the growth of diverse bacterial strains, subsequent incubation on radioactive test media, and the use of autoradiography to identify colonies with desired phenotypes amidst a background of other colonies. Since this method permits the use of rich media and spatially separates otherwise competing populations, simultaneous recovery of organisms with different growth rates and nutritional requirements is possible, and large numbers of bacteria can be screened rapidly at one time.

MATERIALS AND METHODS

Media and reagents. R2A agar (Difco Laboratories, Detroit, Mich.), which is a general-purpose medium for cultivation of heterotrophs, was used for initial cultivation of bacteria from soil. After isolation, bacterial strains were routinely cultured using one-quarter-strength trypticase soy agar (TSA). Bacterial phenotypes were assayed in defined aerobic basal (DAB) medium, described in Table 1, with or without benzoate (3 mM).

Soil. Soil for isolation of benzoate degraders was collected in October 1993 from an agricultural plot at Kellogg Biological Station, Hickory Corners, Michigan, and stored at 4°C. A single soil slurry consisting of 1 g of soil and 9 ml of 10 mM phosphate buffer (pH 6.8) was used as a common inoculum source for

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TABLE 1. Composition of defined aerobic basal (DAB) medium

Compound	Concn (mg/liter)
Na_2HPO_4	866
KH_2PO_4	531
$(NH_4)_2SO_4$	0.3
$MgSO_4$	50
$CaCl_2 \cdot 2H_2O$	5.88
Na_2HPO_4	3.2
$FeSO_4 \cdot 7H_2O$	2.78
$ZnSO_4 \cdot 7H_2O$	1.15
$MnSO_4 \cdot H_2O$	1.69
$CuSO_4 \cdot 5H_2O$	0.375
$Co(NO_3)_2 \cdot 6H_2O$	0.233
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.1236
Pyridoxine-HCl	0.05
Thiamine-HCl	0.025
Nicotinic acid	0.025
p-Aminobenzoic acid	0.025
Biotin	0.01
Folic acid	0.01
Pyridoxal phosphate	0.0005
Riboflavin	0.025
Thioctic acid	0.025
Pantothenic acid	0.025
L-Glutamate	0.94
L-Leucine	0.38
L-Lysine	0.30
L-Serine	0.30
L-Isoleucine	0.26
L-Tyrosine	0.26
L-Valine	0.26
L-Aspartate	0.25
L-Alanine	0.22
L-Phenylalanine	0.2
L-Arginine	0.18
L-Threonine	0.16
L-Methionine	0.14
L-Asparagine	0.1
L-Histidine	0.08
L-Tryptophan	0.04
L-Glycine	0.02
L-Proline	0.02

most probable number (MPN) assays and for isolation of organisms able to degrade benzoate (benzoate-positive organisms) by enrichment or by autoradiographic plating. The phosphate buffer was identical to the buffer used in DAB medium. After being shaken for 30 min at 30°C (at 225 rpm on a rotary shaker), the slurry was serially diluted by transferring 1 ml to 9 ml of sterile phosphate buffer.

Enumeration of bacteria. The MPN values for culturable heterotrophs and benzoate-positive organisms in the soil sample were determined in accordance with the procedure of Cochran (3). Serial dilutions of the soil slurry were used to inoculate replicate tubes (five per dilution) containing either R2A liquid medium (for heterotrophs) or DAB-benzoate medium. MPN tubes were incubated at 30°C for 2 weeks. Cultures which became visually turbid were considered positives. The number of positive tubes at each dilution was scored, and estimates of microbial numbers were obtained from statistical tables (3). Bacterial numbers were also estimated by counting colonies after growth on R2A agar for 7 days. The total number of colonies was used as an estimate of heterotrophic bacterial numbers. The fraction of colonies putatively identified as benzoate positive by autoradiography (described below) was used as an estimate of benzoate-positive organisms in the soil sample.

Batch culture enrichment. An enrichment culture for benzoate-positive organisms was established by adding 0.1 ml of the soil slurry to a 50-ml flask containing 20 ml of DAB–3 mM benzoate medium. The culture was shaken at 30°C in a rotary incubator shaker (225 rpm), and 0.2 ml was transferred to fresh medium every 4 days. After three transfers, the enrichment culture was streaked on R2A agar medium. Colonies with unique morphologies were transferred to 3 ml of DAB-benzoate medium. Cultures which became turbid were tested by high-performance liquid chromatography (HPLC) for benzoate degradation. Positive cultures were streaked on one-quarter-strength TSA to check for purity, then regrown in DAB-benzoate medium to establish 15% glycerol stocks for storage at −80°C.

HPLC analyses. The concentration of benzoate in liquid media was routinely measured by using a Hewlett-Packard series 1050 HPLC apparatus with a LiChrosorb RP-18 column (E. Merck, Darmstadt, Germany) and a 50% methanol–50% phosphoric acid mobile phase. One-milliliter samples of broth cultures were filtered through 0.45-μm-pore-size Acrodisc filters (Gelman, Ann Arbor, Mich.) prior to analysis.

Direct plating method. (i) Production of master filters. For isolation of benzoate-degrading bacteria by direct plating, 0.1- to 0.5-ml volumes of various dilutions of the soil slurry (described above) were spread on sterile, detergent-free, 82-mm-diameter nitrocellulose filters (Millipore HATF; 0.45-μm pore size) atop R2A agar plates. Prior to use, filters were soaked in MilliQ water, sandwiched (in single layers) between squares of Whatman #1 filter paper, and then wrapped in aluminum foil and autoclaved for 25 min. Orientation marks were drawn on the topside of filters with permanent ink just before the filters were transferred to R2A plates. Filters spread with soil dilutions (master filters) were incubated on R2A plates for 7 days at 30°C and then replicated.

(ii) Production of replica filters. Replica filters were produced essentially as described by Hanahan and Meselson (10), by pressing the master filters against sterile HATF nitrocellulose filters. To accomplish this, a master filter was removed from an agar plate and placed colony side up on a sterile square of Whatman #1 paper. A sterile filter was placed on the master filter, both were covered with another square of Whatman #1 paper, and then the stack was compressed with a surface-sterilized Plexiglas weight. Before the master and replica filters were separated, the reference marks on the master filter were copied onto the back of the replica filter to record the orientation of the two filters relative to one another. From each master filter, two or more replicas were produced. Following replication, all filters were transferred to fresh R2A plates and regrown for 24 h at 30°C. The master filter was then photographed and stored at 4°C for further use. The replica filters were transferred to sterile DAB–1% agarose plates and starved for 72 h at 30°C to deplete intracellular carbon reserves.

(iii) Substrate assay with replica filters. Following the starvation period, replica filters were transferred to DAB-agarose plates containing either 1 μCi of $Na_2{}^{35}SO_4$ (Dupont NEN) ml^{-1} (control plate) or 1 μCi of $Na_2{}^{35}SO_4$ ml^{-1} with 3 mM benzoate (test plate). After 3 days of incubation at room temperature, the filters were transferred to 10 mM phosphate-buffered Bacto Agar (1%) medium containing 0.2 M Na_2SO_4 to remove unincorporated $Na_2{}^{35}SO_4$ from the filters. Following a 60-min incubation on the wash medium, the filters were taped onto sheets of paper (20 by 25 cm), air-dried, wrapped with plastic wrap, and exposed to Kodak XAR film (Eastman Kodak Company, Rochester, N.Y.) overnight at −80°C. The film was developed in accordance with the manufacturer's recommendations. Orientation marks on the replica filters were then copied onto the developed films.

(iv) Identification and isolation of colonies from master filters. Putative benzoate-degrading colonies were identified by comparing the intensities of signals from filters incubated on DAB-$Na_2{}^{35}SO_4$-agarose medium with and without benzoate. The locations of these colonies on a master filter were determined by alignment of a given autoradiogram with the corresponding master filter. Colonies corresponding to signals on the film were picked and restreaked on TSA agar to check for purity. Several colonies apparently unable to metabolize benzoate were also picked and restreaked. Purified cultures were then inoculated into sterile DAB-benzoate liquid medium to confirm their phenotypes. Benzoate disappearance was monitored by HPLC, and 15% glycerol stocks of positive cultures were established for storage at −80°C. Cultures unable to degrade benzoate were regrown in 1/10-strength trypticase soy broth in order to establish −80°C glycerol stocks.

Sensitivity of detection. Two bacterial cultures (one benzoate-positive strain and one benzoate-negative strain) were separately grown to stationary phase in 1/10-strength trypticase soy broth and then mixed together at various ratios. From each mixed culture, 0.1 ml was spread on nitrocellulose filters atop R2A agar. Plates were incubated at 30°C for 3 days, then replicated and processed by autoradiography as described above.

REP-PCR. Fingerprints of the genomes of the isolates were obtained by using consensus primers for repeated extragenic palindromic (REP) sequences (25) and PCR conditions described by de Bruijn (7). Each strain was grown to stationary phase in DAB-benzoate medium or in 1/10-strength trypticase soy broth; then cells were disrupted by four freeze-thaw cycles. For each strain, 1 μl of thawed cell lysate was added to 24 μl of PCR reaction mix. PCR reactions were performed in a Perkin-Elmer 9600 thermal cycler. Reaction products were visualized by electrophoresis in 1.5% agarose gels containing 0.5× TAE and ethidium bromide (22). PCR amplification was performed twice for each strain to confirm the reproducibility of fingerprint patterns.

16S rDNA restriction analysis. Amplified ribosomal DNA restriction analysis was carried out as follows. PCR amplification of the genes coding for 16S rRNA (16S rDNA) was performed by using primers 8-27 and 1541-1522 (2) under the reaction conditions described by Laguerre et al. (18). For each 100-μl reaction volume, 1 μl of thawed cell lysate (described above) was used as template. Approximately 10 μl of PCR product was subsequently used for each restriction endonuclease digestion. Amplified 16S rDNA was digested separately with *Hae*III, *Cfo*I, *Msp*I, and *Hin*fI in accordance with the manufacturer's recommendations. Restriction fragments were visualized by electrophoresis in 2.75% Metaphor agarose (FMC) gels containing TAE and ethidium bromide.

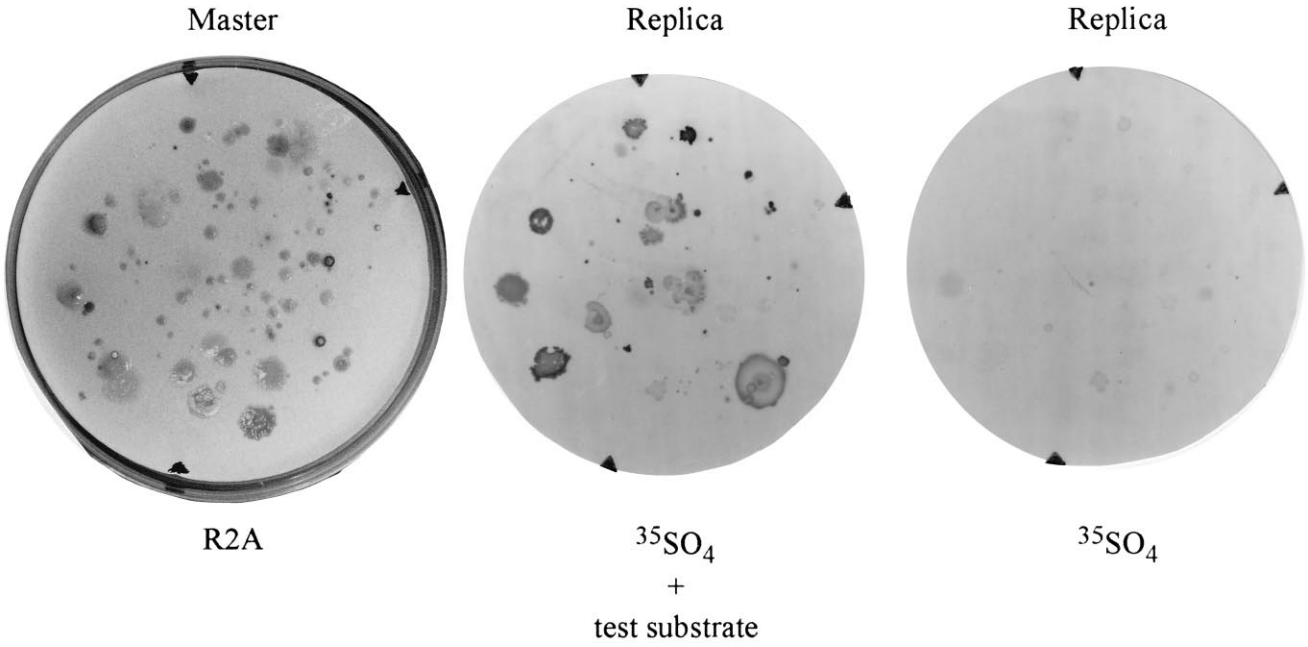

FIG. 1. Autoradiographic detection of benzoate-positive organisms. Shown are a master filter (left) containing heterotrophic bacterial colonies from soil and autoradiograms of two replica filters. Colonies which produce darker autoradiography signals in the presence of benzoate (middle) than in its absence (right) are putative benzoate-positive colonies.

Growth rates. Growth rates of organisms were determined in two ways. A rough comparison of the growth rates of all isolates was performed by determining the number of days required for pinpoint colonies to form on TSA plates. In these experiments, isolates were streaked directly from glycerol stocks. A more precise comparison of growth rates was performed with a subset of isolates representing the three growth rate categories (fast, medium, and slow) observed on TSA plates. Single colonies on TSA plates were transferred to 5 ml of DAB-benzoate medium and grown at 30°C, at 225 rpm, to mid-log phase. Portions of these cultures were diluted in 25-ml volumes of sterile, prewarmed DAB-benzoate medium in 125-ml flasks to an optical density at 600 nm of approximately 0.001. Each isolate was tested in triplicate. Optical density was monitored with a Hewlett-Packard diode array model 8452A spectrophotometer, and doubling times were calculated from least-squares regression analysis of transformed data (17).

RESULTS

Bacterial numbers. Estimates of bacterial numbers in soil were obtained from viable counts on nitrocellulose filters atop R2A agar and from MPNs. According to plate counts, the numbers of heterotrophs and benzoate-positive organisms in the soil were approximately 4.9×10^6 and 1.1×10^5 CFU/g of soil, respectively. Similarly, according to counts from MPN tubes, which were incubated 1 week longer than the plates, the numbers of heterotrophs and benzoate-positive organisms were approximately 7×10^7 and 3.3×10^6 cells/g of soil, respectively. Thus, among the culturable microorganisms in the soil, benzoate-positive organisms represented less than 5% of the heterotrophic community.

Isolation of benzoate-positive organisms. Bacteria able to degrade benzoate were obtained by enrichment and by direct plating with autoradiography. The benzoate enrichment culture was serially transferred four times and then streaked on one-quarter-strength TSA; three prominent colony morphotypes were observed. Only one of the three types was capable of transforming benzoate, as determined by HPLC analyses of cultures grown in DAB-benzoate medium (data not shown). In contrast, a large number of benzoate-positive isolates were identified by autoradiography of a single filter spread with a 2.5×10^{-5} soil dilution (Fig. 1). Of the 125 colonies which grew on the filter, 27 colonies incorporated ^{35}S and produced autoradiographic signals when incubated in the presence of benzoate and not in the absence of benzoate. Thus, these 27 colonies were identified as putative benzoate-positive organisms. Of these 27, 19 were picked and purified by restreaking. All 19 isolates completely transformed benzoate, as determined by HPLC analyses (Table 2). An additional five colonies identified by autoradiography as benzoate negative (no incorporation of ^{35}S in the presence of benzoate) were purified. These five isolates differed from one another in colony morphology and REP-PCR pattern. None of them was able to transform benzoate in DAB-benzoate medium. Thus, for the 24 colonies tested, the HPLC results confirmed the phenotypes indicated by autoradiography.

Diversity of benzoate-positive isolates. Genetic diversity among the benzoate-positive isolates was assessed by comparison of their REP-PCR fingerprints. Among the 19 benzoate-positive isolates obtained by direct plating, 18 distinct patterns were observed (Fig. 2). The fingerprints of the 19 direct-plating isolates also differed from the REP-PCR pattern of the enrichment isolate, E1. It is interesting to note that these 20 benzoate-positive isolates represent at least 14 different species, as determined by restriction analysis of 16S rDNA (data not shown).

These isolates were divided into three groups (fast, medium, and slow) on the basis of the length of time required for pinpoint colonies to develop on one-quarter-strength TSA plates (Table 2). The growth rate of an isolate chosen from each group was measured in DAB-benzoate medium. As illustrated in Fig. 3, the doubling times ranged from 1.4 h (standard deviation, 0.082 h) to 17.1 h (standard deviation, 0.098 h). The doubling time of the isolate obtained by enrichment was 1.97 h (standard deviation, 0.032 h). These data demonstrate that a collection of phenotypically and genotypically diverse isolates was efficiently obtained by autoradiographic plating, in sharp contrast to the enrichment culture, which yielded a single benzoate-positive isolate.

TABLE 2. Genotypic and phenotypic comparison of isolates obtained by enrichment and by direct plating

Isolate	REP-PCR pattern	Phenotype[a]		Growth on 1/4 TSB agar[b]	Generation time, h (SD)[c]
		Putative (autoradiography)	Confirmed (HPLC)		
E1	1	NA	+	F	2.0 (0.082)
DP1	2	+	+	F	ND[d]
DP2	3	+	+	F	ND
DP3	4	+	+	F	1.4 (0.032)
DP4	5	+	+	F	ND
DP5	6	+	+	F	ND
DP6	7	+	+	F	ND
DP7	8	+	+	M	ND
DP8	9	+	+	M	ND
DP9	9	+	+	M	ND
DP10	10	+	+	M	ND
DP11	11	+	+	M	3.4 (0.366)
DP12	12	+	+	M	ND
DP13	13	+	+	M	ND
DP14	14	+	+	M	ND
DP15	15	+	+	S	ND
DP16	16	+	+	S	6.5 (0.311)
DP17	17	+	+	S	17.1 (0.098)
DP18	18	+	+	S	ND
DP19	19	+	+	S	ND
DP20	20	−	−	F	ND
DP21	21	−	−	M	ND
DP22	22	−	−	M	ND
DP23	23	−	−	M	ND
DP24	24	−	−	S	ND

[a] NA, not applicable; +, benzoate positive; −, benzoate negative.
[b] 1/4 TSB, one-quarter-strength TSB. F, fast; M, medium; S, slow.
[c] Doubling times were calculated by least-squares analysis of transformed data.
[d] ND, not determined.

Sensitivity. The sensitivity of the method was measured by detection of strain DP5 (benzoate positive) on plates with a lawn of strain DP24 (benzoate negative). The frequency of DP5 was held constant at 500 CFU/ml, while the frequency of DP24 varied from 0 to 10^8 CFU/ml. Strain DP5 was detected in all mixtures; thus, the method can be used to identify organisms of interest in a large background of colonies formed by other organisms (Fig. 4). Of course, the practical detection limit will depend on the diversity and kind (size and other characteristics) of colonies that develop on agar media after an actual sample has been plated.

DISCUSSION

The autoradiographic method is based on the detection of radionuclide incorporation as an indicator of cell growth. Previous investigators have used the rate of sulfur incorporation into protein as a measure of bacterial growth rate and the total amount of sulfur incorporated as a measure of total microbial growth (5, 8, 16). We have extrapolated this accepted technique to the growth of colonies on nitrocellulose filters on a solidified growth medium. Since most bacterial species are capable of using sulfate as a sole source of sulfur (6, 9), radiolabeled sulfate can be effectively used with a broad range of bacterial species. Other radiolabeled elements, such as phosphorus, that are incorporated into cellular macromolecules (e.g., nucleic acids and phospholipids) can also be successfully used (data not shown).

The data show that the direct plating method facilitates the isolation of diverse bacterial strains of a specific catabolic phenotype. The genetic diversity of benzoate-positive isolates obtained was evident from the differences in REP-PCR genomic fingerprints and amplified ribosomal DNA restriction analyses. Differences among benzoate-positive strains were also reflected in their growth rates, which varied 12.2-fold. The fact that only 2 isolates among the 19 obtained by the autoradiographic method share the same REP-PCR pattern suggests that the total diversity of benzoate-positive populations in the soil is very high and that it greatly exceeded the number of strains sampled. Thus, it is not necessarily surprising that strain E1, isolated by enrichment culture techniques, differed from all 19 isolates obtained by the autoradiographic technique. It could be that strain E1 would have been detected by autoradiography if a larger number of strains were examined. Alternatively, strain E1 may have been initially present in the soil in very low numbers but increased in the liquid batch culture to become numerically dominant. Use of the autoradiographic method provides a way to obtain a greater diversity of isolates than might be obtained by using enrichment cultures; however, some strains with the desired phenotype will not be obtained if they are initially rare relative to other populations with the same phenotype.

The lack of diversity observed in the benzoate enrichment culture is typical of this technique. By design, an enrichment culture is intended to yield only one organism exhibiting a particular catabolic phenotype, although the original sample may have contained a diverse group of organisms possessing the same phenotype. Furthermore, the high substrate concentrations ($\gg K_m$) commonly used in batch culture enrichments generally select for fast-growing organisms (11). However, cul-

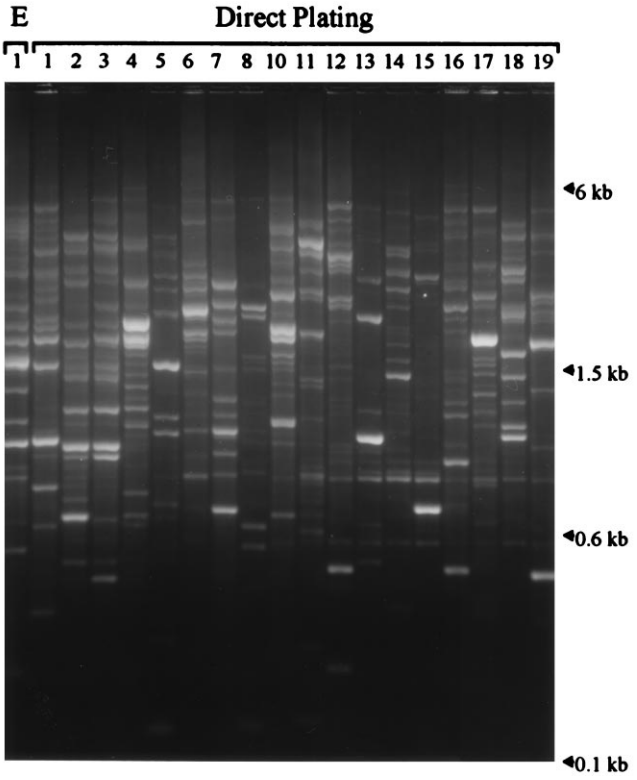

FIG. 2. REP-PCR fingerprint patterns observed among isolates obtained by enrichment and by direct plating. Lane 1, enrichment isolate E1; lanes 2 to 20, direct plating isolates DP1 to DP 19. (Isolate DP9 was omitted; its REP-PCR pattern is identical to that of DP8.)

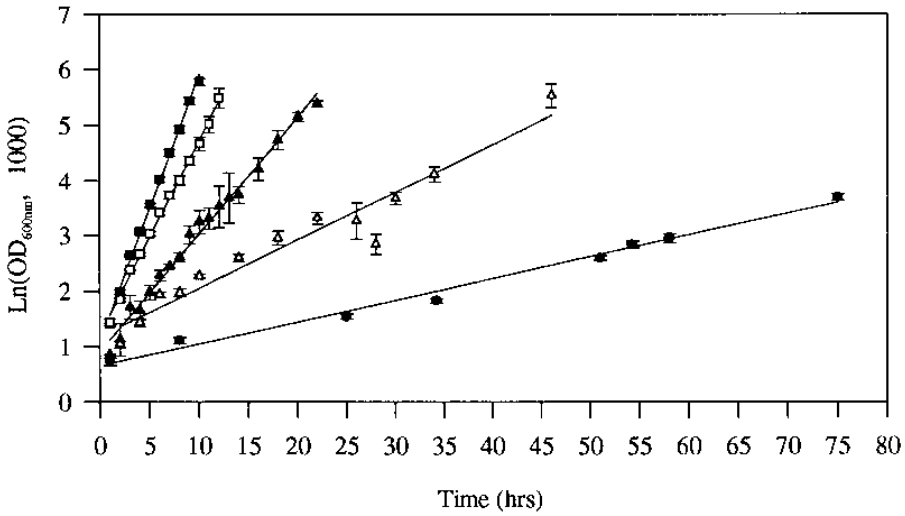

FIG. 3. Growth rates of five benzoate-positive isolates. ■, strain DP3; □, strain E1; ▲, strain DP11; △, strain DP16; ●, strain DP17. Error bars are standard deviations of data from three replicate cultures.

tivation of bacteria on solid medium spatially separates populations and thereby eliminates the competition for resources that would otherwise occur in liquid enrichments. Populations with different growth rates, such as isolate DP3 (doubling time, 1.4 h) and DP17 (doubling time, 17.1 h), can therefore be isolated simultaneously.

Various indicators have been used previously for presumptive identification and differentiation of bacterial colonies on solid media. Metabolism of some compounds (e.g., cellulose or *p*-nitrophenol) visibly alters the opacity or color of the culture medium, and the resulting zones of clearing can be used to identify colonies expressing a specific phenotype (e.g., cellulose or *p*-nitrophenol degradation) (4, 13–15). The use of pH indicators has provided a more general method for identifying colonies which specifically metabolize a test compound (4, 15, 20). Acid production during metabolism produces color changes in and around colonies on a medium with a suitably balanced pH indicator. Although these approaches are suitable in many circumstances, they suffer from a lack of sensitivity. Nonspecific bacterial metabolism (of agar impurities, for example) can produce many false positives with pH-based detection methods, and diffusion of color can obscure many true positives. Detection of small colonies can also be problematic since they produce proportionately smaller visual effects. These problems are overcome with the use of autoradiography. False positives resulting from nonspecific growth are easily detected by comparison of replica filters incubated with and without a test substrate. Likewise, strains that form small colonies can be readily identified and are not overlooked when the autoradiographic method is used, since small amounts of incorporated radionuclides can be sensitively detected. Furthermore, since colonies with the phenotype of interest can be detected in a lawn of colonies, selective media are not required for general cultivation of bacteria.

Use of nonselective media for cultivation of bacteria can substantially reduce the length of time required to isolate organisms. Selective media employing carbon sources that are inefficiently metabolized, slightly toxic, or poorly soluble require relatively long incubation periods to generate visible biomass. Slow growth combined with the need for numerous serial transfers can result in months of incubation before the organism(s) of interest becomes established as a dominant member of the culture and can be efficiently isolated. In contrast, the autoradiographic method employs a nonselective,

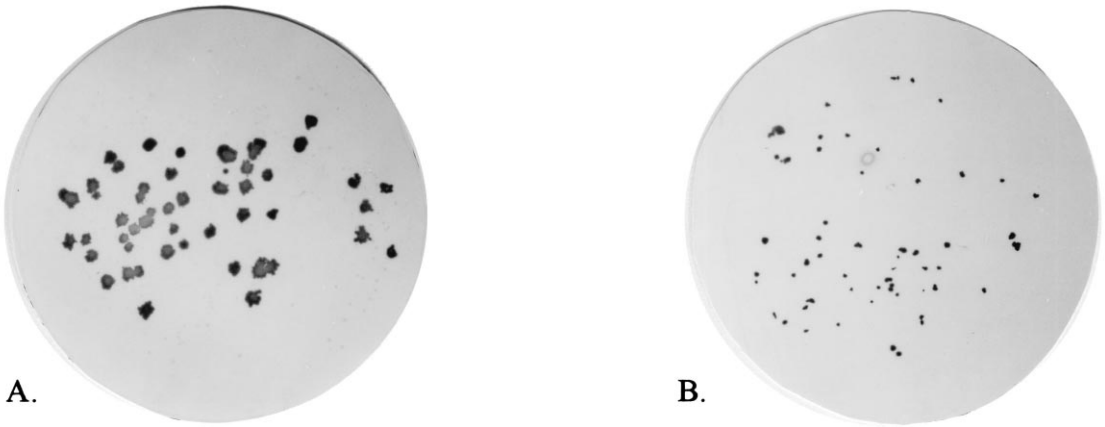

FIG. 4. Sensitivity of autoradiographic detection. Shown are autoradiograms of filters containing a 10^{-1} dilution of (A) strain DP3 (benzoate positive) at 500 CFU/ml and (B) strain DP3 and strain DP20 (benzoate negative) at 500 CFU/ml and 5×10^8 CFU/ml, respectively.

general-purpose medium for rapid cultivation of bacterial colonies ($\geq 10^6$ cells per colony). Bacterial phenotypes can subsequently be assayed on selective medium. Although we have used DAB medium for screening the catabolic phenotypes of bacterial colonies, other media could be employed. The use of media with higher nutrient concentrations is possible as long as differences in the amount of incorporated radionuclide in the presence and absence of a test substrate can be detected. Since bacterial biomass is initially produced on a nonselective medium, extensive metabolism of a specific test compound is not necessary. An estimate of the required amount of growth in the presence of the test compound can be calculated on the basis of the medium composition and a cellular content of 1.1% sulfur (6). For a small bacterial colony with 10^6 cells, one doubling on DAB medium containing 1 μCi of $Na_2{}^{35}SO_4$ per ml would incorporate 18.2 nCi (4×10^4 dpm), which is at least twice the amount necessary to produce a signal on Kodak XAR film in 18 h. Thus, organisms which are able to transform compounds that are recalcitrant, toxic, or only slightly soluble can be rapidly detected.

In summary, we have developed a simple and effective autoradiography method for isolating diverse microbial species with unique catabolic traits. Modifications of the basic autoradiographic technique have also been used to isolate and characterize mutants (21, 23). The method is most advantageous for isolation of diverse populations which occur at low frequencies in a large background of populations that lack the phenotype of interest. Under other circumstances, such as when the desired populations are very abundant relative to others and the test substrate is easily metabolized, traditional plating on selective media may be more suitable.

ACKNOWLEDGMENTS

We sincerely appreciate the helpful suggestions offered by Connie Winans, Sally Sullivan, Neil Burris, and Craig Weaver during the development of this method.

This research was funded by Synergen, Inc., and by a grant (BIR 9120006) from the National Science Foundation.

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