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Rapid Method for Simultaneous Detection of the Arginine Dihydrolase System and Amino Acid Decarboxylases in Microorganisms

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A specific procedure has been developed for the detection of the first two enzymes involved in the arginine dihydrolase system and the detection of the decarboxylases of arginine, glutamic acid, histidine, lysine, ornithine, phenylalanine, tryptophan, and tyrosine. A loopful of growth of each organism from dihydrolase-decarboxylase induction agar medium (or broth) was washed and incubated separately with 0.2-ml samples of three test media supplemented with different amino acids. Each spent test medium was dansylated, and the dansyl derivatives were separated by two-dimensional thin-layer chromatography on polyamide sheets. The end products (citrulline, ornithine, γ-amino-*n*-butyric acid, and amines) produced during incubation were estimated by comparing the fluorescent intensities of end products from the spent test media and of the corresponding parent amino acids from test medium controls after thin-layer chromatography. The method is reproducible, requiring incubation of an organism in three test media for 1 h for simultaneous detection of the first two enzymes involved in the arginine dihydrolase system and of eight amino acid decarboxylases. This method has been successfully applied to gram-positive and gram-negative microorganisms and also to *Mycoplasmatales*. It could simplify and improve the accuracy of the corresponding biochemical tests performed in clinical laboratories for the identification and differentiation of microorganisms, and it may prove particularly useful for the differentiation of species of *Pseudomonas* and *Mycoplasma*.

Enzymes involved in the arginine dihydrolase system and the amino acid decarboxylases are widely distributed in microorganisms (1, 2, 8). The microbial arginine dihydrolase system which converts arginine to ornithine via citrulline consists of three enzyme reactions: arginine + H_2O → citrulline + NH_3 by arginine deiminase; citrulline + P_i → ornithine + carbamyl phosphate by catabolic ornithine carbamoyltransferase; and carbamyl phosphate + ADP → ATP + CO_2 + NH_3 by carbamate kinase (1). Detection of the arginine dihydrolase system and decarboxylases of lysine and ornithine has proven valuable for differentiation within the family *Enterobacteriaceae* and is useful for characterizing certain genera within other families (7). Tests for the arginine dihydrolase system are of value for differentiating aerobic *Pseudomonas* spp. from other nonfermentative gram-negative bacilli (9, 27) as well as within their own genus (22, 25, 27, 29). The methods devised by Møller (17) for identifying *Enterobacteriaceae* by detection of the dihydrolase and decarboxylase systems still serve as the standard methods (7, 12, 16, 20, 23). Procedures for the detection of the bacterial arginine dihydrolase system are based on the elevation of pH (17, 27), on the decrease of arginine concentration (25) after growth of bacteria in a medium containing a high concentration of arginine, or on the identification of the end products (ornithine or citrulline) by one-dimensional thin-layer chromatography (TLC) on cellulose layers (21, 29, 31). Procedures based on the alkalization of a medium can be obfuscated by NH_3 production, by decarboxylation of arginine or other amino acids, or by acid production from sugar fermentation; procedures based on the decrease of arginine concentration in the spent medium can be confused by other catabolic pathways for arginine (1). Although the procedures for identifying end products by one-dimensional TLC provide more reliable results, the arginine dihydrolase system cannot be distinguished from arginase if citrul-

line is absent in the spent medium. Moreover, a suitable solvent system has not been reported for the separation of all possible components (arginine, citrulline, ornithine, putrescine, and agmatine) in the test system (31). Gas-liquid chromatography has also been used for demonstrating ornithine and lysine decarboxylase activities (15) and for detecting arginine metabolites (19). However, the gas-liquid chromatography procedures described were not shown to be capable of detecting the decarboxylases of glutamic acid, histidine, phenylalanine, tryptophan, or tyrosine, and they were less efficient for testing a large number of specimens. The methods for detecting the arginine dihydrolase system and amino acid decarboxylases all have required incubation of the bacteria in media containing high concentrations of each key amino acid tested for periods ranging from 1 to 24 h up to 4 to 10 days (15–17, 19–21, 23, 25, 27, 29, 31).

We previously described a rapid procedure with only two separate incubations for the simultaneous detection of eight bacterial amino acid decarboxylases by identifying the dansyl derivatives of the decarboxylation products in the spent media after 1 h of incubation by TLC on polyamide sheets (5). In this paper, we describe a general procedure for the rapid and specific detection of the arginine dihydrolase system (arginine deiminase and catabolic ornithine carbamoyltransferase) and decarboxylases of arginine, glutamic acid, histidine, lysine, ornithine, phenylalanine, tryptophan, and tyrosine. This method requires only three separate incubations of the microorganisms for 1 h for simultaneous identification of citrulline, ornithine, γ-amino-*n*-butyric acid (GABA), and seven amines, and it has been successfully applied to species of *Enterobacteriaceae*, *Pseudomonadaceae*, *Neisseria*, *Eikenella*, *Streptococcus*, *Staphylococcus*, *Bacillus*, and *Mycoplasmatales*.

MATERIALS AND METHODS

Chemicals. L-Citrulline was obtained from Sigma Chemical Co. (St. Louis, Mo.). Pyridine obtained from MCB Manufacturing Chemists, Inc. (Cincinnati, Ohio) was redistilled and refrigerated. The sources of other chemicals were described previously (5).

Dihydrolase-decarboxylase induction medium. The standard dihydrolase-decarboxylase induction agar medium used in this study was prepared as follows. The ingredients of peptone-starch-dextrose (PSD) agar medium (6) plus 0.1% each L-arginine hydrochloride, L-glutamic acid, L-histidine hydrochloride monohydrate, L-lysine dihydrochloride, L-ornithine hydrochloride, L-phenylalanine, and L-tryptophan and 0.02% L-tyrosine were solubilized by heating at 100°C for 15 min. The pH of the medium was adjusted to 5.5 with 6 N HCl (at about 70°C), and the medium was autoclaved and dispensed in petri dishes (60 by 15 mm; Falcon Plastics, Oxnard, Calif.). This induction agar medium was used throughout this study unless otherwise described.

Dihydrolase-decarboxylase test. Three media were used to test each microorganism for dihydrolase-decarboxylase activity. Medium 1 consisted of PSD broth supplemented with 0.1% L-arginine hydrochloride. Medium 2 consisted of PSD broth supplemented with the same amount of amino acid supplements used for the dihydrolase-decarboxylase induction medium, but without arginine. Medium 3 consisted of PSD broth supplemented with 0.1% L-citrulline. The supplements were added to the autoclaved PSD broth. Each supplemented medium was sterilized by membrane filtration after the pH was adjusted to 5.5 with 6 N HCl. Medium 1 was used only for tests of the arginine dihydrolase system and arginine decarboxylase; medium 2 was used only for tests of decarboxylases of glutamic acid, histidine, lysine, ornithine, phenylalanine, tryptophan, and tyrosine; and medium 3 was used only for tests for catabolic ornithine carbamoyltransferase.

Preparation of inocula for the dihydrolase-decarboxylase test. The microorganisms used in these studies were stock cultures from the American Type Culture Collection and from the Neisseria Reference Laboratory. *Pseudomonas* spp. were generously provided by Stephen A. Morse and Barbara Minshew. *Citrobacter diversus* was obtained from Judith Hale. *Ureaplasma urealyticum* type 8 (cloned 8 times) was obtained from Maurice C. Shepard. Each microorganism was streaked on a dihydrolase-decarboxylase induction agar medium plate and was grown aerobically at 37°C for 12 h unless otherwise described. One loopful (diameter, 2 mm) of growth from the microorganism grown on an agar plate was suspended (by blending in a Vortex mixer) in each of three microfuge tubes (400 μl; Stockwell Scientific, Monterey Park, Calif.) containing 0.2 ml of 0.85% NaCl and centrifuged in a microfuge (model 152; Beckman Instruments, Inc., Fullerton, Calif.) for 1 min. The supernatant was removed. Washing of each pellet was repeated once to remove the metabolites from the spent induction medium. Then, 0.2 ml each of media 1, 2, and 3 was added separately to the three pellets and incubated aerobically at 37°C for 1 h after blending in a Vortex mixer, along with uninoculated test medium controls. Species of *Enterobacteriaceae* were also grown in the standard induction broth and other induction agar media, including Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) and brain heart infusion agar medium (Difco Laboratories, Detroit, Mich.), with the same pH and amino acid supplements as described for the standard induction agar medium. Species of *Neisseria* were grown as described (3). Species of *Eikenella* were grown on chocolate agar without amino acid supplements at pH 7.3 (no growth at pH 5.5) for 48 h in a CO_2 incubator as described for *Neisseria* spp. *Mycoplasmatales* organisms were grown in soy peptone fresh yeast dialysate broth (13) supplemented with the following additives for the indicated organisms: *Mycoplasma hominis*, 10% agamma horse serum; *Acholeplasma laidlawii*, 2% agamma horse serum; and *Ureaplasma urealyticum*, 2% agamma horse serum–30 mM urea–100 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.3 (14). Organisms grown in broth were concentrated by centrifugation at 12,000 × *g* for 10 min

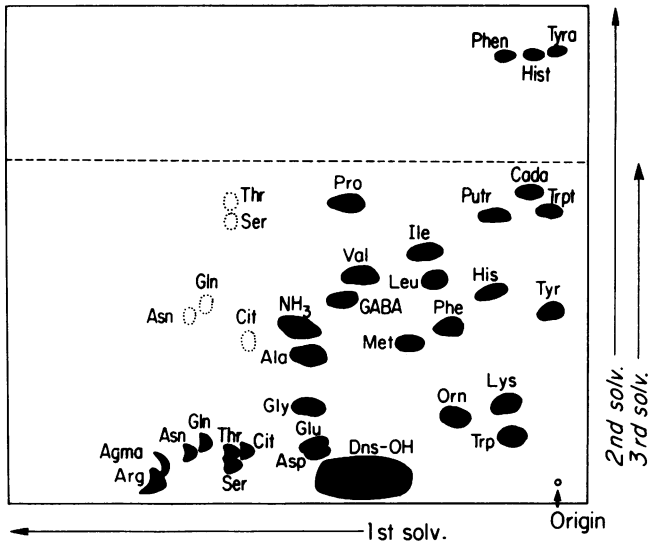

FIG. 1. Separation of dansyl amines, GABA, citrulline, and other amino acids by two-dimensional TLC on polyamide sheets. Solvent systems used: first solvent system, water-formic acid (100:1.5); second solvent system, benzene-glacial acetic acid (9:1); third solvent system, benzene-pyridine-glacial acetic acid (15:4:1). The solid spots are the positions of the dansyl derivatives after two-dimensional chromatography in the first and second solvent systems, and the open spots are those after chromatography in the third solvent system. The direction of each chromatographic system is indicated by an arrow with the solvent front at its tip. Abbreviations: Agma, agmatine; Cada, cadaverine; Cit, citrulline; Dns-OH, 1-dimethylaminonaphthalene-5-sulfonic acid; Hist, histamine; Orn, ornithine; Phen, phenethylamine; Putr, putrescine; Trpt, tryptamine; Tyra, tyramine; Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

(except *Mycoplasma* spp., 8,000 × *g* for 30 min), and pellets were washed twice with 0.85% NaCl by blending in a Vortex mixer and centrifuging to remove the metabolites from the spent induction media. A heavy suspension was made in 0.85% NaCl, and loopfuls of each suspension were separately incubated with the three test media as described above.

Dansylation of the spent test media. After incubation, all inoculated tubes were centrifuged in a microfuge for 1 min to pellet the organisms. Supernatant (10 μl) from each tube (including test medium controls) was removed and placed separately in another microfuge tube containing 40 μl of 1 M $NaHCO_3$. This mixture was blended briefly in a Vortex mixer, and 50 μl of dansyl chloride solution (5 mg per ml of acetone) was added to the mixture. The microfuge tubes were capped tightly, blended in a Vortex mixer, and incubated at 37°C for 1 h.

Separation of dansyl derivatives by TLC on polyamide sheets. After incubation, the reaction mixture was centifuged for 1 min. The dansylated supernatant (4 μl) from spent test medium 1, which had been incubated with a test organism, was spotted on one side (front side) of a Chen-Chin polyamide layer sheet (15 by 15 cm; Accurate Chemical & Scientific Corp., Hicksville, N.Y.), and the dansylated supernatant (4 μl) from spent test medium 1 which had been incubated with another test organism was spotted at the same position on the opposite side (back side) of the same sheet. Note that each 4-μl sample of the dansylated supernatant after centrifugation contained 0.4 μl of spent test medium. TLC was carried out by the procedure described by Woods and Wang (30), except that the second-dimension chromatography was run to only about two-thirds the length of the sheet. The positions of ornithine and agmatine were then identified. Dansyl citrulline could only be separated from dansyl derivatives of threonine, serine, asparagine, and glutamine after chromatography in a third solvent system (benzene-pyridine-glacial acetic acid, 15:4:1) in the same direction as the second solvent system to about half the length of the sheet. Dansyl agmatine was slightly further separated from dansyl arginine after chromatography in the third solvent system (R_f of dansyl agmatine at the third solvent system was about 0.05, and the position of dansyl arginine remained unchanged). However, better separation could be achieved by using the fourth solvent system (ethylacetate-glacial acetic acid-methanol, 20:1:1) as described previously (5; R_f of dansyl agmatine in the fourth solvent system was about 0.2, and the position of dansyl arginine remained unchanged).

Dansyl derivatives of spent test medium 2 and of spent test medium 3, incubated with the same organisms, were applied at the same position on opposite sides of the same sheet as described above and were chromatographed similarly, except that chromatography on the third and fourth solvent systems was not required because these two test media were not used to detect arginine deiminase or arginine decarboxylase

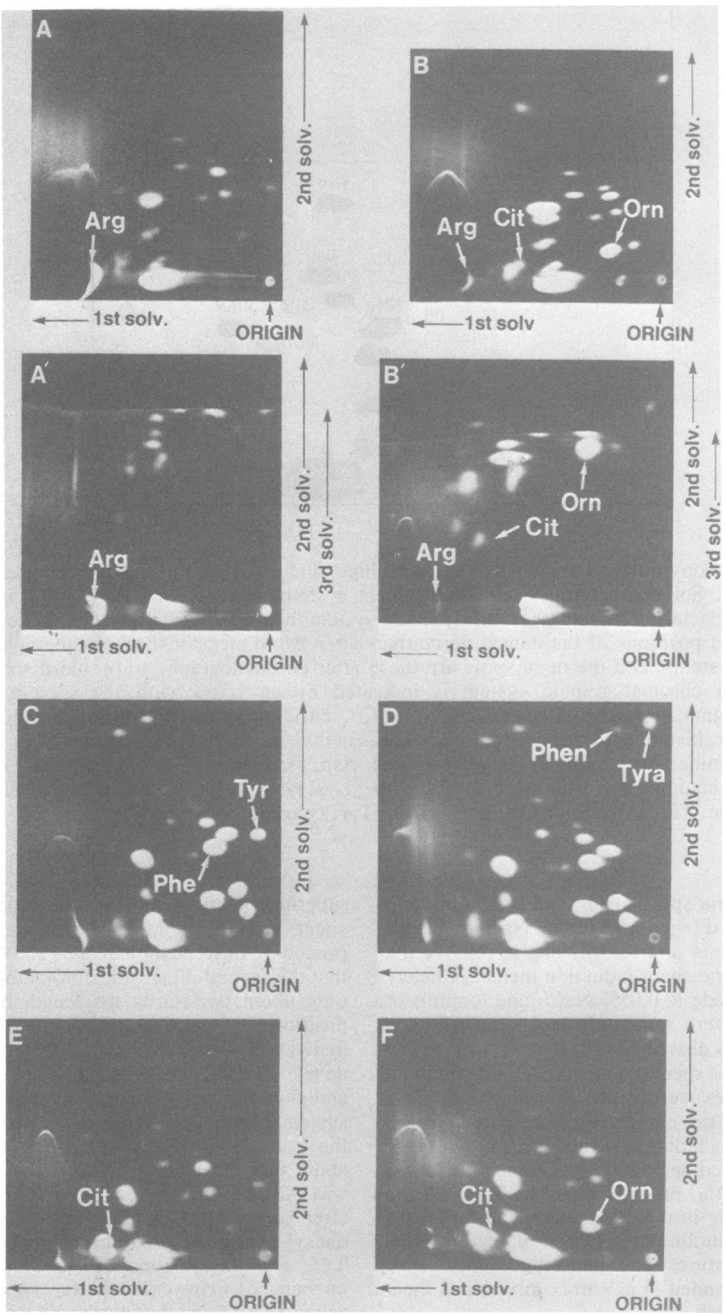

FIG. 2. Separation of dansyl derivatives of 0.4 μl each of test medium 1 control (A, A'), test medium 2 control (C), test medium 3 control (E), spent test medium 1 (B, B'), spent test medium 2 (D), and spent test medium 3 (F) incubated with *Streptococcus faecalis* strain ATCC 12984 for 1 h at 37°C, by two-dimensional chromatography on polyamide sheets with the system shown in Fig. 1 and described in the legend. After TLC in the first and second solvent systems, further TLC in the third solvent system (Fig. 1) was used to identify dansyl citrulline (A' and B'). See legend to Fig. 1 for abbreviations.

activity. The positions of ornithine, GABA, and the amines were then identified as described previously (5). Each dansylated uninoculated test medium control was spotted on the sheet and chromatographed as described for its corresponding dansylated spent test medium. Dansyl amino acids were identified as described previously (5). Only a set of controls for the experiments of each day was required.

TABLE 1. Activities of the first two enzymes involved in the arginine dihydrolase system and amino acid decarboxylases in various representative microorganisms determined by identification of end products by TLC on polyamide sheets after incubation for 1 h

Microorganism[a]	Activity[b]									
	Arginine dihydrolase enzymes			Amino acid decarboxylases						
	AD	OCT	AD + OCT	Arg	Glu	His	Lys	Orn	Phe	Tyr
Gram-negative enteric										
Citrobacter diversus JH 3481								S (1/1)		
Citrobacter freundii NRL 5329; ATCC 10787				S (1/2), M (1/2)				S (1/2)		
Enterobacter aerogenes NRL 5325, 9817	W (1/2)	W (1/2)	M (1/2)		W (1/2)		S (1/2), W (1/2)	S (2/2)		
Enterobacter agglomerans ATCC 29915										
Enterobacter cloacae NRL 5335, 9818		W (1/2)	W (1/2)	W (2/2)		S (1/2)	S (1/2)	S (2/2)		
Enterobacter hafniae NRL 9815				M (1/1)	M (1/1)		S (1/1)	S (1/1)		
Escherichia coli NRL 9813; ATCC 26, 27549				W (1/3), M (2/3)	W (1/3), M (2/3)		S (2/3)	W (1/3), M (2/3)		
Klebsiella ozaenae NRL 9977										
Klebsiella pneumoniae NRL 9976							S (1/1)			
Proteus mirabilis ATCC 14273					W (1/1)			S (1/1)		
Morganella morganii NRL 5334					W (1/1)	M (1/1)		S (1/1)	W (1/1)	W (1/1)
Providencia stuartii NRL 5328						W (1/1)				W (1/1)
Salmonella sp. group C2 NRL 5317				M (1/1)			S (1/1)	S (1/1)		
Salmonella sp. group D NRL 5316				M (1/1)			S (1/1)	S (1/1)		
Salmonella typhimurium ATCC 13311				M (1/1)			S (1/1)	S (1/1)		
Serratia marcescens ATCC 17991							S (1/1)	S (1/1)		
Shigella sp. group B NRL 5327				W (1/1)	S (1/1)					
Shigella sp. group D NRL 5326				W (1/1)	S (1/1)			S (1/1)		
Shigella dysenteriae ATCC 13313				W (1/1)	S (1/1)					
Shigella sonnei ATCC 11060				M (1/1)	M (1/1)			S (1/1)		

TABLE 1—*Continued*

Microorganism[a]	Activity[b]									
	Arginine dihydrolase enzymes			Amino acid decarboxylases						
	AD	OCT	AD + OCT	Arg	Glu	His	Lys	Orn	Phe	Tyr
Gram-negative nonenteric										
Eikenella corrodens ATCC 23834, 1073							S (2/2)	S (2/2)		
Pseudomonas aeruginosa SM 31302-31320	M (19/19)	M (19/19)	S (19/19)							
Pseudomonas cepaciae BM 1, 2							S (2/2)	S (1/2)		
Pseudomonas fluorescens MS 3	W (1/1)	M (1/1)	S (1/1)							
Pseudomonas maltophilia MS 4, 5		W (2/2)	W (2/2)				S (2/2)			
Pseudomonas putida MS 6, 7; ATCC 25571	M (2/3), W (1/3)	M (3/3)	S (3/3)							
Neisseria gonorrhoeae NRL F62, 33, B, Mel, 7122										
Neisseria meningitidis NRL 8833, 3008, 30012, 30017, 9212										
Neisseria flava NRL 7350, 9478, 9999										
Gram-positive										
Bacillus subtilis ATCC 14807, 14415, 14416	W (3/3)	W (3/3)	W (3/3)							
Bacillus licheniformis ATCC 6598, 14409	W (2/2)	M (2/2)	S (2/2)							
Staphylococcus aureus ATCC 25923, 12598	W (2/2)	W (2/2)	M (2/2)		W (1/1)					
Streptococcus faecalis ATCC 11420, 12984	M (2/2)	M (2/2)	S (2/2)						W (1/2), M (1/2)	M (2/2)

TABLE 1—*Continued*

Microorganism[a]	Activity[b]									
	Arginine dihydrolase enzymes			Amino acid decarboxylases						
	AD	OCT	AD + OCT	Arg	Glu	His	Lys	Orn	Phe	Tyr
Mycoplasmatales										
Acholeplasma laidlawii ATCC 14192								S (1/1)		
Mycoplasma hominis ATCC 14027, 15056, 27545	S (3/3)	M (3/3)	S (3/3)							
Ureaplasma urealyticum MS type 8; ATCC 27813-27815										

[a] Microorganisms were grown on dihydrolase-decarboxylase induction media aerobically at 37°C for 12 h and suspended in the test media for 1 h as described. Species of *Eikenella, Neisseria*, and *Mycoplasma* were grown under conditions as described. Strain numbers are those of the American Type Culture Collection (ATCC), the Neisseria Reference Laboratory (NRL), Stephen A. Morse (SM), Barbara Minshew (BM), Judith Hale (JH), and Maurice C. Shepard (MS).

[b] The presence of the enzymes in microorganisms was detected by identification of the end products in the spent test medium after incubation by TLC on polyamide sheets. The activity of each enzyme was recorded as follows: S, strong, product had about the same fluorescent intensity under UV light as its parent amino acid in the control; W, weak, product was easily identified, but its fluorescent intensity was much weaker than that of its parent amino acid in the control; M, moderate, fluorescent intensity of the product was intermediate between those described for S and W. AD, Arginine deiminase; OCT, catabolic ornithine carbamoyltransferase; AD + OCT, ornithine detected in the spent test medium 1 was recorded in this column only if ornithine was also detected in spent test medium 3; Arg, arginine; Glu, glutamic acid; His, histidine; Lys, lysine; Orn, ornithine; Phe, phenylalanine; Tyr, tyrosine. Numbers in parentheses show number of organisms having a positive test/number tested.

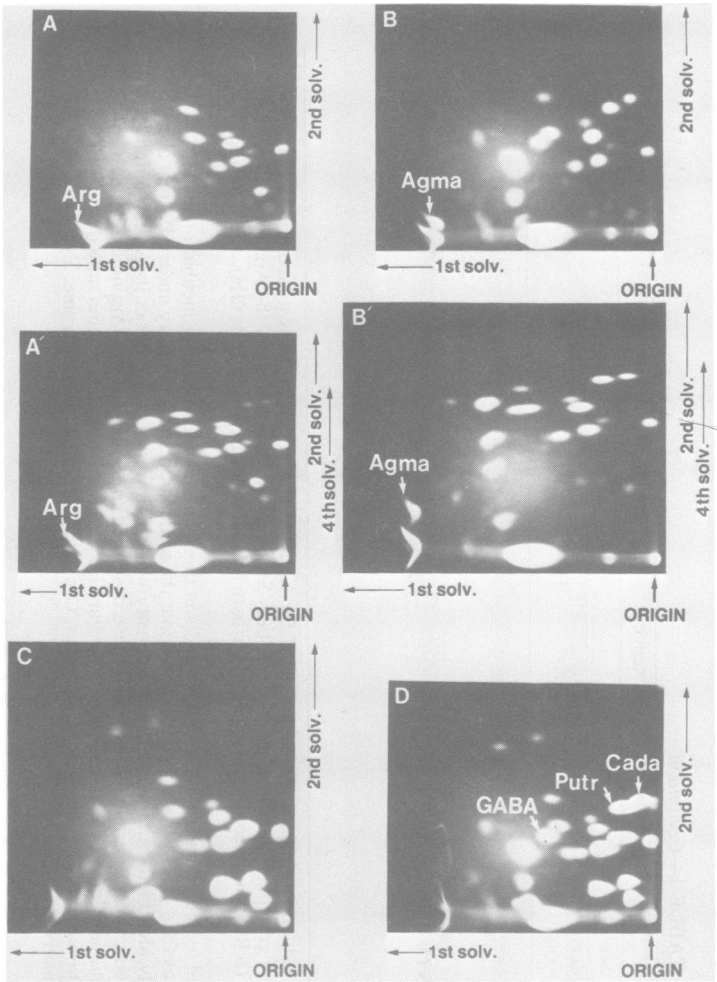

FIG. 3. Separation of dansyl derivatives of 0.4 μl each of test medium 1 control (A, A'), test medium 2 control (C), spent test medium 1 (B, B'), and spent test medium 2 (D) incubated with *Enterobacter hafniae* for 1 h at 37°C, by two-dimensional TLC on polyamide sheets by the system shown in Fig. 1 and described in the legend. After TLC in the first and second solvent systems, further TLC in the fourth solvent system, ethyl acetate-glacial acetic acid-methanol (20:1:1), was used for further separation of dansyl agmatine from dansyl arginine (A', B'). See legend to Fig. 1 for abbreviations.

RESULTS

Detection of the arginine dihydrolase system and of amino acid decarboxylases by identification of dansyl end products from the spent test media on polyamide sheets after two-dimensional chromatography. The relative positions of dansyl derivatives of citrulline, ornithine, GABA, amines, and other amino acids from the spent test media after two-dimensional chromatography (Fig. 1) were determined as described previously (5). Dansyl citrulline in the dansylated spent test medium 1 could be detected after further chromatography in the third solvent system (Fig. 1), whereas dansyl agmatine in the dansylated spent test medium 1 could be identified clearly after further chromatography in the third and fourth solvent systems.

TLC methods were applied for the detection of the first two enzymes involved in the arginine dihydrolase system (arginine deiminase and catabolic ornithine carbamoyltransferase) and of decarboxylases of phenylalanine and tyrosine of *Streptococcus faecalis* (Fig. 2). Citrulline was produced by *Streptococcus faecalis* during incubation in medium 1 for 1 h at 37°C (compare 2A' and B'), and ornithine was produced during incubation in both media 1 and 3 for 1 h at 37°C (compare 2A and B, E and F), showing the presence of arginine deiminase (resulting in production of citrulline from arginine) and of catabolic ornithine carbamoyltransferase (resulting

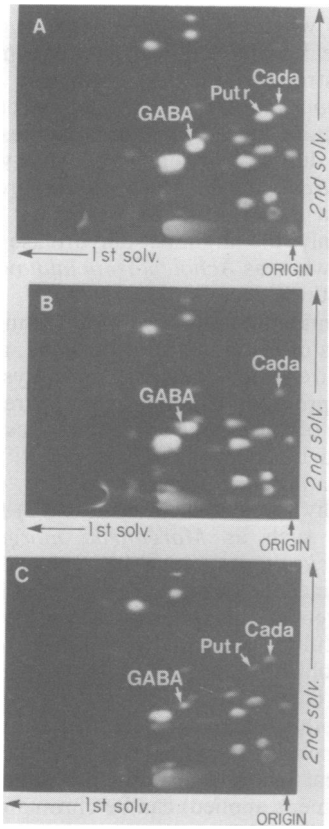

FIG. 4. Production of decarboxylases of glutamic acid, lysine, and ornithine by *Enterobacter hafniae* under different growth conditions. (A) Same conditions used for Fig. 3 (optimum, PSD agar supplemented with eight amino acids as described and pH adjusted to 5.5). (B) pH 7.0 with amino acid supplements; same strong glutamic acid decarboxylase activity as shown in (A), but no ornithine decarboxylase activity or weak lysine decarboxylase activity. (C) pH 7.0 without amino acid supplement; weak activities of decarboxylases of glutamic acid, lysine, and ornithine. The incubation conditions for the detection of amino acid decarboxylases were the same as for Fig. 3. See legend to Fig. 1 for abbreviations.

in production of ornithine from citrulline) in *Streptococcus faecalis*. Phenethylamine and tyramine were produced during incubation in medium 2 for 1 h at 37°C (compare 2C and D), indicating the presence of both phenylalanine and tyrosine decarboxylases in the same organism.

The same methods were applied for the detection of four decarboxylases produced by *Enterobacter hafniae* (Fig. 3). Agmatine was produced by *Enterobacter hafniae* during incubation in medium 1 for 1 h at 37°C (compare 3A and B, A′ and B′), indicating the presence of arginine decarboxylase. Cadaverine, GABA, and putrescine were produced by the same organism during incubation in medium 2 for 1 h at 37°C (compare Fig. 3C and D), demonstrating the presence of decarboxylases of lysine (resulting in production of cadaverine), glutamic acid (resulting in production of GABA), and ornithine (resulting in production of putrescine). Note that dansyl derivatives of all test medium controls and spent test media were spotted on the same side of sheets shown in Fig. 2 and 3 for easy illustration and comparison with Fig. 1, whereas those spotted as described above were for the routine tests for dihydrolase-decarboxylase activity.

Activities of enzymes involved in the arginine dihydrolase system and amino acid decarboxylases in selected microorganisms. To determine the dihydrolase-decarboxylase activity in each microorganism, the fluorescent intensities (detected on the polyamide sheets after two-dimensional chromatography) of the dansyl derivatives of the end products in the spent test media were compared with those of the corresponding parent amino acids in the test medium controls. Although dansyl glutamic acid could not separate from dansyl aspartic acid after two-dimensional chromatography in the first two solvent systems, the amount of aspartic acid present was negligible compared with the amount of glutamic acid (parent amino acid of GABA) present in the uninoculated test medium 2 control, which was supplemented with 0.1% glutamic acid (4). If necessary, dansyl glutamic acid could be easily separated from dansyl aspartic acid with the fourth solvent system (28). The activity of each enzyme was rated as strong if its end product produced during incubation had about the same fluorescent intensity under UV light as its parent amino acid in the test medium control; weak if its end product was easily identified but its fluorescent intensity was much weaker than that of its parent amino acid in the control; or moderate if the fluorescent intensity of the end product was intermediate between those described for strong and weak (Table 1). The results obtained for species of *Enterobacteriaceae* grown in the PSD induction agar (Table 1) were similar to those obtained with growth in PSD induction broth or in other induction media, as described above (data not shown).

DISCUSSION

The procedures described in this report allow simultaneous detection of the first two enzymes involved in the arginine dihydrolase system and of eight amino acid decarboxylases by three 1-h incubations per organism, instead of one incubation for each enzyme as tested by existing methods. This method can also be applied for the detection of the dihydrolase and decarboxylase

systems in species of *Enterobacteriaceae* grown in induction broth or other induction agar media, and it may prove generally applicable for other microorganisms. Prolonged incubation of the test media did not improve detection of the existing enzymes involved in the arginine dihydrolase system (data not shown) or of the amino acid decarboxylases as previously described (5); instead, it increased the possibilities of detection of the biosynthetic amino acid decarboxylases such as ornithine, lysine, and arginine decarboxylases (18).

Use of test medium 3 for detecting the arginine dihydrolase system in microorganisms is necessary because the arginine dihydrolase system cannot be distinguished from arginase if citrulline is not detected in the spent test medium 1 (presumably owing to the high turnover rate of the intermediate citrulline by catabolic ornithine carbamoyltransferase). The production of ornithine in both spent test medium 1 and spent test medium 3 is a clear proof of the arginine dihydrolase system in the microorganism tested. Less ornithine was usually found in the spent test medium 3 than in the spent test medium 1 (Table 1), presumably because of the lower permeability of citrulline into the microbial cells (24, 26).

Amino acid decarboxylases in microorganisms are substrate and acid inducible (8). For example, both amino acid supplements and low pH are required for optimum production of amino acid decarboxylases by *Enterobacter hafniae* (Fig. 4). When we detected the arginine dihydrolase system in the microorganisms listed in Table 1 by using the induction medium and test media 1 and 3 at pH 7.0, the activities of the two enzymes (arginine deiminase and catabolic ornithine carbamoyltransferase) were either slightly increased or remained the same, but in a few cases citrulline was not detected, presumably because catabolic ornithine carbamoyltransferase has a higher activity at neutral pH (data not shown). Therefore, the induction medium and the three test media described above are required for the simultaneous detection of the dihydrolase and decarboxylase systems in microorganisms. The results listed in Table 1 agree with those obtained by identifying the end products of the arginine dihydrolase enzymes and decarboxylases of arginine, lysine, and ornithine with one-dimensional TLC on cellulose plates (10, 11, 21, 29, 31, 32) and with gas-liquid chromatography (15, 19).

This method may prove useful for differentiating species of *Pseudomonas* and *Mycoplasmatales*. As shown in Table 1, preliminary results indicate that *Pseudomonas aeruginosa, Pseudomonas fluorescens*, and *Pseudomonas putida* show only strong arginine dihydrolase enzyme activity, whereas *Pseudomonas maltophilia* shows weak arginine dihydrolase enzyme activity but strong lysine decarboxylase activity; *Pseudomonas cepacia* shows both strong lysine and ornithine decarboxylase activities but no arginine dihydrolase enzyme activity. *Ureaplasma urealyticum* shows no dihydrolase-decarboxylase activity. *Mycoplasma hominis* shows only the arginine dihydrolase enzyme activity, whereas *Acholeplasma laidlawii* shows only ornithine decarboxylase activity.

Conventional methods for detecting decarboxylases of histidine, phenylalanine, and tyrosine require tedious manometric assays (8). Our method provides a simple procedure for the detection of these three decarboxylases and may provide a biochemical test for identifying and differentiating microorganisms. For example, the lengthy tyrosine-clearing test (12) for some bacteria, such as *Morganella morganii* and *Providencia stuartii*, may be replaced by this method since the tyrosine clearing may be due to the conversion of water-insoluble tyrosine to water-soluble tyramine (solubility increases over 20-fold after conversion) by tyrosine decarboxylase (see Table 1).

The method described here is rapid, sensitive, and inexpensive, and thus it is potentially useful for clinical laboratories. Six polyamide sheets (12 specimens applied) can be chromatographed in a glass jar (13 by 16 by 26 cm) and air dried. Approximately 1 h is required to complete chromatography for the first and second solvent systems and for the third and fourth solvent systems (2 h for the all solvent systems). Polyamide sheets can be washed and reused at least 10 times as described previously (5). Because of the hematoxic, hepatotoxic, and carcinogenic potential of benzene, a chemical exhaust hood should be used for working with the chromatographic jars and for hanging the polyamide sheets to dry. The third solvent system described in this report allows the separation of dansyl derivatives of asparagine, citrulline, glutamine, serine, and threonine, and it may be useful for rapid qualitative analyses of free amino acids in physiological fluids and in the exopeptidase digests of proteins or peptides.

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