JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1978, p. 313-319
0095-1137/78/0008-0313$02.00/0
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Vol. 8, No. 3
Printed in U.S.A.

Carbon Substrate Utilization Studies of Some Cultures of *Alcaligenes denitrificans, Alcaligenes faecalis,* and *Alcaligenes odorans* Isolated from Clinical Specimens

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Received for publication 19 June 1978

One hundred and sixty-two cultures of *Alcaligenes* species (*A. denitrificans, A. faecalis,* and *A. odorans*) of clinical origin were characterized by routine diagnostic and carbon substrate utilization techniques. The microorganisms were tested for their ability to utilize a total of 188 substrates. Substrate utilization was assayed by (i) growth stimulation and (ii) substrate alkalinization. The *A. denitrificans* and *A. odorans* cultures had unique substrate utilization profiles for each species. The *A. faecalis* isolates were redefined by colonial morphology into two biotypes: (i) biotype I, morphologically and biochemically similar to the *A. denitrificans* cultures and (ii) biotype II, morphologically similar to the *A. odorans* cultures.

Recognition and identification of nonfermentative, gram-negative bacterial rods are prominent problems in the clinical microbiology laboratory, primarily because very few positive reactions are obtained when these microorganisms are examined by conventional diagnostic procedures. In particular, some of these bacteria do not oxidatively acidify carbohydrate substrates, and thus the phenotypic markers which form an integral part of dichotomous keys used for the identification of these bacteria are ineffective.

Nutritional characterization, by the use of carbon substrate utilization (CSU) tests, has been used in a number of laboratories as a characterization tool. Stanier et al. (15), Ralston et al (12), Baumann et al. (1, 2), Otto and Pickett (11), and Gilardi (5, 6) have used this technique to characterize bacterial species. Although useful information has been obtained with this procedure, it is not easily adapted for use in the clinical diagnostic laboratory. Problems with inoculum standardization, consumption of large amounts of media, and growth end point evaluation make this procedure somewhat suspect. Riley et al. (13) were able to control some of these factors by incorporating the Steers replicator (16) into their CSU experiments. With this replicator, they found that a relatively large number of cultures could be processed accurately and rapidly.

Recently, Oberhofer and Rowen (9) and Otto and Pickett (11) devised new media for the study of CSU profiles. In both instances, the alkalinization of the growth medium was used as a reaction end point. This alkalinization resulted in a color change which is much simpler to interpret than relative differences in colonial growth.

This report concerns the determination of CSU profiles for the gram-negative, glucose-nonoxidative microorganisms *Alcaligenes denitrificans, A. odorans,* and *A. faecalis.* The modified Stanier technique (13) and the media of Oberhofer and Rowen (9) and Otto and Pickett (11) were investigated.

MATERIALS AND METHODS

Bacteria. The bacterial cultures were obtained from the stock culture collection maintained by the Special Bacteriology Section at the Center for Disease Control. They were identified from clinical isolates that were submitted to this laboratory for further investigation (Table 1).

Growth stimulation studies. The technique of Stanier et al. (15), as previously described (13), was used. The bacterial cultures were grown on tryptone-glucose-yeast extract agar slants (7) for 18 to 24 h. The cell mass was washed off the slants with sterile basal salts solution. After the cellular growth was suspended, the optical density of the suspensions was adjusted to 0.35 to 0.40 at a wavelength of 550 μm (4.8 to 5.2 μg of dry weight per ml). These suspensions were then diluted 1/10 in sterile basal salts solution before being used to fill the replicator seed plate. After drying, the substrate plates were inoculated in duplicate with the replicator. The inoculum on each plate's surface was allowed to dry before the culture was incubated at 35°C for 48 h.

The final concentrations of the substrates were 0.2% for carbohydrates and 0.1% for all other substrates.

TABLE 1. *Sources of isolation of the Alcaligenes cultures*

Source	*A. denitrificans* (39 strains)	*A. faecalis* (63 strains)	*A. odorans* (60 strains)
Abscesses, ulcers		1	3
Blood	4	7	
Cerebrospinal fluid	3	1	1
Ear	1	13	9
Environment	4	3	
Eye	3		1
Feces	1	2	1
Finger	2		
Mastoid			2
Mouthwash		5	
Peritoneal fluid		3	
Pleural fluid	2		
Sputum	3	6	1
Urine	3	5	24
Wounds		4	7
Miscellaneous	9	7	6
Unknown	4	6	5

The substrates used were acetamide, acetate, *cis*-aconitate, *trans*-aconitate, adipate, adonitol, β-alanine, D-alanine, L-alanine, allantoin, *m*-aminobenzoate, *p*-aminobenzoate, γ-aminobutyrate, DL-2-aminobutyrate, 2-aminoethanol, δ-aminovalerate, α-amylamine, aniline, anthranilate, D-arabinose, L-arabinose, arginine, asparagine, aspartate, azelate, benzoate, benzylamine, benzylformate, betaine, borate, 1,4-butanediol, butanol, butylamine, butyramide, butyrate, caprate, caproate, caprylate, catechol, cellobiose, cetrimide, citrate, DL-citrulline, creatine, creatinine, cysteine, cystine, dextrin, 1,3-dihydroxy-2-propanone, docosanoate, dulcitol, erythritol, ethanol, DL-ethionine, ethylene glycol, ethylene glycol monomethyl ether, folate, formate, fructose, fucose, galactose, gentisate, glucosamine, gluconate, glucose, glutamate, glutamine, glutarate, DL-glycerate, glycerol, glycine, glycolate, glyoxylate, heptanoate, 1,6-hexanediol, hippurate, histamine, histidine, *m*-hydroxybenzoate, *p*-hydroxybenzoate, DL-β-hydroxybutyrate, β-hydroxy-β-methylglutarate, hydroxyproline, indoleacetate, inositol, inulin, isobutanol, isobutyrate, isoleucine, isophthalate, isopropanol, isovalerate, isovaline, itaconate, 2-ketogluconate, α-ketoglutarate, kynurenate, DL-lactate, lactose, laevulinate, laurate, DL-leucine, linoleate, linolenate, lysine, D-malate, L-malate, maleate, malonamide, malonate, maltose, mandelate, mannitol, mannose, melezitose, melibiose, mesaconate, methanol, methionine, methylamine, mucate, myristate, naphthalene, nicotinamide, nicotinate, DL-norleucine, DL-norvaline, octadecanoate, DL-ornithine, oxalate, pantothenate, pelargonate, pentadecanoate, pentadecanol, phenol, phenylacetate, phenylalanine, phthalate, pimelate, potassium hydrogen phthalate, proline, propanol, propionamide, propionate, propylene glycol, protocatechuate, putrescine, pyruvate, quinate, raffinose, ribose, L-rhamnose, salicin, salicylate, sarcosine, sebacate, serine, sodium thiocynate, sorbitol, sorbose, spermine, suberate, succinamide, succinate, sucrose, tartarate, *m*-tartarate, terephthalate, testosterone, trehalose, threonine, tributyrin, tryptamine, D-tryptophan, DL-trytophan, Tween 20, Tween 40, Tween 60, Tween 80, tyrosine, undecane, undecanoate, urea, uric acid, valeramide, valerate, DL-valine, and xylose.

Substrate alkalinization. The alkalinization technique was performed by the procedure of Oberhofer and Rowen (9). The substrates were added to the basal medium, Simmons citrate base (Difco Laboratories), to a final concentration of 0.5% (the final concentration of mucate was 0.1%). The filter-sterilized substrates were aseptically added to the sterile melted agar base (mucate and saccharate were sterilized by autoclaving at 15 pounds [ca. 680.228 g] and 121°C for 15 min). The completed media (pH 6.8) were dispensed in 3-ml aliquots into screw-capped tubes (13 by 100 mm) and allowed to cool on slant boards. The slants were inoculated with one drop of an 18- to 24-h heart infusion broth (Difco) culture and incubated at 35°C. The incubated slants were examined at 1, 2, and 7 days. A positive result (alkalinization of the substrate) was recorded when the color of the medium changed to blue.

The Otto and Pickett medium was prepared and tested as described (11). Subsequently, some modifications were made: (i) the substrates were filter-sterilized; (ii) the agar content was raised to 1.5% to facilitate slant preparation; and (iii) the medium was dispensed in 3-ml aliquots into screw-capped tubes (13 by 100 mm).

Diagnostic studies. The biochemical tests were those routinely used in the Special Bacteriology Section for the characterization of microorganisms submitted for identification. The methods for preparing the media and performing the tests have been previously described (4, 8, 14). All agar slant culture media were inoculated with one drop of an 18- to 24-h heart infusion broth culture. Flagella stains were performed by using Clark's modification (3) of the Leifson technique.

RESULTS

Some of the biochemical characteristics determined for each of the bacterial cultures are listed in Table 2. Each of the peritrichously flagellated cultures was oxidase positive and grew on MacConkey agar. None of the carbohydrate oxidative-fermentative media was acidified by any of the *Alcaligenes* species. Denitrification was observed in all of the *A. denitrificans* cultures and 43% of the *A. faecalis* cultures. However, only the *A. denitrificans* bacteria were able to reduce nitrate beyond the oxidation level of nitrite. Nitrite, but not nitrate, was reduced by each of the *A. odorans* cultures. Most of the *Alcaligenes* organisms displayed no proteolytic activity and demonstrated motility in semisolid agar tubes.

One hundred and eighty-eight substrates were examined for growth stimulation of the *Alcaligenes* microorganisms (Table 3). Fourteen of these substrates (acetate, D-alanine, L-alanine, aspartate, citrate, glutamate, histidine, DL-lac-

TABLE 2. *Some characteristics of the Alcaligenes species*

Test or substrate[a]	*A. denitrificans* (39 strains)	*A. odorans* (60 strains)	*A. faecalis* (63 strains)
Acidification of:			
Glucose OF	0[b]	0	0
Other sugars[c]	0	0	0
Growth on:			
MacConkey agar	100	100	100
SS agar	65	100	79
TGY at 25°C	100	100	100
TGY at 35°C	100	100	100
TGY at 42°C	52	20	75
Oxidase	100	100	100
Catalase	100	98	100
Citrate alkalinization	90	100	95
Urea hydrolysis	15	3	6
Nitrite reduction	100	100	0
Nitrate reduction	100	0	43
Gas from nitrate	100	0	0
Motility	100	97	97
Gelatin hydrolysis	0	20	0
Litmus milk peptonization	0	0	0
Chromogenesis	0	0	0
Lysine decarboxylase	0	0	0
Ornithine decarboxylase	0	0	0
Arginine dehydrolase	0	0	0
Esculin hydrolysis	0	0	0

[a] OF, Oxidative-fermentative; SS, salmonella-shigella; TGY, tryptone-glucose-yeast extract.

[b] Percentage of cultures producing positive reaction.

[c] Xylose, mannitol, lactose, sucrose, maltose, glycerol, salicin, L-arabinose, adonitol, dulcitol, galactose, fructose, mannose, rhamnose, trehalose, raffinose, sorbitol, inositol, cellobiose, inulin, dextrin, glycogen, erythritol, melibiose, melezitose, and starch.

tate, laurate, L-malate, phenylalanine, proline, pyruvate, and tyrosine) stimulated the growth of 90% of all of the *Alcaligenes* cultures tested. They consisted primarily of amino acids and short-chain fatty acids. Ninety-six of the substrates did not enhance bacterial growth. Moreover, seven of the substrates (*p*-aminobenzoate, catechol, cetrimide, indoleacetate, isophthalate, suberate, and terephthalate) either partially or completely inhibited cellular growth. When four of the substrates (*p*-phenyl phenol, palmitate, stearate, and tridecanoate) were incorporated into the basal medium, the plates were too opaque to provide accurate growth determinations. The remaining 78 substrates produced various reactions among the *Alcaligenes* species.

Utilization results for 21 substrates provided a potential basis for differentiation among the *Alcaligenes* species (Table 4). More than 95% of the *A. odorans* cultures utilized butyramide, glycolate, methionine, phenol, DL-ethionine, DL-leucine, and phenylacetate, but no more than 33% of the other bacteria utilized these substrates. At least 80% of the *A. denitrificans* and *A. faecalis* cultures but less than 13% of the *A. odorans* cultures utilized γ-aminobutyrate and β-alanine. Moreover, the *A. denitrificans* cultures utilized glutarate (100%), gluconate (80%), DL-citrulline (80%), and isoleucine (78%), but less than 33% of the *A. faecalis* organisms were able to metabolize these substrates. The *A. faecalis* organisms were characterized by stimulation levels below 90%.

The substrates acetamide, β-alanine, malonate, nicotinamide, propionate, saccharate, and tartrate were used to determine which of the substrate alkalinization methods was most useful for our purposes. Each of the procedures was tested in screw-capped tubes (13 by 100 mm) because of the false positive reactions observed when compartmentalized petri dishes were used. These results were due to cross-contamination of adjacent substrates by the ammonia released by the metabolizing microorganisms.

The carbon substrate alkalinization profiles of 20 cultures each of *A. denitrificans, A. faecalis,* and *A. odorans* were determined (Table 5). The bacterial cultures alkalinized each of the substrates tested. Only nicotinamide (*A. denitrificans*), saccharate (*A. odorans*), and tartrate (*A. odorans*) were not utilized by cultures of each species. Little difference was observed when reactivity patterns of the bacteria on the two types of substrate media were compared. Incubating the slants of the Otto and Pickett (11) media for longer intervals resulted in a reaction reversion in some of the saccharate tubes inoculated with *A. denitrificans* and *A. faecalis* cultures. After 6 days of incubation, the percentage of positive reactions dropped from 90 to 50 (*A. denitrificans*) and from 60 to 10 (*A. faecalis*). No reaction reversions were noted in the Oberhofer and Rowen (9) media.

The Oberhofer and Rowen medium was used to determine substrate alkalinization patterns of 162 *Alcaligenes* cultures (Table 6). Twenty-one substrates which appeared to be capable of establishing unique CSU profiles for each species were tested. The *A. denitrificans* cultures gave positive reactions (≥80%) on nine substrates (butyramide, formate, malate, mesaconate, *m*-tartarate, propionamide, saccharate, serine, and mucate), negative reactions on one (nicotinamide), and variable reactions on the remaining substrates. Reactions of *A. odorans* cultures were positive on 11 substrates (acetamide, butyramide, butyrate, formamide, formate, malonamide, malate, malonate, nicotinamide, propionamide, and propionate), negative on 9, and var-

TABLE 3. *Substrate utilization by the Alcaligenes species (modified Stanier technique)*

Substrate[a]	*A. denitrificans* (39 strains)	*A. faecalis* (63 strains)	*A. odorans* (60 strains)
Acetamide	23[b]	63	100
Acetate	92	94	100
cis-Aconitate	100	95	67
trans-Aconitate	44	5	0
Adipate	97	70	3
β-Alanine	80	87	12
D-Alanine	90	95	100
L-Alanine	90	95	100
γ-Aminobutyrate	82	80	2
DL-2-Aminobutyrate	15	41	65
Anthranilate	36	41	93
Asparagine	100	52	83
Aspartate	100	100	90
1,4-Butanediol	64	21	0
Butanol	56	22	7
Butyramide	23	27	100
Caprate	36	40	0
Caproate	36	71	72
Caprylate	38	67	67
Citrate	90	95	100
DL-Citrulline	80	25	0
Cysteine	97	51	93
Ethanol	59	6	83
DL-Ethionine	28	0	97
Formate	80	0	68
Gentisate	36	5	0
Glutamate	95	100	100
Glutamine	100	84	100
DL-Glycerate	100	68	100
Glycolate	33	32	100
Glyoxylate	77	20	8
Gluconate	80	32	3
Glutarate	100	32	93
Glycine	49	40	100
Heptanoate	36	41	18
1,6-Hexanediol	51	14	0
Hippurate	74	36	88
Histidine	92	100	97
p-Hydroxybenzoate	54	17	17
β-Hydroxy-β-methylglutarate	97	51	10
DL-β-Hydroxybutyrate	100	100	83
Isobutyrate	31	44	28
Isoleucine	79	9	100
Isovalerate	41	22	78
Isovaline	64	56	93
Itaconate	74	48	3
α-Ketoglutarate	100	100	83
Kynurenate	28	52	52
DL-Lactate	100	100	97
Laevulinate	38	16	0
Laurate	100	100	100
DL-Leucine	30	25	97
Lysine	59	19	12
D-Malate	95	60	2
L-Malate	100	100	100
Maleate	92	95	83
Malonate	79	8	90
Mesaconate	82	48	3
Methionine	23	0	100
Mucate	97	67	3
Nicotinate	21	32	17
DL-Norleucine	69	59	100
DL-Norvaline	33	21	93
Pantothenate	46	10	7
Pelargonate	33	52	0
Phenol	23	33	100
Phenyl acetate	33	32	97
Phenylalanine	100	100	100
Pimelate	90	57	0
Proline	92	100	100
Propanol	77	22	68
Propionamide	36	60	100
Propionate	85	94	100
Protocatechuate	49	63	8
Pyruvate	100	100	100
Quinate	0	41	3
Serine	51	38	0
Succinate	0	0	32
Tartarate	33	10	0
m-Tartarate	92	60	2
Threonine	72	11	0
Tributyrin	51	21	0
Tryptamine	28	37	83
D-Tryptophan	85	71	50
DL-Tryptophan	85	79	100
Tyrosine	100	100	100
Undecanoate	41	41	50
Uric acid	36	0	70
Valeramide	69	84	100
Valerate	31	22	63
DL-Valine	56	6	23
DL-Ornithine	23	21	93

[a] Ninety-six of the substrates that were examined provided no growth enhancement for any of the *Alcaligenes* species.

[b] Percentage of cultures producing positive reactions after 2 days of incubation.

iable on 1 (β-alanine). The *A. faecalis* microorganisms were positive on 4 substrates (β-alanine, butyrate, malate, and propionate) and gave variable reactions on the other 17 substrates.

A. denitrificans and *A. odorans* cultures had high levels of activity (80 to 100%) on several substrates in each of the substrate utilization media. *A. faecalis* cultures, however, utilized substrates at levels between 40 and 60%. Observation of the colonial morphology of the *A. faecalis* cultures on rabbit blood agar plates indicated two distinct types of growth. The mor-

phology of *A. faecalis* type I colonies was similar to that of *A. denitrificans*. The colonies were small in diameter (≤0.5 mm), low convex, glistening, and had an entire edge. The morphology of *A. faecalis* type II colonies was similar to that of *A. odorans*, but did not produce the dark green color on rabbit blood agar. The type II colonies were larger in diameter (1.5 to 2.0 mm), umbonate with a spreading periphery, and granular. Forty of the *A. faecalis* cultures were designated as type I, and 23 culture were designated as type II.

Reevaluation of the substrate utilization data in Tables 4 and 6 provided supporting evidence for the separation of the *A. faecalis* cultures into two types (Table 7). The *A. faecalis* type I organisms utilized a greater number of substrates than did type II, and the activity percent levels were higher in most cases. The *A. faecalis* type II organisms were more reactive only on propionate, β-alanine, and butyrate.

The CSU profiles of the *A. faecalis* type I and type II cultures were compared with those of *A. denitrificans* and *A. odorans* microorganisms (Table 7). The *A. faecalis* type I cultures had a CSU profile quite similar to that of the *A. denitrificans* cultures. However, there were notable

TABLE 4. *Substrates potentially differential for the Alcaligenes species*

Substrate	*A. denitrificans* (39 strains)	*A. faecalis* (63 strains)	*A. odorans* (60 strains)
Adipate	97[a]	70	4
Butyramide	24	27	100
γ-Aminobutyrate	82	80	2
Glycolate	33	32	100
D-Malate	95	61	2
m-Tartarate	92	60	2
Methionine	23	0	100
Mucate	97	67	4
Phenol	23	33	100
Propionamide	37	60	100
Pimelate	90	57	0
DL-Ethionine	28	0	97
Glutarate	100	32	93
Isoleucine	78	9	100
DL-Leucine	30	25	97
Phenylacetate	33	32	97
DL-Norleucine	69	59	100
β-Alanine	80	87	12
Cysteine	97	51	93
DL-Citrulline	80	25	0
Gluconate	80	33	3

[a] Percentage of cultures producing positive reactions.

TABLE 6. *Substrate alkalinization reactions of the Alcaligenes cultures on Oberhofer and Rowen media*

Substrate	*A. denitrificans* (40 strains)	*A. faecalis* (63 strains)	*A. odorans* (64 strains)
Acetamide	53[a]	33	100
Adipate	78	54	0
β-Alanine	75	89	50
Azelate	65	51	0
Butyramide	81	51	100
Formamide	58	32	100
Formate	100	65	100
Malate	100	100	88
Malonamide	50	33	88
Malonate	55	40	100
Mesaconate	83	62	0
Mucate	83	49	0
Nicotinamide	0	32	100
Pimelate	75	54	0
Propionamide	83	33	98
Propionate	55	87	100
Saccharate	83	54	0
Serine	100	63	9
m-Tartarate	98	56	0
Tartrate	68	21	0
Butyrate	36	95	100

[a] Percentage of cultures producing positive reactions after 2 days of incubation.

TABLE 5. *Comparison of substrate alkalinization by the Alcaligenes species on Oberhofer and Rowen and Otto and Pickett media*

Substrate	*A. denitrificans*[a]		*A. faecalis*[a]		*A. odorans*[a]	
	OP[b]	OR[b]	OP	OR	OP	OR
Acetamide	40[c]	55	20	30	100	100
β-Alanine	80	75	90	90	50	50
Malonate	60	55	55	40	100	100
Nicotinamide	0	0	50	35	100	100
Propionate	65	65	90	95	100	100
Saccharate	90	90	55	60	0	0
Tartrate	50	65	30	30	0	0

[a] Twenty cultures of each species were tested.
[b] OP, Otto and Pickett media; OR, Oberhofer and Rowen media.
[c] Percentage of cultures producing positive reactions.

TABLE 7. *Selected substrate utilization by A. faecalis type I and type II, A. denitrificans, and A. odorans cultures*

Substrate	*A. denitrificans* (40 strains)	*A. odorans* (60 strains)	*A. faecalis*	
			Type I (40 strains)	Type II (23 strains)
Modified Stanier				
Adipate	97[a]	4	98	13
m-Tartarate	92	2	93	4
Mucate	97	4	98	13
Pimelate	90	0	90	0
Propionate	56	100	37	91
Malate	100	78	100	100
β-Alanine	75	50	83	96
Substrate alkalinization				
Adipate	78	0	88	0
Butyramide	81	100	83	0
Butyrate	36	100	70	100
Formamide	58	100	50	0
Formate	100	100	100	4
Mesaconate	83	0	98	0
m-Tartarate	97	0	88	0
Nicotinamide	0	100	48	0
Propionamide	81	99	53	0
Saccharate	81	0	95	0
Serine	100	9	100	4
Tartrate	67	0	33	0
Pimelate	75	0	85	0
Mucate	83	0	78	0

[a] Percentage of cultures producing positive reactions after 2 days of incubation.

differences in that the *A. denitrificans* organisms were more reactive on propionamide and tartrate, and the *A. faecalis* type I cultures were more reactive on butyrate and nicotinamide. CSU profiles of the *A. faecalis* type II and *A. odorans* cultures were similar when determined by the modified Stanier method, but little similarity was noted when they were determined by substrate alkalinization.

DISCUSSION

The *Alcaligenes* species were able to utilize the substrates in each of the systems tested. However, differentiation of these bacteria by the use of the CSU profiles was not absolute. Rather, with the modified Stanier technique, the *Alcaligenes* species produced identical growth stimulation profiles on 61% (14 positives at >90%, and 99 negative reactions) of the substrates tested. Short-chain fatty acids, amino acids, and one amide, valeramide, were the compounds that provided growth stimulation for each of the microorganisms. The unutilized substrates varied from carbohydrates to long-chain fatty acids and ring structure-containing compounds.

The CSU pattern of the *A. odorans* cultures was quite different from those of the other *Alcaligenes* species. At least 95% of the *A. odorans* microorganisms utilized butyramide, glycolate, methionine, phenol, DL-ethionine, DL-leucine, and phenylacetate, whereas most of the other *Alcaligenes* organisms did not utilize these substrates. Cultures of *A. faecalis* and *A. denitrificans* produced CSU patterns which were more nearly related to each other than to the *A. odorans* pattern. However, *A. denitrificans* cultures did utilize glutarate (100%) DL-citrulline (80%), gluconate (80%), and malonate (79%) at higher levels than did the *A. faecalis* microorganisms (≤33%).

The CSU profiles determined from the substrate alkalinization studies were no more effective in differentiating the *Alcaligenes* species than those obtained with the modified Stanier system. The *A. denitrificans* cultures utilized mesaconate (83%), *m*-tartarate (97%), saccharate (81%), and mucate (83%), but none of these substrates was utilized by any of the *A. odorans* cultures. The *A. odorans* cultures utilized nicotinamide (100%; *A. denitrificans*, 0%), malonamide (87%), propionamide (99%), and formamide (100%), but no more than 33% of the *A. faecalis* organisms utilized these substrates. The *A. faecalis* microorganisms utilized butyrate (95%), but only 36% of the *A. denitrificans* cultures utilized this substrate.

The *A. faecalis* cultures consisted of two distinct groups as determined by (i) colonial morphology, and (ii) CSU profiles. The profile of the group I organisms was quite similar to that of the *A. denitrificans* cultures. With the modified Stanier technique, the group II organisms had a profile quite similar to that of the *A. odorans* cultures. However, the results obtained for the group II cultures with the substrate alkalinization technique were unlike those of any of the other organisms used in this study.

Although use of CSU profiles did not allow absolute differentiation of the organisms, these patterns did serve to separate the *Alcaligenes* species from phenotypically closely related groups (Table 8). When the substrate alkalinization method is used, the Group IVc(2) and Group IVe cultures can be differentiated from the *Alcaligenes* organisms by testing with the substrates butyramide and formate. Cultures of *Bordetella bronchiseptica* can be separated by testing for utilization of saccharate and formate. *Pseudomonas diminuta* and *P. alcaligenes* cultures can be differentiated from the *Alcaligenes* organisms by testing with the substrates butyramide and D-malate.

Reading and recording the CSU results were much easier with the alkalinization procedure than with the modified Stanier technique, be-

TABLE 8. *Comparison of substrate alkalinization patterns of the Alcaligenes species and other similar organisms which are isolated from clinical specimens*

Bacteria	Substrate alkalinization of:[a]					
	Butyramide	Formate	Nicotinamide	Saccharate	Pimelate	D-Malate
A. denitrificans	+	+	−	+	+(−)	+
A odorans	+	+	+	−	−	+(−)
A. faecalis I	+	+	−	+	+	+
A. faecalis II	−	−	−	−	−	+
B. bronchiseptica	+(−)	−	−	+	−	+
Group IVc(2)	−	+	+	+	+	+
Group IVe	−	+	−	−	−	−
P. diminuta	−	−	−	−	−	−
P. alcaligenes	−	+(−)	−	−	−	+

[a] +, 80 to 100% alkalinization; +(−), 70 to 80% alkalinization; −, 50% or less alkalinization.

cause it was simpler to observe a color change than to compare colonial growth. Moreover, the preparation, storage, and inoculation of the media were simplified.

Both Otto and Pickett (11) and Oberhofer and Rowen (9) substrate media provided satisfactory results in recording substrate utilization. However, the Otto and Pickett medium is more difficult to prepare than the Oberhofer and Rowen medium, and we have stored the latter for up to 4 months at 4°C with no apparent loss in reaction sensitivity.

The results we obtained with both substrate alkalinization media agreed with most published results (10, 11). However, observations of culture reactions on tartrate and saccharate were quite different from previously recorded results. Other investigators (11) have reported that these substrates provide different results for each of the *Alcaligenes* species, but experimental observations in our laboratory did not support these findings. This variance in results is probably due to the small number of cultures which the other investigators used in their study.

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