

Nutritional Requirements of *Acinetobacter* Strains Isolated from Soil, Water, and Sewage

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One hundred five strains of *Acinetobacter* were isolated from water, soil, and sewage on nonselective complex media, and their nutritional properties were studied. Only one of these strains requires growth factors in order to grow in a mineral medium containing a single carbon source.

Most strains of *Acinetobacter* studied grow well in simple mineral media containing a single organic compound, such as acetate or lactate, as a source of carbon and energy (1, 2, 4, 6). Enrichment procedures designed for isolation of acinetobacters have generally been successful when such simple media, at a moderately acid pH, were used with good aeration (1, 8). For routine isolation of acinetobacters in hospital diagnostic laboratories, however, complex media are generally employed. Studies of the nutritional requirements of many such hospital strains have revealed that only a very small proportion of them fail to grow in simple media lacking growth factors such as amino acids, vitamins, purines, and pyrimidines (2, 4, 6). The chief aim of the present investigation was to determine whether it would be possible to obtain growth factor-requiring acinetobacters from soil, water, and sewage if all such strains were isolated from these sources by using only complex growth factor-containing media.

Samples of soil, water, and sewage collected chiefly within 15 miles of Ann Arbor, Mich., were suspended in 0.8% saline, and various dilutions were plated on Antibiotic Medium 3 (Penassay agar; Difco) adjusted to pH 6.0, Sellers agar (Difco), and Herellea agar (Difco). After incubation for 1 or 2 days at 30 C, wet mounts of paste from well isolated colonies were examined for nonmotile coccobacilli. Bacteria from such colonies, which also stained gram-negative, were then tested for reaction in the oxidase test (7). Oxidase-negative organisms were considered to be presumptive acinetobacters. A total of 114 such strains were isolated, and deoxyribonucleic acid (DNA) from

each strain was used in the *Acinetobacter* transformation assay (6). It has been shown in another study from this laboratory that DNA from any strain of *Acinetobacter* is capable of transforming suitable auxotrophs of a competent *Acinetobacter* strain to prototrophy, thus establishing a genetic relationship between the tested strain and the competent strain (6).

DNA species from 105 of the 114 strains isolated were effective in the transformation assay, thus unequivocally establishing these strains as acinetobacters. The remaining nine strains were reexamined to check for possible errors in the original tests. Six of these nine strains were found to be motile, one was shown to be gram positive, and another proved to be oxidase positive. The one remaining non-*Acinetobacter* strain was found to be a facultative anaerobe capable of producing acid and growing anaerobically in a medium containing glucose. Although acinetobacters are able to form acid aerobically from sugars, these organisms are all aerobes and cannot ferment sugars anaerobically (2, 4, 5). Of the 105 *Acinetobacter* strains isolated, 26 were derived from soil

TABLE 1. *Distribution and classification of Acinetobacter strains^a*

Strains	No. of strains isolated			
	Soil	Water	Sewage	Total ^b
All strains	26	56	23	105
Group A	16	15	1	32
Group B	10	41	21	72

^a Strains were grouped according to the scheme of Baumann et al. (2).

^b The one auxotrophic strain in this collection has not been placed in either group A or B.

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TABLE 2. Phenotypic grouping of isolated *Acinetobacter* strains

Group or subgroup ^b	No. of strains in group ^c	No. of substrates used by strains in each group	Growth in media containing: ^a													
			Acetate	DL-Lactate	L-Malate	2,3-Butanediol	D (-)-Quinate	Malonate	Adipate	β -Alanine	Putrescine	Aspartate	D-Glucose	D-Ribose	D-Xylose	L-Arabinose
A	32	7-14	100	100	97	97	88	75	94	81	69	94	38	22	22	28
B	72	1-7	100	58	52	26	45	44	1	1	0	56	0	0	0	0
A1	21	8-14	100	100	95	100	86	95	100	86	95	95	48	33	33	48
A2	9	7-9	100	100	100	100	78	22	100	78	0	100	22	0	0	0
A3	2	7-8	100	100	100	50	100	100	0	50	100	50	0	0	0	0
B1	25	5-7	100	100	80	60	80	92	0	0	0	100	0	0	0	0
B2	25	1-5	100	60	20	20	4	16	0	4	0	0	0	0	0	0
B4	22	2-5	100	9	55	0	55	23	5	0	0	73	0	0	0	0

^a The numbers are percentages of strains in each group which grow with a particular carbon source. Strains that mutated spontaneously to utilization of a particular compound were scored as growing with this substrate.

^b The groups and subgroups are those described by Baumann et al. (2).

^c The carbon sources used by the one auxotrophic strain in this collection of bacteria were not determined.

samples, 56 came from various rivers, ponds, and swamps, and 23 were isolated from several samples of sewage obtained from the Ann Arbor Sewage Processing Plant.

The possible growth factor requirements of the 105 strains of *Acinetobacter* isolated in this study were then examined by streaking each organism on a sector of an acetate-mineral agar plate (6). It was found that 104 of the 105 strains tested were able to grow on this simple medium. The one strain which failed to grow in the acetate-mineral medium, unless a trace of yeast extract was also included, was obtained from a sewage sample and may, therefore, represent an organism of recent human origin. This strain appears to require more than one growth factor since it fails to grow on any of the growth factor-containing plates used in the determination of auxotrophic requirements (3). The defect in this strain is not the inability to use a particular carbon source since the organism failed to grow on mineral agar plates containing any one of a series of organic compounds which serve as growth substrates for a variety of acinetobacters. Upon prolonged incubation of this growth factor-requiring strain on an acetate-mineral agar plate, however, revertant colonies did eventually appear spontaneously, these revertants being able to grow readily on this medium as well as on others, each of these containing only a single organic carbon source. It thus appears that the large majority of *Acinetobacter* strains normally occurring in water, soil, and sewage do not require growth factors and can grow in a mineral medium

containing acetate or some other organic source of carbon and energy.

The phenotypic characteristics of 106 strains of *Acinetobacter* placed these organisms into two major groups, which were designated A and B, each group being further subdivided into several subgroups (2). Strains in group A are able to dissimilate more compounds than strains in group B. The 105 strains of *Acinetobacter* isolated in the present study were grouped according to the scheme of Baumann et al. (2). Table 1 shows that 62% of the strains isolated from soil belong to group A, whereas 73% of strains isolated from water and 96% of strains isolated from sewage belong to group B. Table 2 is a compilation of the ability of strains in both groups to grow in mineral media containing 1 of 14 different carbon substrates, these results being used as the basis for classification of each strain into a particular subgroup. With the exception of subgroup B3, into which none of the isolated strains could be classified, representatives of all the other subgroups defined by Baumann et al. (2) were found in this collection of *Acinetobacter* strains.

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