Interspecies Transformation of *Acinetobacter*: Genetic Evidence for a Ubiquitous Genus

ELLIOT JUNI

Department of Microbiology, University of Michigan, Ann Arbor, Michigan 48104

The availability of a strain of *Acinetobacter* competent for transformation has made it possible to demonstrate the genetic relatedness of a large variety of gram-negative, oxidase-negative, nonmotile, and aerobic coccobacilli originally classified into eleven different genera. Deoxyribonucleic acid (DNA) species from 265 such strains are capable of transforming stable auxotrophs of the competent *Acinetobacter* to prototrophy. The compositions of these DNA species vary from 40 to 46.8% guanine plus cytosine. Strains with widely differing phenotypic properties are also included in this collection of acinetobacters. DNA species from all oxidase-positive strains of *Moraxella* and from a variety of common bacteria are unable to transform the competent *Acinetobacter*. Although acinetobacters are usually considered to be unable to reduce nitrate to nitrite, six strains known to carry out this reduction have been shown to be authentic acinetobacters since their DNA species readily transform the competent *Acinetobacter* auxotrophs to prototrophy. In contrast to previous findings that acinetobacters rarely grow with glucose as a sole carbon source, the results of the present study show that 17 of the 265 strains grow readily in a glucose-mineral medium, and 48 other strains can mutate spontaneously to grow in such a medium. A second competent strain of *Acinetobacter*, originally unable to use glucose, D-xylose, or D-ribose as carbon sources, has been transformed for ability to dissimilate these compounds using DNA species from strains that normally grow on these sugars. Although most of the 265 *Acinetobacter* strains studied were originally grown on complex media when isolated from human sources, only nine of these strains require growth factors in order to grow in a mineral medium containing a single carbon and energy source. A simple transformation assay has been devised for rapid examination of large numbers of strains to determine whether or not they are acinetobacters. This assay, which is suitable for routine diagnostic work, includes a procedure for preparation of crude transforming DNA from a small quantity of bacterial paste. Samples of DNA prepared from *Acinetobacter* cultures that had died on slants and plates were still able to effect transformation of the competent auxotrophs to prototrophy.

Several laboratories have suggested that there is a close taxonomic relationship among species of gram-negative, oxidase-negative bacteria usually referred to as either *Moraxella, Herellea, Mima, Bacterium, Achromobacter,* or *Acinetobacter* (4, 29, 46, 54). Organisms previously placed in 15 different genera have been classified as members of the genus *Acinetobacter* on the basis of similarities in their phenotypic properties (4).

The finding that one particular strain of *Acinetobacter* is competent for genetic transformation (32) suggested the possibility of using this property for establishing genetic relatedness among the bacteria of this group (30, 39, 43). It has been demonstrated that deoxyribonucleic acids (DNA species) from all 265 strains of *Acinetobacter* studied are able to transform auxotrophs of the competent strain to prototrophy. A simple diagnostic procedure has been devised to facilitate the testing of large numbers of strains for genetic relatedness to the competent strain.

The results of the present investigation confirm the conclusion, drawn from taxonomic studies, that bacteria previously grouped into a

variety of genera are all, in fact, members of one widely distributed genus, *Acinetobacter*. A preliminary report of this work has been presented (E. Juni, Bacteriol. Proc., p. 39, 1971).

MATERIALS AND METHODS

Bacteria. The oxidase-negative strains used were as follows:

1. *Acinetobacter calcoaceticus*, strain BD4, isolated from soil by 2,3-butanediol enrichment.

2. *Herellea* sp., ATCC 9955.

3. *Mima polymorpha*, ATCC 9957.

4. *Mima polymorpha*, ATCC 9957, received as strain RH482 from R. Hugh.

5. *Mima polymorpha*, ATCC 14291, received as strain RH2419 from R. Hugh.

6. *Mima polymorpha*, ATCC 14292, received as strain RH2420 from R. Hugh.

7. *Micrococcus cerificans*, ATCC 14987, received as strain RH2338 from R. Hugh.

8. *Bacterium anitratum*, ATCC 15149.

9. *Bacterium anitratum*, ATCC 15150.

10. *Bacterium anitratum*, ATCC 15151.

11. *Acinetobacter lwoffi*, ATCC 15309, received as strain RH2393 from R. Hugh.

12. *Neisseria winogradskyi*, ATCC 17902.

13. *Bacterium anitratum*, ATCC 17903.

14. *Achromobacter mucosus*, ATCC 17904.

15. *Achromobacter conjunctivae*, ATCC 17905.

16. *Achromobacter haemolyticus*, ATCC 17906.

17. *Achromobacter haemolyticus* subsp. *alcaligenes*, ATCC 17907.

18. *Achromobacter citroalcaligenes*, ATCC 17908.

19. *Achromobacter metalcaligenes*, ATCC 17909.

20. *Achromobacter metalcaligenes*, ATCC 17910.

21. *Bacterium anitratum*, ATCC 17912.

22. *Bacterium anitratum*, ATCC 17913.

23. *Achromobacter winogradski*, ATCC 17922, received as strain RH2382 from R. Hugh.

24. *Alcaligenes metalcaligenes*, ATCC 17923.

25. *Bacterium anitratum*, ATCC 17924.

26. *Achromobacter lwoffi*, ATCC 17925.

27. *Diplococcus mucosus*, ATCC 17957.

28. *Mima polymorpha*, ATCC 17959.

29. *Herellea vaginicola*, ATCC 17961.

30. *Moraxella lwoffi* subsp. *nonliquefaciens*, ATCC 17968.

31. *Moraxella lwoffi* subsp. *nonliquefaciens*, ATCC 17969.

32. *Moraxella lwoffi* subsp. *nonliquefaciens* ATCC 17976.

33. *Moraxella lwoffi* subsp. *liquefaciens*, ATCC 17977.

34. *Moraxella glucidolytica* subsp. *nonliquefaciens*, ATCC 17978.

35. *Moraxella glucidolytica* subsp. *liquefaciens*, ATCC 17979.

36. *Moraxella lwoffi*, ATCC 17984.

37. *Moraxella lwoffi* subsp. *bacteroides*, ATCC 17985.

38. *Moraxella lwoffi*, ATCC 17986.

39. *Moraxella lwoffi* subsp. *brevis*, ATCC 17987.

40. *Alcaligenes haemolysans*, ATCC 17988.

41. *Herellea caseolytica*, ATCC 19002.

42. *Herellea vaginicola*, ATCC 19003.

43. *Herellea vaginicola*, ATCC 19004.

44. *Herellea saponiphilum*, ATCC 19194.

45. *Bacterium anitratum*, ATCC 19606, received as strain RH2208 from R. Hugh.

46. *Moraxella calcoacetica*, ATCC 23055.

47.-52. *Achromobacter* sp., strains MJT/F4/3/16, MJT/F4/18/30, MJT/F5/5, MJT/F5/14, MJT/F5/122, and MJT/F5/284, respectively, received from M. J. Thornley.

53.-60. *Herellea vaginicola*, CDC strains B5W4, B5W99, 154BK, 730, 755W, 7104A, 9372, and 9391, respectively, received from W. B. Cherry.

61.-65. *Mima polymorpha*, CDC strains 47-8639, 47-8818, 676, 7345, 7833, respectively, received from W. B. Cherry.

66.-71. *Herellea vaginicola*, CDC strains A3862, A9924, B2937, B3504, 7764 and 7811, respectively, received from R. E. Weaver.

72. *Bacterium anitratum*, strain RH454, received from R. Hugh.

73. *Moraxella lwoffi*, strain RH462, received from R. Hugh, identical to strain NCTC 7976.

74. *Mima polymorpha*, strain RH469, received from R. Hugh, identical to strain King 2478.

75. *Mima polymorpha*, strain RH471, received from R. Hugh, identical to strain King 2617.

76. *Bacterium anitratum*, strain RH2230, received from R. Hugh.

77. *Bacterium anitratum*, strain RH2239, received from R. Hugh.

78. *Acinetobacter* sp., strain AI-3, isolated from a pond in Urbana, Illinois by acetoin enrichment.

79. *Acinetobacter* sp., strain 420, isolated as an air contaminant on a nutrient agar plate.

80. *Acinetobacter* sp., strain 520, isolated as an air contaminant on a 2,3-butanediol-mineral agar plate.

81. *Acinetobacter* sp., strain 620, isolated as an air contaminant on an L-arabinose-mineral agar plate.

82. *Acinetobacter* sp., strain 23B3-11, isolated from soil by 2,3-butanediol enrichment.

83. *Bacterium anitratum*, strain UM39-1, obtained from the stock culture collection of the Department of Microbiology, University of Michigan.

84.-86. *Acinetobacter* sp., strains Gil 1, Gil 2, and Gil 3 respectively, obtained by streaking water from an industrial lagoon on acetate-mineral agar plates.

87.-91. *Acinetobacter* sp., strains Gil 5, Gil 6, Gil 9, Gil 10 and Gil 11, respectively, obtained by streaking water from an industrial lagoon on lactate-mineral agar plates.

92. *Acinetobacter* sp., strain Gil 7, obtained by streaking water from an industrial lagoon on a 2,3-butanediol-mineral agar plate.

93. *Acinetobacter* sp., strain Gil 8, obtained by streaking water from an industrial lagoon on a malate-mineral agar plate.

94. *Acinetobacter* sp., strain GAH-10X, obtained by streaking a soil suspension on a 2,3-butanediol-mineral agar plate.

95.-106. *Acinetobacter* sp., EB strains 1, 2, 3, 4, 6, 7, 8, 12, 14, 15, 16, and 17, respectively, received

from E. M. Britt, Diagnostic Laboratory, St. Joseph Mercy Hospital, Ann Arbor, Mich.

107.-181. *Herellea vaginicola*, KK strains 1, 3, 4, 5, 7, 8, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 57, 60, 62, 70, 71, 72, 73, 74, 78, 79, 80, 83, 85, 86, 90, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, and 118, respectively, received from K. A. Klippert, Diagnostic Laboratory, University of Michigan Hospital, Ann Arbor, Mich.

182.-205. *Mima polymorpha*, KK strains, 2, 6, 11, 12, 24, 25, 26, 51, 53, 54, 55, 56, 59, 64, 65, 75, 76, 77, 87, 88, 89, 115, 116, and 117, respectively, received from K. A. Klippert, Diagnostic Laboratory, University of Michigan Hospital, Ann Arbor, Mich.

206.-218. *Acinetobacter lwoffii*, strains 4, 6, 13, 15, 16, 17, 18, 19, 22, 23, 24, 25 and 26, respectively, received from M. Pintér.

219.-223. *Acinetobacter haemolysans*, strains 9, 10, 12, 113, and 125, respectively, received from M. Pintér.

224.-230. *Acinetobacter anitratus*, strains 27, 104, 105, 106, 107, 109, and 111, respectively, received from M. Pintér.

231.-237. *Acinetobacter anitratum*, strains 23, 441, 567, 639, 803, 825, and 831, respectively, received from G. L. Gilardi.

238.-246. *Acinetobacter lwoffi*, strains 250, 505, 530, 538, 583, 589, 661, 662, and 700, respectively, received from G. L. Gilardi.

247.-251. *Acinetobacter haemolyticus*, subsp. *haemolyticus*, strains 465, 470, 477, 492 and 728, respectively, received from G. L. Gilardi.

252.-253. *Acinetobacter haemolyticus*, subsp. *alcaligenes*, strains 506 and 612, received from G. L. Gilardi.

254.-264. *Herellea vaginicola*, strains Sp 3493, Sp 3498, Sp 3540, Sp 3556, Sp 3560, Sp 3586, Sp 3590, Sp 3615, G 7594, T 6541, and T 6564, respectively, received from J. A. Washington II.

265. *Vibrio* 01, identical to strain NCIB 8250 (ATCC 11171) received from C. W. Fewson.

The oxidase-positive strains used were as follows:
266. *Mima polymorpha*, ATCC 10973.
267. *Micrococcus cryophilus*, ATCC 12226.
268. *Flavobacterium meningosepticum*, ATCC 13253, received as strain RH 540 from R. Hugh.
269. *Micrococcus cryophilus*, ATCC 15174.
270. *Moraxella duplex*, subsp. *liquefaciens*, ATCC 17952.
271. *Moraxella nonliquefaciens*, ATCC 17953.
272. *Moraxella nonliquefaciens*, ATCC 17955.
273. *Moraxella nonliquefaciens*, ATCC 19961, received from B. W. Catlin.
274. *Moraxella nonliquefaciens*, ATCC 19962, received from B. W. Catlin.
275.-278. *Achromobacter* sp., strains MJT F4/11/5, MJT F5/158, MJT F5/199A, and MJT F5/211, received from M. J. Thornley.
279. *Mima polymorpha* var. *oxidans*, strain CDC 8375, received from W. B. Cherry.
280. *Acinetobacter* sp. n., strain A 189 (Brisou 1919), isolated from seawater, received from H. Lautrop.

281. *Acinetobacter winogradskyi*, strain A 191 (Brisou 2299), isolated from seawater, received from H. Lautrop.
282. *Acinetobacter* sp. n., strain A 192 (Brisou 1922), isolated from seawater, received from H. Lautrop.
283. *Acinetobacter anitratum*, strain A 193 (Brisou 1924), isolated from seawater, received from H. Lautrop.
284.-287. "*Acinetobacter*-like," strains WM 32, A 273, A 280, and A 285, respectively, isolated from pathological materials, produce acid aerobically from glucose and maltose, received from H. Lautrop.
288. Strain PAR, amine-utilizing soil organism, received from E. R. Leadbetter.
289. *Neisseria catarrhalis*, obtained from the stock culture collection of the Department of Microbiology, University of Michigan.
290.-291. *Acinetobacter lwoffi*, strains 632 and 633, respectively, obtained from G. L. Gilardi.

The exceptional strains used were as follows:
292. Oxidase-negative mutant of strain 272.
293. "Acinetobacter-like" strain isolated from Great Salt Lake mud by C. F. Gonzalez and W. A. Taber (ATCC 27042), received from W. A. Taber.

Media. Minimal agar was prepared by mixing equal parts of 3% agar and S-2 salts solution of Monod and Wollman (44) followed by addition of the appropriate amount of a 50% solution of the carbon source to give a final concentration of 0.25%. Carbon sources suitable for growth of the competent strain of *Acinetobacter* (strain BD413) include D,L-lactic acid, L-malic acid, glucose, and 2,3-butanediol. When required, amino acid growth factors were each added to give a final concentration of 100 μg/ml. Strains unable to grow on the above media were cultivated on heart infusion agar (Difco) plates.

Preparation of transforming DNA. To assay DNA species from a large number of bacterial strains, a simplified method was devised for the preparation of crude transforming DNA. A loopful of cell paste, gathered from the surface of a suitable growth medium (usually heart infusion agar), is suspended in 0.5 ml of a sterile solution containing 0.05% sodium dodecyl sulfate in standard saline citrate (0.15 M NaCl, 0.015 M Na₃ citrate), contained in a 16-mm test tube. Suspension is facilitated by stirring in an orbital mixer, care being taken to avoid adherence of particles of cell paste to portions of the test tube that are not immersed in liquid. The suspended cells are then heated in a 60 C water bath for 1 hr; this procedure lyses the cells and results in a sterile crude DNA solution. Such DNA preparations can be stored at 3 to 5 C for periods longer than a year and will still retain the ability to transform competent cells. The exact amount of cell paste used in this procedure is not critical for the transformation test described below.

DNA used for the determination of base composition was prepared by the method of Marmur (42).

Competent auxotrophs used in the transformation assay. Cells of the wild-type culture of competent *Acinetobacter* (strain 1) possess large polysaccharide capsules and are not completely suitable for many studies since the presence of these capsules

prevents cells from packing during centrifugation (32). A mutant of strain 1 (strain BD413) was shown to have what appears to be an extremely small capsule (32), and cells from this strain form a solid pellet upon centrifugation. Since strain BD413 is also competent for genetic transformation, auxotrophic mutants of this strain have been used for all transformation studies in this laboratory.

Auxotrophic mutants were obtained by N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis (1). Strain Ivl-10 requires 100 μg of isoleucine, valine, and leucine per ml to grow in minimal medium containing a single source of carbon and energy. The defect in this strain appears to be the result of a single mutation since attempts to transform this auxotroph to independence from the requirement for any one of the three amino acids have been uniformly unsuccessful. Furthermore, the ability to transform this auxotroph to prototrophy with good efficiency, using DNA from the parent strain (strain BD413), implies that the lesion in strain Ivl-10 must be confined to one small region of the chromosome.

Strain trpE27 is a tryptophan auxotroph and lacks a functional anthranilate synthetase. The particular auxotroph used in the present studies was prepared by transforming the defective anthranilate synthetase gene trpE27 into strain Bdl-35, a mutant of strain BD413 which is unable to use 2,3-butanediol as a carbon source, and by isolating tryptophan-requiring cells from among transformants able to grow with 2,3-butanediol.

Test for stability of the auxotrophic mutants. The stability of a series of auxotrophs was tested by attempting to revert them to prototrophy with chemical mutagens using the "auxanographic" procedure of Iyer and Szybalski (28). Auxotrophs that readily revert spontaneously when plated on minimal medium were not further analyzed by this method.

Transformation assay. A small amount of bacterial paste of a stable auxotroph (strain Ivl-10 or strain trpE27), just visible to the naked eye, is applied to a sector of a plate containing a medium which will support growth of the auxotroph. Complete media such as heart infusion agar or a lactate-mineral medium supplemented with growth factor quantities (100 μg/ml) of the required nutrients are both satisfactory for this purpose. A loopful of the crude DNA preparation, described above, is used to suspend and spread the cell paste in an area of about 5 to 10 mm in diameter. Cell paste of the auxotroph is also spread on another sector of the same plate to serve as a non-DNA-treated control. A third sector of the plate is streaked with a loopful of the DNA preparation employed to test its sterility. A dozen or more DNA samples can be tested conveniently on one plate.

After incubation for 12 to 24 hr at 35 C, growth from each of the inoculated areas is streaked heavily on a sector of a lactate-mineral agar plate which is incubated at 35 C. Any utilizable carbon source may be used in place of lactic acid. After 12 to 24 hr, all auxotrophic cells transformed to prototrophy will appear as colonies visible to the naked eye. A low-power lens or dissecting microscope can be used for recognition of transformant colonies after shorter periods of incubation. Auxotrophs of the competent organism treated with DNA from an authentic strain of Acinetobacter will result in the appearance of prototrophic transformant colonies, whereas cells mixed with DNA from an inrelated organism, or control cells containing no DNA, will show no colonies on the minimal plate.

Determination of DNA composition. The base composition of DNA from the competent strain BD413 was calculated from the buoyant density in a cesium chloride density gradient (40).

Bacteriological tests. Nitrate reduction was performed in test tubes sealed with paraffin as described by Skerman (51). The growth medium used in this test was nutrient broth (Difco) containing 0.1% $NaNO_3$. The oxidase test was carried out by using the method of Kovacs (35). For determination of bacterial motility, cells grown on a semisolid medium for 12 to 14 hr were suspended in a small drop of water on a glass slide, covered with a cover slip, and observed with the $43\times$ objective of a phase contrast microscope immediately after preparation of the wet mount. To avoid dehydration of semisolid media during prolonged incubation, plates were stored in plastic bags.

RESULTS

Interspecies transformation. When it was first discovered by Juni and Janik (32) that Acinetobacter calcoaceticus (strain 1) is competent for genetic transformation of encapsulation markers, several amino acid auxotrophs were obtained by mutagenesis to study the quantitative aspects of this transformation. It was noted (E. Juni and A. Janik, Bacteriol. Proc., p. 59, 1969) that in addition to homologous DNA, heterologous DNA species from several other strains of Acinetobacter are also able to transform a competent auxotroph (strain Ivl-10) to prototrophy. Several laboratories (4, 29, 46, 54) have stressed the possible relationship of bacteria that are gram-negative cocci or short rods, strict aerobes, nonmotile, and give a negative oxidase test. Such organisms also appear to be generally unable to reduce nitrate to nitrite.

It has been shown that DNA from each of the 265 oxidase-negative strains examined is able to transform the extremely stable competent auxotroph, strain Ivl-10, to prototrophy. In addition, a tryptophan auxotroph (strain trpE27) of the competent strain has also been used. Figures 1 and 2 show an application of the transformation assay in the testing of DNA species from five presumed acinetobacters which were isolated in hospital diagnostic laboratories. Both auxotrophic strains were used in this particular assay. DNA from each orga-
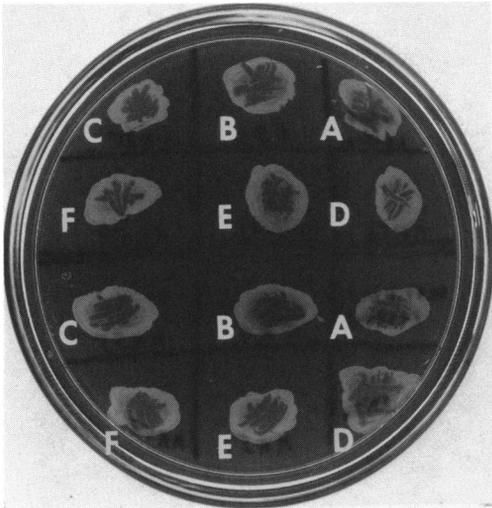
FIG. 1. *Transformation of Acinetobacter auxotrophs to prototrophy during growth on a semisolid medium. On the top half of the plate, small amounts of cell paste of auxotrophic strain Ivl-10 were placed in each of the six squares. A loopful of DNA from each of five presumptive acinetobacters was used to suspend and spread the paste in a suitably labeled square. Cell paste in sector A was spread with a sterile loop and constitutes the non-DNA-treated control. DNA species used to suspend the cell paste in the remaining squares were derived from the following strains: B, 96; C, 192; D, 193; E, 145; and F, a strain tentatively designated KK-58. The same procedure was used for auxotrophic strain trpE27, which was spread in the six corresponding squares on the bottom half of the plate. Growth took place for 12 hr at 35 C. The marks in the center of each growth smear resulted from removal of cell paste, with a loop, for streaking on the lactate-mineral agar plates in Fig. 2. The medium in the above plate is lactate-mineral agar which contains 100 μg each of L-isoleucine, L-valine, L-leucine, and L-trypotophan per ml.*

nism was used to suspend and spread the auxotrophs over a sector of a plate containing the growth factors required by the two auxotrophs (Fig. 1). Generous portions of the resulting growth of each auxotroph was streaked on sectors of lactate-mineral agar plates which were incubated to permit growth of cells transformed to prototrophy (Fig. 2). Failure of the DNA sample used in sector F of each plate (Fig. 2) to transform either auxotroph to prototrophy indicates that the organism from which this DNA was derived is not an *Acinetobacter*. Further microscopic examination of this strain showed it to be a motile rod.

Transformation of the tryptophan auxotroph (strain *trpE27*) with DNA species from the four *Acinetobacter* strains tested results in

significantly more prototrophic recombinant colonies than for the case when the Ivl-10 auxotroph is transformed to prototrophy (Fig. 2). Although the transformation assay used in these studies is essentially a qualitative test, the results have been found to be remarkably reproducible, thus permitting semiquantitative estimates to be made. It appears that the genetic defect in strain *trpE27* may reside in a
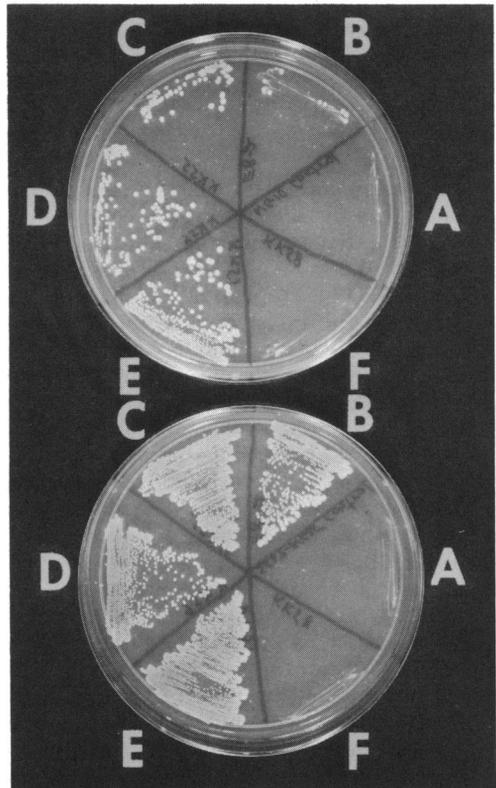
FIG. 2. *Transformation as evidenced by the growth of prototrophic recombinant colonies on minimal agar. The plate on the top shows the transformed colonies of strain Ivl-10 whereas the plate on the bottom shows the transformed colonies of strain trpE27. Bacterial paste from each of the growth smears in Fig. 1 was streaked on a sector of the above lactate-mineral agar plate. Sector A of each plate was streaked with paste from the respective non-DNA-treated control smear. No colonies are seen on these sectors, only some of the original cell paste that was streaked being visible. The remaining sectors were streaked with the cell paste-DNA mixture on the plate in Fig. 1 from similarly lettered squares. Both plates were incubated for 24 hr at 35 C. The extremely small particles seen throughout the plates are crystals that appeared in the medium after storing the plates for several weeks before they were used.*

so-called conserved gene (16, 19) since DNA species from a wide variety of *Acinetobacter* strains transform this auxotroph to prototrophy with approximately the same efficiency as is found using homologous DNA. In spite of the fact that strain Ivl-10 is not transformed with good efficiency by DNA species from some acinetobacters (Fig. 2), it has been found to be transformed to at least some extent by DNA from each of the 265 strains tested. In control experiments, treatment of crude DNA preparations with deoxyribonuclease prior to mixing with the competent auxotrophs results in no prototrophic transformant colonies. Quantitative studies concerned with transformation of auxotrophs of *Acinetobacter* have been reported (32).

As is true for other transformation systems (50), there is a distinct interval of competence for transformation during one short period of normal growth; for *Acinetobacter* growing in liquid media, a sharp peak of competence occurs when the culture is about to enter the stationary phase (A. Janik and E. Juni, *unpublished data*). If transformation on semisolid media also takes place when growth is nearly completed, it is to be expected that any auxotrophic cells transformed to prototrophy will not have an opportunity to divide and give rise to prototrophic progeny. This possibility was investigated by allowing transformation of Ivl-10 to take place on a series of plates containing decreasing but equal concentrations of isoleucine, valine, and leucine. Any prototrophic transformants that arise during growth on plates containing suitable DNA and growth-limiting concentrations of the required amino acids should continue to grow when the auxotrophic parent cells are no longer able to do so. For this experiment, DNA from strain 96 was used since it was already demonstrated that very few transformant colonies were obtained in the standard transformation assay with this DNA (Fig. 2). When the concentrations of isoleucine, valine, and leucine were decreased in the media in which transformation took place, the total number of prototrophic recombinant cells increased markedly (Fig. 3). For each concentration of amino acids used, a control smear of Ivl-10 was also included to check for possible spontaneous revertants to prototrophy. In no case were such revertants observed. Since control smears contained amounts of bacterial paste clearly visible to the naked eye (Fig. 2), the spontaneous reversion rate of Ivl-10 must be less than 10^{-8} or 10^{-9}.

Stability of auxotrophs used in the trans-
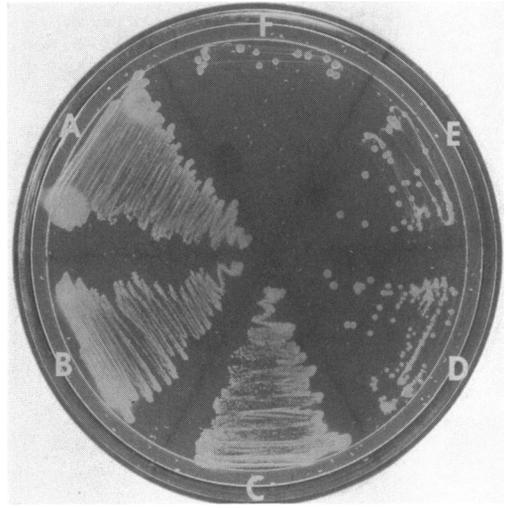
FIG. 3. *Effect of amino acid concentration on transformation of auxotrophic strain Ivl-10 to prototrophy. DNA from strain 96 was used to transform strain Ivl-10 to prototrophy during growth on separate plates containing the following equal concentrations of isoleucine, valine, and leucine: A, 1.0 µg/ml; B, 3.5 µg/ml; C, 8.5 µg/ml; D, 13.5 µg/ml; E, 38.5 µg/ml; and F, 100 µg/ml. The plate shown above was incubated at 35 C for 24 hr. The extremely small particles distributed throughout this plate are crystals that appeared in the medium after storing the plate for several weeks prior to use.*

formation assay. The control smears without DNA (Fig. 2, sector A of each plate) for the auxotrophs used in the transformation assay show only some of the original paste spread on the plates. Such control smears are routinely used for each assay. To date, not a single revertant colony has ever been observed on a control smear of Ivl-10. Although *trpE27* is extremely stable, one or two revertant colonies have been observed in about 50 control smears. Other auxotrophs have been observed to revert to prototrophy spontaneously in control smears, however, and are consequently not suitable for this type of assay.

The stability of the two auxotrophs was also investigated by attempting to revert them to prototrophy with chemical mutagens. In the presence of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, strain Ivl-10 gave rise to only three revertant colonies on a heavily seeded lactate-mineral agar plate containing 1% brain heart infusion broth (Difco). Treatment with diethyl sulfate resulted in only a single revertant, whereas no revertants were found when methyl methanesulfonate was the mutagen. By contrast, when strain *trpE27* was subjected to the

same three mutagens, large numbers of prototrophic revertants were obtained.

Nontransforming DNA species. Without exception, DNA species from the 25 gram-negative, oxidase-positive, and nonmotile coccobacilli failed to transform either of the two stable competent auxotrophs of *Acinetobacter* to prototrophy. Bövre (7) reported that DNA species from some streptomycin-resistant strains of *Acinetobacter* were able to transform competent oxidase-positive strains of *Moraxella* to drug resistance at extremely low frequencies. We have confirmed this finding and also have shown that DNA from streptomycin-resistant strains of *Moraxella osloensis* are capable of transforming the competent *Acinetobacter* strain to streptomycin resistance at very low frequencies (E. Juni and G. A. Heym, Bacteriol. Proc., p. 58, 1972).

The lack of genetic relatedness between *Acinetobacter* and other bacteria was demonstrated by the complete inability to transform either of the competent auxotrophs to prototrophy when using DNA species obtained from strains of *Alcaligenes faecalis*, *Pseudomonas* sp., *Rhizobium meliloti*, *Escherichia coli*, *Streptococcus faecalis*, *Bacillus licheniformis*, *Acetobacter suboxydans*, *Aerobacter aerogenes*, *Serratia marcescens*, and *Haemophilus influenzae*.

Several strains, originally designated as acinetobacters, were found to be genetically unrelated to the competent strain as determined by the transformation assay. Careful examination of these strains revealed that they were either gram-positive, oxidase-positive, motile, or capable of forming acid from glucose anaerobically.

Since the finding of a positive oxidase reaction implies that the organism tested contains cytochrome *c* (3), attempts were made to mutagenize an oxidase-positive strain of *Moraxella* to loss of its functional cytochrome *c* component. A culture of strain 272 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and the surviving cells were plated on heart infusion agar. Upon flooding the plate with oxidase reagent, a colony was observed which failed to turn purple. This mutant culture grows slower but appears identical to the parent strain in every other respect, except that it no longer gives a positive oxidase test. It would appear that this mutant strain can still obtain enough energy for growth using oxidative mechanisms not involving cytochrome *c*. DNA from this oxidase-negative mutant of *Moraxella* fails to transform the competent *Acinetobacter* auxotrophs to proto-

trophy. The phenotypic properties of some *Moraxella* species are very similar to those of certain *Acinetobacter* strains (3, 10), and the ability to mutate from the oxidase-positive to the oxidase-negative state implies that even the lack of an oxidase-positive characteristic does not insure that the organism under study is an *Acinetobacter*.

Strain 292, which gives a weak oxidase-positive reaction, might, at first sight, be considered to be an *Acinetobacter*. The inability of DNA from this strain to transform the competent *Acinetobacter* auxotrophs to prototrophy, however, shows clearly that it is not a member of this genus. Strain 292 was isolated from Great Salt Lake mud and grows with ethylene glycol as the sole carbon source in a medium containing 12% sodium chloride (C. F. Gonzalez and W. A. Taber, Bacteriol. Proc., p. 17, 1971). As far as I am aware, no authentic *Acinetobacter* strains have been isolated from seawater. In a recent study of 291 strains of aerobic, oxidase-negative, gram-negative bacteria, Gilardi (22) has shown that none of these organisms is capable of growing in media supplemented with 6.5% sodium chloride. Furthermore, of the 106 strains of *Acinetobacter* studied by Baumann et al. (4), none was able to grow with ethylene glycol as the source of carbon and energy.

Nitrate reduction by certain Acinetobacter strains. Acinetobacters are generally considered to be unable to reduce nitrate to nitrite. A report has appeared (49), however, describing such a reduction by a few acinetobacters. Six strains labeled *Herellea vaginicola* (strains 66–71) are indeed able to reduce nitrate to nitrite. DNA from each of these six strains transforms the competent *Acinetobacter* auxotrophs to prototrophy, thus verifying that these strains are acinetobacters. These strains cannot use nitrate as an alternate electron acceptor in place of molecular oxygen, however, since they do not grow anaerobically in nitrate broth.

The two exceptional organisms studied (strains 292 and 293) are both able to reduce nitrate to nitrite. Strain 292, the oxidase-negative mutant of the oxidase-positive strain 272, thus resembles its parent since strain 272 is also able to reduce nitrate to nitrite.

Distribution of acinetobacters in nature. Most of the strains of *Acinetobacter* examined in the present study were originally isolated from clinical specimens. It is well known, nevertheless, that acinetobacters commonly occur in water and soil (2, 20, 31, 37, 55). Acinetobacters have appeared as air contaminants on

the surfaces of various semisolid media. They have also been found to be present in a variety of food products (52).

DNA compositions of various Acinetobacter strains. Table 1 illustrates the fairly wide variation in DNA composition for DNA species from some of the *Acinetobacter* strains investigated. The fact that DNA species from all the bacteria tested are able to transform auxotrophs of the competent strain to prototrophy proves that it is not necessary for genetically interacting species to have very similar DNA compositions, as was previously believed to be required for such interspecies recombinational events to take place (30, 43). Strains 207, 208, 211, 212, 213, 215, 216, and 218 have DNA species with guanine plus cytosine (GC) values ranging from 46.3 to 46.8 moles % (18, 47). DNA from competent strain 1 has a GC value of 41.4 moles %.

The acinetobacters tested (Table 1) include most of the strains from each of the six DNA-DNA homology groups of Johnson et al. (29) as well as strains from each of the seven phenotypic subgroups of Baumann et al. (4).

Ability of acinetobacters to use glucose as a carbon source. Previous taxonomic studies have emphasized that acinetobacters generally cannot use glucose as the sole source of carbon and energy for growth in a defined mineral medium (4, 22, 54). Baumann et al. (4) have demonstrated that only 1 of 106 *Acinetobacter* strains was able to grow with glucose or gluconate as the carbon source. Since the competent strain (strain BD413) is able to grow in a glucose-mineral medium, I tested the ability of the other acinetobacters to grow in this medium by routinely smearing sectors of glucose-mineral agar plates with generous samples of bacterial paste followed by incubation at room temperature in a plastic bag for at least a month. Of the 265 *Acinetobacter* cultures examined, 17 were able to grow readily on the glucose plates whereas 48 other strains gave rise to mutant colonies which grew well upon reisolation on this medium (Table 2). When such mutants were stored on complex media lacking glucose and subsequently transferred to glucose-mineral medium, nearly all such cultures were able to grow. Strain 59 was an exception to this rule since mutant colonies, isolated on glucose plates and then transferred to complex media, reverted rapidly to inability to grow on glucose plates.

Transformation of ability to use glucose. The only sugars capable of being used as sole carbon and energy sources by any strain of *Acinetobacter* are D-glucose, D-ribose, L-arabinose, and D-xylose (4). Table 3 shows the utilization of these four sugars by three strains of *Acinetobacter*. Strain BD413 is the microencapsulated mutant of competent strain 1 (32), whose auxotrophic mutants have been used in the above studies. Strain 83 has also been found to be competent for genetic transformation, whereas strain 78 is not. In an attempt to transform strain 83 for ability to use glucose as a carbon source, DNA from strain BD413 was mixed with bacterial paste of strain 83 and spread on a sector of a glucose-mineral agar plate containing 0.1% casein hydrolysate and 0.1% yeast extract. After incubation for 2 weeks, there were 12 distinct colonies growing in the mass of cell paste. In the control smear of strain 83, to which no DNA had been added, there were three large colonies. Six of the colonies from the smear containing DNA from strain BD413 were each streaked on a sector of a glucose-mineral agar plate and incubated at room temperature for 3 days. As may be seen in Fig. 4 streakings of five of these six colonies showed excellent growth whereas the sixth colony (Fig. 4A) grew very poorly. Spontaneous mutants able to grow better than the bulk of cells from the sixth colony also appear on this sector. When these mutant colonies were streaked on a glucose-mineral agar plate, they grew as well as the five colonies streaked on the plate in Fig. 4.

The three colonies on the control area containing strain 83 but no DNA were also streaked on sectors of a glucose-mineral agar plate. In all cases there was very poor growth but rapidly growing mutant colonies were also observed in the thick mass of streaked cells as for streak A in Fig. 4. When strain 83 is streaked on glucose-mineral agar, no growth at all takes place. It appears that strain 83 is capable of spontaneous mutation to glucose utilization in at least two steps. First there is a mutation to slow growth on glucose followed by a second mutation permitting more rapid growth. When DNA from strain BD413 was present during incubation of strain 83, interspecies transformation was probably responsible for the large proportion of colonies able to grow rapidly with glucose as the carbon source (Fig. 4). When DNA from the glucose-positive transformant of strain 83 was incubated with wild-type strain 83, there was a quantitatively more significant transformation to glucose utilization, as expected when homologous DNA is used (Fig. 5B).

Transformation of ability to use pentoses. A more critical test for ability to effect interspecies transformation of sugar utilizing markers is evident for transformation of genes involving pentose utilization. Although strain

TABLE 1. *Acinetobacter strains used in the present study which have been classified into DNA-DNA homology groups by Johnson et al. (29) and into phenotypic groups by Baumann et al. (4) and DNA composition*

Strain no. (present study)	ATCC no.	Strain no. of Baumann et al.	DNA-DNA homology group	Phenotypic group or subgroup	GC moles %	Ref. for DNA composition[a]
2	9955	49	1	A1	42	(29)
3	9957	25	5	B2	44	(29)
5	14291	26		B2		
6	14292	27		B2		
7	14987	61	2	A1	40	(29)
8	15149		1		43	(29)
9	15150		1		42	(29)
10	15151		1		42	(29)
12	17902	90	1	A1	42	(29)
13	17903		1		41	(29)
14	17904	65	1	A1	42	(29)
15	17905	30	3	B3	44	(29)
16	17906	40	2	B4	43	(29)
17	17907	44	2	B4	43	(29)
18	17908	10	6	B1	42	(29)
19	17909	8	4	B1	44	(29)
20	17910		5		46	(29)
21	17912	38	2	B4	42	(29)
22	17913		2		43	(29)
23	17922		1	A[b]	42	(29)
24	17923		4		45	(29)
25	17924		6		45	(29)
26	17925		5		46	(29)
27	17957		1		41	(29)
28	17959	100	1	A3	42	(29)
29	17961	55	1	A1	41	(29)
30	17968		5		45	(29)
31	17969		4		44	(29)
32	17976	98	2			
33	17977		2		43	(29)
34	17978		1		42	(29)
35	17979		6		42	(29)
36	17984		5		45	(29)
37	17985		5	B2[b]	43	(8)
38	17986		5	B2[b]	44	(29)
39	17987		5		43	(29)
40	17988	34	3	B3	41	(8)
41	19002		6		40	(29)
42	19003		1		42	(29)
43	19004		1		41	(29)
44	19194		2		41	(29)
46	23055	46	1	A		
64		35		B3		
65		1		B		
265	11171	94	1	A2	40	(29)

[a] Data in the column to the left are taken from these references.

[b] Personal communication from M. Doudoroff.

83 can grow with L-arabinose as the carbon source, it cannot use D-ribose or D-xylose for this purpose (Table 3). Furthermore, prolonged incubation of large numbers of cells in a D-ri-

bose or D-xylose-mineral medium containing 0.1% casein hydrolysate and 0.1% yeast extract does not result in appearance of spontaneous mutants capable of utilizing either of these two pentoses. In studies using the mutagens *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and diethyl sulfate, no mutants capable of using D-ribose or D-xylose were obtained. When strain 83 was grown in the presence of DNA from strain 78, however, transformant colonies able to use these sugars were observed. In the experiment shown in Fig. 6, wild-type strain 83, its slow-growing and glucose-utilizing mutant, and the transformant of strain 83 that grows rapidly with glucose as the carbon source were each mixed with DNA from strain 78 on sectors of a D-xylose mineral agar plate containing

TABLE 2. *Strains of Acinetobacter capable of using glucose as a carbon source for growth[a]*

Strains able to use glucose	Strain no.
Without prior mutation	1, 8, 34, 40, 55, 98, 111, 149, 150, 151, 156, 160, 161, 167, 169, 177, 178
After prior mutation	7, 9, 23, 27, 45, 57, 59, 76, 79, 83, 108, 109, 110, 117, 119, 121, 122, 123, 134, 139, 141, 144, 145, 147, 159, 174, 176, 228, 256, 258, 260

[a] Growth was tested on glucose-mineral agar plates.

TABLE 3. *Utilization of sugars as carbon sources by several strains of Acinetobacter*

Strain no.	Carbon source			
	D-Glucose	D-Ribose	D-xylose	L-Arabinose
1	+	−	−	−
78	−	+	+	+
83	−[a]	−	−	+
83glu[+b]	+	−	−	+
83rib[+c]	−	+	+	+
83xyl[+d]	−	+	+	+
83glu[+]xyl[+e]	+	+	+	+

[a] Wild-type strain 83 cannot grow on glucose but can mutate to glucose utilization (Fig. 4, Table 4).

[b] Transformant of strain 83 able to grow with glucose as the carbon source (Fig. 4).

[c] Transformant of strain 83 able to grow with D-ribose as the carbon source (Fig. 6).

[d] Transformant of strain 83 able to grow with D-xylose as the carbon source (Fig. 6).

[e] Transformant of strain 83glu[+] able to grow with D-xylose as the carbon source (Fig. 6).
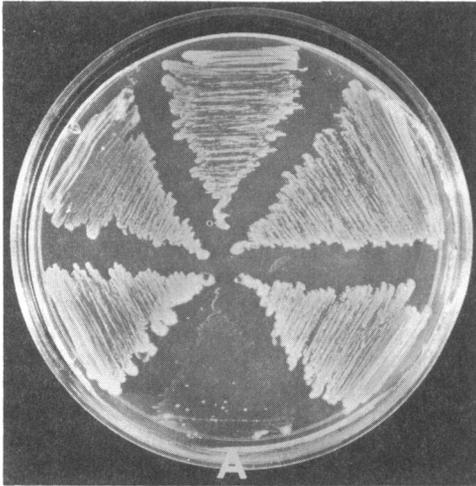
FIG. 4. *Growth of transformant colonies of strain 83 on glucose-mineral medium. Bacterial paste from each of six presumed glucose-utilizing transformant colonies obtained after incubation with DNA from strain BD413 was streaked on a sector of a glucose-mineral agar plate which was incubated at 30 C for 3 days.*

0.1% casein hydrolysate and 0.1% yeast extract. Transformant colonies capable of growing on D-xylose were obtained in each of the three smears (Fig. 6). D-Xylose-utilizing colonies were picked from each smear on this plate, streaked for isolation on D-xylose-mineral agar plates, and each tested for ability to grow with glucose as the carbon source. The responses of the D-xylose-utilizing transformants to growth on glucose-mineral agar were in all cases the same as those for the strains from which they were derived. These results demonstrate that ability to dissimilate D-xylose is independent of ability to dissimilate glucose. The results also show that the isolated D-xylose-utilizing colonies were not contaminants.

In a similar experiment, DNA from strain 78 was used to transform strain 83 and its two derivative strains for ability to grow with D-ribose as sole carbon source. The pattern of transformation observed was the same as that illustrated in Fig. 6 for transformation to D-xylose utilization. Transformants able to grow with D-xylose were tested for ability to use D-ribose and vice versa. Each transformant was found to be capable of using both D-xylose and

FIG. 5. *Transformation of strain 83 for glucose utilization with homologous DNA. Sector B of the glucose-mineral agar plate, supplemented with 0.1% yeast extract and 0.1% casein hydrolysate, was spread with cell paste of strain 83 suspended in DNA from one of the glucose-utilizing transformants of the same strain (Fig. 4). Cell paste of strain 83 was suspended in 0.85% sodium chloride solution and spread on sector A of the plate (non-DNA-treated control). The sterility of the DNA preparation used in this experiment was checked by spreading a sample of this DNA on sector C of the plate. The plate was incubated at 30 C for 2 days.*

FIG. 6. *Interspecies transformation of strain 83 for ability to grow with D-xylose as the carbon source. Recipient cell paste was suspended in DNA from strain 78 and streaked on a D-xylose-mineral agar plate supplemented with 0.1% yeast extract and 0.1% casein hydrolysate. The plate was incubated at 30 C for 3 days. The recipient strain used in the smear on sector C was wild-type strain 83. A glucose-utilizing transformant of strain 83 (Fig. 4) was used in the smear on sector A. The smear on sector B was made using the slow glucose-utilizing mutant of strain 83.*

D-ribose, these results indicating that inability of wild-type strain 83 to grow with these two pentoses is probably due to a defect in an enzymatic step common to the respective pathways for degradation of these sugars. In addition, it was shown that all pentose-utilizing transformants retain the ability to grow with L-arabinose as carbon source, as expected.

Growth factor requirements of acinetobacters. Each of the strains listed above was tested for ability to grow on mineral agar media containing single carbon sources such as acetate, lactate, malate, and 2,3-butanediol. Inability to grow on any such medium was taken as evidence for the requirement of one or more growth factors. Of the 265 *Acinetobacter* strains tested, only nine strains were demonstrated to require growth factors. These strains are 4, 13, 30, 35, 53, 72, 73, 75, and 207. The growth factor requirements of acinetobacters isolated from soil and water are discussed in another report (55).

In their study of 106 strains of *Acinetobacter*, Baumann et al. (4) report that all these organisms grow well on mineral media containing acetate as the carbon source. All the prototrophic strains examined in the present study are able to use acetate as the sole carbon source with the exception of strains 12 and 74. The latter two strains do give rise to spontaneous mutants capable of growing with acetate when wild-type cells are incubated on acetate-mineral agar plates for 2 weeks. These two strains are, nevertheless, able to grow on lactate-mineral agar plates without prior mutational changes. The fact that strain 12 grows readily with 2,3-butanediol as the carbon source implies that its inability to grow immediately with acetate results from a nonfunctional mechanism for uptake of acetate. Since bacteria growing with 2,3-butanediol as the carbon source convert this substrate to acetate, which is the actual growth substrate (31), strain 12 is thus able to grow with acetate produced intracellularly.

Use of DNA from nonviable cells in the transformation assay. All cultures received for testing were first grown to obtain cells for preparation of DNA to be used in the transformation assay. Nine of the cultures tested failed to grow when paste from the original slants was streaked on heart infusion agar. Seven of these cultures had been in transit for over a month and probably died on the slants during this long period of incubation at unknown temperatures. These strains were tested in the transformation assay, nevertheless, by preparing DNA species from the dead cell paste on the original slants. In each case, DNA from nonviable cells was able to transform the competent *Acinetobacter* auxotrophs to prototrophy. Subsequent tests of duplicate cultures containing viable cells showed that all of these strains grow well on heart infusion agar. The results of this experiment demonstrate that although the nine cultures originally tested were dead, the processes leading to nonviability did not result in extensive destruction of cellular DNA.

DISCUSSION

The classification of gram-negative, nonfermenting bacteria has presented taxonomists with many difficulties (4, 25, 46, 54). One phenotypic characteristic that serves to separate these organisms into two groups is the oxidase test. According to the data presented by Baumann et al. (3), it seems fairly certain that a positive oxidase test is indicative of the presence of cytochrome *c*, this cytochrome being absent from extracts of bacteria which give a negative oxidase test. Genetic transformation of many of the gram-negative, oxidase-positive bacteria has been demonstrated (5, 7, 9), and evidence of extensive interspecies transformation for many members of this group has been reported (7, 12, 14, 15). It is now clear, however, that there are several distinct groups of oxidase-positive bacteria each of which is genetically unrelated, or at best distantly related, to the other groups (4, 7, 10).

Until recently it has not been possible to study genetic relationships among the gram-negative, oxidase-negative bacteria. In 1969, Juni and Janik (32) reported the discovery of a competent oxidase-negative strain of an organism identified as *Bacterium anitratum*. It was also shown in the same year that DNA from other oxidase-negative strains could transform auxotrophs of the competent strain to prototrophy (E. Juni and A. Janik, Bacteriol. Proc., p. 59, 1969). The present study demonstrates that 265 strains of gram-negative, oxidase-negative, nonmotile bacteria examined are genetically related to the competent strain and are, consequently, all members of the same genus.

Besides their normal occurrence in water and soil, some *Acinetobacter* species are also found to reside as commensals in the eye, ear, respiratory tract and vagina of the human body (17, 21, 25, 48), possibly as a consequence of the fact that most of these bacteria grow well at 37 C, having optimal growth temperatures near 35 C. It has been demonstrated that acinetobacters can be the causative agents of

diseases in man such as septicemia, meningitis, endocarditis, osteomyelitis, pneumonia, empyema, and genitourinary tract infections, particularly in debilitated individuals (17, 21, 25). Some of these organisms appear to be responsible for spontaneous abortion in many different kinds of animals (11). *Acinetobacter* species have also been isolated from a variety of foods (36, 52, 54).

The present study indicates that bacteria previously classified into eleven different genera are, in fact, all members of the genus *Acinetobacter*. It is evident that previous classification of acinetobacters into such a large number of genera has served to obscure the natural relationships of these organisms. Although genetic evidence for relatedness of all species of *Acinetobacter* is the best criterion for classifying these organisms in the same genus (30, 39, 43), it should be pointed out that recent taxonomic studies involving comparison of phenotypic properties (4, 22, 54) and DNA-DNA homology (29) have also provided strong evidence for the same conclusion.

Thornley (54) has grouped a large number of strains of gram-negative or gram-variable, nonmotile coccoid rods into five phenons based upon a computer analysis of the similarities of a number of phenotypic properties. Strains in phenons two, three, and four were classified as *Acinetobacter*. When several strains from these phenons were tested in the transformation assay it was found that all the oxidase-negative strains (strains 47–52) reside in phenon 4, whereas the oxidase-positive species (strains 275–278) were distributed among the three phenons. It appears that in spite of many similar phenotypic properties, the oxidase-positive bacteria in Thornley's study are not closely related to the oxidase-negative organisms since only DNA species from the latter group of bacteria were found to transform the competent *Acinetobacter* auxotrophs to prototrophy. The weak genetic interaction, described above, between *Acinetobacter* and certain moraxellas may, nevertheless, indicate that the oxidase-negative acinetobacters and the oxidase-positive moraxellas could have evolved from a common ancestor.

As I examined more strains it became clear that the results obtained with the transformation test were demonstrating an unequivocal relationship among the various oxidase-negative strains. The fact that these strains have marked differences in phenotypic properties prompted a consideration that it might be possible to find a set of such properties which is common to all acinetobacters. All the strains

of *Acinetobacter* listed above are gram-negative, oxidase-negative, nonmotile, and aerobic coccobacilli.

Acinetobacter strains are also considered to be unable to reduce nitrate to nitrite (4, 22), although it is known that many such strains are able to grow in defined media with nitrate or nitrite as sole source of nitrogen (4, 33). Under such conditions, nitrate must be reduced to ammonia, presumably through the intermediary formation of nitrite, so that cells can synthesize the amino groups of amino acids and other nitrogenous compounds. It has been shown that suspensions of *Acinetobacter* cells, which are grown in media containing nitrate as the source of nitrogen, possess nitrate reductase and nitrite reductase activities (34). When such cells are grown in the presence of amino acids, however, these reductase activities are completely repressed (34). Since the test for ability to reduce nitrate to nitrite is generally performed by growing cells in complex, amino acid-containing media, it is understandable why such assays for appearance of nitrite are usually negative. The six strains of *Acinetobacter*, which have been demonstrated in the present study to reduce nitrate to nitrite, may represent mutants in which the ability of amino acids to repress synthesis of nitrate reductase is lost. Such constitutive derepressed mutants would be expected to reduce nitrate to nitrite regardless of the composition of the growth medium. It is, therefore, clear that the nitrate reduction test cannot be used as an unquivocal aid in the diagnosis of presumptive acinetobacters. There is no evidence that any strain of *Acinetobacter*, including any of the six strains able to reduce nitrate to nitrite, can carry out dissimilatory nitrate reduction where nitrate replaces oxygen as the final electron acceptor, thus permitting growth to occur in the absence of air (45).

Although others have generally found that strains of *Acinetobacter* do not use glucose, or other hexoses, as a source of carbon and energy for growth (4, 22, 54), approximately 6% of the strains used in the present study are able to grow in a glucose-mineral medium. Furthermore, another 18% of these strains were shown to mutate spontaneously to glucose utilization when incubated on glucose-mineral agar plates for several weeks. The fact that 24% of the cultures under study can either grow or mutate to grow with glucose as the carbon source implies that ability of acinetobacters to use glucose is considerably more widespread than previously assumed to be the case. Metabolic studies of

strain 1 have shown that glucose is oxidized rapidly to gluconate which accumulates in the growth medium (53). Gluconate is then degraded more slowly through a series of enzymatic steps, the first of which is phosphorylation of gluconate to 6-phosphogluconate by gluconokinase (53). 6-Phosphogluconate is then metabolized to pyruvate and glyceraldehyde-3-phosphate via 2 keto-3-deoxy-6-phosphogluconate (53).

Bacteriologists have made use of the ability of some acinetobacters to form acid (gluconic acid) aerobically from glucose as a basis for distinguishing *Bacterium anitratum* (*Herellea vaginicola*), which forms acid, from strains of *Mima polymorpha* (*Moraxella lwoffi*), which do not form acid (22, 26, 27, 49). In the present study there is a total of 67 strains that have been named either *Mima polymorpha*, *Acinetobacter lwoffi*, or *Moraxella lwoffi* and must, consequently, be unable to form acid from glucose. It is of interest that none of these 67 strains is among the group of 48 strains which were shown to mutate spontaneously to glucose utilization. It seems likely, therefore, that in addition to lacking a functional glucose oxidase, an enzyme already demonstrated in *Acinetobacter* (23, 24), strains which are unable to form acid from glucose may also lack some or all of the other enzymes required for conversion of glucose to pyruvate and glyceraldehyde-3-phosphate. The failure of any of the 67 non-acid-forming strains to mutate to ability to grow with glucose as the carbon source may, in fact, be a consequence of the complete absence in such strains of genes that direct the synthesis of the enzymes for glucose utilization. This hypothesis is currently under investigation in this laboratory.

The ability to transform strain 83 so that it can grow with glucose, D-xylose, or D-ribose as the carbon source shows that this strain probably contains most of the genetic information for synthesis of all the enzymes required for utilization of these sugars, with one or more of these genes giving rise to nonfunctional enzymes, possibly as a result of prior mutation of genes which formerly directed the synthesis of functional enzymes. Pathways for pentose utilization by acinetobacters have been discussed by Baumann et al. (4).

Baumann et al. (4) have pointed out that *Acinetobacter* strains can mutate to auxotrophy when incubated in complex media for long periods of time. Although only 9 of the 265 strains examined were found to have growth factor requirements, prolonged incubation of these strains on acetate-mineral agar

failed to reveal any spontaneous revertants to prototrophy. One of the growth factor-requiring strains (strain 4) was found to have the same ATCC number (9957) as did strain 3, which is prototrophic. Although these two strains were both obtained, at different times, from the American Type Culture Collection, it does not appear that one strain is a recent derivative of the other since antiserum against strain 1 readily agglutinates cells of strain 3 but fails to agglutinate cells of strain 4. In this connection it should be mentioned that the evolution of *Acinetobacter* has resulted in species with many different serotypes (41).

The present study has demonstrated the genetic relatedness of a large group of gram-negative aerobes previously classified into a variety of genera. Baumann et al. (4) have suggested that all these organisms be placed in the genus *Acinetobacter*. At the last meeting of the Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria of the International Committee on Nomenclature of Bacteria, it was proposed that organisms previously referred to by names which contain the specific epithet *anitratum* or *lwoffi* be included in the genus *Acinetobacter* (38). Further studies are required before all acinetobacters can be grouped into clearly definable species.

LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. Baumann, P. 1968. Isolation of *Acinetobacter* from soil and water. J. Bacteriol. **96**:39–42.
3. Baumann, P., M. Doudoroff, and R. Y. Stanier. 1968. Study of the *Moraxella* group. I. Genus *Moraxella* and the *Neisseria catarrhalis* group. J. Bacteriol. **95**:58–73.
4. Baumann, P., M. Doudoroff, and R. Y. Stanier. 1968. A study of the *Moraxella* group. II. Oxidative-negative

species (genus *Acinetobacter*). J. Bacteriol. **95**:1520–1541.

5. Bövre, K. 1964. Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. 1. A method for quantitative transformation in *Moraxella* and *Neisseria*, with streptomycin resistance as the genetic marker. Acta Pathol. Microbiol. Scand. **61**:457–473.

6. Bövre, K. 1967. Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. 8. The relative position of some oxidase-negative immotile diplobacilli (*Achromobacter*) in the transformation system. Acta Pathol. Microbiol. Scand. **69**:109–122.

7. Bövre, K. 1967. Transformation and DNA base composition in taxonomy, with special reference to recent studies in *Moraxella* and *Neisseria*. Acta Pathol. Microbiol. Scand. **69**:123–144.

8. Bövre, K., M. Fiandt, and W. Szybalski. 1969. DNA base composition of *Neisseria*, *Moraxella*, and *Acinetobacter*, as determined by measurement of buoyant density in CsCl gradients. Can. J. Microbiol. **15**:335–338.

9. Bövre, K., and S. D. Henriksen. 1962. An approach to transformation studies in *Moraxella*. Acta Pathol. Microbiol. Scand. **56**:223–228.

10. Bövre, K., and S. D. Henriksen. 1967. A new *Moraxella* species, *Moraxella osloensis*, and a revised description of *Moraxella nonliquefaciens*. Int. J. Syst. Bacteriol. **17**:127–135.

11. Carter, G. R., T. T. Isoun, and K. K. Keahey. 1970. Occurrence of *Mima* and *Herellea* species in clinical specimens from various animals. J. Amer. Vet. Med. Ass. **156**:1313–1318.

12. Catlin, B. W. 1964. Reciprocal genetic transformation between *Neisseria catarrhalis* and *Moraxella nonliquefaciens*. J. Gen. Microbiol. **37**:369–379.

13. Catlin, B. W., and L. S. Cunningham. 1961. Transforming activities and base contents of deoxyribonucleate preparations from various *Neisseria*. J. Gen. Microbiol. **26**:303–312.

14. Catlin, B. W., and L. S. Cunningham. 1964. Genetic transformation of *Neisseria catarrhalis* by deoxyribonucleate preparations having different average base compositions. J. Gen. Microbiol. **37**:341–352.

15. Catlin, B. W., and L. S. Cunningham. 1964. Transforming activities and base composition of deoxyribonucleates from strains of *Moraxella* and *Mima*. J. Gen. Microbiol. **37**:353–367.

16. Chilton, M. D., and B. J. McCarthy. 1969. Genetic and base sequence homologies in bacilli. Genetics **62**:697–710.

17. Daly, A. K., B. Postic, and E. H. Kass. 1962. Infections due to organisms of the genus *Herellea*. Arch. Intern. Med. **110**:580–591.

18. DeLey, J. 1968. DNA base composition and taxonomy of some *Acinetobacter* strains. Antonie van Leeuwenhoek; J. Microbiol. Serol. **34**:109–114.

19. Dubnau, D., I. Smith, P. Morell, and J. Marmur. 1965. Gene conservation in *Bacillus* species. I. The location of genes concerned with the synthesis of ribosomal components and soluble RNA. Proc. Nat. Acad. Sci. U.S.A. **54**:491–498.

20. Finnerty, W. R., E. Hawtrey, and R. E. Kallio. 1962. Alkane-oxidizing micrococci. Z. Allg. Mikrobiol. **2**:169–177.

21. Gardner, P., W. B. Griffin, M. N. Swartz, and L. J. Kunz. 1970. Nonfermentative gram-negative bacilli of nosocomial interest. Amer. J. Med. **48**:735–749.

22. Gilardi, G. L. 1969. Characterization of the oxidase-negative, gram-negative coccobacilli (the *Achromobacter-Acinetobacter* group). Antonie van Leeuwenhoek; J. Microbiol. Serol. **35**:421–429.

23. Hauge, J. G. 1966. Glucose dehydrogenases-particulate. I. *Pseudomonas* species and *Bacterium anitratum*, p. 92–98. In W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.

24. Hauge, J. G. 1966. Glucose dehydrogenase-soluble. II. *Bacterium anitratum*, p. 107–111. In W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.

25. Henderson, A. 1965. The *Moraxella lwoffi* group of bacteria; a review. Antonie van Leeuwenhoek; J. Microbiol. Serol. **31**:395–413.

26. Hugh, R., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. **66**:24–26.

27. Hugh, R., and R. Reese. 1968. A comparison of 120 strains of *Bacterium anitratum* Schaub and Hauber with the type strain of this species. Int. J. Syst. Bacteriol. **18**:207–229.

28. Iyer, V. N., and W. Szybalski. 1958. Two simple methods for the detection of chemical mutagens. Appl. Microbiol. **6**:23–29.

29. Johnson, J. L., R. S. Anderson, and E. J. Ordal. 1970. Nucleic acid homologies among oxidase-negative *Moraxella* species. J. Bacteriol. **101**:568–573.

30. Jones, D., and P. H. A. Sneath. 1970. Genetic transfer and bacterial taxonomy. Bacteriol. Rev. **34**:40–81.

31. Juni, E., and G. A. Heym. 1956. A cyclic pathway for the bacterial dissimilation of 2,3-butanediol, acetylmethylcarbinol, and diacetyl. I. General aspects of the 2,3-butanediol cycle. J. Bacteriol. **71**:425–432.

32. Juni, E., and A. Janik. 1969. Transformation of *Acinetobacter calco-aceticus* (*Bacterium anitratum*). J. Bacteriol. **98**:281–288.

33. Jyssum, K., and P. E. Joner. 1965. Growth of *Bacterium anitratum* (B5W) with nitrate or nitrite as nitrogen source. Acta Pathol. Microbiol. Scand. **64**:381–386.

34. Jyssum, K., and P. E. Joner. 1965. Regulation of the nitrogen assimilation from nitrate and nitrite in *Bacterium anitratum* (B5W). Acta Pathol. Microbiol. Scand. **64**:387–397.

35. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) **178**:703.

36. Laycock, R. A., and L. W. Regier. 1970. Pseudomonads and achromobacters in the spoilage of irradiated haddock of different preirradiation quality. Appl. Microbiol. **20**:333–341.

37. Lemoigne, M., H. Girard, and G. Jacobelli. 1952. Bacterie du sol utilisant facilement le 2-3 butanediol. Ann. Inst. Pasteur **82**:389–398.

38. Lessel, E. F. 1971. Minutes of the meeting. International committee on nomenclature of bacteria. Subcommittee on the taxonomy of *Moraxella* and allied bacteria. Int. J. Syst. Bacteriol. **21**:213–214.

39. Mandel, M. 1969. New approaches to bacterial taxonomy: perspective and prospects. Annu. Rev. Microbiol. **23**:239–274.

40. Mandel, M., C. L. Schildkraut, and J. Marmur. 1968. Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA, p. 184–195. in L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12, part B. Academic Press Inc., New York.

41. Marcus, B. B., S. B. Samuels, B. Pittman, and W. B. Cherry. 1969. A serologic study of *Herellea vaginicola* and its identification by immunofluorescent staining. Amer. J. Clin. Pathol. **52**:309–319.

42. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208–218.

43. Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. Annu. Rev. Micro-

biol. **17:**329-372.

44. Monod, J., and E. Wollman. 1947. L'inhibition de la croissance de l'adaptation enzymatique chez les bacte'ries par le bacteriophage. Ann. Inst. Pasteur **73:** 937-956.

45. Nason, A. 1962. Enzymatic pathways of nitrate, nitrite, and hydroxylamine metabolisms. Bacteriol. Rev. **26:** 16-41.

46. Pintér, M., and I. Bende. 1968. Biochemical similarity of *Acinetobacter lwoffii* and *Acinetobacter anitratus.* Path. Microbiol. **31:**41-50.

47. Pintér, M., and J. DeLey. 1969. Overall similarity and DNA base composition of some *Acinetobacter* strains. Antonie van Leeuwenhoek; J. Microbiol. Serol. **35:**209-214.

48. Rosebury, T. 1962. Microorganisms indigenous to man. McGraw-Hill Book Company, New York.

49. Samuels, S. B., B. Pittman, and W. B. Cherry. 1969. Practical physiological schema for the identification of *Herellea vaginicola* and its differentiation from similar organisms. Appl. Microbiol. **18:**1015-1024.

50. Schaeffer, P. 1964. Transformation, p. 87-153. In I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 5. Academic Press Inc., New York.

51. Skerman, V. B. D. 1959. A guide to the identification of the genera of bacteria, p. 149. The Williams and Wilkins Company, Baltimore.

52. Snodgrass, C. J., and J. A. Koburger. 1967. The isolation and characterization of the tribe *Mimeae* in foodstuffs. J. Food Sci. **32:**584-591.

53. Taylor, W. H., and E. Juni. 1961. Pathways for biosynthesis of a bacterial capsular polysaccharide. II. Carbohydrate metabolism and terminal oxidation mechanisms of a capsule-producing coccus. J. Bacteriol. **81:** 694-703.

54. Thornley, M. J. 1967. A taxonomic study of *Acinetobacter* and related genera. J. Gen. Microbiol. **49:**211-257.

55. Warskow, A. L., and E. Juni. 1972. Nutritional requirements of *Acinetobacter* strains isolated from soil, water, and sewage. J. Bacteriol. **112:**1014-1016.