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Genetic Transformation Assays for Identification of Strains of *Moraxella urethralis*

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Studies of 31 strains of *Moraxella urethralis* have shown that 20 of them are competent for genetic transformation. This finding has led to the development of transformation assays for identification of newly isolated strains of this organism. Crude deoxyribonucleic acid (DNA) samples from all strains of *M. urethralis* readily transform auxotrophic mutants of competent strains to prototrophy, whereas DNA samples from unrelated bacteria such as *Acinetobacter*, *Moraxella*, and *Neisseria* species uniformly fail to elicit positive transformation of mutant tester strains. One of the competent strains of *M. urethralis* investigated is a naturally occurring mutant defective in its ability to utilize citrate as a carbon and energy source. DNA samples from 29 of the 30 remaining strains of *M. urethralis* examined in the present study transform this mutant to citrate utilization; the one nonreacting strain is citrate negative and probably possesses the same genetic lesion as the citrate-negative mutant. Three organisms originally identified as strains of *M. urethralis*, because of their phenotypic properties, are probably incorrectly designated, since DNA samples from these strains failed to transform any of the tester mutant strains used in the present study. The transformation assay for *M. urethralis* is very simple and can be performed readily in a clinical laboratory. The entire procedure can be carried out in less than 24 h.

Considerable difficulties have been encountered by many workers attempting to classify the various kinds of gram-negative, oxidase-positive, aerobic, and nonmotile coccobacilli frequently encountered in several natural environments. Some of these organisms have been assigned to one of the five species of *Moraxella* based upon their conventional taxonomic properties as well as on strong interstrain genetic interactions (2, 9). In 1970 Lautrop et al. (13) suggested that a group of bacteria, isolated primarily from urine and from the female genital tract and previously classified as strains of *Mima polymorpha* var. *oxidans*, might be members of a new species of *Moraxella*, which they designated as *Moraxella urethralis*. The finding that many strains of *M. urethralis* are competent for genetic transformation (E. Juni, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, D9, p. 53) has made it possible to devise a transformation assay for detection and identification of suspected strains of this organism similar to the assay systems devised for diagnosis of strains of *Acinetobacter* (11), *Moraxella osloensis* (12), and *Neisseria gonorrhoeae* (10). It is the purpose of this paper to describe transformation assays for *M. urethralis* suitable for routine use in a clinical diagnostic laboratory.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study are listed in Table 1. With the exception of strain 1, all cultures designated *M. urethralis* were received from R. E. Weaver. Cultures were maintained by adding 0.5 ml of a heavy bacterial suspension in buffer or media into a sterile screw-capped test tube containing 1.0 ml of sterile glycerol and, after complete mixing, storing at −20°C. *Neisseria* cultures can be maintained in a similar manner but require lower storage temperatures (−40 to −45°C). Cultures stored in this manner have been found to remain viable for several years. When required, 0.1 ml, or occasionally a loopful, of any of these suspensions is placed on a suitable growth medium plate and incubated overnight at 37°C. Bacterial suspensions stored in 67% glycerol do not freeze at the temperatures used, and small samples may be conveniently and rapidly removed to growth media when required. This procedure is based upon unpublished information received from R. R. Brubaker.

Growth media. All strains of *M. urethralis* grow well on heart infusion agar (Difco) and were routinely cultured on this medium. Citrate-yeast extract-mineral medium, used for detection of citrate-positive transformants of strain 1, was prepared by adding the following chemicals, one at a time, to approximately 800 ml of distilled water until completely dissolved: citric acid, 2 g; KH_2PO_4, 1.5 g; Na_2HPO_4, 13.5 g (or $Na_2HPO_4 \cdot 7H_2O$, 25.5 g);

TABLE 1. *Strains tested*

Strain no.	Tentative identification	Original strain designation	Competent[a]	Citrate utilization	Donor DNA transforms: Strain 1	Donor DNA transforms: *trp-22*	Donor DNA transforms: *hyx-27*	Isolated from:
1	*M. urethralis*	ATCC 17960	+	−	−	+	+	Ear
2	*M. urethralis*	C 136	+	+	+	+	+	Chorioamnion
3	*M. urethralis*	B 279	−	+	+	+	+	Leg wound
4	*M. urethralis*	A 324	−	−	−	−	−	Urine
5	*M. urethralis*	B 344	+	+	+	+	+	Urine
6	*M. urethralis*	B 583	+	+	+	+	+	Leg
7	*M. urethralis*	A 948	+	+	+	+	+	Urine
8	*M. urethralis*	C 1098	+	+	+	+	+	Vagina
9	*M. urethralis*	C 1199	+	−	−	+	+	Urine
10	*M. urethralis*	C 1591	−	−	+	+	+	Urine
11	*M. urethralis*	B 1882	+	+	+	+	+	Urine
12	*M. urethralis*	B 1883	+	+	+	+	+	Urine
13	*M. urethralis*	B 2209	+	+	+	+	+	Vagina
14	*M. urethralis*	A 2225	−	+	+	+	+	Urine
15	*M. urethralis*	B 2630	+	+	+	+	+	Placenta
16	*M. urethralis*	A 2837	+	+	+	+	+	Vagina
17	*M. urethralis*	A 3719	+	+	+	+	+	Urine
18	*M. urethralis*	A 4435	−	+	+	+	+	
19	*M. urethralis*	A 5021	+	+	+	+	+	Urine
20	*M. urethralis*	B 5129	−	−	−	−	−	Mastoid
21	*M. urethralis*	B 5889	+	+	+	+	+	Urine
22	*M. urethralis*	A 6571	−	+	+	+	+	
23	*M. urethralis*	B 6745	+	+	+	+	+	Urine
24	*M. urethralis*	B 6786	−	+	+	+	+	Genital
25	*M. urethralis*	B 7286	+	+	+	+	+	Cervix
26	*M. urethralis*	B 7306	−	+	+	+	+	Urine
27	*M. urethralis*	A 7354	−	+	+	+	+	Vagina
28	*M. urethralis*	B 8393	+	+	+	+	+	Urine
29	*M. urethralis*	A 8620	−	+	+	+	+	
30	*M. urethralis*	C 8990	+	+	+	+	+	Urine
31	*M. urethralis*	A 9198	−	+	+	+	+	
32	*M. urethralis*	B 9265	−	+	+	+	+	Urine
33	*M. urethralis*	B 9412	+	−	+	+	+	Urine
34	*M. urethralis*	B 9546	−	−	−	−	−	Genital
35	*Acinetobacter* sp.	BD 413	+	+	NT[b]	−	−	Soil
36	*Achromobacter* sp.	MJT F5/158	NT	−	NT	−	NT	
37	*Achromobacter* sp.	MJT F4/11/5	NT	−	NT	−	NT	
38	*Achromobacter* sp.	MJT F5/199A	NT	−	NT	−	NT	Poultry
39	*Achromobacter* sp.	MJT F5/211	NT	−	NT	−	NT	
40	*M. osloensis*	ATCC 19961	+	+	NT	−	−	
41	*M. osloensis*	NCTC 10749	NT	NT	NT	−	−	
42	*M. osolensis*	ATCC 10973	NT	NT	NT	−	−	
43	*M. phenylpyruvica*	ATCC 17955	NT	−	NT	−	−	
44	*M. phenylpyruvica*	ATCC 23333	NT	NT	NT	−	−	Blood
45	*M. phenylpyruvica*	ATCC 23334	NT	NT	NT	−	NT	Blood
46	*M. phenylpyruvica*	ATCC 23335	NT	NT	NT	−	NT	Scalp lesion
47	*M. phneylpyruvica*	B 5856	NT	NT	NT	−	−	
48	*M. nonliquefaciens*	ATCC 17953	NT	NT	NT	−	−	Nose
49	*M. nonliquefaciens*	ATCC 19975	NT	NT	NT	−	−	Nose
50	*F. meningosepticum*	ATCC 13253	NT	NT	NT	−	NT	Spinal fluid
51	*M. cryophilus*	ATCC 15174	NT	NT	NT	−	NT	Pork sausage
52	*B. catarrhalis*	ATCC 23246	+	−	NT	−	−	
53	*B. catarrhalis*	ATCC 25238	+	−	NT	−	−	
54	Group II j (ref. 16)	B 5939	NT	NT	NT	−	−	
55	*N. gonorrhoeae*	ATCC 10874	NT	NT	NT	−	−	
56	*N. meningitidis*	ATCC 13077	NT	NT	NT	−	−	Spinal fluid
57	*N. meningitidis*	ATCC 13090	NT	NT	NT	−	−	Spinal fluid
58	*N. perflava*	ATCC 14799	NT	NT	NT	−	−	
59	*N. flavescens*	ATCC 13120	NT	NT	NT	−	−	Spinal fluid
60	*N. lactamica*	ATCC 23970	NT	NT	NT	−	−	Nasopharynx

[a] The competency of a given strain was tested by the ability of that strain to be transformed to streptomycin resistance by DNA from a high-level streptomycin-resistant mutant of strain 8.

[b] NT, Not tested.

$MgSO_4$, 0.1 g (or $MgSO_4 \cdot 7H_2O$, 0.2 g); NH_4Cl, 2 g; $CaCl_2$, 1 ml of a 1% solution; $FeSO_4 \cdot 7H_2O$, 0.5 ml of a freshly prepared 0.1% solution; and yeast extract (Difco), 0.5 g. The final volume was adjusted to 1 liter with distilled water, and the medium was sterilized by autoclaving for 20 min. Citric acid-yeast extract-mineral agar plates were prepared by pouring a volume of citric acid-yeast extract-mineral liquid medium (medium at room temperature) into an equal volume of recently melted (90 to 100°C) sterile 3% agar, mixing, and pouring approximately 20 ml per plate. The salts mixture used in the liquid citric acid-yeast extract-mineral medium is the S-2 medium of Monod and Wollman (14).

Glutamate-casein hydrolysate plates were prepared by adding the following chemicals, one at a time, to approximately 800 ml of distilled water until completely dissolved: monosodium glutamate, 5 g; NaCl, 10 g; Na_2HPO_4, 5.6 g (or $Na_2HPO_4 \cdot 7H_2O$, 10.6 g); $MgSO_4$, 0.45 g (or $MgSO_4 \cdot 7H_2O$, 0.9 g); and casein hydrolysate (vitamin free, salt free; ICN Pharmaceuticals), 16 g. The final volume was adjusted to 1 liter with distilled water and the medium was sterilized by autoclaving for 20 min. Glutamate-casein hydrolysate agar plates were prepared by pouring a volume of glutamate-casein hydrolysate liquid medium (medium at room temperature) into an equal volume of recently melted (90 to 100°C) sterile 3% agar, mixing, and pouring approximately 20 ml per plate. The casein hydrolysate-salts mixture in the glutamate-casein hydrolysate agar is medium A of Gould et al. (7). After being dried in the inverted position, all plates were stored in double plastic bags, to prevent drying, either at room temperature or in a refrigerator (5°C).

Mutant tester strains used in the transformation assay. Strain 1 (Table 1) is a naturally occurring mutant which, unlike most strains of *M. urethralis*, is normally unable to utilize citrate as a carbon and energy source but can be transformed to grow on a citrate-mineral medium by deoxyribonucleic acid (DNA) from citrate-utilizing strains of this organism. Auxotrophic mutants of strains 1 and 8 (Table 1) were obtained after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1). Mutants able to grow on heart infusion agar but unable to grow on glutamate-casein hydrolysate agar were further characterized to determine their specific nutritional requirements (5). Most of the studies using growth factor-requiring strains reported here were conducted with a tryptophan auxotroph of strain 8 (*trp-22*) and a hypoxanthine auxotroph of strain 1 (*hyx-27*).

Preparation of crude transforming DNA. Crude transforming DNA samples were prepared as described previously (12). A small amount of bacterial cell paste well visible on the edge of a 2-mm bacteriological loop, and taken from a growth area on a heart infusion plate, was transferred to 0.5 ml of sterile lysing solution consisting of 0.05% sodium dodecyl sulfate in standard saline citrate solution (0.15 M sodium chloride, 0.015 M trisodium citrate). The cells were suspended as completely as possible by stirring with an orbital mixer, and the suspension was heated either in a water bath or an electrical heating block at 60 to 70°C for 30 to 60 min. The amount of cell paste used is not critical nor is the exact heating temperature or the time of heating. DNA is released during the heating period and the crude DNA preparation is ready for use immediately after heating.

Most crude DNA samples can be stably stored in the refrigerator (5°C) for many months, although the DNA in an occasional preparation will be degraded slowly by a nuclease, as described in Results. Screw-capped test tubes (13 by 100 mm) containing lysing solution were prepared in advance and used as required. Unless the caps on these tubes are screwed on tightly, concentration of the detergent by evaporation will take place during storage and result in lysis of recipient mutant strains during the transformation assay.

Transformation assay. One of the mutant strains described above was grown on a heart infusion plate for 6 to 18 h at 37°C. Small amounts of mutant cell paste, clearly visible to the naked eye, were transferred to suitably labeled squares on a heart infusion plate, one such square being required for each DNA sample tested. Using a 2-mm-diameter loop, a loopful of crude DNA was used to suspend and spread the cell paste over a small area of the square, such as a circle approximately 1 cm in diameter. To insure that the DNA preparations were sterile, a loopful of each DNA sample was also placed in a separately labeled square of either the same or another heart infusion plate; no growth should occur in this square. Lastly, a control square was spread with cell paste of the non-DNA-treated mutant strain used in the assay.

After incubation of DNA-mutant cell mixtures at 37°C for 2 to 18 h, a generous amount of cell paste from each growth area was streaked to cover uniformly a sector of the appropriate display plate, and this plate was incubated at 37°C. The display plate for transformation of strain 1 to citrate utilization was citric acid-yeast extract-mineral agar. Transformation of auxotrophs to prototrophy requires the use of glutamate-casein hydrolysate agar.

After 20 h of incubation, inoculated display plates may be viewed by using a low-power dissecting microscope. If the DNA sample was derived from a strain of *M. urethralis*, the sector of the display plate streaked with cell paste from the DNA-mutant strain mixture will show numerous colonies, each originating from a mutant cell that had been transformed to grow on the display medium. If incubation is continued for a total of 2 days, transformant colonies will be visible clearly with the naked eye. If no transformant colonies can be observed after 2 days of incubation, it can be concluded that the DNA sample used in a particular test was not derived from a strain of *M. urethralis*.

No colonies should be seen in the sector of the display plate streaked with the non-DNA-treated mutant strain. On a rare occasion this control streak may show one or more spontaneous revertant colonies, however. The very small number of such colonies should never be confused with the massive number of colonies observed for the case of positive transformation. It is possible that the defect in

strain 1 may be a deletion of a small segment of DNA, since this strain has never been observed to revert spontaneously to citrate utilization. If the DNA sample used should prove to be nonsterile, a relatively rare occurrence, the test must be repeated using a new DNA preparation or after reheating of the original DNA preparation.

RESULTS

Discovery of competency in *M. urethralis*. During a study of strain 1 (Table 1) and four organisms subsequently shown to be strains of *M. urethralis* (strains 18, 22, 29, 31; Table 1), it appeared that all these strains might be related because of similarities in several phenotypic properties. Unlike the other strains, however, strain 1 was unable to grow on citrate-mineral agar plates. It was considered that should strain 1 happen to be competent for genetic transformation, it would be possible that DNA samples from other related citrate-positive organisms might be able to transform this competent strain for ability to grow on citrate-mineral agar. Preliminary tests did indeed demonstrate that DNA samples from strains 18, 22, 29, and 31 (Table 1) were able to transform strain 1 (Table 1) to grow on citrate-mineral agar. Further investigation revealed that 20 of the 31 strains of *M. urethralis* analyzed in the present study are competent for genetic transformation (Table 1).

Transformation to citrate utilization as an assay. Table 1 reveals that 27 of the 31 strains of *M. urethralis* examined in the present study are able to grow with citrate as the sole carbon and energy source, a result indicating that citrate utilization is a common property of most strains of this organism. Since strain 1 (Table 1) is competent, and does not revert spontaneously to citrate utilization, a transformation assay was devised making use of the ability of DNA samples from other strains of *M. urethralis* to transform strain 1 to grow on a citrate mineral medium. Figure 1 shows the growth of DNA mixtures of strain 1 for DNA samples from 16 suspected strains of *M. urethralis*. A loopful of each DNA sample was also spread on squares of another heart infusion plate (not illustrated) to prove that the DNA samples used were sterile. After overnight growth of the strain 1-DNA mixtures, some cell paste from each growth area was removed with a loop, as may be seen from the scrape marks in the growth areas of Fig. 1, and streaked on sectors of a citrate-yeast extract-mineral agar plate. Figure 2 illustrates the positive results obtained for DNA samples from 5 of the 16 strains tested. Cells of strain 1 transformed to citrate utilization on the plate in Fig. 1 gave rise to

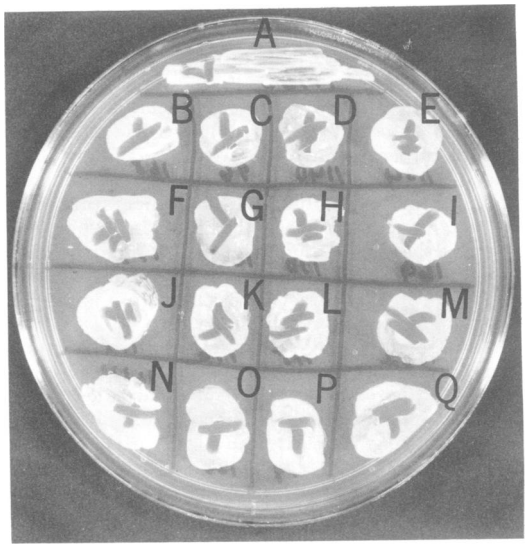

FIG. 1. *Growth of mixtures of strain 1 and DNA samples from other strains of M. urethralis listed in Table 1 at 37°C for 20 h on heart infusion agar. Prior to incubation cell paste of strain 1 was spread in sector A (non-DNA-treated control) and was also mixed with DNA from the following: B, strain 31; C, strain 18; D, strain 26; E, strain 27; F, strain 24; G, strain 15; H, strain 26; I, strain 30; J, strain 13; K, strain 12; L, strain 11; M, strain 19; N, strain 16; O, strain 14; P, strain 7; and Q, strain 4. The scrape marks in each growth area were made when cell paste was removed with a loop and spread on sectors of citrate-yeast extract-mineral agar plates, one of which is shown in Fig. 2.*

colonies on the citrate plate pictured in Fig. 2. The sector of the citrate plate streaked with non-DNA-treated strain 1 (Fig. 2A) shows only the original cell paste, no citrate-utilizing colonies being visible. The few small particles visible in this control sector (Fig. 2A) were shown to be crystals that formed in the medium. Yeast extract (0.025%) was included in this citrate-mineral medium since it accelerates the growth of only those cells transformed to citrate utilization.

DNA samples from all 34 strains received as being either *M. urethralis* or *M. polymorpha* var. *oxidans* were used to transform strain 1 to citrate utilization using the assay procedure shown in Fig. 1 and 2. Positive results similar to those shown in Fig. 2 were obtained with DNA samples derived from 29 of these strains (Table 1). As expected, DNA from strain 1 was unable to transform strain 1 to utilize citrate. DNA samples from strains 4, 20, and 34 also failed to transform strain 1 to citrate utilization. As will be discussed further, it seems

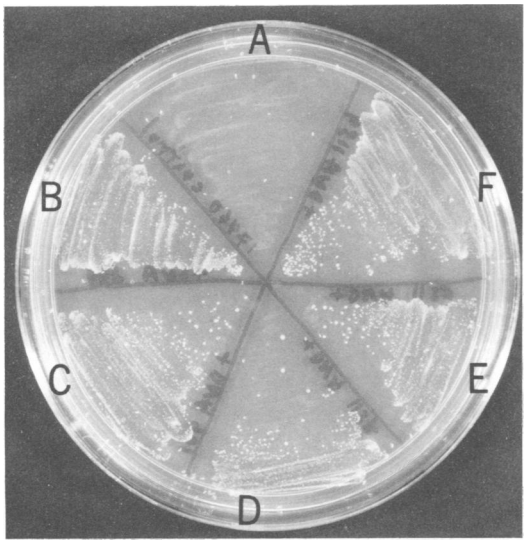

FIG. 2. *Interstrain transformation of strain 1 to citrate utilization. Cell paste from the growth areas of the plate shown in Fig. 1 was spread uniformly on sectors of a citrate-yeast extract-mineral agar plate and incubated at 37°C for 2 days. The letters in this figure correspond to the similarly lettered growth areas of Fig. 1.*

quite certain that these three organisms are, in fact, not strains of *M. urethralis*. In addition, DNA from strain 9 failed to transform strain 1 to citrate utilization in spite of the fact that other results, to be discussed below, show this organism to be a strain of *M. urethralis*. Since strain 9 is normally unable to grow in a citrate-mineral medium (Table 1), it seems quite likely that the genetic lesions in the chromosomal region directing the synthesis of an enzyme required for citrate utilization in strains 1 and 9 must either be either identical or overlapping. By contrast, DNA samples from strains 10 and 33, organisms unable to grow on a citrate-mineral medium (Table 1), readily transformed strain 1 to citrate utilization (Table 1). These findings prove that the genetic defects in strains 10 and 33 are not identical to the genetic defect in strain 1 regarding ability to dissimilate citrate.

Transformation assays using auxotrophic tester strains. A tryptophan-requiring mutant (*trp-22*) of strain 8 was treated with DNA samples in a manner similar to that pictured in Fig. 1. The incubated mutant cell-DNA mixtures were streaked on sectors of a glutamate-casein hydrolysate-mineral medium, and the results obtained are shown in Fig. 3. The non-DNA-treated *trp-22* streaked on sector A of Fig. 3 shows no tryptophan-independent colonies, only the originally streaked cell paste being visible. The massive numbers of colonies on the five other sectors of the plate in Fig. 3, each colony being derived from a mutant cell that was transformed to tryptophan independence, clearly demonstrates the close genetic relationship between the strains from which the DNA samples were derived and strain 8. In this case it was also shown that all DNA samples used were sterile.

When DNA samples from 34 strains designated as *M. urethralis* (Table 1) were tested, it was demonstrated that 31 of them were able to transform mutant *trp-22* of strain 8 to tryptophan independence in a manner identical to that illustrated for five of these samples in Fig. 3. As was the case when transformation of strain 1 to citrate utilization was used as the assay system, DNA samples from strains 4, 20, and 34 all failed to give even a single transformant colony of *trp-22*, thus providing strong evidence that these organisms are not strains of *M. urethralis*. Exactly the same results were obtained using several other auxotrophic mutants derived from strains 1 and 8, such as *hyx-27* (Table 1). The inclusion of casein hydrolysate in the glutamate-mineral medium used in Fig. 3 serves to accelerate the growth of proto-

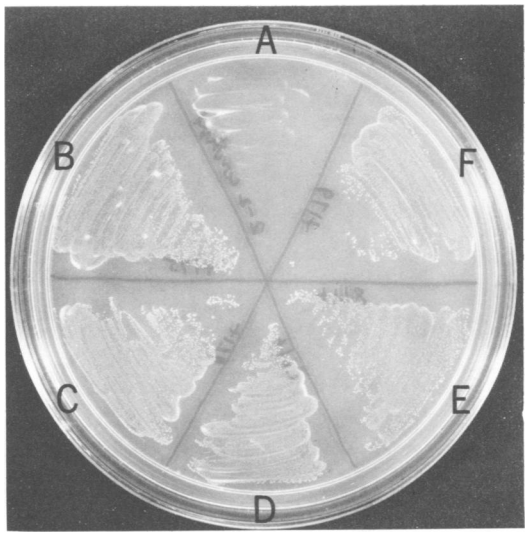

FIG. 3. *Interstrain transformation of trp-22 to prototrophy. Cell paste from the growth of DNA-trp-22 mixtures, similar in appearance to those in Fig. 1, was spread uniformly on sectors of a glutamate-casein hydrolysate-mineral agar plate and incubated at 37°C for 2 days. The DNA samples used were: A, no DNA; B, from strain 12; C, from strain 11; D, from strain 19; E, from strain 16; and F, from strain 14.*

trophically transformed cells of *trp-22* and *hyx-27* but does not permit growth of the untreated auxotrophs.

Effect of time of incubation of mutant cell-DNA mixtures. In the assays described previously, the tester strains (strain 1, *trp-22 hyx-27*) were incubated with the DNA samples being tested for 15 to 20 h before these mixtures were streaked on indicator media. To test the effect of time of incubation of mutant cell-DNA mixtures on the extent of transformation, strain *trp-22* was incubated with DNA from wild-type strain 8 (homologous DNA) for various periods of time ranging from 0 to 7 h. Figure 4 shows the results obtained when these mixtures were streaked on sectors of a single display plate followed by incubation at 37°C for 2 days. It can be seen (Fig. 4) that some transformant colonies are visible for the mutant cell-DNA mixture that was streaked on the display plate immediately after mixing. Prior incubation for 2 h or more resulted in a significant increase in the subsequent number of colonies observed (Fig. 4). Although the plate shown in Fig. 4 was incubated for 2 days, for photographic purposes, minute transformant colonies can be seen by using a low-power dissecting microscope after 18 to 20 h of incubation. It is thus possible to complete the entire transformation assay within 24 h.

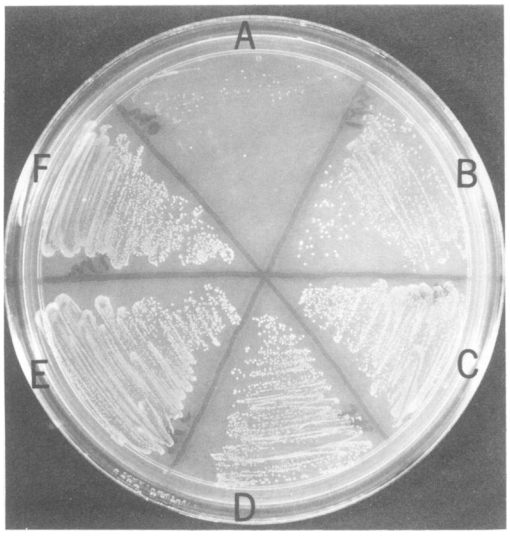

FIG. 4. *Effect of time of incubation of trp-22 with homologous DNA prior to streaking on glutamate-casein hydrolysate-mineral agar indicator medium. DNA from strain 8 was mixed with trp-22 on squares of a heart infusion plate at different times and incubated at 37°C. These DNA-mutant cell mixtures were then streaked on sectors of a glutamate-casein hydrolysate-mineral agar plate shown in this figure, which was incubated at 37°C for 2 days. The times of incubation of the DNA-mutant cell mixtures on the heart infusion plate (not shown) were: A, 0 h; B, 1 h; C, 2 h; D, 3 h; E, 5 h; and F, 7 h. The colonies seen on this plate were each derived from those cells of trp-22 that were transformed to prototrophy.*

Specificity of the transformation assay for *M. urethralis*. All DNA samples that reacted positively in the transformation assays described above gave strong reactions, similar to that obtained using homologous DNA, thus indicating that there is a close relationship between the strains from which they were derived and the indicator strains. To test the specificity of the transformation assay for *M. urethralis*, DNA samples derived from strains of other organisms that resemble *M. urethralis* in some ways were also analyzed. For all DNA samples from strains 35 through 60 (Table 1) no transformant colonies appeared with any of the mutant strains used. An occasional spontaneous revertant colony arising from one of the mutant strains should not be taken as an indication of positive transformation. In such a case where there is a possible question of the results, the entire transformation should be repeated in duplicate. Although similar studies with other organisms have shown that weak genetic interactions are possible (2, 9), the results of the present study indicate that when there is close genetic relatedness of strains strong reactions occur, such as those illustrated in Fig. 2 and 3, whereas there is no interaction at all for unrelated strains, such as strains 35 through 60 (Table 1).

Stability of crude DNA preparations. Previous studies from this laboratory of crude DNA preparations from *Acinetobacter* (11), *M. osloensis* (12), and *N. gonorrhoeae* (10) have revealed that the DNA in such solutions remains stable and capable of transforming suitable mutant strains after storage of these preparations in the refrigerator (5°C) for several years. In the present study DNA preparations from strain 1 (Table 1) showed a marked instability upon storage in the refrigerator, as evidenced by a gradual decrease in the ability of the aged DNA solutions to transform the mutant tester strains. Freshly prepared crude DNA from strain 1 readily transformed auxotrophs of strains 1 and 8 to prototrophy, but this ability to transform was virtually completely lost after several weeks of storage. It would thus appear that, unlike other strains of *M. urethralis*, strain 1 may contain a nuclease that is resistant to heating in the presence of 0.05% sodium dodecyl sulfate. Since DNA samples are usually assayed shortly after they are prepared, the slow deterioration of DNA from

strain 1, or from other strains possibly producing a similar nuclease, is of no practical consequence for routine application of the transformation assay for *M. urethralis*. It should be pointed out, however, that crude DNA preparations from some strains of *M. urethralis* have retained their transforming ability after storage in the refrigerator for 3 years.

When it was found that DNA samples from strains 4, 20, and 34 (Table 1) uniformly failed to transform all of the mutant tester strains available, it was considered that these negative results might have been the consequence of the action of strong nucleases in these strains, which destroyed the DNA as soon as it was released from the cells after lysis by the detergent. To test this possibility, cell paste from strain 8, a typical strain of *M. urethralis*, was added to lysing solution together with cell paste from either strain 4, 20, or 34, and the mixed suspension was heated as described in Materials and Methods. These mixed DNA solutions were then tested for their ability to transform mutant tester strains. In all cases good transformation was obtained, thus providing evidence that nucleases in strains 4, 20, and 34 are probably not leading to immediate destruction of DNA and consequent inability of crude DNA preparations from these strains to transform the mutant tester strains. If nucleases had been responsible for destruction of DNA, it would be expected that such nucleases might also degrade the DNA from strain 8 as well as homologous DNA.

DISCUSSION

The identification of clinically isolated gram-negative coccobacilli has frequently been difficult, if not impossible, largely because of the paucity of distinguishing phenotypic characteristics. Gram-negative coccobacilli that are also oxidase positive, and are not clearly strains of *Moraxella*, have frequently been classified as strains of *M. polymorpha* var. *oxidans*. It is now well recognized that this "catchall" designation has included organisms subsequently demonstrated to be members of several different genera (8, 9). In 1970 Lautrop et al. (13) proposed that a group of such organisms, which included strain ATCC 17960, had sufficient similarities in phenotypic properties to justify placing all these strains in the same taxon. These workers suggested that an appropriate designation might be *M. urethralis* since the collection of some 20 such strains that they studied was isolated chiefly from urine and from the female genital tract. Cultures of *M. urethralis* appear to be identical to those previously delegated to group M-4 (16) in the taxonomic scheme used by the Center for Disease Control in Atlanta, Ga.

When streaked on complex media, such as heart infusion agar, colonies of *M. urethralis* are characteristically whiter and more opaque than colonies of other moraxellas (13, 15). *M. urethralis* is an oxidase-positive, catalase-positive aerobe, which does not form acid from any sugar (13, 15). It shares with *M. osloensis* the property of being able to grow in simple mineral media containing a single organic carbon and energy source. Compounds such as glutamate, citrate, and acetate serve as carbon sources for most strains. Poly-β-hydroxybutyrate inclusion granules are also detected in these bacteria (13). *M. urethralis* is sensitive to low levels of penicillin, as are the other moraxellas (13).

In 1974 Riley et al. (15) reported on the characterization of 56 strains of *M. urethralis*. They found that these strains reduce nitrite, but not nitrate, and that all of them are able to deaminate phenylalanine to phenylpyruvic acid. Although Lautrop et al. (13) concluded that strains of *M. urethralis* are unable to deaminate phenylalanine, this apparent conflict with the results of Lautrop et al. (13) is probably the result of the use of different methods for determination of this particular characteristic. Riley et al. (15) also emphasize the fact that it is relatively easy to confuse *M. urethralis* with certain strains of *M. osloensis* and *M. phenylpyruvica*. The introduction of a transformation assay for *M. urethralis*, as described here, now makes it possible to diagnose definitively all strains of *M. urethralis*. The transformation assay is certainly as simple to perform and interpret as virtually any test used in a clinical laboratory and is readily adaptable for routine clinical application.

Other workers have used the ability to demonstrate interstrain transformation of a streptomycin resistance marker as evidence for genetic relationship of interacting strains of *Moraxella* (2, 9) and *Branhamella* (4). Studies in this laboratory of several strains of *M. urethralis* have shown that all of them give rise readily to spontaneous mutants resistant to high levels of streptomycin. Attempts to transform such markers into competent streptomycin-sensitive strains of *M. urethralis* have most frequently been unsuccessful, although occasional mutant markers of this type are readily transformed into competent strains. The rapid rate of spontaneous mutation to streptomycin resistance in *M. urethralis* and the inability to transform most of such markers into competent strains strongly suggest that many mutants of this organism resistant to high levels of strepto-

mycin arise through a multistep mutational process. If such mutations leading to resistance to high levels of streptomycin are not genetically linked, it would be expected that not all of these markers would be transferred and recombined simultaneously into competent streptomycin-sensitive strains. Inability to transform multistep, high-level streptomycin resistance in *M. urethralis* may have been responsible for the original finding that all strains of *M. urethralis* are not competent (9). Twenty of the 31 strains of *M. urethralis* tested proved to be competent for genetic transformation (Table 1). At least one of these competent strains (strain 1, Table 1) was also examined in the study of Lautrop et al. (13). Although high-level streptomycin resistance markers are generally highly conserved (6), a fact that justifies the use of such markers in demonstrating interspecies relatedness, the use of this marker can lead to erroneous conclusions, as in the case for *M. urethralis*.

In spite of the fact that strains of *M. urethralis* have several properties in common with other moraxellas, it is still not firmly established that this organism is a true *Moraxella*. The DNA composition of *M. urethralis* has been reported to be 46 mol% guanine plus cytosine (G+C) (13), a value slightly higher than those for most moraxellas (40 to 45 mol% G+C) (2, 3, 9) and somewhat lower than those for strains of *Neisseria* (46.5 to 53 mol% G+C) (2, 3, 9). Streptomycin resistance markers from several strains of *M. urethralis* were not transformed into various competent moraxellas and neisserias (13). In light of the possible multistep nature of most streptomycin-resistant mutants of *M. urethralis*, as discussed above, these results are open to question and similar experiments should be performed using a streptomycin-resistant marker from *M. urethralis*, which has first been demonstrated to transform readily a competent strain of this organism.

As shown in this study, where transformation of strain 1 to citrate utilization was the basis of the assay used, the use of only a single carbon source marker can lead to false results if the DNA sample from the strain being tested is also defective in the identical region of the gene that is being modified by transformation. Since all strains of *M. urethralis* examined are able to grow in a simple mineral medium containing a single carbon and energy source, it seems highly improbable that naturally occurring strains will be found having the same genetic defect as that of a chemically induced auxotrophic mutant tester strain that requires either an amino acid, a purine, or a pyrimidine, for example. For this reason mutants of the latter type would appear to be most useful when testing suspected strains of *M. urethralis* using the transformation assay. When the tester strain is strain 1, which is a naturally occurring mutant strain having a defect in one of the genes involved in citrate utilization, negative transformation results would only be suspect if the strain under test is also citrate negative. In such a case, the additional use of one of the auxotrophic tester strains would serve as a check of the original diagnosis.

The three strains examined in the present study, which were originally classified as *M. urethralis* but whose DNA samples failed to transform all competent mutant tester strains used (strains 4, 20, and 34), have not yet been properly identified and will be investigated further. Except for the facts that these three strains are all citrate negative and that strain 4 gives a weak phenylalanine deaminase reaction, all other key phenotypic reactions are the same for these strains as for strains of authentic *M. urethralis*.

Now that a simple and definitive transformation procedure is available for identification of strains of *M. urethralis* it should be possible to assess the role, if any, of this bacterium in infectious processes.

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