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Simple Genetic Transformation Assay for Rapid Diagnosis of *Moraxella osloensis*

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A genetic transformation assay for unequivocal identification of strains of *Moraxella osloensis* is described. In this assay a stable tryptophan auxotroph is transformed to prototrophy by deoxyribonucleic acid (DNA) samples from other strains of *M. osloensis* but not by DNA samples from unrelated bacteria. The test is simple to perform and definitive results can be obtained in less than 24 h. The procedure, which is suitable for routine diagnosis in a clinical laboratory, involves a rapid method for preparation of crude transforming DNA from small quantities of bacterial cells and permits simultaneous examination of large numbers of isolated cultures. The assay was shown to correctly identify 27 strains previously classified as *M. osloensis*. Forty-five other gram-negative, oxidase-positive, nonmotile coccobacilli, which might be confused with *M. osloensis* unless subject to more extensive testing, were shown to be unrelated genetically to *M. osloensis*. The transformation assay clearly distinguishes *M. osloensis* from *Acinetobacter*. Although most strains of *M. osloensis* are nonfastidious, being able to grow in a mineral medium supplemented with a single organic carbon source, one of the strains tested was only able to grow on fairly complex media and could not be transformed to grow on simple media. Inability to alkalize Simmons citrate agar was shown not to be characteristic of all strains of *M. osloensis*.

Several reports have appeared in the literature implicating strains of *Moraxella* as the causative agents of disease in man (5, 14, 16, 19, 20, 21, 22, 33, 43, 45, 46, 48) and in animals (13, 44). Taxonomic (4, 24) and genetic studies (9) of aerobic gram-negative, oxidase-positive, and nonmotile coccobacilli, classified as *Moraxella*, have served to emphasize the existence of several distinct groups of these organisms which appear to be more or less distantly related to each other. To better establish the possible role of moraxellas in disease it is essential to have rapid and positive methods for identification of these bacteria. It has been shown, however, that it is frequently difficult to distinguish various *Moraxella* strains from each other only by examination of their phenotypic properties (12).

In 1962 Bövre and Henriksen (11) reported that many moraxellas are competent for genetic transformation of streptomycin resistance markers. As a result of quantitative interstrain transformation investigations Bovre (7, 8) was able to demonstrate that strains formerly classified as *Moraxella nonliquefaciens* could be divided into at least two distinct and genetically compatible groups. Members of each group showed high ratios of inter- to intrastrain frequencies of transformation to streptomycin resistance. By contrast, very low transformation ratios were observed when strains from each of the groups were compared (8). Although most strains of *M. nonliquefaciens* have complex nutritional requirements, there is a genetically distinct group of *Moraxella* which can grow in a mineral medium containing a single organic carbon source. It has been suggested that strains in this group be classified as *Moraxella osloensis* (12).

In a recent study of *Acinetobacter* it was shown that deoxyribonucleic acid (DNA) samples from all acinetobacters could transform auxotrophs of a genetically competent strain to prototrophy (29). It is possible to use this procedure as a routine diagnostic test for positive identification of newly isolated strains of *Acinetobacter* (29). The present investigation describes a similar simple diagnostic assay for rapid and unequivocal identification of strains of *Moraxella osloensis* suitable for use in a clinical laboratory.

MATERIALS AND METHODS

Bacterial strains. The strains of *M. osloensis* used are listed in Table 1. Each strain bears the designation which the culture or DNA sample had when received.

Growth media. Heart infusion agar (Difco) was used for routine cultivation of all strains studied. One liter of lactic acid-mineral liquid medium was prepared by adding the following chemicals, one at a time, to 800 ml of distilled water until completely dissolved: lactic acid (reagent grade, supplied commercially as approximately 85%), 5 ml; KH_2PO_4, 1.5 g; Na_2HPO_4, 13.5 g (or $Na_2HPO_4 \cdot 7H_2O$, 25.5 g); $MgSO_4$, 0.1 g (or $MgSO_4 \cdot 7H_2O$, 0.2 g); NH_4Cl, 2 g; $CaCl_2$, 1 ml of a 1% solution; and $FeSO_4 \cdot 7H_2O$, 0.5 ml of a freshly prepared 0.1% solution. The final volume was adjusted to 1 liter with distilled water (final pH, 6.65) and sterilized by autoclaving for 20 min. Lactic acid-mineral agar plates were prepared by pouring a volume of lactic acid-mineral liquid medium (medium at room temperature) into an equal volume of recently melted (90 to 100 C) sterile 3% agar, mixing, and pouring 15 to 20 ml per plate. The salts mixture used in this lactic acid-mineral medium is the S-2 medium of Monod and Wollman (39). Preparation of the complete mineral medium, as described above, avoids the precipitation of salts that usually occurs when mixing the components of S-2 medium. After drying in the inverted position all plates are stored in double plastic bags either at room temperature or in a refrigerator (5 C).

Preparation of crude transforming DNA. A small amount of bacterial cell paste on a bacteriological loop, from growth on any suitably plated medium, is carefully placed into 0.5 ml of a lysing solution consisting of sterile 0.05% sodium dodecyl sulfate in standard saline citrate solution (0.15 M sodium chloride, 0.015 M Na_3 citrate) contained in a screw-capped tube (13 by 100 mm), and the cells are suspended uniformly with the aid of an orbital mixer. Care should be taken to avoid placing cell paste on the side of the tube where it cannot come into contact with the detergent solution. The suspended cells are then heated in a deep 60 C water bath for 15 to 60 min, a procedure which sterilizes the contents of the tube by causing cell lysis and the release of intracellular DNA. DNA solutions prepared this way can be stored indefinitely in the refrigerator, if desired, provided that the caps are screwed on tightly. If the lysing solution, or DNA preparation, is permitted to evaporate before use, the concentration of sodium dodecyl sulfate will increase to such an extent that the recipient cells to be transformed will be killed during the transformation assay. The use of screw-capped tubes for containing the lysing solution facilitates preparation of large numbers of such tubes at one time and the tightly capped tubes may be stored indefinitely at room temperature.

Auxotroph used in the transformation assay. Auxotrophs of *M. osloensis* (strain 23) were prepared by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine according to the procedure of Adelberg et al. (1). Strain *trpE*55, a mutant lacking a functional anthranilate synthetase and requiring either anthranilate or tryptophan for growth in lactic acid-mineral medium, was selected as the test organism in the transformation assay since it is relatively stable and reverts spontaneously to prototrophy only rarely.

Transformation assay. A grid of squares is marked on the bottom of a heart infusion plate containing as many as 36 squares, as required. A small amount of cell paste of auxotroph *trpE*55, grown overnight on a heart infusion plate at 34 to 35 C, is placed in the center of one of the squares. The quantity of cell paste used is not critical, an amount just visible to the naked eye being sufficient. A sterile loopful (2 mm diameter loop) of crude DNA to be tested is used to suspend the cells previously placed on the plate and the DNA-cell mixture is spread in a circular area somewhat smaller than the confines of the marked square. A second loopful of DNA is spread over the area of another square to serve as a sterility control; no growth should be visible in this square after incubation. A small amount of cell paste of *trpE*55 is spread over the surface of a third square, the subsequent growth in this square being used to check the stability of the auxotroph. Several DNA samples may be tested on the same plate, each sample being mixed with *trpE*55 on a separate square, as described above. For each DNA sample a DNA sterility square is also prepared. Only a single square with *trpE*55 alone (non-DNA-treated control) need be made per plate. After incubation at 34 to 35 C for 2 to 24 h (see Results for a discussion of incubation time) a generous portion of each growth area is streaked on a pie-shaped sector of a lactic acid-mineral agar plate. As many as eight sectors can be used conveniently per plate. One sector of each plate should be streaked with *trpE*55 non-DNA-treated control cells. The streaked plate is incubated for 15 to 48 h at 34 to 35 C. After incubation the streaked areas are observed for colonies derived from cells of *trpE*55 that were transformed to prototrophy during growth in the presence of DNA on the heart infusion plate. The absence of prototrophic transformant colonies after 48 h of incubation indicates that the organism being tested is not a strain of *M. osloensis*.

Since *M. osloensis* grows relatively slowly, observation of transformant colonies at 15 h, or sooner, may require use of a low-power dissecting microscope. After 24 h or more of incubation at 34 to 35 C prototrophic transformant colonies will be clearly visible to the naked eye. The streak of non-DNA-treated *trpE*55 control cells should show no prototrophic colonies. An extremely rare occasional colony on the control sector is the result of spontaneous reversion of *trpE*55 to prototrophy.

Bacteriological tests. The oxidase test was carried out by using the method of Kovacs (30). Ability to alkalize citrate medium was determined by streaking cultures on Simmons citrate agar (Difco) plates and incubating at 34 to 35 C. Each culture was streaked so that both isolated colonies and massive growth could be observed on the same plate.

TABLE 1. *Strains tested*

Organism	Strain	Received from	Isolated from
1. *Mima* Z4	[a]	M. Mandel	
2. *M. polymorpha*	RH486	R. Hugh	
3. *M. osloensis*	C572	R. E. Weaver	
4. *M. osloensis*	A608	R. E. Weaver	Blood
5. *M. osloensis*	8134	R. E. Weaver	
6. *M. osloensis*	8292	R. E. Weaver	Blood
7. *M. polymorpha* var. *oxidans*	8375	W. B. Cherry	
8. *M. osloensis*	B9400	R. E. Weaver	
9. *M. osloensis*	B9762	R. E. Weaver	
10. *M. osloensis*	B9777	R. E. Weaver	
11. *M. nonliquefaciens*	CDC 9870[a]	M. Mandel	
12. *M. osloensis*	9893	R. E. Weaver	Nose
13. *M. polymorpha* var. *oxidans*	ATCC 10973[a]	W. B. Cherry	
14. *M. osloensis*	ATCC 15276	ATCC	Spinal fluid
15. *M. osloensis*	ATCC 17974	ATCC	Ganglion of child
16. *M. osloensis*	ATCC 19954[a]	M. Mandel	Urine
17. *M. osloensis*	ATCC 19955[a]	ATCC	Urine
18. *M. osloensis*	ATCC 19956[a]		Meningitis case
19. *M. osloensis*	ATCC 19957[a]	ATCC	
20. *M. osloensis*	ATCC 19958[a]		
21. *M. osloensis*	ATCC 19959	ATCC	
22. *M. osloensis*	ATCC 19960[a]		
23. *M. nonliquefaciens*	ATCC 19961 (19116/51)[a]	B. W. Catlin	
24. *M. nonliquefaciens*	ATCC 19962	B. W. Catlin	Patient with gonorrhea-like syndrome
25. *M. osloensis*	ATCC 19963	ATCC	
26. *M. osloensis*	ATCC 19964	ATCC	
27. *M. osloensis*	ATCC 19965	ATCC	Ulcer that developed after vein stripping
28. *M. osloensis*	ATCC 19976 (type strain)	S. D. Henriksen, K. Bövre, and R. E. Weaver	Cerebrospinal fluid
29. *Achromobacter* sp.	MJT F/4/11/5	M. J. Thornley	
30. *Achromobacter* sp.	MJT F5/158	M. J. Thornley	
31. *Achromobacter* sp.	MJT F5/199A	M. J. Thornley	
32. *Achromobacter* sp.	MJT F5/211	M. J. Thornley	
33. *Neisseria catarrhalis*	41	Stock culture collection, Dept. of Microbiology, Univ. of Michigan	
34. *Acinetobacter lwoffi*	632	G. L. Gilardi	
35. *Acinetobacter lwoffi*	633	G. L. Gilardi	
36. *Mima polymorpha* var. *oxidans*	A4435	R. E. Weaver	
37. *Mima polymorpha* var. *oxidans*	A6571(1)	R. E. Weaver	
38. *Mima polymorpha* var. *oxidans*	A8620	R. E. Weaver	
39. *Mima polymorpha* var. *oxidans*	A9198(2)	R. E. Weaver	
40. *Micrococcus cryophilus*	ATCC 12226	ATCC	
41. *Flavobacterium meningosepticum*	ATCC 13253 as strain RH540	R. Hugh	
42. *Micrococcus cryophilus*	ATCC 15174	ATCC	
43. *Moraxella nonliquefaciens*	ATCC 17953	E. J. Ordal	
44. *Moraxella nonliquefaciens*	ATCC 17955	E. J. Ordal	
45. *Mima polymorpha* var. *oxidans*	ATCC 17960	ATCC	
46. *Moraxella nonliquefaciens*	ATCC 19975 (Neotype strain)	S. D. Henriksen and K. Bövre	
47. *Moraxella phenylpyruvica*	ATCC 23333 (Neotype strain)	S. D. Henriksen and K. Bövre	

[a] DNA sample from M. Mandel.

RESULTS

Analysis of DNA samples by the transformation assay. DNA samples from all 47 cultures listed in Materials and Methods were tested for ability to transform *trpE*55, a tryptophan auxotroph of *M. osloensis*, strain 23, to prototrophy. Figure 1 shows the growth of *trpE*55 after mixing with DNA samples from each of five different strains of *M. osloensis*, as well as growth of *trpE*55 in the absence of added DNA. Cell paste from each growth area was streaked heavily on a sector of a lactic acid-mineral agar plate. Growth of cells of *trpE*55 that were transformed to prototrophy may be seen in Fig. 2. It is evident that DNA from each of the five strains of *M. osloensis* readily transformed *trpE*55 to prototrophy. Sector A of Fig. 2, which contains a streaking of non-DNA-treated *trpE*55 (grown on square A, Fig. 1), shows no prototrophic colonies, only the amount of cell paste originally streaked on this sector being visible. Similar results were obtained with DNA samples from strains 1 to 28, all of which had been previously identified as strains of *M. osloensis*.

DNA samples from strains 29 to 47 all failed to give even a single prototrophic transformant colony when used to treat *trpE*55 in the manner described above. All of these strains are gram-negative, oxidase-positive coccobacilli that can be readily confused with *M. osloensis* unless subjected to further testing. In addition to strains 29–47, a series of 27 oxidase-positive *Moraxella*-like organisms were also examined. These bacteria were selected for study by the Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria of the International Committee on Nomenclature of Bacteria because their taxonomic position and relationship to *Moraxella* have not yet been determined. Unlike authentic *Moraxella* species, most of these organisms form acid aerobically from sugars and some of them also fail to grow at 35 C. DNA samples from all these 27 strains failed to transform *trpE*55 to prototrophy. DNA samples from a variety of oxidase-negative acinetobacters likewise failed to transform *trpE*55 to prototrophy. All the *M. osloensis* strains examined in the study of Baumann et al. (4) and seven of the 10 strains tested by Bövre (8) are included in the series of strains analyzed in the transformation assay described above.

Optimum conditions for the transforma-

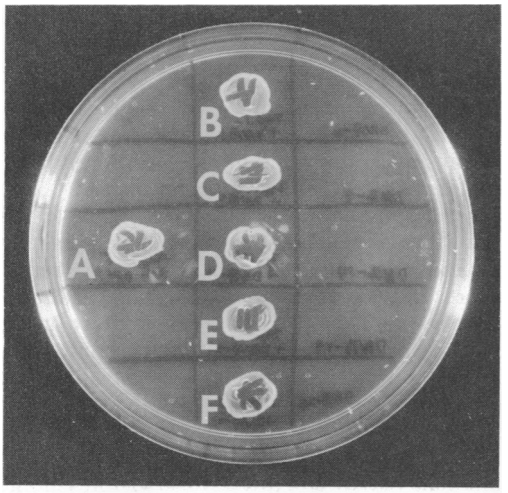

FIG. 1. *Growth of mixtures of trpE55 and DNA on heart infusion agar for 21 h at 34 to 35 C. The growth areas contained: A, no DNA; B, DNA from strain 6; C, DNA from strain 9; D, DNA from strain 14; E, DNA from strain 19; and F, DNA from strain 26. Each DNA sample used was also spread in the square immediately to the right of the respective trpE55-DNA mixture to verify that the DNA preparation was sterile. The marks in the central portion of the growth areas were made when cell paste was removed with a loop and spread on sectors of the lactic acid-mineral agar plate shown in Fig. 2.*

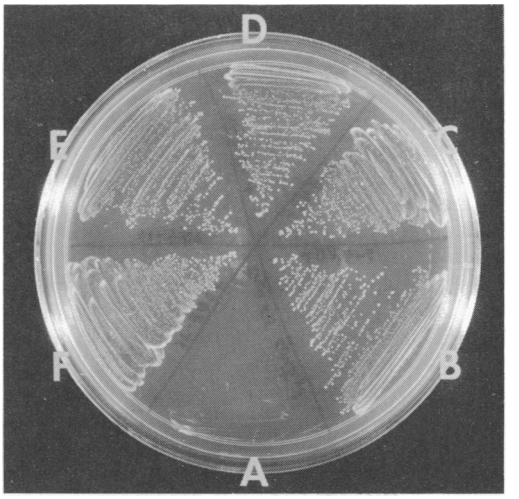

FIG. 2. *Interstrain transformation of trpE55. Cell paste from the growth areas of the plate shown in Fig. 1 were spread on sectors of a lactic acid-mineral agar plate and incubated for 48 h at 34 to 35 C. The letters in this figure correspond to the similarly lettered growth areas of Fig. 1. The growth observed is that of prototrophically transformed cells of trpE55 which are now able to synthesize their own tryptophan and are capable of growing on this simple medium. Only the original amount of cell paste of trpE55 streaked in sector A (non-DNA-treated control) is visible, this auxotroph being unable to grow in the absence of tryptophan.*

tion assay. In the first step of the transformation assay, cell paste from growth on any suitable medium of the organism to be tested, is used for the preparation of crude transforming DNA. The amount of cell paste taken is not critical and even the cell material in part of a single small colony is sufficient for this purpose, as much as possible of the colony being transferred with a loop to the lysing solution. The time of heating can vary considerably and may be extended for several hours with no damage to the DNA. Although 60 C is the recommended temperature for heating, temperatures from 55 to 70 C may be used since even the highest value is below the melting temperature for DNA from *M. osloensis*, which has a DNA composition of 43 to 43.5 mole % guanine plus cytosine (35). Cells incubated in the lysing solution are killed within a few minutes, even at room temperature. Heating this cell suspension, however, does accelerate lysis and release of intracellular DNA. When crude DNA is mixed with cells of *trpE*55 on a plate, as in the transformation assay described above, detergent (sodium dodecyl sulfate) in the DNA solution is absorbed into the agar rapidly enough so that only a few of the recipient cells are killed. The large molecules of DNA are retained on the agar surface until they are subsequently taken up by the competent cells.

The next step in the transformation assay involves growth of *trpE*55 in the presence of DNA. During growth on heart infusion agar the cells pass through a competency phase where DNA is taken up. If the DNA used is derived from a strain of *M. osloensis* it can recombine with the chromosomal DNA of the recipient *trpE*55 cells, some of which will be transformed to prototrophy. Since the interval of competency is not known for growth of a competent strain on semisolid media, a test was performed where *trpE*55 was mixed with DNA from *M. osloensis*, strain 19, and incubated at 34 to 35 C for various periods of time before the cell paste-DNA mixture was streaked on sectors of a lactic acid-mineral agar plate. The results of this study are shown in Fig. 3. It can be seen that prototrophic transformant colonies appeared even when the cell paste was streaked on lactic acid-mineral agar immediately after mixing with DNA. The number of transformant colonies increased significantly, however, when incubation of the cell paste-DNA mixture was extended to at least 2.5 h before streaking on the minimal agar plate (Fig. 3B). Although incubation with DNA prior to streaking was only continued for 12.5 h in the test illustrated in Fig. 3, it has been shown that incubation

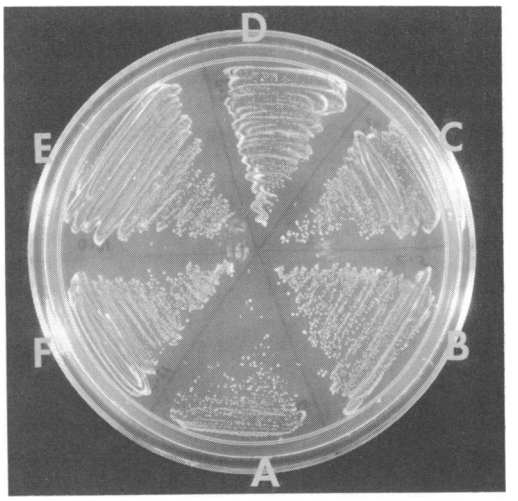

FIG. 3. *Effect of time of incubation of trpE55 with DNA from another strain of M. osloensis prior to streaking on lactic acid-mineral agar. DNA from strain 19 was mixed with trpE55 on squares of a heart infusion agar plate at different times and incubated at 34 to 35 C. The DNA-cell paste mixtures were then streaked on sectors of a lactic acid-mineral agar plate and incubated for 48 h. The colonies seen on this plate are derived from prototrophically transformed cells of trpE55. The times of incubation of the DNA-trpE55 mixtures on the original heart infusion agar plate (not shown) were: A, 0 h; B, 2.5 h; C, 5 h; D, 7.5 h; E, 10 h; and F, 12.5 h.*

periods as long as 24 to 48 h also result in maximum numbers of transformant colonies. Because it is desirable in clinical procedures to perform the entire transformation assay in as short a time period as possible, it is clear that the step in which incubation of cell paste of *trpE*55 with DNA occurs can be extremely short. It is recommended that this incubation time be at least 2 to 5 h in order to assure reasonably large numbers of prototrophic transformants for DNA samples from strains of *M. osloensis*.

The assay step requiring the longest period of time is the one in which prototrophic transformant cells of *trpE*55 grow to form visible colonies on sectors of the lactic acid-mineral agar plate (Fig. 2). Since *M. osloensis* has a longer generation time than organisms such as *Acinetobacter* or *Escherichia*, it is important that the test be performed in such a way as to permit a maximum time for growth. If assays are started early in the morning the lactic acid-mineral agar plate can be streaked within a few hours. This will make it possible to look for prototrophic transformant colonies by the next morning. Early recognition of prototrophic colonies is

greatly facilitated by the use of a low-power dissecting microscope. The inclusion on the lactic acid-mineral agar plate of a sector streaked with *trpE*55 which has not been treated with DNA (non-DNA-treated control, Fig. 2A) is most useful for comparative purposes when looking for such early transformant colonies. Furthermore, the non-DNA-treated *trpE*55 control serves to insure that the sample of *trpE*55 used in the test has not reverted grossly to prototrophy. This control also shows that *trpE*55 is not contaminated with other bacteria capable of growing on the lactic acid-mineral agar plate.

An incubation temperature of 34 to 35 C is suggested for growth of *trpE*55 on heart infusion and lactic acid-mineral agar. Although *M. osloensis* does grow well at 37 C, it is similar to *Acinetobacter* in that the optimal temperature for growth is slightly below body temperature. Plates may also be incubated at room temperature. In this case, however, growth will be somewhat slower than is obtained at the optimal temperature. Although transformant colonies are visible to the naked eye after 24 h of incubation, the plates used in Fig. 2 and 3 were incubated for a total of 48 h for photographic purposes.

Mutants suitable for the transformation assay. *trpE*55 was chosen as a test organism for the transformation assay because of its inherent stability and also because of the high efficiency with which it is transformed to prototrophy by DNA samples from other strains of *M. osloensis*. The ability of heterologous *M. osloensis* DNA samples to transform auxotrophs of strain 23 requiring either leucine, arginine, or histidine for growth in lactic acid-mineral medium has also been demonstrated.

Growth factor and carbon source requirements of M. osloensis strains. It has been reported that strains of *M. osloensis* are all capable of growing in a simple acetate-mineral medium (4, 12) and should thus show no requirement for growth factors. All the strains of *M. osloensis* examined in the present study, with one exception, have indeed been shown to be able to grow in simple mineral media supplemented with acetic acid. The one exceptional strain (strain 2) will only grow on complex media such as heart infusion or antibiotic medium 3 (Difco) agar. Virtually no growth of this strain takes place on nutrient agar (Difco) or on lactic acid-mineral agar supplemented with vitamin-free casein hydrolysate. Strain 2, received from R. Hugh, was described as being identical with ATCC 10973. A culture of ATCC 10973 (strain 13), received from W. B. Cherry, was able to grow on an acetate-mineral medium and appears to be identical with the same strain tested by Baumann et al. (4). It seemed possible that strain 2 might be a spontaneous auxotrophic mutant of strain 13. Since strain 2 is competent for genetic transformation, as shown by its ability to be transformed to streptomycin resistance using DNA samples from streptomycin-resistant mutants derived from several strains of *M. osloensis*, an attempt was made to transform strain 2 to prototrophy with DNA from strain 13. This experiment was not successful, however, not even a single prototrophic transformant of strain 2 being obtained. Furthermore, attempts to isolate a spontaneous prototrophic revertant of strain 2 were also unsuccessful.

It is generally considered that all strains of *M. osloensis* either grow slowly or not at all on Simmons citrate agar (12). In all cases reported no strain of *M. osloensis* has been observed to be able to alkalize this medium. All strains of *M. osloensis* examined in the present report were streaked on Simmons citrate agar since it was reasoned that even strains that grow slowly on this medium might give some evidence of alkalization. The results listed in Table 2 show that most of the strains tested do not grow on Simmons citrate agar. Several strains appear to grow slowly on this medium with two strains showing weak alkalization after prolonged incubation. By contrast, strain 23 grows well on this plate giving strong alkalization in less than 24 h.

DISCUSSION

The suitability of genetic transformation as a means of establishing taxonomic relationships among various strains of *Moraxella* was first reported in 1962 by Bövre and Henriksen (11). Using interstrain transformation of streptomycin-resistance markers Bövre (9) was able to show that there appear to be several distinct groups of *Moraxella*, members of a particular group showing high ratios of inter- to intraspecies transformation (range, 0.3 to 1.0), the corresponding ratios for transformation between *Moraxella* strains from different groups being considerably lower (usually less than 10^{-4}).

In a transformation study of various strains originally designated *M. nonliquefaciens*, high transformation ratios (0.34 to 0.99) were found for 20 of the 22 strains investigated, ratios less than 2×10^{-5} being obtained for interstrain transformation between the remaining two strains and representatives of the major class of organisms (7). One of these unusual strains (strain 19116/51) was considered to possibly represent a new taxonomic group (7). In 1965

Bövre (8) showed that nine other independently isolated strains belong to the "19116/51" group since there were high ratios of inter- to intraspecies transformation of streptomycin resistance (range, 0.32 to 1.0) among the various members of this group. On the basis of these studies, Bövre and Henriksen (12) proposed that strains belonging to the "19116/51" group be designated *M. osloensis*. This suggestion necessitated a new, more restrictive, definition of the *M. nonliquefaciens* group (12). Unfortunately, the phenotypic properties used to distinguish strains of *M. osloensis* from strains of *M. nonliquefaciens* frequently overlap making it necessary to rely on the results of transformation studies for definitive diagnosis (12). Interstrain transformation of organisms now known to be *M. osloensis* has also been shown by Catlin and Cunningham (15).

Both *M. osloensis* and *M. nonliquefaciens* are aerobic, gram-negative, oxidase-positive, nonmotile, nonsporeforming coccobacilli which do not produce acid from hexoses. Unlike *M. nonliquefaciens*, *M. osloensis* can grow in Hugh and Leifson's medium (8, 12). Strains of *M. osloensis* are also somewhat more stable to heating than are strains of *M. nonliquefaciens* (12). The DNA base compositions of strains of *M. nonliquefaciens* range from 40 to 42 mole % guanine plus cytosine, whereas the corresponding range for strains of *M. osloensis* is 43 to 43.5 mole % guanine plus cytosine (12). It is quite clear that phenotypic characteristics alone cannot be used with certainty to identify strains of *M. osloensis*. Although strains of *M. osloensis* are said to grow on Simmons citrate medium without alkalization (12), the results of the present study (Table 2) reveal that only some strains are able to grow on this medium, a few of these giving rise to an alkaline reaction. It has been reported recently that only a maximum of 10% of *M. osloensis* strains grow in citrate media when several passages are required (H. Lautrop, personal communication cited in ref. 10).

TABLE 2. *Growth of strains of M. osloensis on Simmons citrate agar*

Type of growth	Strain no.
No growth	3, 6, 7, 8, 9, 14, 15, 17, 19, 21, 24, 28
Poor growth	4, 5, 10, 12[a], 13, 25[b], 26, 27
Good growth	23[c]

[a] Alkalization after 3 to 4 days.
[b] Alkalization after 10 days.
[c] Alkalization within 24 h.

The finding that one strain of *M. osloensis* (strain 2) is not, unlike the other strains studied, able to grow in simple mineral media supplemented with a single carbon source serves to emphasize the fact that nonfastidiousness cannot be taken as an absolute criterion for diagnosis of strains of *M. osloensis*. Furthermore, a new species, *M. urethralis*, has been described (32) which grows on a simple acetate- or hydroxy butyrate-mineral medium and is not genetically related to *M. osloensis*. Strain 45, originally described as *Mima polymorpha* var. *oxidans*, has been classified as *M. urethralis* (32). It is now clear that organisms formerly described as *Mima polymorpha* var. *oxidans* (2, 17, 23, 42) may, upon genetic analysis, prove to be strains of either *M. osloensis*, *M. nonliquefaciens*, or *M. urethralis*.

To date, streptomycin resistance has been the principal marker used in transformation studies of *Moraxella* strains. In order to perform transformation with this marker it is first necessary to isolate a streptomycin-resistant mutant of the strain under study and then prepare DNA from this mutant for transformation of another competent streptomycin-sensitive strain (6). Furthermore, the methods used for isolation of transforming DNA are quite time consuming and require rather large quantities of cells (6). The fact that most strains of *M. osloensis* grow in a simple mineral medium supplemented with a single carbon source, such as acetic or lactic acid, makes it possible to isolate auxotrophic mutants and use these as markers for transformation studies. The development of a simple and rapid procedure for the preparation of crude and sterile transforming DNA samples from a large number of bacterial strains has already proven useful in the development of a transformation assay for *Acinetobacter* strains (29).

In the present study this transformation assay has been modified for use in the routine diagnosis of strains of *M. osloensis*. It is generally acknowledged that genetic interaction, as evidenced by ready interstrain transformation, is among the best means of establishing taxonomic relatedness (28, 34, 36). This is particularly true when the characteristics transformed are nonribosomal auxotrophic markers. In order to transform a competent auxotroph to prototrophy with DNA from another strain it is essential that the base sequence of donor DNA in the region of the marker be nearly identical with that of recipient chromosomal DNA in the same region in order for recombination to take place (9, 25). Organisms that are genetically unrelated must have considerably different

DNA base sequences in the corresponding regions of their respective chromosomes since it has been well documented that in such cases there can be virtually no DNA-DNA hybridization (27, 28, 34, 36–38). For example, there is almost no homology between DNA species from *M. osloensis* and *Acinetobacter* (26). It has been shown, however, that ribosomal ribonucleic acid (rRNA)-DNA hybridization can occur using ribosomal RNA species from strains unrelated to those used as the source of DNA (26, 40, 47). This finding has led to the conclusion that the base sequences of ribosomal RNA species must be highly conserved in a wide variety of living forms, possibly because any extensive compositional changes in ribosomal components may result in poorly functioning ribosomes (18, 47). Although there is little or no measurable homology between DNA samples from *M. osloensis* and *Acinetobacter*, there is, nevertheless, good intergeneric rRNA-DNA homology for these two organisms (26).

Streptomycin resistance has been shown to result from mutational alteration of a ribosomal protein (41). Since the amino acid sequences of ribosomal proteins are also more conserved than the amino acid sequences of other proteins (18), the use of the streptomycin resistance marker in interstrain crosses may reveal distant relationships which might not otherwise be evident if less conserved genes, as represented by auxotrophic markers, were used in such crosses. The fact that DNA samples from all 27 strains of *M. osloensis* were able to readily transform four different unlinked auxotrophic markers of strain 23 in the present study must be taken as strong evidence for the close genetic relatedness of all these strains. It will be of interest to continue testing DNA samples from new isolates of *M. osloensis* for ability to transform several auxotrophic markers of strain 23 to determine whether strains have evolved in nature having extensive chromosomal alterations such that interstrain recombination of certain markers has become quantitatively less efficient. Such evolutionary changes have been observed in a study of several strains of *Acinetobacter* (E. Juni, unpublished data).

All strains of *M. osloensis* analyzed in this study were obtained from human materials. The natural sources for these organisms appear to be the genitourinary tract, spinal fluid, blood, the pleural cavity, and more rarely the nose and respiratory tract (12). Oxidase-positive moraxellas have never been isolated from soil or water (3). Relatively few reports have appeared specifically implicating *M. osloensis* in human disease. In all probability this is a result of difficulties frequently encountered in identification of *M. osloensis* in clinical laboratories. One report recently appeared in which an authenticated strain of *M. osloensis* was shown to be the causative agent of septic arthritis and vaginal discharge in a young girl (20). It is also possible that other infections caused by organisms described as *Mima polymorpha* var. *oxidans* may in fact be cases where *M. osloenis* is the infectious agent (5, 14, 19, 21, 22, 31, 33, 43, 45). Introduction of the transformation assay as a routine diagnostic procedure for unequivocal identification of strains of *M. osloensis*, as described in this report, should help in assessing the distribution and clinical significance of this organism.

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