Transforming Activities and Base Composition of Deoxyribonucleates from Strains of *Moraxella* and *Mima*

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SUMMARY

Fourteen strains of *Moraxella* and possibly allied organisms were studied: *M. liquefaciens*, *M. bovis*, *M. nonliquefaciens*, oxidase-positive and oxidase-negative strains of *Mima polymorpha* and *Herellea vaginicola*. Classical bacteriological characteristics, capacity to undergo genetic transformation (resistance to 500 µg. dihydrostreptomycin/ml.), and DNA base composition were examined. *Moraxella nonliquefaciens* and *Mima polymorpha* var. *oxidans* were transformable (2 strains at frequencies of 1-4%). Attempts to elicit transformation of the remaining organisms were unsuccessful. The ability of DNA preparations from *M. polymorpha* var. *oxidans* to elicit high frequencies of transformation of *Moraxella nonliquefaciens*, and *vice versa*, indicated their close relationship. Moreover, their DNA base contents were indistinguishable (about 44.4% guanine + cytosine, 6 strains). The G + C contents averaged 43.1% for 3 oxidase-negative *Mima polymorpha* strains and 39.8% for 2 *Herellea vaginicola* strains.

INTRODUCTION

Transformation of *Moraxella* was described by Bøvre & Henriksen (1962). Our own studies of this group were initiated earlier as a result of finding that genetic transformation of *Neisseria catarrhalis* was elicited by DNA preparations from certain strains of the so-called Mima-*Herellea* group. The development of a reciprocal transformation system became essential. Furthermore, elucidation of possible interrelations of these 'Neisseria-like' organisms which might be gained from transformation studies was clearly needed.

Mima polymorpha was described briefly by De Bord in 1939, and later somewhat more completely (De Bord, 1942) as a non-motile, Gram-negative pleomorphic rod which tended on solid media to exhibit diplococcal forms morphologically indistinguishable from *Neisseria gonorrhoeae*. As with *N. catarrhalis*, carbohydrates were not attacked. Though many isolates were oxidase-negative, some were oxidase-positive (*M. polymorpha* var. *oxidans*). De Bord (1942) also proposed the designation *Herellea vaginicola* for another morphologically similar form which was oxidase-negative, and produced acid without gas from glucose and from some other carbohydrates.

Henriksen (1952) and Murray & Truant (1954) considered that *Mima polymorpha* var. *oxidans* might be the same as *Moraxella nonliquefaciens*. Serological analyses provided conflicting evidence (Cary, 1961; Mitchell & Burrell, 1964). Reviewing the standing in nomenclature of subdivisions within the tribe Mimeae, Henriksen (1963)

concluded that the name *Mima polymorpha* var. *oxidans* is illegitimate because it is a later synonym of *Moraxella nonliquefaciens*, that *Herellea vaginicola* is a *nomen dubium*, and that the tribe Mimeae has no place in the taxonomic system. On the other hand, Lindberg & Moschides (1962) proposed that the species 'vaginicola' and 'polymorpha' be combined in the genus *Mima*. Problems associated with these controversial 'Neisseria-like' bacilli have been reviewed by Rosebury (1962), and various solutions of the problem of their taxonomic position have been proposed (e.g. Piéchaud, 1961; Lautrop, 1961; Stenzel & Mannheim, 1968). An *ad hoc* committee has been organized to study the nomenclature and classification of 'Bacterium anitratum', the moraxellas, the Mimeae and allied organisms (Lautrop, 1963).

When strains of *Mima polymorpha* var. *oxidans* obtained locally were found to undergo transformation, authentic strains of *Moraxella nonliquefaciens* and other possibly related organisms were obtained for comparison. Characteristics of the particular strains, their genetic competence, and the base composition of their DNA were investigated. A transformation procedure was developed which provided a genetic approach to problems concerning relationships of these enigmatic bacteria. Evidence obtained bears on two specific questions. Is there a genetic basis for the opinion that strains of *Mima polymorpha* var. *oxidans* and *Moraxella nonliquefaciens* are similar? Are strains of *Mima polymorpha* var. *oxidans* closely related to *M. polymorpha* or to *Herellea vaginicola*?

METHODS

Of the 14 strains investigated, 6 were classified as species of *Moraxella*: *M. liquefaciens* (NCTC 7911) obtained from the National Collection of Type Cultures, London; *M. bovis* (NCTC 9426) obtained from Professor S. D. Henriksen, Oslo; and 4 strains of *M. nonliquefaciens* also from Professor Henriksen (11865/52, 7146/51, 18522/51, 19116/51; hereafter the last two numbers will be deleted from these strain designations). Two strains designated *Mima polymorpha* var. *oxidans* were received from the Milwaukee Health Department (MHD 2/10 from a gonorrhoea-like syndrome; MHD 307 from an ulcer which developed after vein stripping). Two strains designated *M. polymorpha* were from the American Type Culture Collection, Washington, D.C. (ATCC 10973 which, although oxidase-positive, did not carry the varietal designation; and ATCC 9957). Two strains of *M. polymorpha* were obtained locally from specimens of spinal fluid (Raschig, non-fatal meningitis; MHD 12/13, fatal meningitis). Two strains designated *Herellea vaginicola* were received from the Milwaukee Health Department (MHD 11/30, throat culture; MHD 305, a stock culture obtained for reference purposes 5 yr. earlier from the Communicable Disease Center, U.S. Public Health Service).

When each strain was received, it was subcultured on heart infusion + yeast-extract agar with supplements (HIY-1 agar, Catlin & Cunningham, 1961) and on blood agar. Cultures were examined for homogeneity, and at least two typical colonies were separately subcultured. To decrease population changes thereafter, subcultures were made by picking ten to twenty young isolated typical colonies.

Storage of cultures and media have been described in the preceding communication (Catlin & Cunningham, 1964). Differential media were prepared in quantities large enough for testing all strains at one time. In addition, Loeffler medium (slopes,

Baltimore Biological Laboratory) was streaked, incubated at 35° for 4 weeks, and examined periodically for evidence of digestion of the inspissated serum.

Previously described methods (Catlin & Cunningham, 1964) were used for preparing transforming DNA, for chemical determination of base composition, and for transformation tests. Minor modifications which were found to increase transformation frequencies of the moraxellas will be described in the Results section.

Dihydrostreptomycin sulphate (Squibb) will be referred to as DHS, and strains genetically resistant to its antibacterial action as *str-r*. Spontaneous mutants resistant to at least 500 µg. DHS/ml. were obtained without difficulty from all but one of the parent strains. A resistant mutant of *Moraxella nonliquefaciens* 19116 was not recovered from any of twelve independent flask cultures. However, *str-r* strains were readily obtained by transformation. One of these, designated 19116 *str-r* 18522, was elicited by treatment of the parent strain 19116 with DNA from *M. nonliquefaciens* 18522 *str-r*, and was recovered from agar containing DHS/500 µg. ml. The identity of all *str-r* mutants and transformants from which DNA was extracted was confirmed by microscopic appearance, typical oxidase reaction, antibiotic susceptibilities, and typical reactions in selected media (glucose, gelatin). All characteristics examined were identical with those of the parent except for streptomycin resistance and, occasionally, morphology.

The temperature of circulating water baths (Bronwill and Magni Whirl) was controlled to $\pm 0.1^\circ$. Petri dish cultures for transformation experiments were placed in humidified air incubators (New Brunswick model G27 with circulating fans, and National water-jacketed) and were not stacked until they warmed; thereafter their temperature varied less than 0.5°. The growth response of *Moraxella nonliquefaciens* on agar medium was related in an ill-defined way to the moisture content, as noted by Henriksen (1952). Therefore, cultures of recipient organisms were incubated in jars on moist bibulous paper.

RESULTS

Bacteriological characteristics

All strains were Gram-negative rods which tended to occur in pairs and in short chains. They were non-motile and did not form spores. Some of the paired rods observed in young cultures on moist HIY-1 agar were so broad and short that they resembled *Neisseria*, as has been observed repeatedly (e.g. discussion in Rosebury, 1962). Three of the strains of *Moraxella nonliquefaciens* received from Professor Henriksen were examined earlier by Murray & Traunt (1954). They emphasized a useful morphological difference between the genera *Moraxella* and *Neisseria*, namely, that cellular division of moraxellas proceeds in a single plane, with resulting chain formation, whereas the division of neisseriae occurs in two planes.

The reactions of each strain were examined at least twice with similar results (Table 1). *Herellea vaginicola* strain MHD 11/30 exhibited the oxidative attack of carbohydrates typical of this disputed group. The carbohydrates tested were incorporated in the basal media of Elrod & Braun and Hugh & Leifson previously described (Catlin & Cunningham, 1964). Acid was produced from glucose, galactose, rhamnose, arabinose, xylose and lactose; whereas maltose, sucrose and fructose media remained alkaline, as did the carbohydrate-free controls.

Table 1. *Characteristics of the organisms*

Reaction or test*	<i>Moraxella</i> sp.														<i>Mima polymorpha</i>														<i>Herellea vaginicola</i>																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
	<i>nonliquefaciens</i> 11865				<i>nonliquefaciens</i> 7146				<i>nonliquefaciens</i> 18522				<i>nonliquefaciens</i> 19116				Var. <i>oxidans</i> MHD 2/10				Var. <i>oxidans</i> MHD 307				ATCC 9957				Raschig				MHD 12/13				MHD 11/30				MHD 305																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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* In addition, all strains gave the following reactions: catalase production +, indole production —, nitrite disappearance —. See text for details and for carbohydrates tested. Symbols: +, positive result; —, negative result; Sl, slight reduction; S, susceptible; R, resistant.

Strain MHD 305 of *Herellea vaginicola*, however, was atypical in failing to acidify any of the carbohydrate-containing media, including the supposedly decisive 10 % lactose agar slope (Schaub & Hauber, 1948; Garrison & Dodd, 1962). This strain, originally a typical representative of *H. vaginicola*, had been maintained for 5 yr. at room temperature as a sealed stab culture and transferred annually. A subculture, recently obtained for the present work, showed mucoid and smooth types of colonies, which were alike in the reactions examined. The smooth type was selected for transformation experiments.

Moraxella nonliquefaciens strain 11865 on HIY-1 agar produced punctiform colonies in 48 hr. Growth was greatly improved by addition to the medium of 10 % (v/v) serum (horse or calf) or 0.2 % (v/v) Tween 80 (Mann Research Laboratories, New York). A loopful of Tween 80 (diluted 1/5, neutralized, autoclaved) was added to each differential medium to stimulate growth. A diffusible yellowish pigment developed in areas of luxuriant growth on HIY-1 agar containing serum or Tween. Pigment production was not detected with any of the other strains.

Transformation

Preliminary examination. All strains were examined for capacity to become transformed to high-degree streptomycin resistance. Organisms grown at 35° (and in most cases also at 30°) were exposed to preparations of DNA (final concentration 25 µg./ml.) extracted from *str-r* strains corresponding to the recipient. Results obtained with each of the 14 strains are included in Table 1. Negative results signify merely that transformation was not detected under the conditions employed.

The transformation method was modified in various ways as required by special features of the strains. Horse serum (10 %, v/v) was added to the culture medium for *Moraxella nonliquefaciens* strain 11865. Three of the strains, *M. nonliquefaciens* 18522 and 7146 and *Herellea vaginicola* MHD 11/30, were resistant to low concentrations of DHS (Table 1), and exhibited high frequencies of spontaneous mutation conferring resistance to DHS 500 µg./ml. The parent strains were screened and, from each, a colony was selected which gave the lowest background of spontaneous *str-r* mutants. These were used as recipients in transformation tests carried out with DHS 500 and 1000 µg./ml. However, evidence of transformation was obtained only for strain 18522; even in this case, only about an eightfold difference was found between numbers of *str-r* colonies on test plates and on corresponding controls. The strain appeared, therefore, to be unsatisfactory for quantitative work involving the *str-r* marker.

The 4 remaining transformable strains, *Moraxella nonliquefaciens* 19116 and all 3 oxidase-positive strains designated *Mima polymorpha*, were examined under various conditions as a basis for developing reliable transformation test methods. The temperature and time of incubation exerted profound influences on transformation to DHS resistance. Four consecutive steps of the over-all process were examined: (i) incubation of recipients to obtain the maximum proportion of competent cells; (ii) incubation of organisms with DNA; (iii) incubation required to achieve phenotypic expression, that is, the development of a physiological state of in-susceptibility to the challenge dose of the antibiotic; (iv) incubation to allow colony-formation by the genetically transformed organisms.

Characteristics of the oxidase-positive Mima polymorpha strains. Transformation reactions involving the oxidase-positive *Mima polymorpha* strains ATCC 10973 and MHD 807 were similar in most respects. Transformation frequencies, which were relatively low for both strains, were increased three- to five-fold by carrying out the first two incubation steps at 30° rather than at 36°. This is shown for ATCC 10973 in Table 2.

Table 2. *Influence of incubation temperature and DNA concentration on % transformation (resistance to DHS 500 µg./ml.) of Mima polymorpha ATCC 10973*

Incubation temperatures for		% transformation elicited by homologous DNA	
Growth of recipient organisms (14 hr)	DNA treatment (30 min.)*	50 µg./ml.	10 µg./ml.
30°	30°	0.00290	0.00087
30°	36°	0.00202	0.00053
33.5°	30°	0.00141	0.00027
33.5°	36°	0.00099	0.00028
36°	30°	0.00143	0.00031
36°	36°	0.00100	0.00023

* Reaction terminated by addition of deoxyribonuclease. Subsequent incubation, 36° for all plates (steps iii and iv, described in text).

Table 3. *Influence of temperature and time of incubation on % transformation (resistance to DHS 500 µg./ml.) of Mima polymorpha var. oxidans MHD 2/10*

Incubation			Time before addition of DHS (hr)	% transformation elicited by homologous DNA (25 µg./ml.)
Growth of recipient organisms (12 hr)	Temperatures for DNA treatment (30 min.)	Phenotypic expression*		
30°	30°	30°	5	0.98
			6	1.19
			7	1.38
			8	1.37
		36°	5	1.24
			6	1.44
			7	1.54
			8	1.52
36°	36°	36°	5	1.19
			6	1.24
			7	1.32
			8	1.40

* Subsequent incubation, 36° for all plates (step iv, text).

Transformation of MHD 2/10, which occurred at a far higher frequency, was less strongly influenced by differences of incubation temperature within this range. Nevertheless, 30° for the first two incubations, followed by 36°, gave the highest frequency. This is illustrated in Table 3, which shows in addition that a period of 7 hr intervening between initiation of exposure to DNA and application of anti-biotic was adequate for maximum expression of the altered phenotype.

The following standardized modifications of transformation procedure were

adopted, therefore, for all 3 oxidase-positive strains of *Mima polymorpha*. (i) Recipient organisms were incubated at 30° for 11–12·5 hr on HIY-1 agar in a humid atmosphere. Organisms taken from agar at this time were suspended in supplemented heart infusion broth (HI-1; Catlin & Cunningham, 1961) at 30° and were immediately exposed to DNA. (ii) Reaction mixtures were incubated at 30°, usually for 30 min. before addition of deoxyribonuclease. (iii) Samples were plated promptly and incubated at 35°. A total period of 7 hr was allowed between the time of mixing recipients with DNA and time of removal of plates from 35° for overlaying DHS-containing agar. (iv) Plates were incubated thereafter at 35° for 4–6 days before counting transformant colonies.

Characteristics of transformation of Moraxella nonliquefaciens strain 19116. In exploratory tests with homologous DNA this strain appeared to be little affected by differences of incubation temperatures, and results were consistent within and between independent tests. However, equivocal results were obtained in tests of strain 19116 with DNA preparations from *Mima polymorpha* strains ATCC 10973 and MHD 307. Variations of numbers of colonies on replicate transformant plates and irregularities of distribution and size of colonies suggested that minor differences of antibiotic concentration had a marked effect. Accordingly, a study was made of the degrees of resistance conferred by various transforming DNA preparations.

Table 4. *Influence of concentration of DHS on % transformation of the oxidase-positive strains*

DNA preparation (25 µg./ml.)	DHS (µg./ ml.)*	% transformation of			
		<i>Moraxella nonlique- faciens</i> 19116	<i>Mima polymorpha</i>		
			MHD 307	ATCC 10973	MHD 2/10
(1) <i>Mima polymorpha</i>	25	0·670	0·0063	0·0011	5·31
MHD 2/10 <i>str-r</i>	600	0·605	0·0062	0·0011	5·15
(2) <i>Moraxella nonliquefaciens</i>	25	1·898	0·0075	0·0017	2·51
19116 <i>str-r</i> 18522	600	1·650	0·0070	0·0012	2·40
(3) <i>Mima polymorpha</i>	25	0·685	0·0299	0·0018	2·76
MHD 307 <i>str-r</i>	600	0·014	0·0281	0·0023	2·82
(4) <i>Mima polymorpha</i>	25	0·670	0·0161	0·0057	1·74
ATCC 10973 <i>str-r</i>	600	0·009	0·0170	0·0070	1·75
(5) <i>Moraxella nonliquefaciens</i>	25	1·260	0·0214	0·0024	(No test)
19116 <i>str-r</i> 307	600	0·019	0·0202	0·0019	(No test)
(6) <i>Moraxella nonliquefaciens</i>	25	1·400	0·0117	0·0024	1·58
19116 <i>str-r</i> 10973	600	0·035	0·0114	0·0028	1·66

* Transformant assay plates after receiving antibiotic challenge dose were incubated at 35° (step iv; other details in text).

Although all *str-r* mutants were obtained by selective growth in an environment containing 500 µg. DHS/ml., all of those examined in this connexion were resistant to at least 600 µg. DHS/ml. This higher concentration was used for the experiments reported in Tables 4 and 5 because it more clearly revealed a difference between *Moraxella nonliquefaciens* 19116 and the 3 strains of *Mima polymorpha*. A satisfactory low concentration for transformation tests with these strains was found to

be 25 μ g. DHS/ml. Resistant mutants rarely appeared on control plates containing either concentration of DHS.

The results given in Table 4 were obtained using the standard transformation procedure devised for *Mima polymorpha*. The antibiotic concentration within the range examined did not significantly affect the numbers of transformants produced by 3 of the 4 recipient strains. Only *Moraxella nonliquefaciens* 19116 showed significant differences, and only in those tests where the *str-r* factor originated in strains MHD 307 or ATCC 10973 (Table 4, DNAs 3–6). Transformant colonies elicited by treatment of strain 19116 with DNA preparations 3 and 4 were far more numerous in agar containing 25 μ g. DHS/ml. than 600 μ g. DHS/ml. The discrepancy was about the same in the tests with DNA preparations 5 and 6, which were extracted from transformants of strain 19116 treated in a previous experiment with DNA preparations 3 and 4 respectively. The strains had been isolated at 35° as single transformant colonies in agar containing 1000 μ g. DHS/ml. Microscopic observation revealed that the cells were highly pleomorphic when subcultured at 35° on medium either with or without DHS. Both morphology and growth were more typical when these transformant strains were incubated at 30°.

On the other hand, transformants of *Moraxella nonliquefaciens* 19116 elicited by treatment with DNA preparations 1 and 2 (Table 4) exhibited typical cellular morphology when cultivated at 35° (or 30°) on medium containing either 25 or 600 μ g. DHS/ml. These observations of the various transformants of strain 19116 suggested that the aberrant response of some might be related to the temperature of incubation of the transformation assay plates.

In an exploratory test, strain 19116 recipient organisms were treated with DNA from *Mima polymorpha* ATCC 10973, all incubation steps (i–iv, above) being carried out at 30°. After 14 hr incubation of transformant plates (step iv), minute colonies were visible in medium containing 600 μ g. DHS/ml. At this time half of these plates were transferred to 37° for a 6 day period of additional incubation; none of these colonies increased in diameter, whereas colonies on the plates retained at 30° grew to typical size.

A quantitative study was made of the influence of varying the temperature of incubation for three steps of the standard transformation test procedure. Results given in Table 5 clearly show that when the *str-r* marker of DNA from *Mima polymorpha* ATCC 10973 *str-r* was introduced into *Moraxella nonliquefaciens* 19116, the temperature of incubation of the transformant assay plates determined whether or not transformants could grow into countable colonies in the presence of DHS. (In this experiment, the incubation temperature was the same for steps iii and iv: either 29° or 36°). At 29° approximately the same number of colonies developed in medium containing 25 μ g. DHS/ml. and 600 μ g. DHS/ml. Incubation at 36° (steps iii and iv, Table 5) prevented the development of most of the colonies in agar containing either DHS concentration. However, in other experiments involving the same recipient and donor DNA (e.g. Table 4, DNA prep. 4) which were carried out at 35°, many more colonies developed in medium containing 25 μ g. DHS/ml. than 600 μ g. DHS/ml.

When DNA from *Moraxella nonliquefaciens* 19116 *str-r* 18522 served to introduce the *str-r* factor into strain 19116 recipients, the temperature at which transformant assay plates were incubated had little effect on the concentration of DHS tolerated.

Also, a higher frequency of transformation was elicited, as expected for homologous DNA. For purposes of comparison, Table 5 gives results also of a similar experiment with recipient cells of *Mima polymorpha* ATCC 10973 (used at a 200 times higher concentration). In confirmation of previous results (Table 4), the recovery of *str-r* transformants was not significantly affected by concentration of DHS.

Table 5. *Influence of incubation temperatures on the tolerance of transformants to DHS*

Temperature of incubation for				Transformation of			
				<i>Moraxella nonliquefaciens</i> 19116*		<i>Mima polymorpha</i> ATCC 10973†	
				By DNA preparation from		By DNA preparation from	
Trans-formant assay plates (steps iii, iv)	Growth of recipient organisms (step i)	Mixture: cells + DNA (25 µg./ml.) (step ii)	Conc. DHS (µg./ml.)	ATCC 10973 <i>str-r</i>	19116 <i>str-r</i> 18522	ATCC 10973 <i>str-r</i>	19116 <i>str-r</i> 18522
29°	29°	29°	25	219	458	74	7
			600	174	441	92	2
29°	29°	36°	25	296	680	28	4
			600	265	697	37	2
29°	36°	29°	25	190	397	34	6
			600	189	362	47	4
29°	36°	36°	25	151	285	21	5
			600	168	299	21	2
36°	29°	29°	25	9	495	166	24
			600	1	386	145	17
36°	29°	36°	25	3	661	48	10
			600	0	655	40	14
36°	36°	29°	25	1	426	66	12
			600	0	318	63	12
36°	36°	36°	25	5	336	28	6
			600	0	291	38	1

* Values: number of transformant colonies/ 2.5×10^4 DNA-treated recipient organisms.

† Values: number of transformant colonies/ 5.0×10^6 DNA-treated recipient organisms.

In subsequent transformation tests of *Moraxella nonliquefaciens* 19116 (Table 6) all incubation steps (i-iv above) were carried out at 30°.

Influence of DNA concentration and time of exposure. Figures 1 and 2 show the response of recipient organisms of *Mima polymorpha* var. *oxidans* MHD 2/10 (curve 1, both Figs.) and *Moraxella nonliquefaciens* 19116 (curves 2, 3) to DNA preparations from the corresponding *str-r* strains. These tests were carried out (using incubation temperatures established for *Mima polymorpha* strains: 30°-30°-35°-35°) during attempts to elucidate the aberrant response of strain 19116 (Table 4). As in previous tests of *Moraxella nonliquefaciens* strain 19116 treated with DNA from 19116 *str-r* 10973, the recovery of transformants cultivated at 35° in the presence of 600 µg. DHS/ml. (values not plotted) was low and somewhat erratic compared with % transformation obtained using the lower challenge dose (curve 2, both Figs.).

The % transformation of both recipients increased linearly with increasing concentration of DNA over a range of at least 1000-fold (Fig. 1). Notable was the finding (see also in Table 2) that a high DNA concentration (about 50 $\mu\text{g./ml.}$) was required for maximum yields of transformants. In contrast, the 'saturating' concentration of transforming DNA was only about 0.1 $\mu\text{g. DNA/ml.}$ for a strain of *Streptococcus* (*Diplococcus*) *pneumoniae* (Hotchkiss, 1957), and about 5 $\mu\text{g. DNA/ml.}$ for 2 strains of *Neisseria meningitidis* (Catlin, 1960).

Fig. 1. Percentage transformation as a function of concentration of DNA. Exposure time, 30 min. Curve 1, *Mima polymorpha* var. *oxidans* MHD 2/10 treated with DNA from strain MHD 2/10 *str-r*; challenge dose, 600 $\mu\text{g. DHS/ml.}$ Curve 2, *Moraxella nonliquefaciens* 19116 treated with DNA from strain 19116 *str-r* 10973; challenge dose, 25 $\mu\text{g. DHS/ml.}$ Curve 3, *M. nonliquefaciens* 19116 treated with DNA from strain 19116 *str-r* 18522; challenge dose, 25 $\mu\text{g. DHS/ml.}$ and 600 $\mu\text{g. DHS/ml.}$

Figure 2 shows that % transformation of both strains increased rapidly with time of exposure to DNA. Transformation events were initiated in about 0.5 % of the cells of the two recipient populations within the first 5 min. Continued exposure to the DNA for an additional 25 min. effected only about a fourfold increase of % transformation.

Tests of DNAs from the various organisms. DNA preparations from each of the 14 strains were examined in at least three separate transformation experiments with each of the four recipients. Table 6 gives results of a single experiment for each

DNA preparations from 3 of the strains designated *Moraxella nonliquefaciens* and from all 3 oxidase-positive *Mima polymorpha* strains exhibited high transforming activity for two of the recipients (*Moraxella nonliquefaciens* 19116 and *Mima polymorpha* MHD 2/10). On the other hand, DNAs from *Moraxella liquefaciens* and *M. bovis*, and from the oxidase-negative *Mima polymorpha* and *Herellea vaginicola* strains had practically no transforming activity for any of the four recipients in any of the tests.

Fig. 2. Percentage transformation as a function of time of exposure of recipient organisms to 50 μ g. DNA/ml. Exposure was terminated at a given time by diluting an aliquot of the reaction mixture in broth containing deoxyribonuclease. Curves 1, 2, 3 as for Fig. 1.

Results of tests of *Moraxella nonliquefaciens* 19116 and of *Mima polymorpha* var. *oxidans* MHD 2/10 were reproducible; % transformation obtained in repeated tests varied only by about a factor of 2. In contrast, uncontrollable variation by as much as 10-fold was encountered in tests of the other 2 recipients. For example, compare the selected data given in Table 4 with those of Table 6 for *M. polymorpha* strain MHD 307. In spite of differences of % transformation of these recipients, however, the relative transforming activities of the various DNA preparations were fairly similar in repeated tests.

Table 6. *Transforming activity and base composition of DNA preparations*

Source of DNA*	% transformation Oxidase-positive recipient strain				Mole % guanine + cytosine†
	<i>Moraxella nonliquefaciens</i> 19116	<i>Mima polymorpha</i> MHD 2/10	<i>Mima polymorpha</i> MHD 307	<i>Mima polymorpha</i> ATCC 10973	
<i>Moraxella</i>					
<i>liquefaciens</i>	NCTC 7911	< 0.00006	0.00008	0.00001	< 0.00001
<i>bovis</i>	NCTC 9426	< 0.00006	0.00004	< 0.00001	< 0.00001
<i>nonliquefaciens</i>	11865	< 0.00006	0.00004	< 0.00001	< 0.00001
	7146	1.453	2.292	0.00102	0.00040
	18522	1.041	1.784	< 0.00001	< 0.00001
	19116	2.024	1.860	0.00155	0.00058
<i>Mima polymorpha</i>					
(oxidase +)	MHD 2/10	0.370	3.550	0.00057	0.00025
(oxidase +)	MHD 307	0.714	2.075	0.00320	0.00117
(oxidase +)	ATCC 10973	0.775	1.388	0.00214	0.00338
(oxidase -)	ATCC 9957	< 0.00006	0.00004	< 0.00001	< 0.00001
(oxidase -)	Raschig	< 0.00006	< 0.00004	0.00001	< 0.00001
(oxidase -)	MHD 12/13	< 0.00006	< 0.00004	< 0.00001	< 0.00001
<i>Herellea vaginicola</i>					
	MHD 11/30	< 0.00006	< 0.00004	< 0.00001	< 0.00001
	MHD 305	< 0.00006	< 0.00004	< 0.00001	< 0.00001
					39.9
					39.8, 39.7

* Preparations were from spontaneous *str-r* mutants of all strains, except *Moraxella nonliquefaciens* 19116 *str-r* 18522 (described in Methods). Treatment with DNA solutions (25 µg./ml.) at 30° for 30 min.; challenge dose dihydrostreptomycin 500 µg./ml.

† Values are results of separate determinations.

Base composition of the DNAs

DNA preparations which were used in the transformation experiments were analysed for base content. The results of separate determinations are given in Table 6. The values of % guanine + cytosine were practically the same for all *Moraxella* strains studied, except *M. nonliquefaciens* 11865. The wide divergence of values obtained for this organism suggests that it was incorrectly named. The oxidase-positive *Mima polymorpha* strains were indistinguishable from *Moraxella nonliquefaciens* by the criterion of % G + C. However, the oxidase-negative *Mima* strains form a second, distinct group; and the 2 *Herellea* strains form a third.

DISCUSSION

Our results independently confirm the finding of Bövre & Henriksen (1962) that *Moraxella nonliquefaciens* is transformable. It is of interest that transformation of different recently isolated strains can be obtained by using somewhat different techniques. Like Bövre & Henriksen, we failed to demonstrate transformation of *M. liquefaciens* strain NCTC 7911.

Genetic evidence obtained in this study corroborates the opinion of Henriksen (1952) and Murray & Truant (1954) that the organism named *Mima polymorpha* var. *oxidans* by De Bord (1942) is, in fact, *Moraxella nonliquefaciens* (Henriksen, 1960, 1963). Similarities of the base composition and transforming activity of DNA preparations from strains designated *M. nonliquefaciens* 7146, 18522 and 19116 and *Mima polymorpha* var. *oxidans* MHD 2/10, MHD 307 and ATCC 10973 establish their close genetic relationship. Accordingly, strains MHD 2/10, MHD 307, and ATCC 10973 will be designated *Moraxella nonliquefaciens* in future references to them.

On the other hand, no genetic relationship was found between *Moraxella nonliquefaciens* and either *Mima polymorpha* or *Herellea vaginicola*. The guanine + cytosine contents of DNA preparations from the 3 oxidase-negative *Mima* strains (43.1 %) and the 2 *Herellea* strains (39.8 %) were distinctly lower than the value characteristic of all the moraxellae (about 44.4 %). Furthermore, none of these DNA preparations elicited transformation of competent strains of *Moraxella nonliquefaciens*.

Incidental findings illustrate certain obstacles which might be encountered in any such genetic study. They emphasize the importance of using a number of recipients of each bacterial species, and preferably more than a single genetic marker, if the analyses are to be applied to taxonomic problems. Thus, (i) a strain selected for investigation may be named incorrectly. A DNA preparation from the strain designated *Moraxella nonliquefaciens* 11865 not only failed to elicit transformation of any of 4 transformable strains of this species, but its G + C content (42.5 %) was lower than that of any of the 8 moraxellas.

(ii) A strain may be unsuitable for use as recipient. It may not undergo detectable transformation or, as in the case of *Moraxella nonliquefaciens* strains 7146 and 18522, high background levels of spontaneous mutation of a given marker may interfere.

(iii) DNA from a given donor may elicit unequal frequencies of transformation of various recipients of the same species in respect to any given genetic marker being investigated. Two separate DNA preparations from *Moraxella nonliquefaciens* 18522

failed to elicit detectable transformation of strains ATCC 10973 and MHD 307 in any test, although they exhibited high transforming activity for strains 19116 and MHD 2/10 (Table 6).

(iv) Environmental conditions which are optimal for transformation in one instance may be deleterious in a second. Test conditions which were suitable for detecting transformation of *Moraxella nonliquefaciens* 19116 following exposure to certain DNA preparations (from *str-r* strains of 19116, 7146, 18522, and MHD 2/10) were found to be unsuitable for detecting transformation of the same recipient following treatment with other DNA preparations (from *str-r* strains of ATCC 10973 and MHD 307). In the latter case, transformants developed a temperature-sensitive condition in which, within given limits, tolerance to dihydrostreptomycin was inversely related to the temperature of incubation (Table 5). The genotype of the recipient was important, as well as that of the donor. Thus, introduced into strain 19116, the *str-r* factor of either ATCC 10973 or of MHD 307 was essentially lethal for transformants cultivated at 36–37° in a medium containing 600 µg. DHS/ml. Introduced into strain MHD 2/10, the factor gave rise to transformants capable of apparently normal growth under the same conditions (Table 4). These findings illustrate the practical necessity for investigating the effect of environmental conditions on the response of each recipient strain.

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