A NEW MORAXELLA SPECIES, MORAXELLA OSLOENSIS,
AND A REVISED DESCRIPTION OF
MORAXELLA NONLIQUEFACIENS

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ABSTRACT. Organisms corresponding to the
description of Moraxella nonliquefaciens can
be subdivided into several taxa. A subgroup
with comparatively fastidious growth re-
quirements is retained in the species M. non-
liquefaciens, and a revised description of
this species is given. The strain 4663/62 is
proposed as a neotype strain. A second, more
robust subgroup is described and given the
name M. osloensis. The strain A1920 is des-
ignated as the type strain. The use in taxon-
omy of such criteria as DNA base composition
and compatibility in transformation is briefly
discussed.

The results of recent studies of a collection of Moraxella
strains by transformation experiments, by analysis of the
DNA base composition, and by more conventional methods
(Bøvre 1964, 1965, 1967) indicate that organisms possessing
the characteristics hitherto considered typical of M. non-
liquefaciens, can be subdivided into several subgroups,
which may deserve recognition as separate taxa. Two of
these subgroups, one of which is the 752/52 group mentioned
in a previous paper (Bøvre 1967), have not yet been charac-
terized in sufficient detail to be described. The other two,
however, are sufficiently well defined and homogeneous
to be described as separate species. One of these is the
19116/51 group studied in previous papers (Bøvre 1965,
1967), whereas the second, for reasons which will be given
in the discussion, is considered to represent M. nonlique-
faciens.
MATERIALS AND METHODS

Most of the strains and methods used have been presented in papers already referred to, likewise most of the results. Some supplementary studies were made with 11 strains each of the 19116/51 group and of *M. nonliquefaciens* (as defined in this paper) according to the following methods:

**Heat resistance:** A "Heto" water bath, which showed very stable temperatures, was used at four-degree intervals from 45°C to 65°C. Racks of culture tubes with c. 4 ml of glucose broth were preincubated at the desired temperature for at least 30 min. before the start of each experiment. The temperature inside a control tube was checked with a thermometer. Heavy suspensions in glucose broth of the strains to be tested were made from 24 h. blood agar cultures. The suspensions were sufficiently heavy to produce an easily visible turbidity when two drops were added to a glucose broth tube. Suspensions used in the same set of experiments were of approximately equal turbidity without being accurately standardized. The culture tubes were inoculated with two drops of suspension with Pasteur pipettes. Great care had to be exercised to avoid depositing any organisms on the inside walls of the culture tubes, as the results otherwise were erratic. Tubes were removed from the water bath after 10, 20 and 30 min., rapidly cooled in running tap water and put in an incubator at 33°C for 24 h. One loopful from each tube was inoculated on blood agar plates, which were checked for growth after 24 h. at 33°C in a moist atmosphere.

**Growth in the defined medium of Audureau:** The medium was prepared as described (Audureau 1940) with 1% ethanol as source of carbon. Tubes of this medium were inoculated with the strains by means of a loop and incubated at 33°C. The tubes were checked daily for visible growth, and when growth appeared, one loopful was transferred to a new tube and so on for a total of 5 transfers. Tubes which showed no sign of growth were incubated for 4 days before being discarded.

**Longevity:** Blood agar plates were inoculated and incubated at 33°C for 24 h. and then kept at room temperature. With intervals of some days subcultures were made on new blood agar plates, as long as viable organisms were found to remain in the original culture.
RESULTS

Table 1 shows the results of heat resistance tests. The strains of the 19116/51 group show a uniformly higher heat resistance than the nonliquefaciens strains. The latter are killed by exposures to 49°C for 10 min., or less, whereas heat exposures to 53°C for 20 min. up to 65°C for 10 min. are necessary to kill different strains of the 19116/51 group. There is no overlapping between the two collections of strains tested, but the interval between the heat exposures needed to kill the most resistant strains of M. nonliquefaciens and the least resistant strain of the 19116/51 group respectively is small. Furthermore, it has been our experience that it is not easy to obtain exactly reproducible results by this technique. This test, therefore, although it reveals a clear difference between the two kinds of organism, may be difficult to use in routine diagnosis.

Table 1. Heat Sensitivity of Moraxella Strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Species</th>
<th>Survives:</th>
<th>Killed by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3828/60, 672/58</td>
<td>n</td>
<td>45°, 20'</td>
<td>45°, 30'</td>
</tr>
<tr>
<td>4663/62, 2770/60, 4863/62</td>
<td>n</td>
<td>45°, 30'</td>
<td>49°, 10'</td>
</tr>
<tr>
<td>4378/62, 826/61, 3326/66</td>
<td>n</td>
<td>45°, 30'</td>
<td>53°, 10'</td>
</tr>
<tr>
<td>2918/66, 7784, 13334/62</td>
<td>n</td>
<td>45°, 30'</td>
<td>57°, 10'</td>
</tr>
<tr>
<td>4608</td>
<td>o</td>
<td>53°, 10'</td>
<td>53°, 20'</td>
</tr>
<tr>
<td>9893</td>
<td>o</td>
<td>53°, 10'</td>
<td>57°, 20'</td>
</tr>
<tr>
<td>8292</td>
<td>o</td>
<td>57°, 10'</td>
<td>57°, 20'</td>
</tr>
<tr>
<td>5893, 8134, A1920</td>
<td>o</td>
<td>57°, 20'</td>
<td>61°, 10'</td>
</tr>
<tr>
<td>B 8198, 5873</td>
<td>o</td>
<td>61°, 10'</td>
<td>61°, 20'</td>
</tr>
<tr>
<td>10973, 5718</td>
<td>o</td>
<td>61°, 20'</td>
<td>61°, 30'</td>
</tr>
<tr>
<td>19116/51</td>
<td>o</td>
<td>61°, 30'</td>
<td>65°, 10'</td>
</tr>
</tbody>
</table>

x : n = Moraxella nonliquefaciens
$\dagger$ : o = Moraxella osloensis

None of the strains of M. nonliquefaciens showed any growth in Audureau's medium. Nine strains of the 19116/51 group showed growth for 5 transfers. Two of the strains seemed to grow only for 2 transfers.

All strains studied remained alive on blood agar cultures at room temperature for at least 27 days. All strains were nonviable after 40 days.
The results reported in the papers already referred to and in this paper show that the two kinds of organism differ from one another in the following characters: ability to grow in Hugh and Leifson's medium, in Koser's citrate medium, in Simmons' citrate agar and in Audureau's medium with ethanol as source of carbon, in heat resistance, in DNA base composition and in genetic compatibility as measured by streptomycin resistance transformation.

We consider these differences sufficient to separate the two groups of strains into two species, and we choose the specific epithet osloensis for the 19116/51 group.

**Revised description of Moraxella nonliquefaciens**

**Micromorphology:** Plump rods measuring c. 1 to 2 μ x 1 to several μ, with obtuse, often nearly square ends, often very short diplobacilli, occasionally occurring in short chains. Diplococcus-like forms are frequent. May show variations in size and shape, often with giant forms and filamentous forms, especially in old cultures. Gram-negative with some tendency to resist decolorization and to irregular staining. Nonmotile, no endospores. May be encapsulated.

**Colonies:** On blood agar a little more than 1 mm in diameter after 24 h., low convex or nearly flat, semitranslucent to opaque, often with a domed opaque center and a flat, translucent periphery. Unpigmented, soft or friable consistency. No haemolysis. Some strains are strongly mucoid with large, domed, shiny and viscous colonies.

**Relation to oxygen:** Strict aerobe.

**Temperature:** Slight growth at room temperature, optimal growth at c. 33°C to 37°C, but growth at the latter temperature sometimes fails or is inhibited if the atmosphere is dry. This is particularly characteristic of the mucoid strains. Sensitive to heat, being killed by exposure to 49°C for 10 min. or less.

**Growth requirements:** Comparatively fastidious. Growth may fail in some plain media, e.g. the M.R.-V.P. medium, and regularly fails in Hugh and Leifson's medium, Koser's and Simmons' citrate media and Audureau's medium with ethanol as carbon source.

**Longevity:** Keeps alive on blood agar plates at room temperature for at least 27 days.

**Biochemical reactions:** Catalase reaction positive. Oxidase reaction positive with dimethyl- and tetramethylparaphenylenediamine. Nitrates reduced to nitrites. Acid and
gas not produced from the usual sugars. Gelatin and serum not liquefied. Indol and \( \text{H}_2\text{S} \) not produced. Some strains split urea immediately after isolation, but this property is lost in subculture.

**Antibiotic sensitivity**: Uniformly sensitive to penicillin, streptomycin, chloramphenicol, tetracyclines and macrolides.

**Habitat**: Respiratory tract of man. Very common in the nose, where it is most easily detected. Apparently rare in other localities.

**Pathogenicity**: Usually nonpathogenic. May occasionally cause minor infections (sinusitis). The mucoid form apparently occurs nearly exclusively—or exclusively—in the nose or bronchi in cases of ozaena, but the relation to the etiology of this disease is extremely doubtful.

**DNA base composition**: Percentage of guanine + cytosine 40-42, most often 41 (CsCl buoyant density method).

**Genetic compatibilities**: All strains mutually compatible in streptomycin resistance transformation with ratios of inter-strain to intra-strain transformants close to 1 (0.34 to 0.99). Less, but distinctly compatible with *M. lacunata*, *M. liquefaciens* and *M. bovis* (ratios from 1 x 10\(^{-3}\) to 7.5 x 10\(^{-3}\)). Some degree of compatibility with *Neisseria ovis* (maximum ratio 1 x 10\(^{-4}\)) and *N. catarrhalis*. Nearly incompatible with *M. osloensis*.

**Description of Moraxella osloensis nov. sp.**

**Micromorphology**: Often as for *M. nonliquefaciens*. Some strains show a more fusiform or lanceolate shape of the cells, others show a preponderance of diplococcal cells. Nonmotile, nonspore-producing, nonencapsulated.

**Colonies**: Usually slightly smaller than those of *M. nonliquefaciens* with circular periphery and even, glistening surface. Soft or coherent consistency. Unpigmented. Non-phaemolytic.

**Relation to oxygen**: Strict aerobe.

**Temperature**: Grows within the same temperature range as *M. nonliquefaciens*, but is distinctly more resistant to exposure to high temperatures, being killed by exposures from 53°C for 20 min. to 65°C for 10 min.

**Growth requirements**: Less fastidious than *M. nonliquefaciens* with better growth on plain media. Grows on Hugh and Leifson's medium, in Koser's and Simmons' citrate media (without alkalizing the latter) and in Audureau's
medium with ethanol as source of carbon. Growth in the three latter media may be somewhat irregular, and repetition of tests is advisable.

**Longevity:** Keeps alive on blood agar plates at room temperature for at least 27 days.

**Biochemical reactions:** Nitrates may or may not be reduced to nitrites. No urease activity, except for irregular reactions which may be observed in fresh isolates. Other biochemical reactions as for *M. nonliquefaciens.*

**Antibiotic sensitivity:** Usually as for *M. nonliquefaciens.*

**Habitat:** Uncertain. Strains have been isolated from genito-urinary tract, blood, spinal fluid, chest fluid, and nose, but seems to be rare in respiratory tract.

**Pathogenicity:** Some strains have been isolated from serious pathological conditions in man, but it is probable that the organism usually is nonpathogenic.

**DNA base composition:** Percentage of guanine + cytosine 43 to 43. 5 (CsCl buoyant density method).

**Genetic compatibilities:** Very high mutual compatibility in streptomycin resistance transformation between strains (ratios of inter-strain to intra-strain transformants 0.32 to 1.0). Very slight compatibilities with *M. lacunata, M. liquefaciens, M. bovis* and *M. nonliquefaciens* (maximum ratio 4 x 10^-5), and with Neisseria species.

**DISCUSSION**

None of the strains studied by Scarlett (1916) or by Oliver and Wherry (1921) exist, and nobody can be sure whether the strains studied by these authors belonged to one or the other of the two species, *M. nonliquefaciens* and *M. osloensis.* *M. nonliquefaciens,* as defined by us, is by far the most common *Moraxella* species in the respiratory tract, and for this reason it seems most probable that it was this species that the early authors studied as their strains were isolated from eyes (Scarlett) and from sputum (Oliver and Wherry). Another reason for letting the fastidious species retain the epithet *nonliquefaciens,* is the fact that this species shows the greatest relationship to *M. lacunata, M. liquefaciens* and *M. bovis,* and is most likely to remain in the genus *Moraxella.* In view of the apparently much more distant relationship of *M. osloensis* to these 4 species, it seems possible that one may later decide to remove it to a different genus. For these reasons we prefer to remove the non-fastidious group of strains to a new species.
Specific epithets are most often based upon some characteristic property of the species. But, in this case the differences between the two species in conventional criteria are rather vague and slight, and there is a possibility that if one of these differences were used as the basis of the epithet, the property in question might later be found to be shared by other *Moraxella* species which remain to be described. Also some of the organisms, whose relationship to *Moraxella* is under discussion ("*Moraxella* lwoffii, "*Moraxella* glucidolytica") share these properties with *M. osloensis*. For these reasons we found it to be preferable to base the specific epithet upon the place where the first description originated, Oslo, and decided to choose the specific epithet *osloensis*.

It is probable that some of the strains studied by one of us (Henriksen 1947) and described under the, at least partly, illegitimate name, *Diplobacillus variabilis*, may have been *M. osloensis*, but, since this is uncertain, we feel that the former name can have no standing in nomenclature. Also, the specific epithet is unsuitable, as variability seems to be a common characteristic of *Moraxella* species.

For diagnostic purposes the behaviour in Hugh and Leifson's medium appears most useful. Cultivation in citrate media and Audureau's medium also mostly gives useful results, but results may sometimes be variable, and may have to be checked by repeated tests. It seems possible that in some instances transformation experiments might be necessary to establish a diagnosis beyond doubt.

The differences in genetic compatibility as measured by transformation of streptomycin resistance and in DNA base composition appear to be most significant, and without these differences the creation of a new species might have appeared very questionable. But in our opinion these differences indicate that the two taxa should at least be separated in different species. Indeed, one might discuss whether the two species should be placed in the same genus. For the time being we feel that it is most sensible to keep the two species in the same genus.

At the present time it is difficult to define bacterial taxa in terms of nucleic acid composition and genetic compatibility, but it seems beyond doubt that DNA base composition data and appropriately measured transformation compatibilities are of a more fundamental biological nature than most individual phenotypic expressions used in ordinary diagnostic work. The practical difficulties involved in the
use of the former criteria will preclude their use in routine identification except for application in special cases. However, the genetic tests may prove to be of great value in the further development and evaluation of conventional criteria for diagnostic purposes (see Bövre 1967).

Each species should have a type or neotype strain. For *M. osloensis* we select the strain A1920. This is a typical and transformable strain, which was isolated by the late Miss Elizabeth O. King, Atlanta, from cerebrospinal fluid some years ago.

For *M. nonliquefaciens* Sneath and Skerman (1966) listed the strain 18522/51, a strain first isolated by one of us, as a paratype. According to the transformation studies of Catlin and Cunningham (1964) this strain is compatible with 19116/51, and consequently must belong to *M. osloensis*. The choice of the strain 18522/51 as a type strain for *M. nonliquefaciens* is therefore incompatible with the classification proposed by us. According to the rules of nomenclature, when a species is divided in two, the old name follows the species which includes the type, which would necessitate that the 19116/51 group retained the specific epithet *nonliquefaciens*.

For reasons which we have already given, we feel that this would be undesirable, and that another strain should be selected as a neotype strain of *M. nonliquefaciens*. We propose that the strain 4663/62 be selected as neotype strain of *M. nonliquefaciens*. This is a strain that has been studied very extensively (see Bövre 1964, 1967), and which has been found to be quite typical in all respects. It is transformable. It was isolated from a nose culture in 1962.

Sneath and Skerman (1966) state: "No taxonomic or nomenclatural changes are proposed in this article" (l.c. p. 13), and it is clear that their mention of the strain 18522/51 does not constitute a formal proposal of a neotype. This matter, therefore, need only be referred to the Judicial Commission if objection is raised to our proposal.

Both strains proposed as type strain and neotype strain have been deposited in the National Collection of Type Cultures (where the number NCTC 10464 is being allotted to strain 4663/62, and the number NCTC 10465 to the strain A1920), and in the American Type Culture Collection where the numbers 19975 and 19976 have been assigned.

New cultural, biochemical and serological properties may be discovered in the type strains and their relatives and added to those listed. It is not expected that such
characteristics will prove consistent in all strains of the two species, in view of the present knowledge of bacterial variation and evolution. Selected cases of deviation may be evaluated by means of genetic compatibility tests with the two transformable type strains or other proved members of the species. This will allow for a controlled flexibility of the definition of the two species and prevent undue subdivision.

REFERENCES