New *Moraxella* Strain Isolated from Angular Conjunctivitis

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A new *Moraxella* strain was isolated from angular conjunctivitis. It is characterized by the ability to produce acid from several carbohydrates, by its ability to produce indole, and by a lack of catalase activity.

In the course of an investigation on the frequency of *Moraxella* species on healthy and inflamed conjunctival membranes with the selective medium for *Moraxella*, a strain was isolated from angular conjunctivitis which differed in many respects from the known *Moraxella* species.

**MATERIALS AND METHODS**

The methods followed for morphological, cultural, and biochemical identification were essentially those recommended for *Moraxella* (2). Locomotion was studied with a modified moist chamber technique (9), by using the medium of Halvorsen (5) with 10% (v/v) horse serum. To study cell habit and the cytoplasmic structure, cells were fixed in situ through the agar with Bouin's fluid (8), stained with 0.01% thionine, and mounted in water. The capsule stain of White (11) was used. Heat resistance was tested with the capillary tube method in a water bath. All biochemical tests were carried out with and without the addition of 10% (v/v) horse serum to the media. A heavy inoculum and aeration were used in fluid media where appropriate. Gelatinase activity was also studied by incorporating 3% gelatin in the *Moraxella* base medium (10). The oxidase reaction was performed by smearing the culture across filter paper impregnated with a 1% aqueous solution of dimethyl-p-phenylene-diamine hydrochloride and 0.5% tetramethyl-p-phenylene-diamine hydrochloride. The catalase reaction was done by running 1 ml of 3% hydrogen peroxide down on the colonies on a serum-meat infusion-agar slope and by mixing washed packed bacteria with 3% hydrogen peroxide. One per cent carbohydrates were incorporated in asces-agar slants. Hemolysis was studied in 5% blood-agar media of 5 mm thickness and on yeast extract-agar with 1- and 2-mm 5% blood-agar overlays, and the media were incubated aerobically and under increased CO₂ pressure. Incubation temperatures were 33 and 37°C. Deoxyribonucleic acid (DNA) extraction was done by the technique of Bøvre et al. (1). The guanine plus cytosine (GC) contents were determined by measurements of buoyant density in CsCl gradients.

**RESULTS**

Morphological, cultural, and biochemical characteristics. Microscopically the cells appeared to be straight rods with rounded ends, 2 to 5 μm in length and 0.50 to 1 μm in breadth, usually arranged in pairs and during the phase of active growth often in chains (Fig. 1). On prolonged incubation, pleomorphism of the cells was observed. Among the aberrant forms, ovoid bodies and filaments were found which usually did not stain well. Circular or oval areas in the cytoplasm that did not stain with Gram stain were usually prominent in these cells. Flagellar motility was not observed, but the peculiar locomotion usually found with *Moraxella* and commonly referred to as “gliding” motility was present. The cells were gram-negative, but a number of cells were slow in decolorizing. Capsules were present (Fig. 2).

After 24 hr of incubation on blood-agar, the colony size was on the order of 0.1 to 0.5 mm in diameter, circular in shape, with a low hemispherical or low conical elevation. After 72 hr of incubation, the diameter increased to 1 to 1.5 mm in diameter; isolated colonies were often twice as large. The colony tended to differentiate into a raised slightly opaque center and a flattened translucent periphery. The structure was amorphous to finely granular, and on horse blood-agar medium it was occasionally coarsely granular. Silvery opalescence was noted in some instances on sheep, rabbit, and human blood-agar medium. The colony was smooth and glistening, but sometimes the surface had a delicate silver-beaten appearance. The consistency of the colonies was butyrous. The colonies were easily emulsified. The emulsion had a tendency to autoagglutinate in saline.

No beta hemolysis was found on conventional human, sheep, horse, or rabbit blood-agar media.
Some beta hemolysis was observed, however, after 3 days of incubation on the 1-mm rabbit blood-agar overlay medium. Alpha hemolysis was found on sheep blood-agar and sometimes on horse blood-agar media. The strain resisted a temperature of 45°C for 60 min. For temperatures of 46.5, 48, 49.5, and 51°C, resistance was 45, 25, 10, and 5 min, respectively. At 52.5°C, the strain was killed within 5 min. The organisms survived for 5 to 9 days at 37°C, for 11 to 21 days at 33°C, for 14 to 21 days at 20°C, and for 32 to 40 days at 4°C. There was no anaerobic growth. Increased CO₂ pressure did not seem to improve growth of the strain.

The temperature for optimal growth appeared to be between 33 and 37°C. No growth was initiated at 20°C. A humid atmosphere enhanced growth markedly. There was no growth in Koser's citrate and Audureau's medium. In the medium of Hugh and Leifson, there was slight growth with weak oxidative acid production from glucose. No growth was observed on MacConkey agar, Endo agar, and Desoxycholate Citrate agar.

Growth on peptone media was scarce; growth on all simple media was improved by the addition of serum, ascites, or blood. There was no apparent need for hematin and nicotinamide adenine dinucleotide.

Very light-brown pigment was formed on Loeffler's medium. The catalase reaction was negative; the oxidase reaction was positive with tetramethyl-p-phenylenediamine hydrochloride. With the dimethyl-p-phenylenediamine reagent, the reaction was dubious when using bacteria incubated for 24 hr. With bacteria incubated for 48 hr, the reaction was positive within 5 min.

Acid was generally formed from carbohydrates slowly. Incubation at 33°C usually gave better results. Strong acid production was observed from glucose, mannose, fructose, and saccharose. Starch, dextrin, and maltose were also attacked but with less acid production. A trace of acid was found with galactose, trehalose, and xylose. No acid was produced from adonitol, arabinose, cellulobiose, dulcitol, glycerol, inulin, lactose, mannitol, melibiose, melitose, mesoinositol, raffinose, rhamnose, salicin, and sorbitol. Ten percent lactose was not attacked.

There was no change in litmus milk. Indole was produced. The methyl red test was negative, and acetyl-methylcarbinol was not produced. No hydrogen sulfide was produced. Nitrate and nitrite were not reduced. No urease activity was found. No ammonia was formed. Methylene blue was not reduced. There was very weak gelatinase activity. Proteolytic activity on Loeffler's medium was also weak. No lecithin-vitellin reaction was found. Starch was slightly hydrolyzed as indicated by the iodine test. Arginine, hippurate, and esculin were not hydrolyzed. Tyrosine and xanthine were not decomposed. Casein was slightly digested. Minimal digestion was found on...
Dorset's egg medium. Malonate was not utilized. No $\beta$-galactosidase activity was found. The phosphatase test was positive. A trace reaction with the gluconate oxidation test was found.

Oxidative deamination of DL-norleucine and L-phenylalanine was found. Reactions with L-leucine, DL-methionine, and L-tryptophan were dubious. No oxidative deamination of L-histidine, DL-isoleucine, and DL-norvaline was observed.

The organisms were highly sensitive to penicillin and its derivatives. With an inoculum of $4 \times 10^8$ viable organisms on media with 0.02 unit penicillin per ml after 4 days of incubation, 100% reduction was found. Sensitivity was also found for chloramphenicol, streptomycin, oxytetracycline, erythromycin, and many other commonly used antibiotics.

Decarboxylase reactions with L-arginine·HCl, L-lysine·HCl, DL-ornithine·HCl, and glutamic acid were negative.

Some of the differential characteristics of the cultural and biochemical tests of the carbohydrate-splitting Moraxella species are presented in Table 1. The buoyant density of the DNA of the strain in CsCl was 1.709 g/cm$^3$. The mole percentage of GC calculated from this result was 49, by using the simplified formula: $\% (G + C) = 1,000 \times (\rho - 1.660)$, where $\rho$ is the buoyant density of DNA (grams per cubic centimeter) measured versus the density of Micrococcus lysodeikticus DNA assumed to be 1.737 g/cm$^3$.

**DISCUSSION**

Three carbohydrate-splitting Moraxella species have been described (3, 4, 7).

Our strain has overall morphological, cultural, and biochemical characteristics in common with these and the other Moraxella species but differs from all known species in its ability to produce indole, with the possible exception of *M. amylolytica*. The original text (3) states that *M. amylolytica* is able to produce indigo base substance, which we interpret as being indole-positive.

With regard to *M. kingii*, our strain also differs in its ability to produce acid from saccharose and fructose. It further differs in its oxidative deamination of phenylalanine, its proteolytic activity and the character of its hemolytic activity, its growth requirements, and its heat resistance. Also, the mole percentage of GC of *M. kingii* is 44.5, whereas for our strain this value is 49.

The taxonomic position of *M. saccharolytica* is somewhat uncertain (6). At any rate, there are significant differences between this species and our strain. *M. saccharolytica* is catalase-positive, produces acid from glycerol, and hydrolyzes esculin. The methyl red test is positive. Serum is not liquefied. It further differs from our strain in its ability to grow in Audureau's defined medium, ability to grow at 22°C as well as at 37°C, ability to grow on simple media, and its resistance to streptomycin, chloramphenicol, and tetracycline.

With regard to *M. amylolytica*, our strain differs notably by its lack of catalase activity, its ability to produce acid from saccharose and fructose, its ability to liquefy serum, and in its inability to reduce nitrate.

It would appear that there are sufficient differences in biochemical characteristics from all known Moraxella species to consider our strain a separate species.

The strain has been deposited in the American Type Culture Collection (strain number 25869) and in the National Collection of Type Cultures (Great Britain; strain number 10717).

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**TABLE 1. Differential characteristics of the carbohydrate-splitting Moraxella species**

| Determination               | *M. saccharolytica* | *M. amylolytica* | *M. kingii* | New Moraxella strain |
|-----------------------------|---------------------|------------------|-------------|----------------------|
| Catalase production        | (+)                 | (+)              |             | (−)                  |
| Serum liquefaction          | (+)                 | (+)              | (−)         | (+)                  |
| Gelatin liquefaction        | (+)                 | (+)              | (−)         | (+)                  |
| β Hemolysis                 | (−)                 | (−)              | (+)         | (−)                  |
| Indole production           | (−)                 | (−)              | (+)         | (−)                  |
| Acid from saccharose        | (+)                 | (+)              | (−)         | (+)                  |
| Acid from fructose          | (+)                 | (+)              | (−)         | (+)                  |
| Acid from glycerol          | (+)                 | (−)              | (−)         | (+)                  |

*a* Weak reaction.

*b* β Hemolysis only with the 1-mm rabbit blood agar overlay medium.
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