

An Unclassified Gram-Negative Rod Isolated From the Pharynx on Thayer-Martin Medium (Selective Agar)

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Received for publication 12 July 1972

An oxidase-positive, small gram-negative rod was isolated on Thayer-Martin medium (TM) inoculated with pharyngeal swabs obtained during surveys to detect *Neisseria* carriers. In one survey, this organism was isolated from 48% of the subjects, and 50 or more colonies were present on the majority of the primary isolation plates. Other characteristics of the organism, which has been given the provisional designation "TM-1," include: delayed production (2 to 10 days) of acid from glucose, formation of gas during nitrate reduction, and the frequent formation of "pits" in the agar surface. On TM, nonpitting colonies of TM-1 are morphologically similar to colonies of *Neisseria gonorrhoeae* and *N. lactamica*. Comparison of the characteristics of TM-1 strains with other similar fastidious gram-negative organisms encountered in clinical laboratories indicates that TM-1 is a distinct species. Further studies are required before proper taxonomic placement can be made.

During a survey to determine the pharyngeal carrier rates of *Neisseria meningitidis* and *N. lactamica* in healthy adult employees at the Center for Disease Control, it was noted that many specimens yielded oxidase-positive colonies of small gram-negative rods on Thayer-Martin medium (TM). Detailed morphological and biochemical studies were made on strains isolated from four individuals. These organisms appeared to be distinct from any species we could recognize, and they were designated "TM-1." In a later survey, pharyngeal swabs were obtained from children and adults at a children's home, and TM-1 strains were isolated from 48% of the subjects. The majority of the TM plates used had more than 50 colonies of this organism.

Although our laboratory has not received any identical cultures isolated from infectious processes, we feel that attention should be called to this organism and its characteristics because of its ability to grow on TM and its positive oxidase reaction. These features could lead to a false presumptive identification as *N. gonorrhoeae* or *N. meningitidis*.

MATERIALS AND METHODS

Population groups surveyed. Pharyngeal speci-

mens from three population groups were examined. Group I specimens came from 207 healthy adult employees at the Center for Disease Control. Group II was comprised of 400 specimens from adults and children surveyed as contacts in an outbreak of meningococcal meningitis. Group III consisted of 108 specimens from children and adults of a children's home who were surveyed after two cases of meningococcal meningitis occurred in the home.

Isolation media. TM (13) consisting of Mueller-Hinton agar with vancomycin, colistin, and nystatin (VCN) inhibitors was used for all the primary isolations. In addition, blood agar plates (BAP) consisting of heart infusion agar (HIA) with 5% rabbit blood were used for primary isolation in group I. The swabs were gently rubbed over one area (approximately one-third of the plate) immediately after the specimens were obtained, and before incubation the plates were cross-streaked with a loop for isolation. Plates were incubated at 35 C in a candle jar and examined at 24 and 48 hr. Colonies were picked and inoculated on BAP and incubated in a candle jar at 35 C.

TM consisting of chocolate agar (TM-chocolate), which was prepared from a GC base, hemoglobin, isovitalex, and VCN, was used for some comparative studies after isolation, as was transgrow medium (9).

Morphological, cultural, and biochemical tests. Hemolytic reactions on BAP were recorded after incubation for 18 to 24 hr. The oxidase test was performed by applying 0.5% aqueous solution of

tetramethyl-*p*-phenylene-diamine dihydrochloride directly to colonies on 18 to 24 hr BAP. Acid production from carbohydrates was observed in fermentation broth base (1) with Andrade's indicator and in cystine Trypticase agar (CTA). One drop of serum from a capillary pipette was added to 3 ml of fermentation broth to test some strains when growth was inadequate in the unenriched medium. Hydrolysis of esculin was determined by the method of Sneath (12). Tests for arginine dihydrolation and for lysine and ornithine decarboxylation were performed by Møller's method (10). The remainder of the biochemical methods employed were outlined by King (7, 8). These included the use of nutrient agar, litmus milk, oxidative versus fermentative (OF) medium, MacConkey agar, 10% glucose agar, Christensen's urea, gelatin, Simmon's citrate agar, cetrimide agar, triple sugar iron agar (TSI), methyl red Voges-Proskauer broth, and tryptone glucose yeast extract agar. The catalase, oxidase, nitrate, indole, H₂S, and motility tests were used. The usual method of inoculating OF media is to stab growth from a 24-hr HIA slant four times to approximately 0.5-inch depth with

an inoculating needle into 6 ml of medium in a tube (16 by 125 mm). In addition to using petrolatum-sealed and -unsealed King (8) medium and Hugh and Leifson (6) OF medium inoculated in the usual manner, we also tested strains in tubes (13 by 100 mm) containing 1 ml of King OF medium, Hugh and Leifson OF medium, CTA, fermentation broth, or fermentation basal medium (11) containing 1% glucose. Inoculum consisted of 0.1 ml of a pool of organisms washed from 48-hr HIA slants. Each HIA slant was washed with 0.1 ml of distilled water. Sealed and unsealed control tubes, without carbohydrates, were used for each determination.

RESULTS

Micromorphology. The gram stains of TM-1 organisms showed moderate to deep gram-negative staining. There were cocci, short rods (ca. 0.4 μ m by 1 to 3 μ m) and occasional long slender rods (ca. 0.4 μ m by 4 to 6 μ m) with rounded ends (Fig. 1).

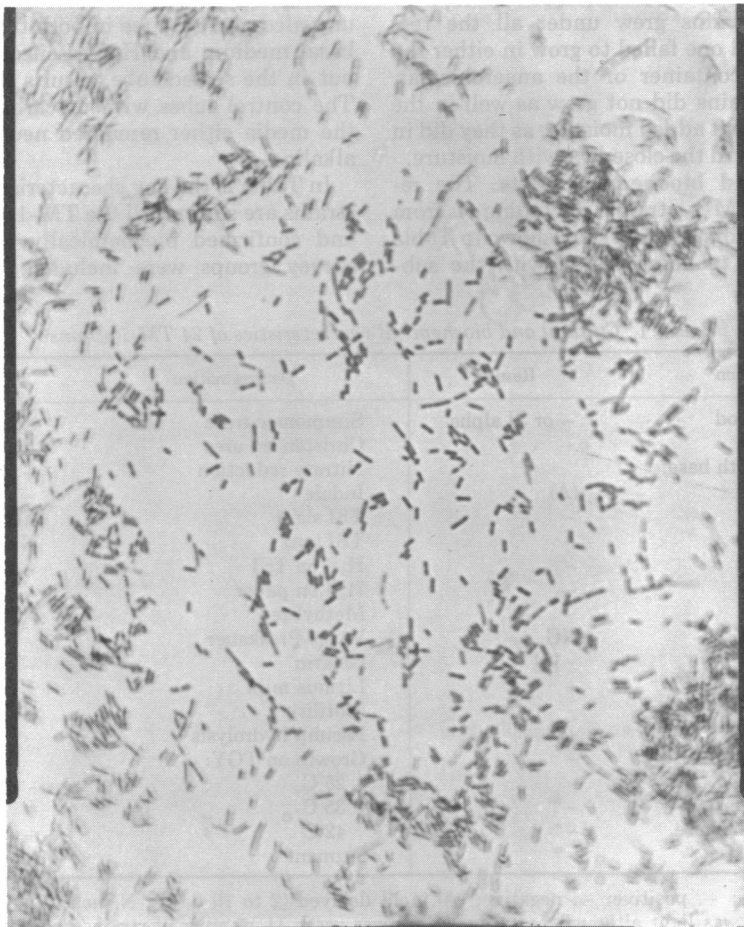


FIG. 1. TM-1 strain 167, gram-stained, blood agar. ($\times 1,125$).

Colonial morphology. After 24 hr on blood agar, colonies were ≤ 0.5 mm in diameter, circular, flat to low convex, and translucent to semi-translucent. Many TM-1 colonies pitted (corroded) the agar surface; some strains formed only nonpitting-type colonies, whereas others had colonies of both types. A pitting and a nonpitting colony from three strains were transferred seven times. Each transfer of the pitting colonies gave rise to both pitting and nonpitting colonies. Pitting colonies were in the majority. The nonpitting substrains remained stable through the seven transfers. At 24 and 48 hr, colonies grown on TM were comparable in appearance to those grown on blood agar, but on TM-chocolate, colonies were larger (0.5 to 1.5 mm) and more opaque.

Oxygen requirements. Four TM-1 strains were tested for growth in an open container in the presence of air, in an anaerobic jar, in a candle jar, in a closed jar with an open tube of water, and in a closed jar without added moisture. Three strains grew under all the test conditions, and one failed to grow in either the open aerobic container or the anaerobic jar. Two of the strains did not grow as well in the closed jar without added moisture as they did in the candle jar and the closed jar with moisture.

Cultural and biochemical tests. The results on 24 TM-1 strains, including 4 from group I and 4 from group III, are shown in Table 1. In addition to being tested with the sub-

strates listed, eight strains were tested in the fermentation broth with glycogen, erythritol, melibiose, melezitose, starch, glycerol, salicin, L-arabinose, adonitol, dulcitol, galactose, levulose, mannose, L-rhamnose, trehalose, raffinose, sorbitol, inositol, cellobiose, inulin, and dextrin. No acid was produced from these additional substrates except for a delayed weak acid reaction of three strains in galactose.

Attempts to determine whether TM-1 strains utilize glucose oxidatively or fermentatively by the use of OF medium inoculated in the usual manner (described in Methods) were unsuccessful because the organisms failed to grow or grew poorly in the medium. Further tests with three strains were made by heavily inoculating small volumes (1 ml) of several different media. After incubation for 1 week, acid was produced only in the unsealed glucose tube in fermentation broth and CTA medium, but was produced in both the unsealed and petrolatum-sealed glucose tube in King OF. Acid was produced in the unsealed glucose tube in both the fermentation basal medium and Hugh-Leifson OF medium, but in the sealed tube, results were equivocal. The control tubes without carbohydrate in all the media either remained neutral or became alkaline.

In Table 2, the key characteristics of 93 TM-1 strains are shown. All the TM-1 strains isolated and confirmed biochemically from the three survey groups were included. Four of these

TABLE 1. *Cultural and biochemical characteristics of 24 TM-1 strains^a*

Determination	Results	Determination	Results
Hemolysis on blood	- or Sl alpha	Simmons citrate	-
Gas from glucose	-	Christensen urea	-
Fermentation broth base:		Nitrate reduction	+, Gas
Glucose	(A)	Indole	-
Xylose	-	TSI slant	Alk [3]
Mannitol	-	TSI butt	N
Lactose	-	H ₂ S on TSI	-
Sucrose	-	H ₂ S on paper	+ [5]
Maltose	-	Methyl red	-*
10% glucose slant	NG	Voges-Proskauer	-*
Catalase	- [3]	Gelatin	-
Oxidase	+	Litmus milk	-
Growth on:		Motility	-
Nutrient agar	+	Esculin hydrolysis	-
MacConkey	-	Growth on TGY:	
Cetrimide	-	25 C	- [9]
Lysine decarboxylase	-*	35 C	+
Ornithine decarboxylase	-*	42 C	+ [10]
Arginine dihydrolase	-*	Pigment	-

^a Abbreviations: +, positive; -, negative; (A), acid delayed (2 to 10 days); N, neutral; *, test definitely negative, growth was light although heavy inoculum was used; [], number of strains deviating from the reported reaction; Alk, alkaline; Sl, slight; NG, no growth.

TABLE 2. Key characteristics of 93 TM-1 strains^a

Determination	Results	Determination	Results
Fermentation broth base:		Nitrate reduction	+, Gas
Glucose	(A)	TSI slant	Alk or N
Xylose	-	TSI butt	N or Alk
Mannitol	-	Indole	-
Lactose	-	Growth on:	
Sucrose	-	MacConkey	-
Maltose	-	Thayer-Martin (TM)	+
Catalase	- [6]	TM-chocolate	+
Oxidase	+		

^a Abbreviations: +, positive; -, negative; (A), acid delayed (2 to 10 days); Alk, alkaline; N, neutral; [], number of strains deviating from the reported reactions.

strains were from group I, 22 were from group II, and 52 were from group III.

In addition to the four strains from group I which were confirmed biochemically, 38 specimens had oxidase-positive, small gram-negative rods of the colonial morphology of TM-1 strains but were not studied further.

The original TM plates from group III were examined in an effort to estimate the number of TM-1 colonies (Table 3).

In Table 4, some characteristics of TM-1 organisms are compared to those of three *Neisseria* species. All four species grow on TM, TM-chocolate, and transgrow medium. A comparison of the colonial morphology of TM-1 nonpitting strains and of the strains of *Neisseria* indicated that TM-1 strains are very similar to some strains of *N. gonorrhoeae* and *N. lactamica* on all three of the above listed media. The colonies of *N. meningitidis* strains are usually larger, but otherwise are similar to nonpitting strains of TM-1 colonies on the same three media.

DISCUSSION

Before encountering TM-1 organisms on TM from several *Neisseria* surveys, we had seen only three of these cultures in our laboratory. All of these organisms came from human pharyngeal sources, with no indication that they were from disease processes or that a selective medium had been used for their isolation. In one survey in which TM and BAP were used, the TM-1 strains were isolated only from the TM plates. This indicated that their presence was more likely to be detected when a selective medium was used. Recently, two strains which were reported to have been isolated on TM during surveys have been referred to our laboratory.

Specimens often have many TM-1 organisms on initial isolation (Table 3). The morphology of

the nonpitting colonies of some strains of TM-1 on TM, TM-chocolate, or transgrow and their positive oxidase reaction may cause them to be confused with *N. gonorrhoeae* and *N. lactamica*. However, TM-1 strains can be differentiated from strains of *Neisseria* species on the basis of gram-stain morphology. The gram stain, therefore, is important in the characterization of organisms on these selective media.

All the TM-1 strains from humans that we

TABLE 3. Number of colonies of TM-1 on Thayer-Martin plates inoculated with pharyngeal swabs (group III)

No. of colonies on plate ^a	No. of plates ^a
5-9	1
10-49	10
50-99	14
≥100	21

^a Colonies could not be counted on 6 of 52 plates because of overgrowth by spreading organisms.

TABLE 4. Some characteristics of TM-1 and *Neisseria* species^a

Characteristics	TM-1	<i>N. gonorrhoeae</i>	<i>N. meningitidis</i>	<i>N. lactamica</i>
Growth on TM, TM-chocolate and transgrow	+	+	+	+
Oxidase	+	+	+	+
Gram-stain morphology	Coccoid to short rods	Diplococci	Diplococci	Diplococci
CTA glucose	N	A(N)	A(Alk)	A
CTA maltose	N	Alk(N)	A(Alk)	A
CTA lactose	N	Alk(N)	Alk	A
Growth on nutrient agar (35 C)	+	-	-(+)	+ or -

^a Abbreviations: +, positive; -, negative; N, neutral; + or -, majority of strains positive; A, acid; Alk, alkaline; (), rare but possible reactions.

studied were from pharyngeal sources except for one from urine. One strain came from the pharynx of a chimpanzee. We have not attempted to isolate TM-1 strains from genitourinary and rectal areas.

The biochemical characteristics of the TM-1 organisms are similar to those of a group of organisms designated in our laboratory as "EF-4" (eugonic fermenter). The EF-4 organisms (14), isolated primarily from human wounds caused by animal bites, can be distinguished by the following: positive catalase and arginine dihydrolase reactions, an acid reaction in the butt of a TSI slant, colonies which are larger (1-2 mm) and more opaque, and no growth on TM.

The TM-1 organisms have similarities to several other species of organisms that form pits in the agar surface. The "corroding" colonies of TM-1 are similar in appearance and consistency to colonies described by Henriksen (2) for *Moraxella kingii* and facultative anaerobic strains of *Bacteroides corrodens*.

Henriksen (4) described *M. kingii* as coccoid to medium-long diplobacteria with square ends, in pairs or chains, and *B. corrodens* as slender, mostly short rods (0.25 to 0.5 by 1-2 μ m), with rounded ends, without any marked tendency to appear as diploforms or chains (3). Comparison shows that the TM-1 organisms share some micromorphological characteristics with *B. corrodens* and *M. kingii*.

The cultural and biochemical characteristics of TM-1 strains are compared to those of similar organisms in Table 5. These data are based on results in our laboratory as well as those described by others (2-5, 14).

The relationship of TM-1 strains to oxygen is similar to that of *M. kingii* (2, 4). Whether there are TM-1 strains which would grow under only strict anaerobic conditions has not been evaluated.

Results of OF utilization of glucose by TM-1 strains grown on several media, sealed and unsealed, were not consistent. On the basis of their reaction on TSI, they appear to be nonfermenters, as do *M. kingii* and *B. corrodens*.

Ten strains each of EF-4, *M. kingii*, and *B. corrodens* (facultative anaerobes) were tested for growth on TM and TM-chocolate. None of the EF-4 or *B. corrodens* strains grew on either medium, but 6 of the 10 *M. kingii* stock strains grew on TM-chocolate and did not grow on TM or on a control plate of Mueller-Hinton medium without VCN.

Overall, TM-1 strains appear to be most similar to the species *M. kingii*; however, we feel that they are sufficiently distinct to form a separate species. Henriksen (3) discussed the "somewhat dubitable relationship of *M. kingii* to other *Moraxella* species." He pointed out the lack of catalase and the ability to produce acid from glucose and maltose as the most conspicuous differences between *M. kingii* and other

TABLE 5. Some cultural and biochemical characteristics of TM-1 strains and similar organisms^a

Characteristics	TM-1 strains	<i>Moraxella kingii</i>	<i>Bacteroides corrodens</i> (facultative anaerobes)	EF-4 species
Corroding of blood agar	+ or -	+ or -	+ or -	- or +
Predominant human sources	Pharyngeal	Pharyngeal and disease processes	Pharyngeal and disease processes	Wounds associated with animal bites
Fermentation of broth base:				
Glucose	(A)	(A)	-	A
Maltose	-	(A)	-	-
Hemolysis on rabbit blood	- or Sl alpha	beta or -	-	-
Nitrate	+ gas	-	-	+ Gas
Triple sugar iron agar				
Slant	Alk or N	N, Alk, or A	Alk or N	Alk or A
Butt	N or Alk	N, Alk, or A	N or Alk	A or N
Litmus milk	-	Peptonize + or -	-	- or Alk
Oxidase	+	+	+	+
Catalase	-	-	-	+
Lysine decarboxylase	-	-	+	-
Ornithine decarboxylase	-	-	+	-
Arginine dihydrolase	-	-	-	+

^a Abbreviations: +, $\geq 90\%$ of strains positive; -, $\geq 90\%$ of strains negative; + or -, majority of strains positive; - or +, majority of strains negative; Sl, slight; (A), acid production delayed (2 to 10 days); A, acid production within 24 to 48 hr; N, neutral; Alk, alkaline.

Moraxella species. TM-1 strains produce acid from glucose, and most strains lack catalase. Other characteristics of TM-1 which differ from those of typical species of *Moraxella* are the cellular morphology, the formation of gas during nitrate reduction, and growth on TM.

Further studies will be necessary before a final proposal concerning the proper taxonomical placement of the organisms can be made.

ACKNOWLEDGMENTS

We thank the Special Pathogens Unit, Epidemiological Services Laboratory Section, Bacterial Disease Branch, Epidemiology Program, Center for Disease Control, for providing the primary isolation plates from population groups II and III. We also thank John C. Feeley, Peter B. Smith, and Wallis L. Jones for their critical review of the manuscript.

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