Identification of *Actinomyces viscosus* from Canine Infections

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*Actinomyces viscosus* is a gram-positive, non-acid-fast, facultative, catalase-positive, filamentous, or diphtheroidal microorganism. It was isolated from six canine infections during a period of 1.5 years. The organism was cultured from exudate and flaky granules aspirated from infectious granulomas and empyemas. All cultures grew well aerobically and anaerobically with the addition of 10% carbon dioxide. They fermented lactose, produced catalase and acetylmethylcarbinol. reduced nitrates, hydrolyzed aesculin, and did not produce gelatinase or urease. These physiological characteristics distinguish *A. viscosus* from other morphologically similar organisms.

Howell (11) and Howell and Jordan (12) isolated a gram-positive filamentous “hamster organism” from periodontal lesions in hamsters. The “hamster strain” was later named *Odonto
tomyces viscosus* (13). It was excluded from the genera *Nocardia* and *Actinomyces* because it produced catalase and was non-acid-fast. Gruter in 1932 (18) and Brion in 1939 (2) reported the isolation of similar filamentous organisms from human gingiva and canine lesions. Unfortunately, physiological studies on these strains were too incomplete to be definitive and the strains were not preserved for future examination. The Subgroup on Taxonomy of the Microaerophilic Actinomycetes (6) recommended that the genus *Actinomyces* include catalase-positive organisms, thus renaming the “hamster strain” *Actinomyces viscosus*. *A. viscosus* and other Actinomycetes will not grow on Sabouraud agar (Difco) but *Nocardia* species grow well (7).

*A. viscosus* is a member of human and hamster periodontal plaque flora (7). Recently, human and canine infections due to this organism have been reported (1, 5, 16). The chronic, suppurative granulatous lesions resembled those seen in actinomycosis and nocardiosis. Six canine infections with *A. viscosus* were seen during the last 18 months at the Michigan State University Veterinary Clinic. They were: two empyemas, one flank abscess, one temporal abscess, and two cervical abscesses. *A. viscosus* was isolated and considered the etiologic agent in each of these cases.

The variability of colonial and cellular morphology of *A. viscosus* makes for difficulty in the differentiation of this species from other filamentous or diphtheroidal microorganisms. The purpose of this study was to delineate a minimal number of physiological tests considered necessary for definitive identification.

**MATERIALS AND METHODS**

***Organisms.*** Cultures of *A. viscosus* strains 295, 470, and 99 were obtained from H. A. McAllister who isolated them from canine clinical specimens. Strain 1097 was cultured from granules obtained from lung tissue, strain 1078 was grown from thoracic fluid from the same animal, and strain 194 was cultured from exudate aspirated from a cervical granuloma. Granules were washed in saline and crushed before culturing. All strains were initially plated on Trypticase soy agar (BBL) with 5% bovine blood. Isolated colonies were then transferred to other media for further examination.

***Morphology.*** Direct smears of granules and exudate were stained by the Gram-stain method and by the modified Kinyoun acid-fast method (4). All strains were transferred from the Trypticase blood agar to 5% bovine blood in brain heart infusion (BHI) agar (Difco), BHI agar, and BHI broth. These media were supplemented with vitamin K hemin solution and yeast extract (9). The strains were incubated with 10% carbon dioxide under aerobic and anaerobic conditions. Anaerobically prepared agar and broth media were made on an Anaerobic Culture Media Apparatus (Bellco Inc.) using the procedures described by Holdeman and Moore (9). Both 15-ml test tubes with 5 ml of broth and 100-ml flat-bottomed prescription bottles with 15 ml of agar media were prepared under an atmosphere of 90% nitrogen and 10% carbon dioxide. They were sealed with rubber stoppers and inoculation of media was done with this same apparatus. Aerobically prepared media had the same composition as the anaerobically prepared media except that resazurin and cysteine hydrochloride were omitted. These media were made without

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the use of an Anaerobic Culture Media Apparatus and were stoppered with Dispo plugs (Scientific Products). Cultures on aerobically prepared medium were incubated in Torbal jars which were flushed with carbon dioxide and air. All cultures were incubated at 37°C and were examined after 24 and 48 h, and 7 days. Gram stains were done on cultures from 48-h broth and supplemented BHI blood agar, and Kinyoun acid-fast stains were made from 4-day aerobic cultures and supplemented BHI blood agar.

Biochemical tests. Catalase production determined by the \( \text{H}_2\text{O}_2 \) slide test (9) was examined with colonies taken from 2-day cultures grown aerobically with \( \text{CO}_2 \) on supplemented BHI blood agar. Other biochemical test media and reagents were prepared as recommended by Holdeman and Moore (9). A basal medium of peptone yeast extract (Difco) with the addition of aesculin, lactose, gelatin, or glucose was prepared. Urea and nitrate media were made without this basal medium (9). Inoculum for all tests was made from 2-day cultures grown aerobically with \( \text{CO}_2 \) on supplemented BHI blood agar. A heavy suspension of the organism was prepared by suspending colonies in physiological saline. Four drops were added to each tube of medium with a Pasteur pipette. All inoculated test media were incubated in a candle jar for 10 days at 37°C. Test reactions were read according to the directions of Holdeman and Moore (9).

RESULTS

Morphology. All strains were gram positive, filamentous, or diphtheroidal and were non-acid-fast. Little or no preference for the addition of 5% bovine blood to supplemented BHI agar was evident but growth on these media was heavier than that on Trypticase blood agar. Cultures grew equally well under aerobic and anaerobic conditions when \( \text{CO}_2 \) was added. Two distinct morphological types were noted on media. One was a smooth entire, convex, glis-

![Fig. 1. Diphtheroidal form of A. viscosus associated with smooth colonies (x500).](#)

![Fig. 2. Filamentous form of A. viscosus associated with rough colonies (x500).](#)
Intermediate forms were also seen (Fig. 1-3).

**Biochemicals.** All strains of *A. viscosus* were catalase positive, reduced nitrates, were urease and gelatinase negative, hydrolyzed aesculin, did not produce acetylmethylcarbinol, and fermented lactose. These reactions were adequate to differentiate *A. viscosus* from other microorganisms shown in Table 1.

**DISCUSSION**

*A. viscosus* is a slow-growing fastidious microorganism which has been found recently in human and canine infections. The organism can not be separated from *Nocardia* or other filamentous or diphtheroidal organisms unless a minimal number of biochemical tests such as those recommended in this report are performed. Because *A. viscosus* may occur in the diphtheroidal form in lesions, it is important that the microbiologist attempt to identify diphtheroids recovered from chronic granulomatous lesions and supplicative processes.

The rough colonial form and related cellular morphology of *A. viscosus* suggest that it belongs in the family *Actinomycetaceae*. There is considerable information (6, 7) on the separation of *A. viscosus* from other members of this family. The smooth form of *A. viscosus* has been isolated from purulent material (1). Cultures with this colonial type and the biochemical reactions of *A. viscosus* resemble rather closely some species of *Corynebacterium*. Some non-hemolytic aerobic *Corynebacterium* species differ from *A. viscosus* only by the inability to hydrolyze aesculin when the scheme of identification suggested in Table 1 is used. The rela-

![Fig. 3. Reversion from rough to smooth colonial morphology (x2).](image)

**Table 1. Expected reactions of various gram-positive filamentous or diphtheroidal microorganisms**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Tests*</th>
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<tbody>
<tr>
<td></td>
<td>Acid-fast</td>
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<tr>
<td><em>Nocardia</em></td>
<td>W</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Bacterionema</em></td>
<td>-</td>
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<tr>
<td><em>Arachnia</em></td>
<td>-</td>
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<tr>
<td><em>Corynebacterium</em></td>
<td>-(W)</td>
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<tr>
<td><em>Erysipelothrix</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>-</td>
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<tr>
<td><em>Rothia</em></td>
<td>-</td>
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<tr>
<td><em>Actinomyces</em> (not <em>A. viscosus</em>)</td>
<td>-</td>
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</tbody>
</table>

* See references 3, 8, 9, 10, 14, 15, 17.

* ND, Not determined; +, positive reaction; -, negative reaction; W, weak; D, delayed; V, varies; ( ), reaction of occasional strain.
tionship of this genus to *A. viscosus* has not been generally emphasized.

Isolation of pure cultures of diphtheroids and filamentous microorganisms, or multiple isolation of these from inflammatory lesions, indicates the necessity for proper speciation. Because antibiotic therapy of infections caused by these species varies, proper identification is of paramount importance.

ACKNOWLEDGMENTS

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LITERATURE CITED


