Isolation of Actinobacillus lignieresii and Actinobacillus equuli from Laboratory Rodents

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Actinobacillus lignieresii and Actinobacillus equuli were cultured from a total of 36 guinea pigs, rats, and mice. The organisms were isolated from the oropharynx, the conjunctiva, and middle ear. Isolates were initially screened by eight biochemical tests to determine whether they were of the genus Actinobacillus. Actinobacillus spp. were then differentiated by fermentation reactions of nine carbohydrates. In the past, actinobacilli may have been mistakenly identified as Pasteurella spp., especially Pasteurella pneumotropica. The importance of realizing that Actinobacillus spp. are frequently isolated from laboratory rodents was stressed.

In 1902 Lignieres and Spitz (6) reported Actinobacillus lignieresii as the causative agent of a granulomatous disease of cattle in Argentina. Subsequently, Thompson (14) confirmed suspicions that actinobacillosis was prevalent among cattle in the United States. Ravaut and Pinoy (12) were the first to describe the pathogenicity of actinobacilli in humans. Others (8, 15) have reported A. lignieresii as a cause of disease in humans even causing death (1). A survey of the literature suggests that A. lignieresii is not well characterized; knowledge of host ranges is incomplete, and it is generally only associated with granulomatous lesions in cattle and sheep (9). Actinobacillus equuli, previously referred to as Shigella equirus or Bacterium viscosum equi, was first reported in 1932 by Dimock and Edwards (2). They reported a 39.8% incidence of A. equuli in foals. Edwards and Taylor (4) reported a case of porcine metritis caused by A. equuli as acute septicemia, glomerular nephritis, and “joint ill” in foals. Finally, Harbourne et al. (5) suggested that A. equuli is a common cause of death in young horses and a rare problem in pigs, but it is of no consequence to other animal species.

Herein, we propose that A. lignieresii and A. equuli or phenotypically similar bacteria are prevalent among laboratory rodents. Also, we submit that microbiologists working with research animals may be misidentifying this organism as Pasteurella spp. or Pasteurella pneumotropica.

During a 6-month period at the University of Missouri Research Animal Diagnostic and Investigative Laboratory, Actinobacillus spp. were isolated from 36 laboratory rodents of sixteen different accessions. (An accession in our laboratory is defined as any specimen, animal, or group of animals from a common source submitted for diagnostic evaluation.) Actinobacillus spp. were readily identified by using the characteristics of the organism described herein. These observations indicate that Actinobacillus spp. are fairly common in laboratory rodents and in many instances are probably being incorrectly identified as Pasteurella spp.

MATERIALS AND METHODS

Organisms. Each primary isolation of an Actinobacillus spp. occurred in the course of routine diagnostic microbiological examinations which included culturing the posterior nasopharynx, conjunctiva, and middle ear, onto Trypticase blood agar (BBL Microbiology Systems, Cockeysville, Md.). Each Actinobacillus isolate was maintained on blood agar plates and preserved by lyophilizing in skim milk. Reference cultures, supplied by American Type Culture Collection (ATCC), were A. equuli (19392), and A. lignieresii (19393).

Biochemical reactions. Colonies were nonhemolytic, small, raised, and greyish white. They stained as pleomorphic gram-negative rods. Isolates with these characteristics and oxidase positive were inoculated into a battery of media consisting of TSI Agar (BBL), MacConkey agar (BBL), urea agar (BBL), tryptone broth (BBL), dextrose agar (Difco Laboratories, Detroit, Mich.) with Andrade’s indicator (Oxoid, K.C. Biologicals, Lenexa, Kan.), nitrate agar (Difco), and API test strips for the α-nitrophenyl-β-D-galactopyranoside test.

If the isolates were regarded as a possible Actinobacillus after initial biochemical tests, fermentative activity was evaluated. The isolates were inoculated into peptone water (Difco) with Andrade’s indicator and each of the following carbohydrates (Difco): mannose, arabinose, xylose, sucrose, maltose, lactose, trehalose, mannitol, and salicin. Methyl red and Voges-Proskauer tests were done in MR-VP broth (Difco).
Confirmation of identity of *Actinobacillus* isolates. Isolates suspected of being *A. lignieresii* or *A. equuli* were sent to the Department of Social Services, Missouri Division of Health (Jefferson City, Mo.) and the National Veterinary Service Diagnostic Bacteriology Laboratory (Ames, Iowa) for confirmation. These isolates were the organisms from which the biochemical data were compiled.

RESULTS

Primary isolates. Isolates of *Actinobacillus* spp. were recovered from 16 accessions which included three species, mice, rats, and guinea pigs (Table 1), from various sources. Primary cultures were collected mostly from the posterior nasopharynx, although *Actinobacillus* spp. were recovered from the tympanic bullae of six animals (involving five accessions) with grossly evident otitis media and from the conjunctival sac of one animal with conjunctivitis. In the six animals with otitis media from which *Actinobacillus* was isolated, the organism was also isolated from the posterior nasopharynx. *Actinobacillus* isolates were cultured from 36 out of 72 total animals involved in the accessions.

Cultural characteristics. The colonies of *Actinobacillus*, freshly isolated on blood agar, were small (0.5 to 2 mm), raised, and greyish white. The colonies were nonhemolytic after 24 h on sheep blood, but α-hemolysis was apparent at 48 h. Microscopically the isolates were small, pleomorphic gram-negative rods.

Differential reactions. The reactions expected of *Actinobacillus* spp. include oxidase production (some strains are weakly positive) and urea hydrolysis within a few hours. Methyl red and Voges-Proskauer tests are negative and TSI Agar has an acid-over-acid reaction. Indole tests (Ehrlich's) are negative (48 h). Nitrate reduction and glucose fermentation occur within 24 h. They are β-galactosidase positive (α-nitrophenyl-β-D-galactopyranoside test) and grow on MacConkey agar.

Table 2 summarizes the fermentative patterns of the two *Actinobacillus* species isolated from the animals of accessions listed in Table 1 and the ATCC reference cultures. These two organisms, *A. lignieresii* and *A. equuli*, were quite similar biochemically, and the biochemical patterns of the clinical isolates were almost identical to those of the reference cultures. Both fermented glucose, xylose, sucrose, maltose, and usually mannitol and arabinose within 24 h. Trehalose was fermented only by *A. equuli*. Lactose fermentation required 24 h for the reference strain and 48 h for the clinical isolates and occurred with *A. equuli* only. Salacin was fermented weakly at 48 h by *A. lignieresii* clinical isolates only.

**DISCUSSION**

The biochemical characteristics of *A. lignieresii* reported by Phillips in 1960 (9) are still used for identification of this agent. The biochemical reactions of the rodent isolates described in this paper essentially agree with those of Phillips. We reported the differential identification schemes because there is confusion in identifying *Actinobacillus* isolates. This is probably due to a paucity of literature on *Actinobacillus* and an incomplete knowledge of the host range for *A.
tinobacillus spp. Originally, our laboratory and other diagnostic laboratories, plus at least one state reference laboratory, misidentified Actinobacillus spp. as Pasteurella spp. This happens because the two genera are culturally very similar and Pasteurella spp. are common in rodents. An immediate impulse is to classify this rodent isolate as a P. pneumotropica. We identified 243 Pasteurella isolates from our possessions in 1979. Since the Actinobacillus agent produces strong urea hydrolysis and a negative indole test, laboratory personnel may be inclined to classify it as Pasteurella ureae. If extensive fermentative screens are performed, the reactions will not justify the P. ureae classification, and then the tendency is to simply identify the isolates as Pasteurella spp. Actinobacillus spp. are differentiated from Pasteurella spp. by four biochemical tests: β-galactosidase and urease activity, no indole production, and growth on MacConkey agar. All four tests must be used in combination since Pasteurella has biotypes showing these reactions but never in the same combination (11). These four characteristics are cited as key properties of the genus Actinobacillus in Bergey’s Manual of Determinative Bacteriology (10).

Our A. equuli isolates quickly fermented xylose and mannitol and lactose at 48 h. However, the A. lignieresii strains did not ferment lactose, and only one clinical isolate did not ferment mannitol. The fermentative reactions of the reference cultures were almost identical to those of the clinical isolates, except that ATCC strain 19393 demonstrated no salicin fermentation. Bergey’s Manual of Determinative Bacteriology does indicate variable reactions to lactose, confirming our observations. This lactose fermentation variability does point out the value of testing for the enzyme β-galactosidase, as all Actinobacillus strains had positive o-nitrophenyl-β-D-galactopyranoside tests. A. lignieresii and A. equuli are differentiated primarily by trehalose fermentation. A. lignieresii is unable to utilize trehalose. The most important factor in identifying Actinobacillus spp. is the use of Andrades’ indicator (5, 9) in peptone or heart infusion broth. Commonly used indicators such as bromocresol purple are not sufficiently sensitive to detect some weak fermenters. The only difference between the features of Actinobacillus spp. as identified by Phillips (9) and our isolates was the weak fermentation of salicin at 48 h which we observed and Phillips did not. However, in the same paper Phillips noted that salicin production had been previously reported for Actinobacillus spp.

The literature, to the best of our knowledge, leads one to believe that Actinobacillus spp. are nonpathogens in laboratory animals (1, 7). Generally, rodents are not considered to be hosts for actinobacilli. One author (16) reported the isolation of A. equuli from rats in France. Recently (13), A. equuli was reported in laboratory animals, but its role in clinical manifestations was unknown. We believe that Actinobacillus spp. are commonly carried by laboratory rodents, based on our observation of the substantial frequency of isolation of these organisms from varied sources. Furthermore, we suspect that Actinobacillus spp. are naturally occurring pathogens of laboratory rodents, based on the isolation

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<th>Carbohydrate</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannitol</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>Trehalose</th>
<th>Salicin</th>
<th>Arabinose</th>
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<td><strong>A. lignieresii</strong></td>
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<td><strong>A. equuli</strong></td>
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<th>Fermentative reaction*</th>
<th>A. lignieresii</th>
<th>A. equuli</th>
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<td>24 h</td>
<td>Clinical isolates&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Strain 19393&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>48 h</td>
<td>Clinical isolates&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Strain 19393&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>+ Positive reaction (fermentation); −, negative reaction (no fermentation); (−), one strain was negative; w, weak acid production; I, some strains were negative at 24 h.
<sup>b</sup>The two clinical A. lignieresii isolates.
<sup>c</sup>ATCC A. lignieresii reference culture.
<sup>d</sup>The 14 clinical isolates of A. equuli.
<sup>e</sup>ATCC A. equuli reference culture.
of the organism from middle ear infections and conjunctivitis. The purpose of this paper is to create awareness among microbiologists concerning the occurrence and possible pathogenicity of *Actinobacillus* spp. in laboratory rodents. Such recognition is a prerequisite to accurate identification of the agent. This awareness, applied in conjunction with the properly selected biochemical battery discussed previously, will lead to the correct identification of a potential human pathogen and allow us to begin better elucidating the roles of *Actinobacillus* spp. and *P. pneumotropica* as rodent pathogens.

ACKNOWLEDGMENTS

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