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 equirulis* 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, Harbourn 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 labo-

ratory 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, Kans.), nitrate agar (Difco), and API test strips for the *o*-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 (*o*-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 stain 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 *Ac*

TABLE 1. Sources of *Actinobacillus* isolates

Accession no.	Species	Strain	No. infected/no. cultured	Culture site ^a	Species isolated
1622	Rat	Long Evans	1/1	NP	<i>A. equuli</i>
1675	Guinea pig	Hartley	2/2	Con	<i>A. equuli</i>
1960	Mouse	B 10 D 2/NSN	7/20	NP	<i>A. equuli</i>
1961	Mouse	B 10 BRSqSh	5/20	NP	<i>A. equuli</i>
1990	Rat	Sprague Dawley	3/4	NP	<i>A. equuli</i>
2236	Rat	Sprague Dawley	1/1	NP	<i>A. lignieresii</i>
2250	Rat	Sprague Dawley	1/2	NP	<i>A. equuli</i>
2283	Mouse	Balb/C	2/2	NP	<i>A. equuli</i>
2325	Rat	Unknown	1/1	ME	<i>A. equuli</i>
2329	Guinea pig	Strain 13	1/1	NP	<i>A. equuli</i>
2345	Rat	Sprague Dawley	1/1	ME	<i>A. lignieresii</i>
2407	Mouse	MRL/L	2/4	NP, ME	<i>A. equuli</i>
2409	Mouse	Balb/C	3/4	NP, ME	<i>A. equuli</i>
2475	Mouse	MRL/L	1/3	ME	<i>A. equuli</i>
2512	Mouse	MRL/L	4/4	NP	<i>A. equuli</i>
(2512) ^b	Mouse	MRL/L	1/4	ME	<i>A. equuli</i>
2513	Rat	Sprague Dawley	1/1	NP	<i>A. equuli</i>

^a NP, Posterior nasopharynx; Con, conjunctiva; ME, Middle ear.

^b These are the same animals as in 2512, with one animal having a positive culture in both the NP and ME.

TABLE 2. Fermentative reactions of *A. lignieresii* and *A. equuli*

Carbohydrate	Fermentative reaction ^a							
	<i>A. lignieresii</i>				<i>A. equuli</i>			
	24 h		48 h		24 h		48 h	
	Clinical iso- lates ^b	Strain 19393 ^c	Clinical iso- lates	Strain 19393	Clinical isolates ^d	Strain 19392 ^e	Clinical isolates	Strain 19392
Glucose	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+
Mannitol	+ (-)	+	+ (-)	+	w	+	+	+
Lactose	-	-	-	-	-	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Trehalose	-	-	-	-	+	+	+	+
Salicin	-	-	w	-	-	-	-	-
Arabinose	+	+	+	+	+ (I)	+	+	+

^a +, Positive reaction (fermentation); -, negative reaction (no fermentation); (-), one strain was negative; w, weak acid production; I, some strains were negative at 24 h.

^b The two clinical *A. lignieresii* isolates.

^c ATCC *A. lignieresii* reference culture.

^d The 14 clinical isolates of *A. equuli*.

^e ATCC *A. equuli* reference culture.

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 accessions 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

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.

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