Mesenteric Lymphadenitis of Swine Caused by *Rhodococcus sputi*

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*Rhodococcus sputi* caused tuberculoid-like lymphadenitis of mesenteric lymph nodes in swine. This is the first study reporting that *R. sputi* can be a pathogen in swine.

Most rhodococci are considered nonpathogens, although *Rhodococcus equi* ([Corynebacterium equi]) causes infections in animals (1, 3, 22), and *Rhodococcus aurantiacus* causes infections in humans (5, 18). *Rhodococcus sputi* (14, 20) has been observed in the sputa of patients with chronic pulmonary disease, but it has not been reported as a pathogen. We report a case of mesenteric lymphadenitis of swine caused by this organism.

Swelling of several mesenteric lymph nodes (25 mm in diameter) of the ileum was observed in a 6-month-old swine. Microscopically the lymph nodes displayed granulomatous lymphadenitis with epithelial cells reminiscent of tuberculosis. A 1-g sample of each node was added to 5 volumes of 1% NaOH, homogenized, and allowed to stand at room temperature for 5 min. The homogenate was centrifuged at 3,000 rpm for 15 min. The centrifugate was mixed with a pipette, and each 0.1-ml sample was inoculated onto an Ogawa egg medium slant. The inoculated tubes were incubated at 37°C for 8 weeks. Growth of colonies appeared in one tube after 4 weeks of incubation and in another after 8 weeks of incubation. Colony pigments ranged from yellowish to reddish. Growth on the isolation medium was slow, but the isolates grew after 3 days on subcultures. No other microorganisms were isolated.

The isolates were examined for a total of 112 characteristics. Of these, 102 were as described previously. Two examinations, acid formation from glucose and mannose, were omitted (16). In addition, the following tests were included: growth at 42°C; glycerol as a carbon source; L-serine as a nitrogen source; succinimide as a nitrogen source; resistance to 5% sodium chloride; resistance to isoniazid (10 μg/ml); resistance to 5-fluorouracil (20 μg/ml); resistance to ofloxacin (1 and 5 μg/ml); and resistance to mitomycin C (5 μg/ml). (The resistance tests were carried out in Ogawa egg medium.) The details of the methods were as described previously (17).

Numerical taxonomy was used to identify the bacterium as described previously (13).

Mycolic acids were determined by the method of Tomiyasu and Yano (10). The cell wall type was determined by the method of Staneck and Roberts (7), the a-yl type was determined by the method of Uchida and Aida (21), and isoprenoid quinones were determined by the method of Tamaoka et al. (8). DNA base composition (moles percent guanine plus cytosine) was determined by the method of Tamaoka and Komagata (9) after isolation of the DNA by the method of Saito and Miura (6).

The isolates occurred as individual, slightly acid-fast, short rods (1 to 3 μm by 0.5 μm). They utilized sucrose as a carbon source in the presence of NH₄⁺, had no β-galactosidase activity, and were susceptible to 5-fluorouracil (20 μg/ml). These characteristics agreed with those for the genus *Rhodococcus* (11, 12, 14, 15, 20). The isolates had mycolic acids with 56 to 66 carbon atoms that resulted in only one spot of alpha-mycolate in thin-layer chromatography. This finding suggested that the isolates belonged to the genus *Rhodococcus* or *Nocardia* (4, 19). The bacterium differed from nocardiae by the absence of a mycelium, the utilization of sucrose, the lack of β-galactosidase activity, and susceptibility to 5-fluorouracil (11, 12, 14, 15, 20).

On initial growth, two isolates had different colony pigments and different growth rates. However, they were similar to each other on further examination. The reactions of the isolates resembled those of *R. sputi* (14, 20) but differed from those of type strain 55001 (ATCC 29627T) in the following ways: alpha-esterase activity was negative; nitrate was not reduced to nitrite; L-serine was not utilized as a simultaneous nitrogen and carbon source; citrate, mannitol, and sorbitol were not utilized as carbon sources; propylene glycol was utilized as a carbon source; and succinimide was utilized as a nitrogen source.

The results of numerical taxonomy, carried out to confirm the identification, are shown in Table 1. The isolates were within the domain of *R. sputi* and outside the domains of other rhodococci. Therefore, they were identified as *R. sputi*.

The results of gas chromatographic and mass spectrometric analyses indicated that both the mycolic acids of the type strain of *R. sputi* and those of strain E12652 (the present isolate) have 56, 58, 60, 62, 64, and 66 carbon atoms and contain two to six double bonds (Fig. 1). These analyses support the identification as *R. sputi*.

Further characteristics of strain E12652 (Japan Collection of Microorganisms, Wako, Saitama, Japan; JCM 6411) which were investigated include the following: the guanine-plus-cytosine content is 65.2 mol%; the cell wall type is IV A; the acyl type is glycolyl; and the menaquinones are MK-9 (H₂) (ca. 50%) and MK-8 (H₂) (ca. 40%), where 9 and 8 are the numbers of isoprene units of the side chain and 2 is the number of hydrogen atoms saturating the side chain.

The isolates were resistant to 5% sodium chloride, isoniazid (10 μg/ml), mitomycin C (5 μg/ml), and ofloxacin (1 μg/ml) but susceptible to 5-fluorouracil (20 μg/ml) and ofloxacin (5 μg/ml).

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The MICs of antituberculosis agents against these isolates determined in Ogawa egg medium were as follows: rifampin, 12.5 µg/ml; streptomycin sulfate, 12.5 µg/ml; ethambutol, >50 µg/ml; isoniazid, >200 µg/ml; ethionamide, >200 µg/ml; kanamycin sulfate, 3.13 µg/ml; enniomycin sulfate, 3.13 µg/ml; sulfadimethoxine, 12.5 µg/ml; kitasamycin, 25 µg/ml; and minocycline, 3.13 µg/ml. MICs were determined by inoculating the medium with a 0.02-ml sample of a 10-µg (wet weight)/ml suspension and reading the results after incubation at 37°C for 5 days.

The present study indicates that R. spuerti can cause tuberculosis-like mesenteric lymphadenitis of swine.

LITERATURE CITED