Deoxyribonucleic Acid Base Composition of Ovine Actinobacilli

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(Received 18 April 1974)

In New Zealand and elsewhere, three groups of organisms predominate in the aetiology of ovine epididymitis. These are *Brucella ovis*, actinobacilli, and a group referred to simply as Gram-negative pleomorphic bacilli (Ek Dahl, Money & Martin, 1968; Watt, 1972). While some of the actinobacilli closely resemble authentic species of the genus, e.g. *Actinobacillus lignieresii*, many are simply referred to as Actinobacillus-like or '*Actinobacillus seminis*'. This binomial was first used by Baynes & Simmons (1960) for a series of apparently rather biochemically inert Gram-negative bacilli, recoverable from ram testicular lesions, which for maximal growth required lowered oxygen tensions and serum enrichment. The organisms appeared to be members of the family Brucellaceae Breed *et al.* and were considered, perhaps wrongly, to be most closely related to the genus *Actinobacillus*. Despite the fact that the original description was not based on a single designated type strain, *A. seminis* has been accepted by Hatt & Zvirbulis (1967) as a validly published and legitimate binomial. Since then, reports of ovine epididymitis associated with the same or similar organisms have been made in a number of countries (Watt, 1972).

Over recent years, techniques have become available for comparing bacterial genomes including the estimation of the deoxyribonucleic acid base ratios (Marmur & Doty, 1962). Organisms with closely related base compositions are believed to be phylogenetically related if they possess similar morphological, physiological, biochemical and other characteristics (Jones & Sneath, 1970). For this reason, the guanine-cytosine base ratio of a group of Actinobacillus-like organisms isolated from ovine genital lesions has been compared with known type strains of *A. lignieresii* and *A. equuli*, in an attempt to demonstrate some sort of relationship between them.

METHODS

Organisms. A total of 13 actinobacilli or Actinobacillus-like strains, details of which are given in Table 1, were maintained on blood agar plates at 37 °C in a candle jar and routinely subcultured every 2 or 3 days.

Each organism, except strain M, was grown on blood agar (BBL trypticase soy agar with 10% human blood) in autoclavable polypropylene instrument trays (20 × 15 × 5 cm) with overlapping lids, containing approximately 200 ml of media. Trays were inoculated with 3 ml of a saline suspension (10⁸ organisms/ml) of the appropriate bacterial strain spread over the surface of the medium by gently shaking and rocking the covered trays, and incubated at 37 °C for 20 h in a cabinet with a front hinged door securely fastened and sealed with a foam rubber strip. Before the door was closed, a lighted candle was placed in the cabinet.

Cells were harvested using sterile saline (0.9% NaCl) and a sterilized glass microscope slide as a scraper. After washing twice in saline, portions (approx. 1 g) were resuspended in

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Table 1. *Details of organisms studied and their DNA base compositions*

Strain no.	Source	Tentative identification	DNA base composition (mol % G + C)
A	Ovine epididymitis, New Zealand	<i>Actinobacillus</i> species	42.2 ± 1.7*
B	Ovine epididymitis, New Zealand	<i>Actinobacillus</i> species	45.9 ± 0.7
C	Ovine epididymitis, New Zealand	<i>Actinobacillus</i> species	47.9 ± 1.2
F	Bovine tongue nodule, New Zealand	<i>A. lignieresii</i>	47.1 ± 0.0
G	Ovine epididymitis, New Zealand	<i>A. seminis</i>	45.0 ± 1.9
H	Vertebral abscess of a lamb, New Zealand	<i>A. lignieresii</i>	41.9 ± 0.6
I	Ovine epididymitis, New Zealand	<i>A. seminis</i>	39.0 ± 0.7
J†	Ovine epididymitis, Australia	<i>A. seminis</i>	48.8 ± 0.7
K	Bovine semen, New Zealand	<i>A. seminis</i>	38.7 ± 0.4
L	Kidney, ureter, bladder of a sheep, New Zealand	<i>Actinobacillus</i> species	42.9 ± 0.4
M‡	NCTC8529, Colindale	<i>A. equuli</i>	41.3 ± 1.0
N‡	NCTC4976, Colindale	<i>A. lignieresii</i>	43.6 ± 0.7
O	Ovine scrotal abscess, New Zealand	<i>A. seminis</i>	37.8 ± 0.8

* Standard deviation, corrected for small groups.

† Original isolate of Baynes & Simmons (1960).

‡ Proposed working types (Sneath & Skerman, 1966).

25 ml saline-EDTA (0.15 M-sodium chloride, 0.1 M-ethylenediaminetetra acetate) pH 9.0 and stored at 4 °C until required.

As strain M (*A. equuli*) adhered strongly to the agar and was difficult to harvest by the above procedure it was grown in liquid medium, which consisted of nutrient broth (Difco) dispensed in 330 ml portions in 600 ml bottles, autoclaved, cooled, inoculated with a loopful of organisms, and incubated at 37 °C on a Griffin Orbital Shaker at 88 oscillations/min. After 20 h the bacteria from each bottle were harvested by centrifuging, pooled, washed twice in saline, and portions (approx. 1 g) resuspended and stored in 25 ml of saline-EDTA pH 9.0 as described above. Blood agar cultures taken from each bottle revealed no contamination.

Extraction of DNA and determination of DNA base composition. The technique of DNA extraction was that of Marmur (1961) with variations as described by Bowie *et al.* (1972).

The DNA base compositions were determined by the 'melting point' method of Marmur & Doty (1962). For producing the DNA melting curves the system of Bowie *et al.* (1972) was used and the DNA dissolved in phosphate-EDTA buffer. At least three melting-point determinations were made on each DNA sample.

RESULTS AND DISCUSSION

Examination of the results (Table 1) reveals a range of % GCs from 37.8 to 48.8. The values for the two type cultures were consistent with the range (40 to 43) previously reported for the genus *Actinobacillus* (Jones & Sneath, 1970; Bailie, Coles & Weide, 1973). As a difference of % GC of 5 usually implies at least a species difference (Jones & Sneath, 1970), it is obvious that the nine isolates considered as probable or confirmed '*A. seminis*' are not of one species, and possibly not of one genus.

An attempt to clarify some of the taxonomic abnormalities within the family Brucellaceae Breed *et al.* has recently been made (Johnson & Sneath, 1973; Sneath & Johnson, 1973). Using a series of 134 characters, a close phenetic relationship was demonstrated between the

three genera *Actinobacillus*, *Pasteurella* and *Haemophilus*, suggesting that they might best be separated from other genera of the family, such as *Brucella*, from which they were phenetically very different. Among the properties listed for actinobacilli were: the anaerobic fermentation of glucose with the production of acid but no gas; the production of acid aerobically from glucose, mannitol and xylose; the formation of the enzymes phosphatase, catalase and urease; the reduction of nitrates to nitrites; and the production of H_2S . They did not require X or V factors or a raised CO_2 tension for growth. Although primary isolation is best accomplished on slightly alkaline blood- or serum-enriched media in an atmosphere of 10% CO_2 , actinobacilli should, on subculture, grow on nutrient agar and produce small colonies on MacConkey plates. Of the 11 probable actinobacilli included in the present investigations, only one (isolate H) came close to fulfilling all these biochemical requirements; the % GC value of this isolate also fell within the required range. Of the remaining ten isolates, three (including J) did not produce acid from glucose and mannitol, three (including J) did not reduce nitrates, and none grew on MacConkey or produced H_2S . Most other tests were negative. These results, as well as those of several other workers (see Wetmore, Thiel, Herman & Harr, 1963; Bailie *et al.* 1973; Johnson & Sneath, 1973) warrant a re-appraisal of the taxonomic position of supposedly *Actinobacillus*-like organisms recoverable from animals. Indeed, it seems that many of the taxonomic problems now becoming apparent stem directly from the failure of earlier investigators to compare their 'new' species with designated type and reference strains.

We wish to thank Miss J. A. Brooks, Miss H. M. McInnes, Dr R. B. Marshall, Mr D. Martinovich and Mr G. C. Simmons for supplying some of the isolates included in this investigation. The advice of Mr M. C. Connell on DNA extraction techniques is also gratefully acknowledged.

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