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THE USEFULNESS OF THE API 20 E CLASSIFICATION SYSTEM IN THE IDENTIFICATION OF *ACTINOBACILLUS ACTINOMYCETEM COMITANS, ACTINOBACILLUS SEMINIS* AND *PASTEURELLA HAEMOLYTICA*

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ABSTRACT

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The prepuces of lambs aged 6–8 months and semen of 2 adult rams were found to be infected with gram-negative, non-motile, non-haemolytic, pleomorphic bacilli. These organisms were compared with those of known strains of *Actinobacillus actinomycetem comitans, Actinobacillus seminis* and *Pasteurella haemolytica*, using the API 20 E classification system. Applying the principles of numerical taxonomy, the majority of suspected strains of *A. seminis* could be classified as *A. actinomycetem comitans* and 3 examples as *Histophilus ovis*. Although some of the suspected strains of *A. seminis* could be classified as *P. haemolytica*, obvious differences between the genera *Actinobacillus* and *Pasteurella* were evident.

INTRODUCTION

Actinobacillus actinomycetem comitans and *Actinobacillus seminis* are bacteria that reportedly cause epididymitis in rams in the USA and Australia (Baynes & Simmons, 1960; De Long, Waldhalm & Hall, 1979). Van Tonder (1979a; 1979 b) concluded that such lesions in rams in the Republic of South Africa were mainly due to *A. seminis* infection, while the most frequent organism isolated by Jansen (1980) from similar cases was *Pasteurella haemolytica*. Some of these strains of *P. haemolytica* showed variation with respect to their ability to produce acids from certain carbohydrates. Using single colonies isolated from rams, he showed that some of these did not metabolize glucose and maltose nor did they haemolyze erythrocytes, as one would have expected from classical cultures of *P. haemolytica*, and they thus resembled *A. seminis* in every respect.

Although the API 20 E system was primarily designed for the rapid identification of Enterobacteriae, Collins & Swanson (1981) tested this system also for the identification of non-Enterobacteriaceae such as *Actinobacillus* spp., *Pasteurella* spp. and *Pseudomonas* spp. Using this method, these authors could correctly identify 62% of their isolates, but at the same time wrongly identify as many as 31%.

Notwithstanding these limitations, the API 20 E system was used to compare phenotypic properties of known strains of *A. actinomycetem comitans, A. seminis* and *P. haemolytica* with those of suspected strains of *A. seminis* found in semen and preputial swabs of rams and ram lambs.

MATERIALS AND METHODS

Preputial swabs were taken from 18 ram lambs aged 6–8 months from 2 farms in the Odendaalsrus and Potchefstroom districts. These swabs were transported to the laboratory in Stuart's transport medium*. Cultures were made immediately on arrival of the swabs at the laboratory on tryptose blood agar medium* containing 5% sheep's blood. Two semen samples from adult rams from different farms in the same area were also plated out on the same medium. All the cultures were incubated for 72 h at 37°C in an atmosphere of 10% CO_2 in air. Single, non-haemolytic colonies of gram-negative, non-motile, pleomorphic bacilli were subcultured by streaking on tryptose blood agar under the same conditions. Eighteen-hour growth from these subcultures was suspended in sterile 0,15 M NaCl to a density of 4 on the McFarland scale. As serum or blood appears to be an essential growth factor for *A. seminis* (Baynes & Simmons, 1960; Worthington & Bosman, 1968), a 2 mℓ volume from each suspension was further diluted 1:4 with sterile 0,15 M NaCl containing 2% human serum. This suspension was transferred to the different reagents of the API 20 E** test strip. Reference strains of *A. actinomycetem comitans*,*** *A. seminis***** and *P. haemolytica****** were subjected to the same procudure. The reactions were read after a 48-hour incubation at 37°C.

Bromthymol blue, which changes to yellow at a pH of 6,0, indicates carbohydrate utilization in the API 20 E system. After a 48-hour incubation, known strains of *A. seminis* and *P. haemolytica* in a suitable medium containing both glucose and serum cause a drop in pH from the original setting of 7,4 to a level varying between 6,1 and 6,5 (J. A. Erasmus, unpublished data, 1982). In the API 20 E system the utilization of a carbohydrate associated with a drop in pH to these levels is indicated by a colour change from blue to yellow-green. A change of this nature was taken as positive in this study.

Organisms were finally compared by estimating similarity coefficients from which a similarity matrix and eventually a dendogram could be drawn (Stanier, Doudoroff & Adelberg, 1972).

RESULTS AND DISCUSSION

The various organisms studied were compared by means of a dendogram (Fig. 1). From the number of tests included in the API 20 E classification system, it was assumed that strains with a similarity of 65–85% belonged to the same genus. Strains with a similarity above 85% were taken as the same species. According to this definition, the known strains of *A. actinomycetem comitans* and *A. seminis* as well as the majority of the suspected strains of *A. seminis* could be classified as belonging to the same genus, but to at least 4 different species. A 100% similarity between the strain of *A. actinomycetem comitans* and strains 6201 and 70/64 of *A. seminus* was noted. Strains V350 and K 3844 C of *A. seminis* could be classified as different, separate species of the genus *Actinobacillus*.

The known strains of *P. haemolytica* formed a separate, fairly heterogeneous genus which contained 4 of the suspected strains of *A. seminis*.

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*** Obtained from Dr W. J. de Long, College of Agriculture, University of Idaho, USA

**** Strain K 3844 C obtained from Mr J.D. Connole, Animal Research Institution, Yeerongpilly, Queensland, Australia. Strains 6201, 70/64 and V350 obtained from Dr E. M. van Tonder, Veterinary Investigation Centre, Middelburg, Cape 5900

***** Obtained from Dr M. M. Henton, Veterinary Research Institute, Onderstepoort 0110

TABLE 1 Phenotypic properties of some strains of *Actinobacillus seminis* and *Pasteurella haemolytica* as per Fig. 1

Test	Organism											
	A. seminis strain						*P. haemolytica*			*P. haemolytica*		
	9.31	1.521	Bos-hoff	K 3884 C	Engel-brecht	1.434	Goat	Sheep	Pig	1.232	I29	KC 282
ONPG	–	–	–	–	–	–	+	+	+	+	+	+
Arginine dihydrolase	–	–	–	–	–	–	–	–	+	–	–	–
Lysine decarboxylase	–	–	–	–	–	–	–	–	–	–	–	–
Ornithine decarboxylase	+	+	+	+	+	+	–	–	+	+	–	–
Citrate	–	–	–	–	–	–	–	–	–	–	–	–
H2S	–	–	–	–	–	–	–	–	–	–	–	–
Urease	–	–	–	–	–	+	–	–	–	–	–	–
Tryptophane deaminase	–	–	–	–	–	–	–	–	–	–	–	–
Indole	–	–	–	–	–	–	–	–	–	–	–	–
VP	+	–	+	+	–	+	–	–	–	+	–	–
Gelatin	–	–	–	–	–	–	–	–	–	–	–	–
Glucose	+	+	–	+	+	+	+	+	+	+	+	+
Mannitol	+	+	–	–	+	+	+	+	+	+	+	+
Inositol	+	+	–	+	–	–	+	+	+	+	+	–
Sorbitol	–	–	–	–	+	–	+	–	+	+	+	–
Rhamnose	–	–	–	–	–	–	–	–	+	–	–	–
Sucrose	–	–	–	–	–	–	+	+	+	+	+	+
Melibiose	–	–	–	–	–	–	–	–	+	–	–	–
Amygdalin	–	–	–	–	–	–	–	–	+	–	–	+
L+ Arabinose	+	+	+	–	–	+	–	–	–	+	+	–
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+

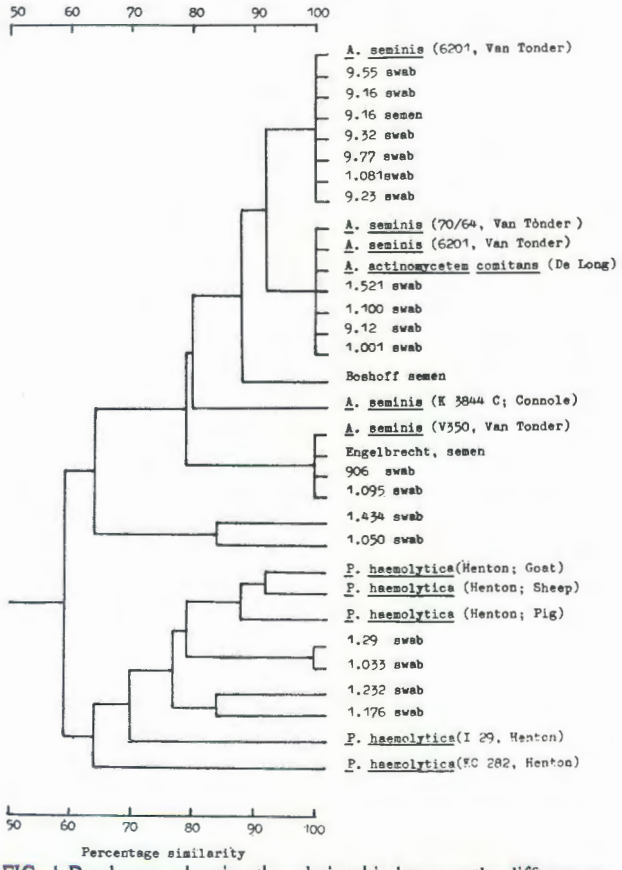

FIG. 1 Dendogram showing the relationship between the different organisms studied

The phonotypic features of the different organisms are summarized in Table 1. Where a 100% similarity between organisms was noted, only 1 example of such a group was presented in Table 1.

Strain 6201 of *A. seminis* appears in 2 groups (Fig. 1) because the progeny of the 1st series of single colonies produced butanediol (VP positive), while the progeny of a 2nd series was completely negative in this respect.

In the presence of serum, *A. seminis* is a biochemically active organism which decarboxylated the amino acid ornithine and reduced nitrate in all instances. One strain only did not metabolize glucose. On the other hand, the known strains of *P. haemolytica* constantly synthetized the enzyme B-galactosidase (ONPG positive). They also reduced nitrates and metabolized the carbohydrates glucose, mannitol and sucrose.

According to the data presented in Fig 1, the swabs and semen samples were infected with 4 different types of organisms. Eleven of the suspected strains of *A. seminis* closely related to the known strain of *A. actinomycetem comitans* as well as to strains 6201 and 70/64 of *A. seminis*, suggesting that these strains should, in fact, be regarded as *A. actinomycetem comitans*. Strain V350, which appears to follow the description of *Histophilis ovis* (Roberts, 1956; Van Tonder, 1979 b), was found, together with 3 of the suspected strains of *A. seminis*, as a separate organism in the genus *Actinobacillus*. Also strain K 3844 C of *A. seminis* is classified as a separate species of the genus *Actinobacillus*.

These findings indicate that the API 20 E classification system can be used as a handy tool in the identification of organisms such as *A. actinomycetem comitans*, *H. ovis* and *P. haemolytica*. The results obtained from

this fairly small sample stressed the fact that some of the gram-negative, non-motile, pleomorphic bacilli found in the semen and prepuce swabs of rams and ram lambs must be regarded as *P. haemolytica*. On the other hand, the majority of such organisms identified could be classified as either *A. actinomycetem comitans* or *H. ovis*, both of which differed markedly from the relevant strains of *P. haemolytica*.

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