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## CHARACTERISTICS OF TOGO STRAINS OF *BRUCELLA ABORTUS* FROM CATTLE

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### Résumé

CARACTÉRISATION DE SOUCHES DE *BRUCELLA ABORTUS* D'ORIGINE BOVINE ISOLÉES AU TOGO. — Trente souches togolaises de brucella d'origine bovine ont été identifiées à *B. abortus* biotype 3 par les épreuves recommandées par le Sous-Comité de la Taxonomie de ce genre bactérien. Deux caractères inhabituels distinguent toutefois ces souches au sein de l'espèce *B. abortus* : leur profil moyen d'oxydation métabolique modifié au niveau de quatre des substrats conventionnels (L-asparagine, L-arabinose, D-galactose et D-xylose) et leur lenteur de croissance sur les milieux usuels. Ces deux caractères originaux sont discutés d'un double point de vue, épidémiologique et taxonomique.

Taxonomy of *Brucella* was established on strains isolated from all over the world, except Africa from where only a few strains have been studied (Thimm and Wundt, 1976). Among these, 161 Senegal strains of *B. abortus* from cattle were unusual in that they grew slowly, were negative to oxidase test and had a specific oxidative pattern on four conventional substrates (Verger *et al.*, 1979). These peculiarities might set in doubt the value of the present identification scheme for *Brucella* species. However, more strains will be needed from others parts of Africa

to validly discuss this point. With this in mind, we report here data on the identification of 30 *B. abortus* strains isolated from cattle in Togo.

### Materials and Methods

#### 1. Bacterial strains

The 30 strains subjected to identification were isolated in the Sio river's valley, near Lomé, from native cattle that showed hygromas. The fluid

obtained by puncture was plated onto WE selective medium (Renoux, 1954) which was incubated at 37°C for four days in air containing 10% v/v carbon dioxide. Colonies resembling those of *Brucella* members were then subcultured onto WE slopes that were sent to Nouzilly for further identification and typing.

## 2. Identification procedures

Methods employed in the identification of the genus *Brucella* were previously reported (Verger *et al.*, 1975).

The strains were then subjected to typing procedures recommended by the Subcommittee on Taxonomy of genus *Brucella* (Stableforth and Jones, 1963; Jones, 1967; Jones and Wundt, 1971) and related in detail by Alton *et al.* (1977).

Phage typing was performed with Tb, BM29 and Bk2 preparations standardized at the routine test dilution (RTD) on their respective propagation strains (Jones *et al.*, 1968; Douglas and Elberg, 1978; Simon, 1979; Ackermann *et al.*, 1981).

Profiles of utilization of 11 selected amino acid and carbohydrate substrates (table 1) were determined for standardized resting cell suspensions by a Warburg constant volume respirometer and the results were expressed as values representing the rate of oxygen consumption in microlitres per milligram of cell nitrogen per hour (QO<sub>2</sub>N). Resting cell suspensions were prepared from a 24 h culture in Roux flasks containing Trypcase Soy agar (Bio-Mérieux) supplemented with 0.1% yeast extract (Difco) (TSAYE medium). Suspensions were standardized on a spectrophotometer (Spectronic 88, Bausch and Lomb, Inc. Rochester, N.Y., U.S.A. wave length 600 nm) according to an absorbance reference curve so that 1 ml of suspension contained 1 mg of cell nitrogen. More complete details for manometric determinations, expression and interpretation of oxidative metabolic profiles of *Brucella* species were previously reported (Philippon, 1970; Verger, 1973; Verger and Grayon, 1977).

## 3. Measurement of growth

For practical convenience this was carried out on two CO<sub>2</sub> independent variants, one of a randomly selected Togolese strain (80-206) and the other of the reference strain *B. abortus* 544. These strains (mean inoculum:  $5 \times 10^7$  viable cells/ml) were grown in 500 ml narrow-necked conical flasks holding 50 ml of three liquid media, i.e. Trypcase Soy broth (TSB), TSB with 0.1% w/v yeast extract (TSByE) and TSByE with 5% v/v

horse serum (TSByES). The cultures were shaken on a New Brunswick rotary shaker (250 cycles/min) which was placed in a 37°C incubation chamber. The growth was measured for two days by hourly viable counts onto TSAyE Petri dishes. The growth parameters i.e. duration of the lag phase, growth rate constant and doubling time of the culture, were calculated according to Meynell and Meynell (1970).

These two CO<sub>2</sub> independent mutants have been previously examined with the usual morphological, biochemical and oxidative tests for conformity to the characteristics of their respective parental strain.

## Results

### 1. Identification (table 1)

Togolese strains were readily identified as *Brucella* members on the basis of the results of the recommended tests for recognition at the genus level.

All the strains were of the smooth form and were lysed at the RTD by the three phages Tb, BM29 and Bk2 which is consistent with the specific lytic pattern of *B. abortus*.

The graphic presentation of manometric determinations, which was very homogeneous among the 30 strains, showed that the mean oxidative profile looked like the most probable one for *B. abortus* (fig. 1). However the general metabolic aspect for this species was altered in Togolese strains on four substrates: L-asparagine, L-arabinose and D-galactose were oxidized at the 1st level (mean QO<sub>2</sub>N below 100) instead of the 2nd; D-xylose was oxidized at the 2nd level (mean QO<sub>2</sub>N between 100 and 300) instead of the 1st.

The Togolese strains were subsequently identified as members of the biotype 3 of *B. abortus* species on the basis of CO<sub>2</sub> requirement, H<sub>2</sub>S production, thionin and basic fuchsin resistance at 20 µg of dye per ml of serum dextrose agar medium and agglutination exclusively with anti-*abortus* monospecific serum.

### 2. Growth characteristics of Togolese strains

After isolation, subcultures on TSAyE medium showed that all Togolese strains grew much slower than did *B. abortus* strains from France and other European countries in identical conditions. It was also noted that, for the same time of incubation, the size of the colonies was significantly increased by the addition of 5% v/v horse

Table 1. — Characterization of 30 Togolese strains isolated from cattle by usual procedures for *Brucella* identification and typing.

CHARACTERS FOR RECOGNIZING						
GENUS						
Coccobacilli	Catalase	+	Acid on glucose	—		
No capsules	Oxidase	+	Citrate utilization	—		
No endospores	Urease	+	Indole	—		
Non motile	Nitrate reaction	+	Methyl red	—		
Gram negative	Hemolysis	—	Voges Proskauer	—		
Strict aerobes						
SPECIES						
	<i>Oxidative pattern</i>					
	oxidative rate (QO <sub>2</sub> N) <sup>a</sup>					
Substrates	observed range	mean ± s.d.				
no substrates (O)	83-182	119 ± 20	<i>Lysis at RTD by phage</i> Tb + BM29 + Bk2 +			
L-glutamic acid (A)	183-460	289 ± 58				
L-alanine (B)	117-370	193 ± 43				
L-asparagine (C)	0- 25	3 ± 6				
L-arginine (D)	0-130	77 ± 26				
DL-ornithine (E)	0-110	40 ± 22				
L-lysine (F)	0- 55	15 ± 18				
L-arabinose (G)	0- 83	31 ± 23				
D-galactose (H)	2-141	57 ± 35				
D-ribose (I)	233-493	366 ± 67				
D-xylose (J)	76-239	155 ± 41				
meso-erythritol (K)	216-494	376 ± 68				
a : the endogenous rate (line O) was subtracted and the values were expressed as microlitres of oxygen taken up per milligram of cell nitrogen per hour.						
BIOTYPE						
CO <sub>2</sub> requirement	+	<i>Growth on media with 20 µg/ml of :</i>		<i>Agglutination with monospecific sera :</i>		
H <sub>2</sub> S production	+	thionin	+	anti-abortus +		
		basic fuchsin	+	anti-melitensis —		

serum in TSAyE but yet was smaller than the size of non African *B. abortus* colonies on TSAyE without serum. It appeared then necessary to express growth in quantitative terms.

Comparative growth parameters of the Togolese strain 80-206 and of *B. abortus* 544 (both CO<sub>2</sub> independent) in the three liquid media TSB, TSB<sub>y</sub>E and TSB<sub>y</sub>ES are shown in table 2. It appears that the pattern of growth of *B. abortus* 544 was identical in the three culture conditions and characterized by a specific growth rate constant ( $\mu = 0.27 \text{ h}^{-1}$ ) approximately twice greater than that of the Togolese strain ( $\mu = 0.14 \text{ h}^{-1}$ ).

It was also observed that yeast extract and serum added to TSB medium did not enhance the exponential growth rate of strain 80-206 — which was identical ( $\mu = 0.14 \text{ h}^{-1}$ ) in the three conditions— but significantly reduced the duration of its lag phase, respectively 14.40, 6.40 and 2.80 h in TSB, TSB<sub>y</sub>E and TSB<sub>y</sub>ES media.

**Discussion**

The classification of the 30 Togolese strains as members of *B. abortus* biotype 3 was consistent with the recommendations for the description of

species and biotypes of the genus *Brucella* (Corbel and Morgan, 1975). However, Togolese strains displayed two unusual characteristics for *B. abortus* species that are worth discussing i.e. oxidative metabolic pattern and growth characteristics.

### 1. Oxidative metabolic profile

Meyer and Cameron (1961 a,b) first demonstrated that for each species of *Brucella* there is a characteristic pattern of oxygen uptake on selected amino acid and carbohydrate substrates. Since then we showed (Verger and Grayon, 1977) that these specific patterns can be graphically presented into three-level oxidative profiles which greatly facilitate the interpretation of manometric determinations (fig. 1). According to this scheme, *B. abortus* is characterized by most probable oxidation of: L-arginine, DL-ornithine, L-lysine and D-xylose at level 1; L-glutamic acid, L-alanine, L-asparagine, L-arabinose and D-galactose at level 2; D-ribose and meso-erythritol at level 3. As shown in the results (table 1 and fig. 1), Togolese strains differ from the most probable profile for *B. abortus* species on four substrates i.e. L-asparagine, L-arabinose, D-galactose and D-xylose which were oxidized at an unusual QO<sub>2</sub>N level. Nevertheless their profile resembled more closely that of *B. abortus* than those of any other species of the genus (fig. 1).

This was not the first time that *Brucella* strains have been recorded to have an atypical pattern of manometric measurements on one or even more substrates. Examples may be found in any species of the genus but are found more frequently in *B. abortus* one (Brown *et al.*, 1972; Luchsinger *et al.*, 1973; Verger, 1973). In particular, an oxidative profile identical to that of the Togolese strains was previously reported for 65 *B. abortus* strains (64 biotypes 3 and one biotype 1) isolated from cattle in various areas of Senegal (Verger *et al.*, 1979). Since then, 29 additional Senegalese strains of *B. abortus* biotype 3 isolated from cattle's hygromas by Akakpo have been subjected to manometric procedures (unpublished results) and revealed the same atypical *B. abortus* oxidative profile which seems therefore to have a relatively widespread distribution in Africa.

These anomalies may incite some research workers to reconsider the value of oxidative metabolic patterns as a criterion for defining *Brucella* species particularly *B. abortus*. In our opinion it is neither an urgent nor a worrying problem. The fluctuations which have been recorded up to now to occur on one or more substrates do not fundamentally alter the general aspect of the most probable species profile even in the case of Togolese and Senegalese strains which could not be oxidatively confused with any other species than *B. abortus* (fig. 1). Moreover observations available in this field are yet relatively too scant to draw final conclusions on the value of the manometric method. Oxidative metabolism tests can therefore always be regarded as valuable criteria for species identification within the genus *Brucella*. On the other hand, the oxidative anomalies up to now reported were of actual epidemiological significance which is obviously of great interest for a genus where known markers are few.

### 2. Growth characteristics

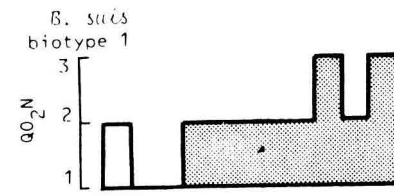
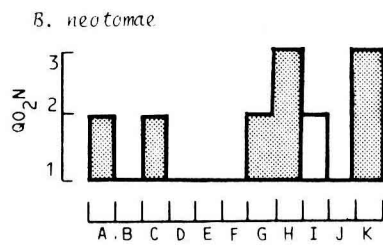
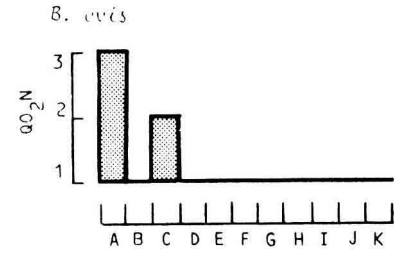
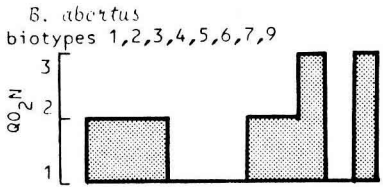
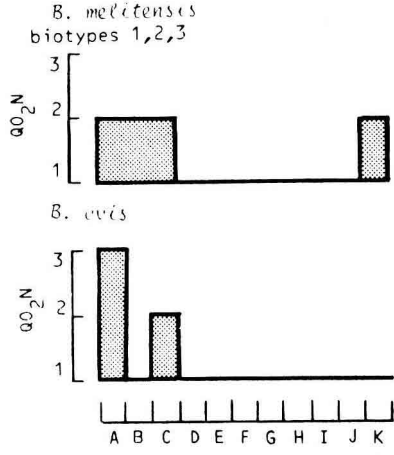
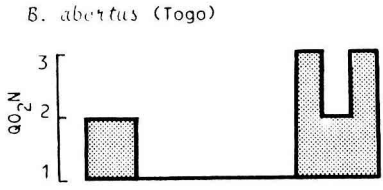
All the Togolese strains like the Senegalese ones previously examined (Verger *et al.*, 1979) were regularly characterized by a very slow growth on usual media for culture of *Brucella*. The kinetics of this growth behaviour clearly shows (table 2) that the observed slowness was essentially due to significant quantitative differences on lag phase and growth rate parameters in comparison with those of the reference strain *B. abortus* 544.

The lag phase of bacterial growth basically corresponds to the time necessary for the cells to build essential intermediates and also to form adaptative enzymes in adequate amounts for cell division. In this regard, and contrary to *B. abortus* 544, the duration of the lag for the Togolese strain was very dependent on the composition of the medium i.e. the poorer the medium, the longer the lag period (table 2). In other words, the cells of this strain are probably unusually deficient in enzymes and coenzymes. These therefore must first be synthesized in the amounts required for optimal operation of the cell's chemical machinery during bacterial division. This synthesis was all the more rapid the better supplemented the medium was. However, even in optimal conditions (TSAyES) the lag of

Fig. 1. — Graphic presentation of manometric determinations: mean oxidative profile of the 30 *B. abortus* strains isolated from cattle in Togo. Comparison with most probable oxidative profiles for *Brucella* species.

Dotted surface: most probable profile whatever the strain may be.

Clear surface: mean profile of the sample but according to the large observed range the substrate may be oxidized at either of the three uptake levels.



QO<sub>2</sub>N level (ml O<sub>2</sub>/mg N/h)

- 1 : QO<sub>2</sub>N < 100
- 2 : 100 ≤ QO<sub>2</sub>N < 300
- 3 : 300 ≤ QO<sub>2</sub>N

Substrates

- A : L-glutamic acid
- B : L-alanine
- C : L-asparagine
- D : L-arginine
- E : DL-ornithine
- F : L-lysine
- G : L-arabinose
- H : D-galactose
- I : D-ribose
- J : D-xylose
- K : meso-erythritol

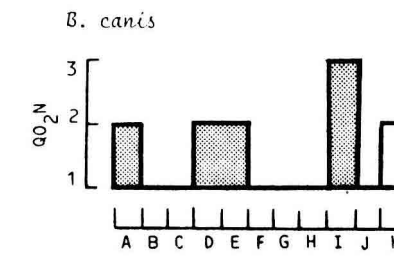
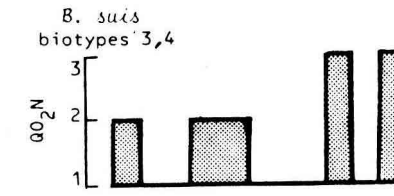
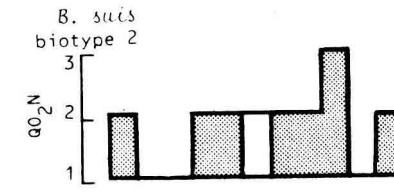


Table 2. — Comparative growth parameters of the Togolese strain 80-206 and of the reference strain *B. abortus* 544 in three liquid media.

strain media <sup>b</sup>	Growth parameters <sup>a</sup>		
	Duration of lag phase (h)	Specific growth rate (h <sup>-1</sup> )	Doubling time of culture (h)
<i>B. abortus</i> 544			
TSByES	2.40	0.27	2.57
TSByE	2.40	0.27	2.57
TSB	2.40	0.27	2.57
<i>B. abortus</i> 80-206			
TSByES	2.80	0.14	4.95
TSByE	6.40	0.14	4.95
TSB	14.40	0.14	4.95

a : mean inoculum  $5 \times 10^7$  viable cells/ml

b : Tryptase Soy broth (TSB), TSB with 0.1% w/v yeast extract (TSByE) and TSBEyE with 5% v/v horse serum (TSByES)

the Togolese strain was slightly longer than that of the 544 strain independent of the three tested media. This behaviour therefore clearly emphasizes the basic enzymatic deficiencies of the 80-206 strain's cells in comparison with those of the 544 strain.

To the logarithmic growth phase of any bacteria corresponds a specific growth rate ( $\mu$ ) which is constant and maximum. This maximum growth rate is characteristic of the organism and also depends, for a given growth temperature, on the adequacy of the medium. In the case of the strain 80-206 cultured at 37°C, the medium did not influence the maximum growth rate which was identical ( $\mu = 0.14 \text{ h}^{-1}$ ) in the three tested media. It seems then that in our experimental culture conditions the organism alone contributed to the maximum growth rate, which was half that of the *B. abortus* 544 reference strain ( $\mu = 0.27 \text{ h}^{-1}$ ). It was not possible however to know what inherent characteristics of the Togolese strain were of importance in this regard.

Published data on growth kinetics of *Brucella* are scarce (Gee and Gerhardt, 1946; Gerhardt and Gee, 1946; Wundt, 1957; Gibby and Gibby, 1964; Dubray, 1972). Moreover the differences in experimental conditions (strains, media, aeration...) do not facilitate a comparison with our results. It seems however that the growth parameters of *B. abortus* 544 are in the range usually reported for *Brucella* members in similar conditions of growth (Gee and Gerhardt, 1946;

Dubray, 1972). Togolese and Senegalese strains can therefore be regarded as significantly slow growing bacteria within the *B. abortus* species.

### 3. Conclusion

The study of African strains obviously leads to a better understanding of the genus *Brucella*. Togolese and Senegalese strains we have studied up to now can unequivocally be classed under the *B. abortus* species as it is defined by the present scheme of taxonomy of the genus *Brucella*. Neither their unusual growth and oxidative characteristics nor the oxidase test negative—for Senegalese strains only—interfered with their classification within this species.

We are therefore of the opinion that a reconsideration of the value of the present criteria for recognizing *Brucella* species is not necessary as of yet. However, there is a definite need for an extensive bacteriological investigation of *Brucella* strains from various areas of Africa.

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### Summary

Thirty Togolese strains of *Brucella* from cattle were classified as members of *B. abortus* biotype 3 by the recommended methods of the Subcommittee on Taxonomy of this genus. However two unusual characteristics distinguish the Togolese strains from the main group of *B. abortus* : their mean oxidative profile altered on four of the conventional substrates (L-asparagine, L-arabinose, D-galactose and D-xylose) and their very slow growth on usual media. These two original characteristics are discussed from an epidemiological and taxonomical point of view.

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