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A Selective Medium for *Brucella* spp.

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SUMMARY: A selective medium for *Brucella* spp. is described which contains 5-nitrofurfurylmethyl ether, bacitracin, polymyxin and Actidione. Organisms normally encountered in culture from faeces or soil are completely suppressed on the medium while brucellas grow quantitatively within 65 hr. of incubation.

A selective medium is essential when the detection of brucellas by direct culture is required from such material as milk, soil, faeces, etc. Felsenfeld *et al.* (1951) devised a medium containing circulin, polymyxin, bacitracin and sulphadiazine to isolate brucellas from chicken faeces. Kuzdas & Morse (1953), using a similar medium, found that 5 days of incubation were necessary to grow these organisms from soil suspensions. Mair (1955) used polymyxin, penicillin and Gentian violet to detect *Brucella abortus* from herd samples of milk, but reported that the medium was sometimes overgrown by contaminant organisms.

The high antibacterial activity of nitrofurans has been reported by many workers (e.g. Dodd & Stillman, 1944; Paul & Bender, 1950; Dodd, Cramer & Ward, 1950). In the present study it was found that 5-nitrofurfurylmethyl ether incorporated in nutrient agar allowed the quantitative growth of *Brucella* spp., while it inhibited the growth of the majority of other Gram-negative organisms, including *Proteus* spp. The medium finally devised enables brucellas to be diagnosed within a 65 hr. incubation period by direct cultivation from material heavily contaminated with other bacteria.

METHODS

Media. The growth of the brucellas was compared on several basal media, e.g. Difco tryptose agar, Albimi agar, 'Oxoid' nutrient agar and a modified Hartley digest agar (TMA) used in this department. Tryptose agar and TMA were identical in their ability to support growth of brucellas in higher concentrations of the inhibitory constituents of the medium than the other basal media tested. The selective medium finally devised consisted of TMA or Tryptose agar at pH 7.4 with 1/30,000 (v/v) 5-nitrofurfurylmethyl ether ('Furaspor'; Menley and James Ltd.); 25 units bacitracin/ml.; 4 units polymyxin B ('Aerosporin'; Burroughs Wellcome and Co.)/ml. and 1/10,000 (w/v) Actidione (The Upjohn Co. U.S.A.). The additions were made to the melted agar cooled to 50° and poured into Petri plates in 20 ml. amounts. The agar plates were dried for 2 hr. at 37° to remove surface moisture and could be stored at 4° for at least 4 weeks without loss in selective properties. The medium was equally selective when bacitracin was replaced by licheniformin.

5-Nitrofurfurylmethyl ether is a viscous yellow liquid soluble at 1/500 in water at room temperature. It is heat labile and completely inactivated by autoclaving at 120°. A 1/500 aqueous solution remains active after several months of storage in the dark at 4°.

Growth tests. The Miles & Misra (1938) technique was used to inoculate the control and test media with pure cultures of brucellas and common contaminating organisms. Inocula were adjusted to give *c.* 200 colonies of brucellas/plate and 2000 of other organisms per plate. *Brucella* spp. were also added to suspensions in water of soil (1 g./20 ml.) and faeces (1 g./100 ml.) so that 0.1 ml. when plated on the media would yield *c.* 100 brucella colonies. Tryptose agar and TMA were used as control media. Incubation for all tests was at 37° for 65 hr. in air and in air + 10 % (v/v) CO₂.

RESULTS

Growth of pure cultures. The 19 strains of the genus *Brucella* examined (*Brucella suis* (6), *B. abortus* (8), *B. melitensis* (3) and two intermediate strains) all gave similar counts on the selective nitrofurant medium and control agar. The results of a typical experiment are summarized in Table 1. Colonies were

Table 1. *Colony counts of Brucella species on the nitrofurant selective medium and on control nutrient agar*

Species		Nitrofurant medium	Control agar
		(Colonies/plate)	
<i>Brucella suis</i>	PS3	290	289
	K/7	198	191
	S/R	269	275
	37	145	158
	B 64	204	208
	B 67	181	221
<i>B. melitensis</i>	MM 6015	212	226
	B 63	141	156
	B 1020	165	178
<i>B. abortus</i>	29	221	198
	B 31	168	157
	B 34	182	186
	B 41	135	136
	B 47	214	225
	B 127	236	221
	B 1019	222	243
	S 19	103	122
Intermediate	B 55	142	154
	B 206	180	175

smaller (0.8–1.0 mm. diam.) on the selective medium than on control agar (1.2 mm. diam.) but were otherwise typical in appearance (Fig. 1). *B. suis* and *B. melitensis* grew equally as well when incubated in air or in the air + CO₂ mixture. Slide-agglutination tests were normal with colonies grown on the selective medium.

E. J. MORRIS--SELECTIVE MEDIUM FOR *BRUCELLA* SPP. PLATE 1

(Facing p. 631)

Growth of the following organisms (showing in parentheses the number of strains tested) was completely inhibited on the selective medium: *Proteus vulgaris* (3), *Escherichia coli* (4), *Aerobacter aerogenes* (2), *Alcaligenes faecalis* (6), *Streptococcus faecalis* (1), *Micrococcus tetragenus* (5), *Staphylococcus aureus* (3), *Pseudomonas aeruginosa* (1), *Serratia marcescens* (2), *Bacillus* spp. (20), *Salmonella* spp. (6), *Achromobacter* spp. (6).

Tests with soil and faecal suspensions. In most instances *Brucella* spp. were grown in pure culture on the selective medium from the many soil suspension mixtures tested. Growth of contaminant organisms in each case was confluent on control agar. Colonies which sometimes developed on the medium were mainly *Achromobacter* species; their numbers (2–20/plate) did not interfere with diagnosis of brucellas. *Brucella* spp. were also isolated without difficulty from the artificially infected suspensions of guinea-pig faeces (Fig. 2). On rare occasions, however, contaminant colonies encountered on the medium from faecal suspensions were unusually high (200/plate). The majority of these colonies were *Alcaligenes* spp. and were suppressed when the medium was incubated in an atmosphere of air + 10 % (v/v) CO₂, while growth of *B. suis* and *B. melitensis* was unaffected by these conditions.

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EXPLANATION OF PLATE

- Fig. 1. Left: colonies of *Brucella suis* on Tryptose agar after 65 hr. incubation at 37° in air. Right: the same inoculum on the selective nitrofurans medium.
- Fig. 2. Left: growth from 0.1 ml. of a faecal suspension on Tryptose agar after 65 hr. incubation at 37° in air. Right: the same inoculum on the selective nitrofurans medium showing a pure growth of *Brucella suis*.

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