AD NUMBER

AD840271

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AUTHORITY

SMUFD D/A ltr, 17 Feb 1972

TRANSLATION NO. 2800

DATE: 21 May 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

INVESTIGATION OF THREE MICROBIOLOGICAL SUBSTRATES CONTAINING WHALE MEAT EXTRACT

Section 144. Biotechnique
FOA Intern rapport
(Defense Research Institute
Internal report C 1256-36)
September 1967 6 sheets
Internal report, not for general distribution

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A new type of substrate from Vato Produkter AB contains as a component part whale meat extract instead of beef extract which is generally used. A comparison between three different Vato substrates, Blood Agar Base, DC Agar and Nutrient Broth with similar substitutes from Oxoid Ltd. shows that whale meat extract can replace beef extract without disadvantage.

Contract No: 144 3600

Investigation of Three Microbiological Substrates Containing Whale Meat Extract

On request from the purchasing center of the county councils, tests were conducted on three microbiological culture substrates: Nutrient Broth, Blood Agar Base and DC Agar, manufactured by Vato Produkter AB, Halmstad. Whale meat extract was used in these substrates instead of the generally used component beef extract.

To be better able to judge the three media, a comparison has been made with similar substrates of the brand Oxoid (Oxoid, Ltd., London).

Nutrient Broth (Vato Produkter AB)

Whale meat extract	6	g/liter
Peptone	10	g/liter
Sodium chloride	6	g/liter
Dipotassium acid phosphate	1	g/liter
Sodium bicarbonate	0.75	g/liter

pH 7.2

Nutrient Broth CM1 (Oxoid Ltd.)

Beef extract	1	g/liter
Peptone	5	g/liter
Sodium chloride	5	g/liter
Yeast extract	2	g/liter

pH 7.4

Blood Agar Base (Vato Produkter AB)

Whale meat extract	10	g/liter
Peptone	10	g/liter
Sodium chloride	5	g/liter
Yeast extract	5	g/liter
Trisodium phosphate	5	g/liter
Agar	15	g/liter

pH 7.2

Blood Agar Base CM 55 (Oxoid Ltd.)

Beef extract	10	g/liter
Peptone	10	g/liter
Sodium chloride	5	g/liter
Agar	15	g/liter

pH 7.5

DC Agar (Vato Produkter AB)

Whale meat extract	5	g/liter
Peptone	5	g/liter
Lactose	10	g/liter
Sodium citrate	5	g/liter
Ferric citrate	1	g/liter
Sodium thiosulfate	5	g/liter
Sodium desoxycholate	2.5	g/liter
Neutral red	0.02	g/liter
Agar	15	g/liter

pH 6.9-7.1

Desoxycholate Citrate Agar CM35 (Oxoid Ltd.)

Beef extract	5	g/liter
Peptone	5	g/liter
Lactose	10	g/liter
Sodium citrate	5	g/liter
Ferric citrate	1	g/liter
Sodium thiosulfate	5	g/liter
Sodium desoxycholate	2.5	g/liter
Neutral red	0.025	"
Agar	15	g/liter

pH 7.3

Test Organisms

Bacillus cereus, Pasteurella pseudotuberculosis, Pseudomonas aerioginosa, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pyogenes.

Test Method

Nutrient Broth. A platinum loop of a day old culture of the test organism in question was introduced into 15 ml sterile broth in a specimen tube which was incubated at 37°C for two days. After one and two days, 3 ml culture were taken out sterilely from the various tubes and the density determined spectrophotometrically at 650 m (Hitachi spectrophotometer 101).

Blood and DC Agar: Day old cultures of the various test organisms were density tested under the microscope by means of a Burker counting chamber and subsequently diluted with sterile physiological NaCl to a density of 10^2 - 10^4 bacteria per ml. From these dilutions, 0.1 ml were laid out on the various plates and allowed to grow at 37°C for two days. Reading with respect to number and estimation of size and appearance were carried out after one and two days.

The results recorded in the table constitute the average value of four tests.

Result

Nutrient Broth. Growth of the test organisms in the two nutrient broths showed no great differences (Table 1). After one day gram-positive bacteria appeared to grow somewhat better in Vato broth while gram-negative grew somewhat better in Oxoid broth. After two days, the culture was somewhat denser in Vato broth, except streptococci.

TABLE 1

Test organism	E_{650} - 1 day		E_{650} - 2 days	
	Vato	Oxoid	Vato	Oxoid
B. cereus	0.34	0.25	0.51	0.41
E. coli	0.29	0.32	0.60	0.46
P. pseudotuberkulosis	0.12	0.14	0.22	0.19
P. aeruginosa	0.25	0.37	0.72	0.55
S. typhimurium	0.27	0.29	0.57	0.54
S. aureus	0.24	0.20	0.29	0.24
S. pyogenes	0.01	0.06	0.04	0.09

Blood Agar. The number of grown colonies from both substrates with the various test organisms was in agreement (Table 2). The colony size was also about alike or in a few cases somewhat larger on the Vato substrate after one and two days incubation.

The hemolysis zone with S. pyogenes was of about the same size on the Vato substrate as on the Oxoid substrate, but the zone was clearer and more distinctly delimited. S. Aureus gave more strongly pigmented colonies with the Vato substrate.

TABLE 2

Test organism	Number of Grown Colonies	
	Vato	Oxoid
B. cereus	10	11
E. coli	43	40
P. pseudotuberkulosis	80	83
P. aeruginosa	27	29
S. aureus	33	30
S. pyogenes	41	42
S. typhimurium	84	81

DC Agar. Growth was obtained only with E. coli, P. aeruginosa and S. typhimurium.

Pseudomonas and coli bacteria gave moderate and irregular growth, but equal for the two DC substrates. With the Salmonella bacteria good growth was obtained on both substrates. The Oxoid substrate gave clarification and discoloration around the colonies.

Comments

Whale meat extract appears to be usable to replace beef extract without disadvantage. The small differences in growth between the Vato and Oxoid substrate that could be observed probably are due to other factors. The nutrient broth from Vato is considerably richer in nitrogen containing material (16 g/l against 8 g/l for Oxoid) which probably explains the generally higher density of broth cultures grown for two days. The stronger pigmentation of S. aureus with Blood Agar Base from Vato is probably due to the fact that yeast extract is included. Oxoid recommends for stronger pigmentation Blood Agar base No. 2 which is richer in nitrogen containing material and where also yeast extract is included.

The media from Vato Produkter AB are packed in bags containing the amount to be added to one half liter water. Thereby the weighing procedure is eliminated, which facilitates preparation.