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Dulcitol–sucrose–salicin–iron–urea agar (DSSIU)—a new medium for differential diagnosis of Salmonellae

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INTRODUCTION

The rapid identification of *Salmonella* is always an attractive subject. Many culture media have been devised to help the biochemical differentiation of *Salmonella* from non-pathogenic enterobacteria (Russell, 1911; Krumwiede, Pratt & McWilliams, 1916; Krumwiede & Kohn, 1917; Kligler, 1918; Hajna, 1945; Silberstein & Rabinowitz, 1950; Bader & Hotz, 1951; Colichon, 1953; Kohn, 1954; Gillies, 1956).

A new differential medium, dulcite–lactose–iron agar (DLI), proposed by Taylor & Silliker (1958) was investigated at the Food Hygiene Laboratory of the Central Public Health Laboratory, Colindale. It was compared with Gillies's (1956) modification of Kohn's (1954) medium, which was in use at the Food Hygiene Laboratory, and also tested with strains from the Laboratory's *Salmonella* collection to determine the proportion of them giving non-typical reactions.

It became clear that the value of DLI medium was limited in a laboratory which habitually uses tetrathionate and selenite broth, Leifson's DCA medium and Wilson and Blair's medium. The new medium had little advantage over DCA which also contains lactose, and was useful only for the investigation of colonies picked up on Wilson and Blair's medium.

Most of the organisms which grow on DCA and Wilson and Blair's medium, but which are not *Salmonella*, are either *Proteus* or 'paracolon' organisms; but *Proteus* on DLI medium resembles the *Arizona* group and the dulcite-negative Salmonellae.

To be useful, DLI medium needed modification, and various formulae were tried. As a result of these tests, a new medium was devised with some characteristics of DLI medium and some of Gillies's modification of Kohn's medium.

This new medium, dulcite–sucrose–salicin–iron–urea agar (DSSIU) has been tested on Salmonellae in the Food Hygiene Laboratory's collection, and typical and atypical reactions have been recorded.

MATERIALS AND METHODS

DSSIU medium is prepared in double-poured slopes, in which the slant and the butt of the slope are of different compositions.

Two solutions, *A* and *B*, are separately prepared.

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Solution A (butt)

Beef extract (Difco)	1·0 g.
Proteose paptone (Difco)	10·0 g.
Sodium chloride	5·0 g.
Agar	16·0 g.
Indicator mixture	27 ml.
Distilled water to	1000 ml.

Solution B (slant)

Beef extract (Difco)	1·0 g.
Proteose peptone (Difco)	10·0 g.
Sodium chloride	5·0 g.
Agar	16·0 g.
Indicator Mixture	27 ml.
Ferrous ammonium sulphate	0·2 g.
Sodium thiosulphate	0·2 g.
Salicin	10·0 g.
Sucrose	10·0 g.
Distilled water to	1000 ml.

Indicator mixture

Solution 1	
Bromthymol blue	0·20 g.
N/ro NaOH	6·4 ml.
Distilled water	100 ml.
Solution 2	
Thymol blue	0·20 g.
N/ro NaOH	8·6 ml.
Distilled water	100 ml.

The indicator mixture, 27 ml. of which is added to both *A* and *B* in accordance with the formulae set out above, comprises 17 ml. of solution 1 and 10 ml. of solution 2.

Solutions *A* and *B* are autoclaved separately for 15 min. at 115° C. and cooled to 55° C. While at this temperature, the two solutions receive the following additions:

To solution *A*, 20 ml. of Seitz-filtered 10% solution of dulcitol and 50 ml. of Seitz-filtered 40% solution of urea; to solution *B*, 50 ml. of Seitz-filtered 40% solution of urea.

The pH of both solutions is adjusted to 7·4. Solution *A* is distributed aseptically in sterile test-tubes in 2–3 ml. amounts and allowed to solidify. Solution *B* is then added in 3–4 ml. amounts and the tubes are then sloped to form half butt and half slant and allowed to cool. In this way the butt of each slope is partly composed of solution *A* and partly of solution *B*, and the slant is solution *B* only (Fig. 1).

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The sugars, especially dulcitol, must be chemically pure, or else false fermentation may occur.

The medium is inoculated by streaking and stabbing. The tube is then incubated at 37° C. for 18–25 hr., and the reactions are read as follows:

The original colour of the medium is dark green. A yellow butt, sometimes with gas bubbles in it, indicates fermentation of dulcitol. Blackening between slant and butt indicates H_2S production. A yellow slant means fermentation of sucrose or salicin.

Urea hydrolysis produces a pure blue colour usually throughout the medium. This is easily distinguished from a greenish blue colour which some alkali-producing organisms show in the slant.

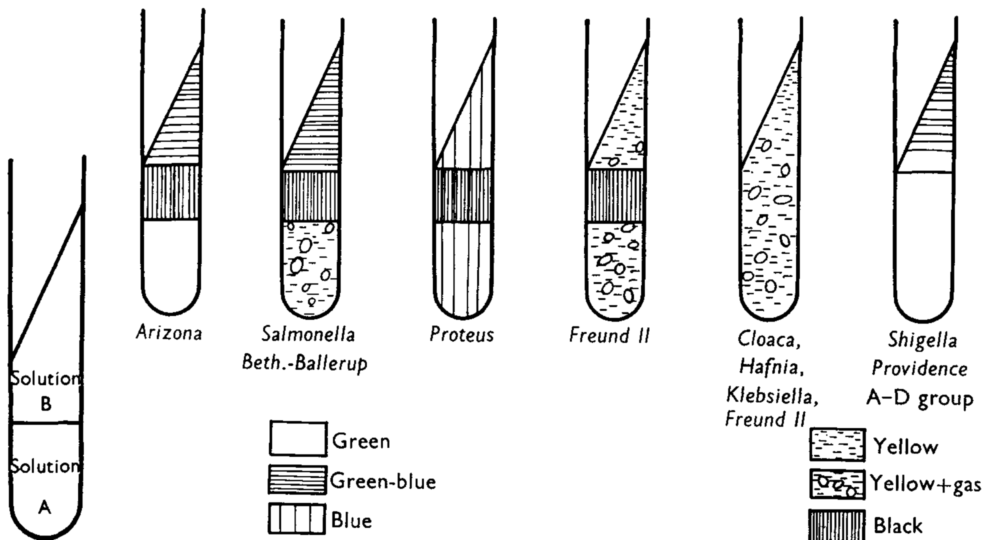

Fig. 1 Fig. 2

RESULTS AND DISCUSSION

According to Kauffmann (1954) dulcitol is fermented by some strains of *Shigella*, *Escherichia coli*, *E. freundii*, *Klebsiella*, *Bethesda–Ballerup*, *Alkalescens–Dispar* group, and by Salmonellae.

Five strains of *Shigella* (Dysentery Reference Laboratory, Colindale) which, according to Kauffmann, should ferment dulcitol in two or more days were found to be inactive on DSSIU medium, producing no change at all. The same was true of four strains of *Alcalescens*, also from the Dysentery Reference Laboratory.

E. coli fermented dulcitol with gas formation, but did not produce H_2S, and sucrose and salicin were often fermented.

Cloaca and *Hafnia* gave positive reactions for sucrose and salicin, with abundant gas formation. *Klebsiella* also fermented sucrose or salicin, and hydrolysed urea after 48 hr.

Proteus caused a very characteristic change in the medium, very often within a few hours.

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Table 1. *Classification of the results of the reactions of* 228 *strains of Salmonellae* (56 *serotypes*) *when tested on the DSSIU medium*

(In accordance with Fig. 2, 204 strains gave typical *Salmonella* reactions within 18 hr. incubation and a further 6 strains showed typical reactions only after 48 hr. incubation. There were, however, 18 strains whose reactions were still atypical at the end of 48 hr. incubation.)

A. *Typical reactions within* 18 *hr. incubation*

Salmonella serotype	No. of strains tested	*Salmonella* serotype	No. of strains tested
S. aberdeen	2	*S. lexington*	1
S. albany	1	*S. marseille*	1
S. amersfoort	1	*S. meleagridis*	2
S. anatum	7	*S. mikawasima*	1
S. bareilly	5	*S. montevideo*	5
S. binza	2	*S. muenchen*	7
S. blockley	10	*S. new brunswick*	1
S. bolton	1	*S. newington*	1
S. bovis morbificans	1	*S. newport*	3
S. california	3	*S. norwich*	1
S. cambridge	1	*S. oranienburg*	11
S. cerro	1	*S. paratyphi B*	14
S. chester	3	*S. paratyphi C*	1
S. chittagong	1	*S. potsdam*	5
S. cholerae-suis	2	*S. rubislaw*	1
S. cubana	1	*S. schwarzengrund*	1
S. denver	1	*S. selandia*	1
S. derby	3	*S. senftenberg*	7
S. duesseldorf	1	*S. shanghai*	1
S. georgia	1	*S. stockholm*	1
S. give	3	*S. tennessee*	6
S. heidelberg	1	*S. thompson*	15
S. hessarek	1	*S. typhimurium*	50
S. infantis	6	*S. uganda*	1
S. kentucky	5	*S. virginia*	1
S. kinshasa	1		

B. *Typical reactions within* 48 *hr. but atypical at* 24 *hr.*

Salmonella serotype	Atypical reaction at 24 hr.	No. of strains
S. hassarek	No dulcite	3
S. new brunswick	No H_2S	1
S. paratyphi B	No dulcite	1
S. worthington	No dulcite	1

C. *Atypical reactions even after* 48 *hr. incubation*

Salmonella serotype	Atypical reaction noted	No. of strains
S. canoga	No H_2S	1
S. cholerae-suis	No H_2S; no dulcite	2
S. gallinarum	No H_2S; no dulcite	1
S. illinois	No H_2S	1
S. newcastle	No H_2S	1
S. oranienburg	No dulcite	3
S. pullorum	No H_2S; no dulcite	2
S. senftenberg	No H_2S	4
S. worthington	No gas in dulcite	1
S. worthington	No dulcite	2

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The only organisms other than Salmonellae which produced the characteristic *Salmonella* reactions were some strains of the *Bethesda–Ballerup* group. These, however, can be differentiated from Salmonellae by the K.C.N. test (Møller, 1954). All *Bethesda–Ballerup* strains grow in the presence of K.C.N., whereas Salmonellae do not.

228 strains of *Salmonella* (56 types) from the Food Hygiene Laboratory collection were tested on DSSIU medium. Of these, 210 strains (51 types) gave quite typical reactions, 204 strains in 18–24 hr. and the remaining 6 strains after 48 hr. incubation; 18 strains (9 types) were atypical in that they either failed to ferment dulcitol or did not produce H_2S even after 48 hr. incubation.

Three strains (*S. cholerae-suis*, *S. pullorum* and *S. gallinarum*) failed both to produce H_2S and to ferment dulcitol. Thus 91 % of the *Salmonella* strains tested gave quite typical reactions.

Proteus and most of the paracolon strains gave characteristic reactions which allowed these organisms to be picked out and discarded at once. In this respect the DSSIU medium is better than DLI medium, on which *Arizona*, *Proteus* and dulcite-negative Salmonellae all give the same picture.

The main advantages of DSSIU over Gillies's modification of Kohn's medium are that only one tube of medium needs to be inoculated, and there is no need to suspend indicator papers above the medium. Moreover, the fermentation of sucrose-salicin, showing in the new medium as a yellow slant, is clearer than in Gillies's medium.

The medium can be stored without deterioration for up to 3 weeks at room temperature and 2 months in the refrigerator. If it is stored for more than 2 months at 4° C., some diffusion of the sugars occurs between butt and slant, so that reactions are less clear cut. If the medium has to be stored for a long time, this deterioration by diffusion can be prevented if, when the tubes are prepared, a thin layer of paraffin wax is poured between the solutions *A* and *B*.

Slide-agglutination tests can be performed on Salmonellae grown on the DSSIU medium.

These studies suggest that the new medium is good enough to warrant its extended trial in routine work in order to find the proportion of newly isolated organisms which fail to give a clear picture and therefore require further biochemical tests for their recognition.

It is possible that, for routine work, a two-tube test may be better, one tube being Gillies's tube I and the other DSSIU medium prepared without urea and with phenol red as indicator. The use of two tubes would permit tests on five 'sugars', but would double the work.

SUMMARY

A new dulcitol–salicin–sucrose–iron–urea medium is described which, on the basis of a single-tube culture, tests the fermentation of three sugars, H_2S production and urea hydrolysis.

On this medium 91 % of 228 Salmonellae gave easily recognized characteristic

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reactions, simulated only by some strains of the *Bethesda–Ballerup* group. Proteus strains and most paracolon strains gave equally characteristic pictures.

The reactions of some other Enterobacteria are described. It is suggested that the medium is good enough to deserve extended trial in routine work.

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