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found parasitic in the alimentary canal in
ruminants and other animals : II. On the
isolation of the bacteria by the use of
galactose-fuchsin agar and Omeliansky agar

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II. On the isolation of the bacteria by the use of
galactose-fuchsin agar and Omeliansky agar

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INTRODUCTION

As described in Report 1,¹⁾ the cellulose-decomposing bacteria in the alimentary canal in ruminants can be isolated in a far shorter period of time when cultured on the author's agar medium (i.e., Omeliansky's medium with agar) compared to time required in any other routine method. The method of isolation which the author used was as follows. A piece of filter paper in Omeliansky's medium was inoculated with an adequate quantity of stomach content, either fresh or air-dried, and kept at 20° to 37°C (30°C suits the purpose in most cases). Two weeks later, when the paper begins to be decomposed and torn into pieces, the liquid part of the medium is diluted with a sterile physiological saline solution 100 to 1000 times its volume; the diluted culture was inoculated on Omeliansky's medium made solid by the addition of agar at a 2 per cent level of agar and cultured at about 37°C; the colonies produced are transferred to another piece of filter paper in Omeliansky's medium; the bacteria in the colonies are tested for the cellulose decomposing power. It has been found, as referred to in Report 1,²⁾ that an addition to the medium of a monosaccharide (xylose, galactose, glucose, etc.) at 0.05 to 0.1 per cent of its volume reduces the time required for the bacterial isolation.

On way of removing the disadvantage (1) of the author's method is to omit the use of Omeliansky's liquid medium for identification. This can be realized when a method is devised which enables us to

distinguish the bacteria under study just as routinely made on Endo's medium. An approach to counteract the disadvantage (2) can be made by furnishing a sufficient amount of cellulose or adequate carbon source to promote the growth of the bacteria if the growth of contaminating bacteria is able to be inhibited by some means.

RESULTS

EXPERIMENT I

Preparation of galactose-fuchsin-added agar medium and discrimination of colonies formed by cellulose-decomposing bacterium on the medium.

The isolation will be simplified if the cellulose-decomposing bacteria can easily be detected by the size and color of their colonies without using Omeliansky's medium; and the bacteria are supplied with sufficient cellulose to promote their growth and make their colonies distinguishable on the one hand, and some substances are added to the medium to inhibit the growth of other forms of bacteria, on the other.

As stated in Report 1,²⁾ the decomposing power of the bacteria is most promoted by saccharides, monosaccharides in particular, and it is conceivable that other growth of forms of bacteria at the same time can be retarded by the acid product of the increased cellulose-decomposing forms. This method of culture appears to suit the purpose better than those of Omeliansky,⁶⁾ Kellermann,⁵⁾ and Bojanovsky,⁴⁾ in which cellulose agar or silica gel is used and so the growth of contaminating organisms is promoted by a supply of some saccharide from the cellulose gel which is split by the cellulose-decomposing bacteria.

Of the three monosaccharides, arabinose, xylose and galactose, which promote the bacterial decomposition of cellulose, the lastnamed can not be, according to Bergey,³⁾ decomposed by a majority of enterococci or enterobacteria. It was, accordingly, chosen as substance to be added to the medium, and for the purpose of chromatic discrimination of the bacteria fuchsin was included, as in Endo's fuchsin and lactose-agar. In practice the author's galactose-fuchsin agar medium is prepared as follows:

K_2HPO_4 —1.0 g,	$(NH_4)_2SO_4$ —1.0 g,	$MgSO_4$ —0.5 g,
$CaCO_3$ —2.0 g,	NaCl—traceable.	

These ingredients are dissolved in 1000 cc of distilled water, added with 0.25–0.5 cc of a galactose stock solution, and further with a sufficient quantity—1.5 cc or so—, of sodium sulfite to deprive the mixture of the red color of fuchsin. The solution thus obtained is

heated to effect better dissolution, filtrated at once at 50°–60°C, and pasteurized for 3 days at 120°–130°C for 20 to 30 minutes a day. This medium should be used within 5 days of preparation, because when left standing at room temperature, it becomes red-colored by oxidation. The way the cellulose-decomposing and the contaminating bacteria form their colonies on the medium will be described below.

(A) The cellulose-decomposing bacteria produce red or pale red pigments when cultured, and form beneath the surface of the medium a round or oval colony which is relatively tiny and granular in shape. The colonies formed on the surface of the medium, are round and moisty. A tiny colony is represented mainly by the *Cellvibrio*, the *Pseudomonas* and the *Cellfalciculla*, and a larger and granular one by the *Cytophaga*. The *Sporocytophaga* which is incapable of decomposing galactose, forms a white transparent colony on the surface of the agar. It is to be noted, however, that the color of a colony does not depend on the genus of the bacteria but rather on the redox potential at the colony.

(B) Generally speaking, common bacteria do not grow well in this medium except cellulose-decomposing one and their colonies are white-colored in most cases. Eleven intragastric strains isolated by Akashi show a white color in a period of 24 hours to 5 days and the 4 galactose-decomposing strains among them which usually grow at a rapid rate and turn red on bouillon agar showed a retarded growth on a medium containing nothing but galactose and inorganic salts. But it must be admitted that there are some exceptional cases where the colonies formed on this medium are red-colored. Such cases will be considered on another occasion. In the majority of cases cellulose-decomposing bacteria are distinguishable from others when cultured on a galactose-fuchsin added agar medium. It is necessary, though, to cultivate these bacteria again on Omeliansky's agar for examination of their cellulose-decomposing power and for preservation of the bacteria thus identified.

EXPERIMENT II

The effectiveness of galactose-fuchsin added agar on isolation of cellulose decomposing bacteria as compared with Omeliansky's agar.

Method: Sample A — fresh stomach contents. Sample B — air-dried stomach contents. Sample C — stomach contents inoculated on a filter paper in Omeliansky's agar medium — medium O — or on the galactose-fuchsin added agar-medium F — till the paper was decomposed and torn into pieces and then left untreated for a month. These material are each diluted 100–1000 fold and smeared separately or grown

by shake culture on medium F and medium O; the colonies formed are inoculated on filter paper in Omeliansky's liquid medium—medium OmL and in a medium-FeL—composed of: $(\text{NH}_4)_2\text{SO}_4$ or NaNO_3 (0.1%) K_2HPO_4 (0.1 %) $\text{MgSO}_4 \cdot \text{HO}$ (0.005 %), FeCl_3 (trace) asparagine (traceable) distilled water (100 cc) and cellulose.

The pigments produced on the filter paper were:

- (α) group—transparent; cream-white, or white,
- (β) group—yolk-yellow, light yellow, fluorescent or orange,
- (γ) group—dark, darkish brown, reddish brown.

Table 1. Results of culture.

Number of experiments	Number of isolated strains in three color groups			Ratio in number of isolated cellulose decomposing bacteria to isolated strains put together
	α	β	γ	
(1) F-agar A- -OmL shake culture	0	8	1	9/9 (100 %)
(2) O-agar A- -OmL shake culture	0	0	4	4/9 (44 %)
(3) F-agar A- -FeL shake culture	2	3	1	6/6 (100 %)
(4) F-agar A- -OmL shake culture	0	0	0	0/9 (0 %)
(5) F-agar A- -FeL smear culture	0	0	0	0/7 (0 %)
(6) F-agar A- -OmL shake culture	0	0	0	0/7 (0 %)
(7) F-agar A- -FeL smear culture	0	0	0	0/4 (0 %)

In case the sample A was used, the most easily distinguishable bacteria were those which were cultured on medium OmL followed by the shake-culture on medium F or on medium O alone. The reason why those grown by smear culture were not separable, remains unknown, though it might be ascribable in part to the kind of the material or the condition of the material used.

Table 2. Results of culture.

Number of experiments	Number of isolated strains in three color groups			Ratio in number of isolated cellulose-decomposing bacteria to all isolated strains
	α	β	γ	
(1) F-agar B-shake culture -OmL	0	11	8	19/28 (67 %)
(2) F-agar B-shake culture -FeL	3	7	0	10/28 (33 %)
(3) O-agar B-shake culture -OmL	1	9	8	18/24 (65 %)
(4) F-agar B-shake culture -FeL	1	9	8	18/24 (65 %)
(5) F-agar B-shake culture -FeL	0	4	2	6/24 (25 %)
(6) F-agar B-shake culture -OmL	0	7	2	9/24 (37 %)
(7) O-agar B-shake culture -OmL	0	5	2	7/10 (70 %)
(8) O-agar B-shake culture -FeL	0	1	2	3/10 (30 %)

Table 2 shows that in the case of sample B those which were cultured by shake culture first on medium F and next on medium OmL, or first on medium O and next on medium F and those which formed yellowish colonies (β), were separable in larger numbers than cultured in other ways.

Table 3 shows that in the case of sample C those cultured first on medium C and next on medium OmL and those formed yellowish colonies were unmistakably separable in far larger numbers than those otherwise cultured.

Table 4 was that the galactose-fuchsin added agar medium is more appropriate for the isolation than is Omliansky's agar medium. The superiority of the former over the latter appears accountable from the fact that the cellulose decomposing bacteria are identifiable mainly by the pigments they produced. No change has so far been observed in the pigment produced by the bacteria while isolation and preservation.

Table 3. Results of culture.

Number of experiments	Number of isolated strains in three color groups			Ratio in number of isolated cellulose-decomposing bacteria to isolated strains put together
	α	β	γ	
(1) F-agar C- smear culture -OmL	13	6	1	20/40 (50 %)
(2) F-agar C- smear culture -FeL	10	4	1	15/40 (37 %)
(3) O-agar C- smear culture -OmL	0	0	4	4/9 (44 %)
(4) O-agar C- smear culture -OmL	0	1	1	2/9 (22 %)
(5) F-agar C- shake culture -OmL	5	9	8	22/41 (53 %)
(6) F-agar C- shake culture -OmL	3	7	8	18/30 (43 %)
(7) O-agar C- shake culture -OmL	1	2	23	26/41 (86 %)
(8) O-agar C- shake culture -FeL	1	2	10	13/30 (42 %)

To sum up: Different cellulose-decomposing bacteria producing different pigments can be separated in relatively numerous numbers by culturing in a galactose-fuchsin-added agar medium or in Omeliansky's agar medium.

(2) The white pigmented forms of the bacteria can be separated by a shake culture on Omeliansky's medium followed by culture on the galactose-fuchsin-added agar medium; the yellow pigmented forms by shake culture or smear culture on a galactose-added agar medium in case of a fresh or air-dried sample; and the brown-pigmented forms by shake culture first on Omeliansky's medium and next on Omeliansky's solid medium. (3) The change in color of a colony formed on a galactose-fuchsin-added medium appears to be nonspecific in some cases.

Table 4. Results of culture.

Number of experiments	Number of isolated strains in three color groups			Ratio in number of isolated cellulose-decomposing bacteria to isolated strains put together
	α	β	γ	
(1) F-agar C-smear culture -OmL	13	6	1	20/40 (50 %)
(2) F-agar C-smear culture -OmL	5	9	1	22/40 (55 %)
(3) F-agar B-smear culture -OmL	7	11	1	19/28 (67 %)
(4) F-agar A-smear culture -OmL	0	8	1	9/9 (100 %)
(5) O-agar C-smear culture -OmL	0	2	23	26/30 (16 %)
(6) A-agar A-smear culture -OmL	0	0	4	4/9 (44 %)
(7) O-agar C-smear culture -FeL	1	2	10	13/30 (43 %)

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REFERENCES

1. Akashi, A. 1954. Jap. J. Zootech. Sci., 25: 193-197.
2. Akashi, A. 1952. Jour. Fac. of Agr., Kyushu Univ., 12: 405.
3. Bergy, D. E. 1949. Manual of Determinative Bact., 6th ed.
4. Bojanovsky, R. 1925. Centralbl. Bact., 11 (64): 223-233.
5. Kellermann, K. J. 1912. Centralbl. Bact., 11 (34): 485-492.
6. Omeliansky, W. L. 1925. Centralbl. Bact., 119 (8): 225-231.