A DIFFERENTIAL BROTH FOR SEPARATING THE LACTIC STREPTOCOCCI[1]

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ABSTRACT

Arginine degradation and citrate utilization, the major differentiating characteristics among lactic streptococci, formed the basis of a differential broth for separating *Streptococcus cremoris*, *Streptococcus lactis*, and *Streptococcus diacetilactis* strains in pure cultures.

The medium contains milk as the sole source of carbohydrate (lactose), arginine and sodium citrate as specific substrates, and a suitable pH indicator (bromcresol purple), in addition to other ingredients. The pH of the medium is adjusted to 6.2 ± 0.05 (which becomes 6.15 ± 0.05 after sterilization) to increase citrate utilization and the broth is dispensed into test tubes containing Durham fermentation tubes. *Streptococcus cremoris* produces a yellow reaction (acid) in the broth. *Streptococcus lactis* initially turns the broth yellow, but on liberation of NH_3 reverses the color back to the original violet hue. *Streptococcus diacetilactis* produces a violet reaction, and CO_2 accumulates in the Durham fermentation tubes from the fermentation of sodium citrate.

The most commonly used starter microorganisms in the dairy industry belong to Sherman's lactic group of the genus *Streptococcus (2)*. *Bergey's Manual of Determinative Bacteriology (1)* recognizes only *Streptococcus lactis* and *Streptococcus cremoris* as members of Sherman's lactic group. Sandine et al. *(7)* have shown that *Streptococcus diacetilactis* also should be included in this group. Routinely, members of this group are differentiated by several biochemical tests *(4, 5, 6)*; the major tests being arginine hydrolysis *(6)* and King's test for diacetyl and acetoin *(4)*. Among the lactic streptococci most strains of S. *lactis* and S. *diacetilactis* can deaminate arginine; S. *cremoris* lacks this ability. *Streptococcus diacetilactis* is the only species able to rapidly utilize citrate to produce CO_2, diacetyl, and its reduced C_4 compounds. Niven et al. *(6)* were the first to differentiate S. *lactis* from S. *cremoris*, on the basis of arginine hydrolysis. This differentiation was based on detection of NH_3 liberated from arginine with Nessler's reagent. Later, Mikolajcik *(5)* described a broth in which arginine hydrolysis could be demonstrated by incorporating an acid-base indicator.

A disadvantage of the media described by Niven et al. *(6)* and Mikolajcik *(5)* was that S. *diacetilactis* also gave a reaction similar to S. *lactis*. Sandine et al. *(7)* described another broth that could be used to separate the citrate-fermenting lactic streptococci from those that do not utilize the tricarboxylic acid, by detection of CO_2 produced in the broth.

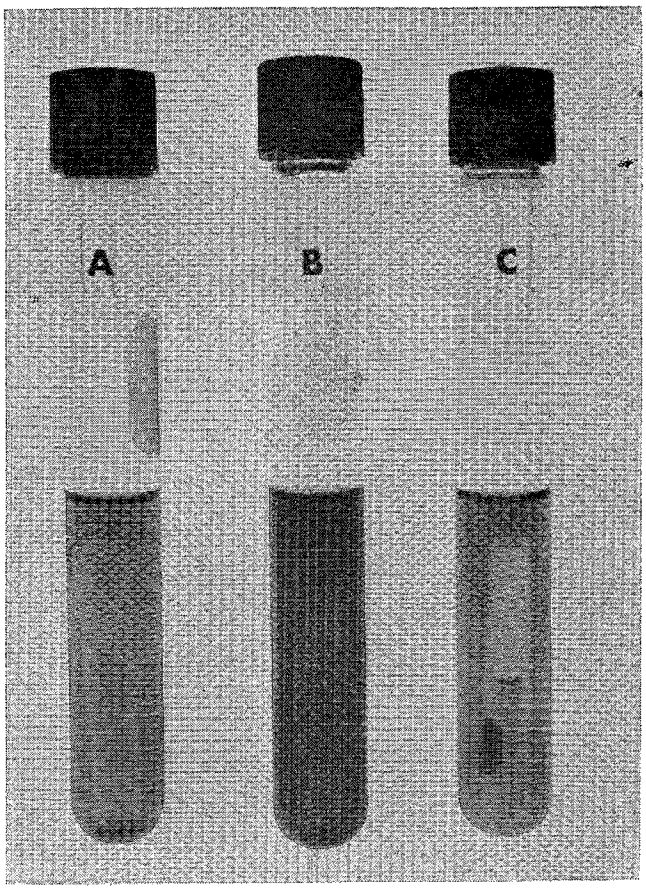

Figure 1. Photograph taken with yellow-sensitive film showing reactions in the differential broth by S. *lactis* C_2F—violet indicator color and no gas (left), S. *cremoris* W—yellow indicator color and no gas (middle), and S. *diacetilactis* 18-16-violet indicator color and gas (right), after 48-hr incubation at 30 C.

Our investigation was undertaken to develop a single test broth that could differentiate between all three species in Sherman's lactic group, namely S. *lactis*, S. *cremoris*, and S. *diacetilactis*. We tried to combine the principles underlying the differential broths of Mikolajcik *(5)* and Sandine et al. *(7)* in the development of such a medium. Suitable modifi-

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TABLE 1. MAJOR BIOCHEMICAL CHARACTERISTICS OF REPRESENTATAVE LACTIC *Streptococcus* AND FOUR *Leuconostoc* STRAINS AND THEIR REACTIONS IN THE DIFFERENTIAL BROTH

Strains	NH_3 in Niven's broth[a]	King's test[b]	Coagulation of milk in 48 hr at 30 C	Reactions in broth		
				Acid[c]	NH_3[d]	Gas[e]
S. lactis						
7963	+	–	+	–	+	–
C_2F	+	–	+	–	+	–
E	+	–	+	–	+	–
7962	+	–	+	–	+	–
S. cremoris						
HP	–	–	+	+	–	–
ML_4	–	–	+	+	–	–
SC_1	–	–	+	+	–	–
SC_3	–	–	+	+	–	–
S. diacetilactis						
18-16	+	+	+	–	+	+
26-2	+	+	+	–	+	+
DRC_3	+	+	+	–	+	+
31-2	+	+	+	–	+	+
Leuconostoc citrovorum						
CAF-B	–	–[f]	–	NC[g]	–	–
Da_3	–	–[f]	–	NC[g]	–	–
Leuconostoc dextranicum						
L.D.	–	–	–	GR[h]	–	+[i]
1145	–	–	–	NC[g]	–	+[i]

[a]Tested with Nessler's reagent.
[b]Colorimetric detection of diactyl-acetoin with α-naphthol.
[c]Yellow color.
[d]Reversal of yellow color to violet (purple).
[e]Collection in Durham fermentation tubes (roughly quantitative).
[f]Tested up to 72 hr of incubation.
[g]No change.
[h]Growth and very slight acid.
[i]Minute amounts of gas observed only after 3 days of incubation.

cations of Mikolajcik broth (5) were made to accommodate utilization of citrate in the presence of the highly basic guanidyl amino acid. To readily observe CO_2 production in the medium, Durham fermentation tubes were placed in the broth in the test tubes.

MATERIALS AND METHODS

Cultures

Fourteen *S. cremoris*, 10 *S. lactis*, 13 *S. diacetilactis*, 2 *Leuconostoc citrovorum*, and 2 *Leuconostoc dextranicum* strains were included in this investigation. They were obtained from culture collections at the Departments of Food Technology, Iowa State University; Microbiology, Oregon State University; and Food Science, University of Wisconsin.

Media

The differential broth contained 0.5% tryptone, 0.5% yeast extract, 0.1% K_2HPO_4, 0.5% arginine, 2% Na citrate, and 0.002% bromcresol purple. Three and one-half milliliters of 11% reconstituted Matriv milk (Galloway-West Co., Fond du Lac, Wis.) were added to every 100 ml of broth and steamed for 15 min. The lactose content of the broth was considered approximately equal to 0.175%. The pH of the mixture was adjusted to 6.2 ± 0.05 *after it was cooled to room temperature*. The broth, in 7-ml quantities, then was dispensed into screw capped test tubes (length 126 mm and neck 10 mm internal diameter, Pyrex No. 9825 containing Durham tubes and was sterilized at 121 C for 15 min.

One loop-full amounts of active 18-hr milk cultures were used to inoculate the tubes. At the time of inculation the pH of the medium should be 6.15 ± 0.05. The test tube caps were tightly closed to prevent escape of liberated ammonia and CO_2, and incubation was at 30 C. Indicator color reactions and CO_2 accumulation were observed at 24-hr intervals for 72 hr.

RESULTS AND DISCUSSION

In most instances, reactions were complete in 24 to 48 hr. After 24-hr incubation, all tubes were tapped a few times to liberate residual gas from the medium.

Streptococcus lactis turned the broth color first to yellow by production of lactic acid and later reversed the reaction to violet because of liberation of NH_3. *Streptococcus cremoris*, on the other hand, produced a deep-yellow color. In addition to reversing the indicator color, *S. diacetilactis* produced copious amounts of CO_2 within 48 hr, which collected in the Durham fermentation tubes. Further, *S. diacetilactis* produced a more intense purple than *S. lactis*. The reactions in the broth are shown in Fig. 1.

Streptococcus diacetilactis failed to produce gas when the pH values were high; i.e., pH ≥6.8. Adjusting the pH of the cooled medium to 6.2 ± 0.05 before sterilization resulted in copious gas production and good differentiation. This is in keeping with the pH optima for citrate permease of S. *diacetilactis* and *Leuconostoc* species as described by Harvey and Collins *(3)*.

A total of 40 strains belonging to the species S. *lactis*, S. *cremoris*, and S. *diacetilactis* were tested in the broth in pure cultures. All gave good growth and excellent differentiation. In addition, four strains of starter *Leuconostoc* species also were tested in this medium. Minute amounts of gas production were observed with two strains of *L. dextranicum* after 3 days of incubation. The two *L. citrovorum* strains failed to produce gas even after prolonged incubation. No appreciable color change in the violet differential broth was observed with the *Leuconostoc* cultures. Results of the reactions in the differential broth along with reactions in previously described biochemical tests *(4, 6)* for 16 representative strains are summarized in Table 1.

Arginine hydrolysis by starter strains grown in this broth can further be confirmed by testing portions of the culture with Nessler's reagent on a porcelain spot plate. Positive results are indicated by a deep-red precipitate.

This differential broth has wide application in the starter-culture industry and in routine laboratory work. The broth also is suitable for qualitative differentiation of individual colonies developing on agar plates containing dilutions of several commercial starter cultures. It simplifies biochemical identification of lactic streptococci.

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REPORT OF THE COMMITTEE ON FOOD PROTECTION, 1969-1970

In previous reports to the membership, this Committee has discussed the desirability of convening a National Conference on Food Protection. It is now possible to state that a National Conference on Food Protection will be held in Denver, Colorado in April 1971. The Food and Drug Administration has contracted with the American Public Health Association to conduct the Conference. A great deal of planning still remains to be done. However, the Conference will run about four days, and will be designed to be an action meeting. Specific proposals are expected to be forthcoming from the Conference to improve food protection programs in this country. Invitations to participate will be sent to all interested governmental, professional, and trade organizations.

Present plans call for work groups to study the following problem areas and recommend solutions.

(a) Control of contamination of raw agricultural and marine products.

(b) Control of contamination of processed foods.

(c) Prevention of mishandling during preparation of foods in commercial and institutional food service operations.

(d) Consumer education to minimize abuse of foods in the home.

(e) Development of an improved system for detection, investigation, and reporting of microbial hazards associated with foods.

(f) Coordination of regulatory activities among the governmental agencies and with industry control programs.

(g) Training and utilization of professional and non-professional manpower.

(h) Development of public acceptance and political support for food protection programs.

(i) Selection and use of criteria for evaluating program effectiveness.

(j) Needs for research, surveillance, and related technical activities in support of a national food protection program.

This, of course, can only be an interim report, since, much remains to be done in planning a Conference of this scope. The Chairman and Vice-Chairman of this Committee have been involved in the preliminary planning, and it is our hope that the entire Committee will become involved in the National Conference on Food Protection.

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