ACETATE AND OLEATE REQUIREMENTS OF THE LACTIC GROUP OF STREPTOCOCCI[1]

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Received for publication October 6, 1949

The need for a chemically defined medium in which strains of *Streptococcus lactis* and *Streptococcus cremoris* would grow was felt in this laboratory while work was being done with bacteriophages active against strains of the lactic group of streptococci.

Storrs and Anderson (1949) have reported that the medium of Niven (1944), modified by the substitution of lactose for glucose and the addition of phosphate for buffering, was inadequate for normal acid production by *S. lactis*. Supplementation with "yeastrel," with fish-meal digest, or with certain incompletely defined fractions of these products stimulated acid production.

The present study describes the results obtained when various supplements were added to the medium of Niven (1944), which was found inadequate for the growth of 9 of 31 strains of *S. lactis* and all 22 strains of *S. cremoris* studied.

METHODS

The cultures employed in this investigation consisted of 22 strains of *S. cremoris* and 3 strains of *S. lactis* from the laboratory stock collection, 8 strains of *S. lactis* isolated in December, 1945, and 20 strains of *S. lactis* isolated for the study. Growth at 40 C and the production of ammonia from L-arginine were used to differentiate *S. lactis* and *S. cremoris*. Stock cultures were carried in litmus milk.

The synthetic medium of Niven (1944), adjusted to pH 6.7 and autoclaved 11 minutes at 15 pounds pressure, was used as the basal medium. Glutamine and asparagine were sterilized by filtration and added aseptically to the autoclaved medium. When supplement I (table 1) was added, this medium permitted the growth of all strains used. After three serial transfers in this supplemental medium, cultures were transferred to duplicate tubes of medium containing the indicated modifications of supplement I (table 1). Turbidity readings were made after 24 hours' incubation at 32 C on the second serial transfer in a medium in which turbidity developed during the first 24-hour incubation period. One drop of the previous culture was used as inoculum in all cases. Turbidity measurements were made with a Klett-Summerson photoelectric colorimeter, using filter no. 54. The colorimeter was adjusted to a reading of 0 with a tube of uninoculated medium, and multiplication is expressed in terms of increase in turbidity.

[1] Journal paper no. J-1694 of the Iowa Agricultural Experiment Station, Project 652.

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TABLE 1

List of supplements

SUPPLEMENT NUMBER	NUTRIENTS (PER 100 ML MEDIUM)
I	Sodium acetate, 0.2 g; sorbitan monooleate,* 0.2 g; recticulogen,† 20 μl; *p*-aminobenzoic acid, 20 μg; folic acid, 1 μg; thymine, 0.5 mg; pyridoxamine, 0.1 mg; pyridoxal, 0.1 mg; nicotinamide, 0.1 mg; inositol, 0.1 mg; and cysteine, 10 mg
II	Supplement I minus sodium acetate, sorbitan monooleate, and reticulogen
III	Supplement I minus sodium acetate and reticulogen
IV	Supplement I minus sorbitan monooleate and reticulogen
V	Reticulogen, 20 μl
VI	Sodium acetate, 0.2 g; and sorbitan monooleate 0.2 g

* A polyoxyethylene derivative of sorbitan monooleate (source of oleic acid radical).

† A commercial liver extract.

TABLE 2

Multiplication of strains of S. cremoris in the synthetic medium of Niven supplemented as indicated

(Average of duplicate determinations)

CULTURE	TURBIDITY READINGS AT 24 HOURS WITH SUPPLEMENT						
	None	I*	II	III	IV	V	VI
FH8	0	74	0	0	5	34	86
6	1	129	0	0	0	98	141
318B/27	3	144	0	0	112	104	110
497	0	131	0	0	0	82	104
144F	0	108	0	0	25	78	108
M1	0	86	8	0	60	16	110
DL	2	138	7	53	108	107	147
H1-2	0	74	0	—	—	45	—†
H1-10	1	83	0	0	21	73	127
ML1	0	94	0	0	28	46	70
Q	0	114	0	0	37	75	108
E8	1	90	0	0	5	60	90
799/11	1	143	0	84	120	93	85
IP5	0	117	0	0	15	94	118
K_2	0	86	0	0	79	54	74
HP	0	80	0	0	0	90	89
KH	0	105	0	10	50	70	126
459	0	101	0	0	39	79	105
573	0	75	0	0	37	84	105
799	1	100	0	0	49	77	61
W4	0	139	0	81	31	110	115
122-1	0	122	0	105	71	103	79

* See table 1.

† Usually growth at 48 hours.

RESULTS

All 22 strains of *S. cremoris* (table 2) failed to become turbid in 24 hours in the medium of Niven until it was supplemented with either sodium acetate and sorbitan monooleate (a source of oleate), or "reticulogen" (a commercial liver extract). When cultures were incubated for as long as 72 hours at 32 C in a medium containing no sodium acetate or sorbitan monooleate, a few cultures occasionally became turbid; however, such turbidity development was very erratic. Even when the basal medium was supplemented with the entire mixture of growth factors minus reticulogen, sodium acetate, and sorbitan monooleate (supplement II), all strains of *S. cremoris* except M1 and DL failed to become turbid within 24 hours. The turbidity readings for M1 and DL were very low. Thus the requirement by this group of cultures for either sodium acetate and sorbitan monooleate or reticulogen was not eliminated by any nutrient or combination of nutrients used in supplement II. When sodium acetate and reticulogen were left out of the supplemented medium, which still contained sorbitan monooleate (supplement III), only one strain, 122-1, developed about as well as in the complete supplement, whereas strains DL, 799/11, KH, and W4 developed somewhat less turbidity than when sodium acetate and reticulogen also were included. With inclusion of sodium acetate and omission of sorbitan monooleate (supplement IV) there was an increase in the number of cultures that became turbid. However, only cultures 318B/27, DL, 799/11, K2, and 122-1 developed turbidity to approximately the same degree as was done in the medium containing both sodium acetate and sorbitan monooleate. In the synthetic medium supplemented only with reticulogen (supplement V), turbidities in general were somewhat less than when the supplement was sodium acetate and sorbitan monooleate (supplement VI); the difference was considerable for many of the cultures. However, turbidities developed by strains 318B/27, 799/11, HP, 799, and W4 were about equal to those developed when the supplement was sodium acetate and sorbitan monooleate, and strains H1-2 and 122-1 actually became more turbid when the supplement was reticulogen. Strain H1-2, an extremely slow culture even in milk, usually became turbid between 36 and 48 hours in the synthetic medium supplemented with sodium acetate and sorbitan monooleate. Cultures 318B/27, 497, ML1, 799/11, 799, W4, and 122-1 developed higher turbidities with the entire mixture of growth factors (supplement I) than with only sodium acetate and sorbitan monooleate (supplement VI), whereas cultures M1, H1-10, KH, and 573 developed slightly lower turbidities when the entire mixture of growth factors was added. Thus, the addition of reticulogen and the several growth factors of the B complex provided little stimulation for most cultures growing in the basal medium supplemented with sodium acetate and sorbitan monooleate. The addition of 0.0004 μg of crystalline vitamin B_{12}[3] per ml to the otherwise unsupplemented medium of Niven did not result in visible turbidity within 24 hours with any of 12 strains of *S. cremoris*.

[3] In the form of cobine, Merck.

Only 9 of the 31 test cultures of *S. lactis* failed to give good turbidity levels at 24 hours on the basal synthetic medium, and these 9 cultures developed satisfactorily when sodium acetate and sorbitan monooleate were added (table 3).

TABLE 3

Multiplication of strains of S. lactis in the synthetic medium of Niven supplemented as indicated

(Average of duplicate determinations)

CULTURE NO.	TURBIDITY READINGS AT 24 HOURS WITH	
	No supplement	Supplement VI*
1	60	—
2	1	127
3	52	—
4	132	—
5	0	131
6	1	126
7	88	—
8	92	—
9	81	—
10	108	—
11	110	—
12	100	—
13	82	—
14	0	136
15	0	103
16	120	—
17	94	—
18	96	—
19	143	—
20	110	—
1-1	80	95
2-1	125	155
3-1	113	183
4-1	0	150
1-8	0	155
4-8	32	123
2-15	0	170
3-15	0	200
712	136	187
565	128	185
W2	132	182

* See table 1.

In the 7 cases in which sodium acetate and sorbitan monooleate were added to cultures of *S. lactis* that did not require this supplement, the development of turbidity during 24 hours was greater than in the unsupplemented medium.

Limited studies with *S. lactis* 565 indicated that the omission from the complete basal medium of one of the following compounds, DL-threonine, glycine, DL-aspartic acid, L-cystine, L-glutamic acid, DL-tryptophan, L-proline, DL-

norleucine, or xanthine, still permitted growth, as indicated by turbidity measurements, equal to that in the complete basal medium. In the case of *S. lactis* W2, omissions of hydroxy-L-proline, L-lysine, DL-threonine, L-tyrosine, asparagine, glycine, DL-aspartic acid, L-cystine, L-glutamic acid, DL-tryptophan, thiamine, riboflavin, adenine, guanine, pyridoxine, or xanthine and possibly DL-phenylalanine, L-proline, DL-norleucine, or uracil from the basal synthetic medium individually did not affect the growth of the organism, as indicated by turbidity readings.

DISCUSSION

The importance of sodium acetate and unsaturated fatty acids in the growth of various lactic acid bacteria has been reported by several workers. Guirard, Snell, and Williams (1946), finding that acetate could be replaced by several fatty acids, keto acids, and sterols for a few lactic acid bacteria, indicated that acetate might serve in the synthesis of cellular lipoidal materials; however, these investigators were unable to replace acetate with unsaturated fatty acids for the strain of *Lactobacillus casei* used. The present work indicates that acetate and oleate are important in the nutrition of many strains of the lactic group of streptococci. This requirement apparently is much more general among the lactic group of streptococci than has been reported for other lactic acid bacteria. No strain of *S. cremoris* tested was able to develop turbidity in the synthetic medium in the absence of both sodium acetate and sorbitan monooleate unless an undefined nutrient different from known members of the B complex of vitamins was included in the medium. Sorbitan monooleate alone permitted growth of only 5 of 22 *S. cremoris* cultures during 24 hours, and, although sodium acetate alone gave better results than sorbitan monooleate alone, both of these nutrients seemed necessary for the good development of turbidity by most cultures. Although sorbitan monooleate without sodium acetate permitted growth of one strain of *S. cremoris*, the stimulation for most cultures provided by the addition of sorbitan monooleate possibly is of the type reported by Guirard, Snell, and Williams (1946), which involves a nutritional relationship between acetate and the synthesis of cell lipoidal materials in bacteria. Sodium acetate seems either to perform an additional function in the metabolism of these organisms or it is transformed by the organisms into some substance that is essential for growth. The fact that very small quantities of the liver extract, reticulogen, may be substituted for sodium acetate and sorbitan monooleate may indicate that the addition of reticulogen provides a growth factor or factors that serve in the formation of acetate or in a metabolic scheme in which some substance formed from acetate also is active.

Bauman and Sauberlich (1948), upon finding that small quantities of reticulogen promoted the growth of *Leuconostoc citrovorum* 8081 in a synthetic medium, attributed the growth-promoting quality to vitamin B_{12}, which was not available at the time of their work. In the current work vitamin B_{12} did not permit any of 12 strains of *S. cremoris* to become turbid during 24 hours.

The species *S. cremoris* often is considered to be a select group containing the

more fastidious strains of the species *S. lactis*. The 29 most recently isolated organisms used in this study were classified as *S. lactis*, whereas those cultures that have been carried in laboratories as milk cultures for many years all classified as *S. cremoris*. This is presumptive evidence that pure cultures of *S. lactis* organisms may become more fastidious under the conditions of laboratory culture, and are finally classified as *S. cremoris*. Whether this change might be due to continued culture in heated milk or whether it might be the result of repeated subculture in the presence of citric-acid-fermenting associates is not apparent from the information available. Nichols and Hoyle (1948) found that 277 strains of the lactic group isolated from commercial starters all were *S. cremoris* and that, of 72 wild strains isolated from samples of sour milk and found suitable for use as starters, 70 were *S. lactis* whereas 2 were *S. cremoris*. In the present study only 9 of 31 strains of *S. lactis* tested required sodium acetate and sorbitan monooleate, whereas all 22 strains of *S. cremoris* required this supplement. This indicates the more fastidious nature of *S. cremoris* organisms in this one requirement.

SUMMARY

A chemically defined medium made by adding sodium acetate and sorbitan monooleate (a source of the oleic acid radical) to the medium of Niven (1944) permitted the growth of all tested strains of the lactic group of streptococci that did not grow in the unsupplemented medium. The addition of sodium acetate and sorbitan monooleate was necessary for the growth of 22 strains of *S. cremoris* and 9 of 31 strains of *S. lactis*. Reticulogen, a commercial liver extract, could be substituted in somewhat smaller quantities for sodium acetate and sorbitan monooleate and also permitted rapid growth of one strain of *S. cremoris* that did not show detectable growth until after 24 hours in the medium supplemented with sodium acetate and sorbitan monooleate. Vitamin B_{12} was not required by these organisms.

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