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End Products and Fermentation Balances for Lactic Streptococci Grown Aerobically on Low Concentrations of Glucose

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Maximum acetate produced aerobically by *Streptococcus diacetilactis* and *Streptococcus cremoris* was 14% of 1 to 7 μmol of glucose/ml in a partially defined medium that contained lipoic acid. Y (glucose) values were 35.3 (*S. diacetilactis*) and 31.4 (*S. cremoris*) with low concentrations (1 to 7 μmol/ml) of glucose in the medium and 21 (*S. diacetilactis*) with higher concentrations (6 to 15 μmol/ml). Y (adenosine 5'-triphosphate) values for the bacteria, determined by taking into account the end products produced, were 15.6 and 13.9 for *S. diacetilactis* and *S. cremoris*, respectively, in the partially defined medium containing 1 to 7 μmol of glucose/ml and higher (21.5 and 18.9, respectively) in a complex medium that contained 2 μmol of glucose/ml. Addition of citrate in addition to glucose did not result in higher molar growth yields.

Species of *Streptococcus* classically use the hexose diphosphate pathway for energy production and ferment carbohydrate chiefly to lactic acid. Resting cells of *Streptococcus diacetilactis* form acetate from pyruvate (6), but growing cells, unlike *Streptococcus faecalis* (10, 11, 16, 24, 25) and lactobacilli (8, 9), are reported unable to utilize pyruvate (or citrate) as a source of energy (6, 11, 22). Homofermentative lactobacilli produce large amounts of acetate from low concentrations of carbohydrate (8, 9). Under aerobic conditions 93% of 1 to 6 μmol of galactose/ml was converted to acetate by *Lactobacillus plantarum* (9). Less acetate at higher concentrations of carbohydrate was attributed to stimulation of lactic dehydrogenase by fructose-1,6-diphosphate (1, 21, 30, 32, 33).

This study was undertaken to determine if supplying minimal glucose under aerobic conditions would enable lactic streptococci to divert appreciable pyruvate to the production of acetate.

MATERIALS AND METHODS

Organisms. The organisms studied were *S. diacetilactis* DRC1 (7, 20, 29) and *Streptococcus cremoris* C13. They were propagated twice weekly in a complex medium. Cultures were incubated overnight at 30°C and stored at 4°C.

Media. The complex medium used for maintaining cultures contained 1% peptone, 1.5% yeast extract, 0.1% Oxoid casein hydrolysate, 0.2% K_2HPO_4, 0.025% $MgSO_4$, 0.2% sodium acetate, and 10 mM glucose, adjusted to pH 6.5. The partially defined medium for growing inocula and experiments was that of Harvey and Collins (13) as modified by Collins and Bruhn (6), except that the glucose content was lower, Oxoid casein hydrolysate (Consolidated Laboratories, Chicago Heights, Ill.) replaced vitamin-free casein hydrolysate, and DL-α-lipoic acid (10 μg/ml) replaced acetate. Oxoid casein hydrolysate contained additional lipoic acid (16). Glucose, the vitamin solution (which contained heat-labile glutamine and asparagine), and citrate (when used) were sterilized by membrane filtration and added aseptically to autoclaved medium. Related components of the medium were together in solutions and stored at 4°C. The complex medium used in the latter part of the work was that of Cambell and Gunsalus (4).

Cultivation method. Medium (100 ml) at 30°C in 250-ml Erlenmeyer flasks in a G76 gyratory water-bath shaker (New Brunswick Scientific Co., New Brunswick, N.J.) was inoculated (1%) with bacteria that had been propagated at 22°C three times in the medium to be used, harvested by filtering with a Sartorius filter (pore size, 0.2 μm), washed twice, and resuspended in an equal volume (10 ml) of the patially defined medium (without glucose). The agitation rate was 120 rpm.

Growth and yield measurements. Growth of the bacteria was followed by periodically measuring optical density (OD) at 600 nm with a Beckman spectrophotometer, model DB, with 1-cm cuvettes, with the corresponding medium as the internal standard. Measurements were made at 20-min intervals as maximal OD was approached, since autolysis of *S. diacetilactis* is known to begin soon after the occurrence of maximal growth (22). Cell mass was determined from a standard curve relating OD to dry weight. OD values of 3.84 and 4.12 represented 1 mg (dry weight) per ml for *S. diacetilactis* and *S. cremoris*, respectively, harvested from the partially defined medium containing 10 μmol of glucose/ml just before the stationary growth phase by membrane filtration, washed three times in cold deionized distilled water, and dried to constant weight. This relationship was linear up to at least an OD value of 0.7.

Analytical methods. Glucose and L-(+)-lactic acid were determined spectrophotometrically with enzyme kits (Calbiochem, Los Angeles, Calif.), acetaldehyde by the method of Sawicki et al. (26) as modified by Lindsay and Day (17), formate by the method of Wood and Gest (34), and acetoin plus diacetyl by the method of Westerfeld (31). Total volatile acids were measured by the procedure of Neish (23), and acetate was determined by subtracting formate from total volatile acids. Citrate was determined by the pyridine acetic anhydride method of Marier and Boulet (19).

RESULTS

Yields and products in partially defined medium. Maximal yields of *S. diacetilactis* and *S. cremoris* in the partially defined medium containing 1 to 7 μmol of glucose/ml are shown in Fig. 1. The relationship between dry weight and glucose concentration for each was linear, showing that growth was limited to utilization of the added glucose. The Y (glucose) values were 35.3 for *S. diacetilactis* and 31.4 for *S. cremoris*. However, with 6 to 15 μmol of glucose/ml, the relationship between dry weight of *S. diacetilactis* and glucose concentration was different; the Y (glucose) value was 21 (Fig. 2). We did not test *S. cremoris* on glucose concentrations higher than 7 μmol/ml.

The possibility that lactic streptococci, similar to homofermentative lactobacilli (8, 9) and *S. faecalis* (10, 16), produce acetate from low concentrations of glucose was tested by determining products in the media in which *S. diacetilactis* had grown. None of the following was found: acetaldehyde, ethanol, formate, glucose, acetoin, and diacetyl. The major product was lactic acid, and there were small amounts of acetate (Table 1). Assuming that *S. cremoris* produced a similar amount of acetate, the Y (adenosine 5′-triphosphate [ATP]) value for *S. cremoris* is 13.9, slightly less than that for *S. diacetilactis* (15.6).

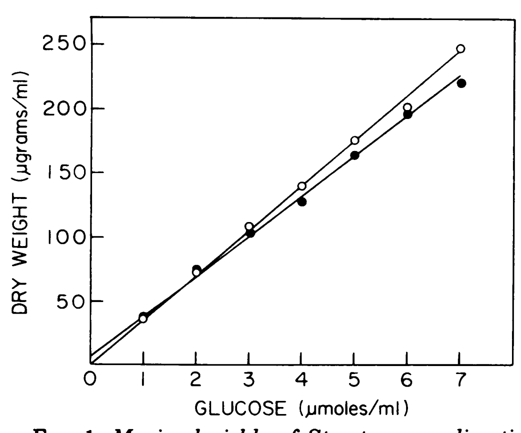

FIG. 1. *Maximal yields of Streptococcus diacetilactis (○) and Streptococcus cremoris (●) at 30°C in partially defined medium containing 1 to 7 μmol of glucose/ml.*

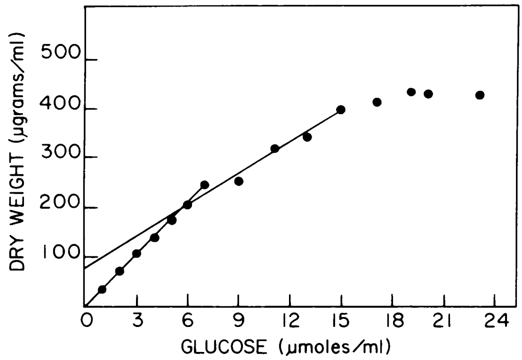

FIG. 2. *Influence of glucose concentration on maximal yields of Streptococcus diacetilactis at 30°C in partially defined medium.*

Complex medium. The streptococci were grown separately in the complex medium of Cambell and Gunsalus (4) containing glucose (10 μmol/ml) and subsequently tested with and without glucose. Each grew in the medium without glucose and produced considerable lactate and acetate (Table 2). Corrected Y (ATP) values were higher in the complex medium than in the partially defined medium, but neither organism produced appreciable acetate from added glucose.

Tests with citrate. Experiments in which some lactic acid bacteria obtained energy from citrate were conducted in complex media (4, 30). We grew *S. diacetilactis* in the media to be used in tests, containing citrate (10 μmol/ml) to induce citrate permease, and subsequently tested it separately in the partially defined medium and the complex medium in which Cambell and Gunsalus (4) had found positive results for three species of *Lactobacillus* (*L. casei*, *L. delbruecki*, and *L. lactis*) and three of *Streptococcus* (*S. faecalis* 10C1, *S. liquefaciens*, and *S. zymogenes*). Citrate was utilized (presumably converted to acetoin), but yields of *S. diacetilactis* were not increased by the addition of citrate to either medium (Fig. 3; Table 3).

DISCUSSION

Lactic streptococci require acetate or lipoic acid for the formation of acetyl-coenzyme A (CoA) from pyruvate (6) and have acetate kinase and phosphotransacetylase (6), the en-

TABLE 1. *End products from low concentrations of glucose and molar growth yield data for Streptococcus diacetilactis*

Glucose concn (μmol/ml)	mol of end products/mol of glucose			Y (glucose) (g [dry wt]/mol)	mol of ATP/mol of glucose[a]	Y (ATP) (g [dry wt]/mol)
	Lactate	Acetate	Total			
3	1.62	0.28	1.90	36.3	2.18	16.7
4	1.66	0.18	1.84	35.0	2.02	17.3
5	1.82	0.38	2.20	34.2	2.58	13.3
Avg	1.70	0.28	1.98	35.2	2.26	15.6

[a] mol of lactate/mol of glucose + 2 (mol of acetate/mol of glucose).

TABLE 2. *Molar growth yields for Streptococcus diacetilactis and Streptococcus cremoris in complex medium*[a]

Determinant	Growth in complex medium			
	S. diacetilactis		*S. cremoris*	
Glucose added (μmol/ml of medium)	0	2	0	2
Glucose utilized (μmol/ml of medium)		1.84		1.79
Lactate produced (μmol/ml of medium)	1.32	4.13	1.01	4.85
Lactate from glucose (μmol/ml of medium)		2.81		3.84
Acetate produced (μmol/ml of medium)	1.03	1.08	0.59	0.67
Acetate from glucose (μmol/ml of medium)		0.05		0.08
Carbon recovery (%)[b]		77.7		109.5
ATP/μmol of glucose[c]		1.58		2.23
Y (glucose) (g [dry wt]/mol)		43.8		38.5
Y (ATP) (g [dry wt]/mol)		27.7		17.3
Corrected Y (ATP) (g [dry wt]/mol)[d]		21.5		18.9

[a] Medium of Cambell and Gunsalus (4).
[b] (μmol of lactate from glucose + μmol of acetate from glucose) ÷ 2 (μmol of glucose utilized ÷ 100).
[c] [μmol of lactate from glucose + 2 (μmol of acetate from glucose)] ÷ μmol of glucose utilized.
[d] Y (ATP) × (% carbon recovery ÷ 100).

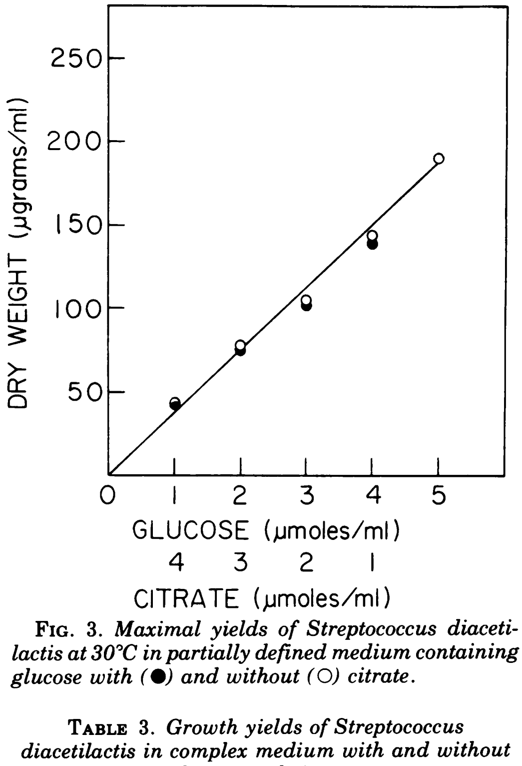

FIG. 3. *Maximal yields of Streptococcus diacetilactis at 30°C in partially defined medium containing glucose with (●) and without (○) citrate.*

TABLE 3. *Growth yields of Streptococcus diacetilactis in complex medium with and without glucose and citrate*[a]

Glucose (μmol/ml)	Citrate (μmol/ml)	Yield[b] (μg [dry wt]/ml)
0	0	132
2	0	215
0	2	128
2	2	209

[a] Medium of Cambell and Gunsalus (4).
[b] Each value is the average of two experiments.

zymes necessary for gain of energy from the conversion of acetyl-CoA to acetate. Although these bacteria normally require most of the pyruvate produced from carbohydrate as a hydrogen acceptor in the reoxidation of nicotinamide adenine dinucleotide, this requirement should be circumvented by the inclusion of citrate in the medium for *S. diacetilactis* (5) or by growing the organism on glucose under aerobic conditions, since *S. diacetilactis* produces reduced nicotinamide adenine dinucleotide oxidase (3). Nevertheless, neither *S. diacetilactis* nor *S. cremoris* converted appreciable glucose to acetate, even with the concentrations of glucose minimal to avoid stimulation of lactic dehydrogenase by fructose-1,6-diphosphate (1, 21, 30, 32, 33). Citrate was utilized by *S. diacetilactis*, but citrate utilization did not increase cell yields. The pyruvate produced from citrate by this organism normally is converted to acetoin, not to lactate (5). Thus, failure of the organism to use citrate as a source of energy shows that failure of the organism to produce appreciable acetate from glucose is not attributable to an unusually active lactic dehydrogenase. Results confirm a suggestion that the formation of acetyl-CoA in growing cells of lactic streptococci is limited (6, 13), or possibly there is a limitation on the synthesis of adenosine diphosphate.

The Y (ATP) values of 15.6 for *S. diacetilactis* DRC_1 and 13.9 for *S. cremoris* C13 are slightly less than the values determined for *S. diacetilactis* 18-16, *S. lactis* C2, and *S. cremoris* K_2E_8 without taking into account their possible production of acetate (22). Different values were obtained by growing the organisms in complex medium (Table 2) and by growing *S. diacetilactis* in partially defined medium with higher concentrations of glucose (Fig. 2). The results in complex medium are consistent with reports for other bacteria (14, 15, 18, 28, 30). Generation of ATP apparently was more closely linked to the synthesis of cellular materials in the complex medium than it was in the partially defined medium, and the linkage in partially defined medium was influenced by the concentration of glucose. The differences in Y (glucose) values for *S. diacetilactis* in the partially defined medium at concentrations of glucose below and above 6 μmol/ml are strikingly similar to those reported for *S. faecalis* by Forrest and Walker (10), but the explanation cannot be the same, because *S. diacetilactis*, unlike *S. faecalis*, did not produce appreciable volatile acids from low concentrations of glucose. Nevertheless, the Y (ATP) values for *S. diacetilactis* and *S. cremoris* in partially defined medium are higher than those determined with similar low concentrations of energy source for five homofermentative species of *Lactobacillus* (8, 9) and for *S. faecalis* (16, 27). Possibly, under optimal growth conditions, the lactic streptococci require less energy for maintenance (28), or they might be otherwise more efficient than lactobacilli and *S. faecalis* in coupling ATP generation to the synthesis of cellular materials (12).

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