JOURNAL OF BACTERIOLOGY, May 1970, p. 369-376
Vol. 102, No. 2

Comparative Metabolism of Vegetative and Sporulating Cultures of *Clostridium thermosaccharolyticum*

EDWARD J. HSU[1] AND Z. JOHN ORDAL

Department of Food Science, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Received for publication 9 February 1970

Cultures of *Clostridium thermosaccharolyticum*, under conditions of restricted growth achieved by slow feeding of glucose, showed a high degree of sporulation. Analysis of the end products showed an accumulation of ethyl alcohol in addition to butyrate and acetate, whereas, in the nonsporulating cultures, acetate and butyrate were the principal products. Incorporation of uniformly labeled ^{14}C-glucose by sporulating cells was three to four times higher than by nonsporulating cells. The efficiency of acetate assimilation into the lipid fraction of sporulating cells was at least two times higher than that of glucose. When starch was used as the carbon source, the growth rate was reduced; sporulation occurred, and the end products and carbon distribution were similar. Alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were preferentially formed by sporulating cells. In vegetative cells, the formation of these enzymes was repressed if the glucose concentration in the medium was increased. The change in enzyme activity appeared to be related to a morphological change in the cells and indicated an altered metabolic pattern for sporulating cells.

Most of the recent research on the biochemical processes involved in the sporulation of bacteria has dealt with the sporulation of the aerobic sporogenic bacteria, although some has been related to the sporulation of the mesophilic anaerobic microorganisms. Recently, the sporulation of the thermophilic anaerobes of which *Clostridium thermosaccharolyticum* is the type species has been studied in this laboratory. L-Arabinose has been found to stimulate the sporulation of *C. thermosaccharolyticum*, whereas glucose represses sporulation (13). However, in none of the published results on sporulation of this organism have the results correlated assimilation of substrate with the morphological change.

We recently demonstrated that *C. thermosaccharolyticum* forms spores under conditions of restricted growth. We also reached the conclusion that sporulation in this organism is not a response to starvation and that an exogenous supply of carbon and energy is essential (7). In batch cultures, good sporulation was also obtained when the supplied carbon source (galactose, starch, or other glucosides) limited the growth rate (8).

[1] Permanent address: Department of Biology, University of Missouri, Kansas City, Mo. 64110.

The present study was prompted by the question of whether sporulation in this organism accompanies a metabolic shift that has been found in many *Bacillus* species (16). Differences in glucose metabolism under the varied physiological conditions that induced or repressed sporulation were studied as reflected by the concomitant change in end-product patterns, enzyme patterns, and assimilation of the carbon source.

MATERIALS AND METHODS

Organism and media. *C. thermosaccharolyticum*, National Canners Association strain 3814, originally obtained from George York, University of California, was used in this investigation.

Stock cultures were prepared by inoculation of pea broth with a vegetative culture. The stock cultures were overlaid with sterile Vaspar immediately after inoculation. After the cultures had been incubated for 8 hr at 56 C, they were stored at 3 C. The stock cultures were transferred at approximately 3-month intervals. For each experiment, a stock culture was removed from the 3 C storage, and was incubated at 56 C overnight and then transferred to pea extract (2% peptone, 10 ppm manganese, and 10% Alaskan seed peas autoclaved and filtered). Three transfers of a 20% inoculum were used to obtain an active culture. The incubation time between each transfer was care-

fully determined to allow the cell mass to increase two times (about 4 hr). A fourth transfer of a 3% inoculum after one generation of growth was made into the test medium. All cultures were incubated at 56 C. Cultures were flushed with oxygen-free nitrogen before, during, and after inoculation.

The majority of the studies were performed in the modified medium of Hsu and Ordal (7) to which 0.2% of various carbohydrates were added. The *p*H was adjusted to 7.0 prior to sterilization. All media were maintained at 56 C and used within 1 day.

Cultivation methods. Batch cultures were grown in large test tubes (50 × 290 mm) fitted with sampling assemblies and partly immersed in a constant-temperature bath. Strictly anaerobic conditions were obtained with the procedure of Bauchop and Elsden (1), except that the amount of alkaline pyrogallol was doubled. Samples were obtained aseptically with sterile syringes through rubber septa in the sampling assemblies.

Continuous-feeding cultures were started as batch cultures; feeding was begun at the point of substrate exhaustion as described previously (7). Experiments were usually terminated when a maximal degree of sporulation was obtained. Contamination was not detected during the several days of operation.

Measurement of growth. Growth was measured by following the changes in optical density (OD) of cultures at 600 nm by use of a Bausch & Lomb Spectronic-20 colorimeter with uninoculated medium as the reference. Dilutions of cultures with the medium were made when necessary. The specific growth rate, k, in hr^{-1}, was computed from the formula

$$k = \frac{2.303\ (\log_{10} X_2 - \log_{10} X_1)}{t_2 - t_1}$$

in which X_1 and X_2 are the OD at times t_1 and t_2, respectively.

Spore and vegetative-cell counts. Direct microscopic counts, made with a Petroff-Hausser counting chamber, were used to determine the total number of spores and vegetative cells. Samples were diluted to a final glycerol concentration of 20% to retard movement during counting. The vegetative cells and spores, after a few minutes of equilibrium, were counted by use of a Zeiss phase-contrast microscope with a phase 3, 100× objective and a 12.5× ocular.

Determination of fermentation products. The supernatant fluid of cultures grown in the presence of labeled substances was analyzed by silica gel column chromatography. The procedures were essentially those of Ramsey (14). Radiobiochemically pure known standards were chromatographed to define the position of elution of the various end products. Residual glucose was determined by the Glucostat method (Worthington Biochemical Corp., Freehold, N.J.).

Measurement of radioactivity. All radioactive samples were counted with a Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co., LaGrange, Ill.), model 314-EX. Correction for quenching was made by the Bush channel ratio method (2). Net counts per minute were computed and converted to disintegrations per minute with an IBM 7094 computer. Each of the chromatographic profiles was recorded by a Cal-Comp computer (California Computer Products, Inc., Anaheim, Calif.). These plots were used to obtain a visual expression of the relative amounts of label found in the various end products.

Samples (0.5 ml) of column fractions were introduced into vials containing 15 ml of scintillation fluid: 0.5% PPO (2,5-diphenyloxazole) and 0.03% dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene] in toluene. For the incorporation studies with labeled substrates, cells were fractionated according to the procedures of Roberts et al. (15). These samples were counted in a scintillation fluid of the following composition: 0.7% PPO, 0.03% dimethyl POPOP, and 10% naphthalene in dioxane.

Preparation of cell-free extracts. After being harvested by centrifugation, the cells were washed twice with 0.01 M tris(hydroxymethyl)aminomethane (Tris) chloride buffer (*p*H 7.0), resuspended in the same buffer, and adjusted to an optical density of about 0.1 at 600 nm. The suspension was then sonically treated for 60 sec at 0 C with a Branson Sonifier (Heat Systems Co., Great Neck, N.Y.). Cellular debris was removed by centrifugation at 27,000 × *g* for 20 min, and the clear supernatant fraction was used for the enzyme assays. Protein was determined by the biuret method (9) with crystalline bovine serum albumin as the standard.

Enzyme assays. Enzyme activities were determined spectrophotometrically by coupling to a pyridine nucleotide-dependent system in such a way that the enzyme to be tested was rate-limiting. Total volume in each cuvette was 3 ml. A unit of enzyme activity was defined as that amount of enzyme catalyzing the formation of 1 μmole of product per min at 30 C. When reduced nicotinamide adenine dinucleotide phosphate (NADPH) was measured, 6.22 per cm^2 per μmole was used as the extinction coefficient. Since our primary concern in this study was a comparison of the metabolic activities of vegetative cells and sporangia, specific enzyme activities were expressed as units of enzyme per OD unit rather than per milligram of protein.

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP-oxidoreductase, EC 1.1.1.49) activity was measured by following NADP reduction (10). The enzyme reaction mixture contained: Tris buffer (*p*H 7.6), 150 μmoles; $MnCl_2$, 5 μmoles; glucose-6-phosphate, 7 μmoles; NADP, 1 μmole; cell-free extract (1 to 2 mg of protein); and water to 3.0 ml.

The 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate: NADP oxidoreductase (decarboxylating), EC 1.1.1.44] activity was determined by the procedure outlined by Horecker and Smyrniotis (6). It differed from the preceding assay in that 6-phosphogluconate replaced glucose-6-phosphate. The reaction mixture consisted of: Tris (*p*H 7.6), 150 μmoles; $MnCl_2$, 5 μmoles; 6-phosphogluconate, 10 μmoles; NADP, 1 μmole; cell-free extract (1 to 2 mg of protein); and water to 3.0 ml.

Alcohol dehydrogenase (alcohol-NADP oxidoreductase, EC 1.1.1.2) activity was assayed by the

method of DeMoss (5). The assay mixture was modified to contain the following: ethyl alcohol, 1,500 μmoles; Tris buffer (*p*H 8.5), 300 μmoles; NADPH, 1 μmole; cell-free extract; and water to 3.0 ml.

RESULTS

Altered end-product pattern during sporulation. Figure 1 shows the metabolic change that occurs as sporulation proceeds. Little or no ethyl alcohol was apparent until the cells elongated and developed sporangia (Fig. 1A). The amount of ethyl alcohol produced became significant as soon as the majority of cells started elongation (Fig. 1B) and became predominant when refractile spores appeared (Fig. 1C). In contrast, in the nonsporulating cultures, such as glucose batch culture, acetate and butyrate were the main products. No detectable amounts of ethyl alcohol were found throughout the entire growth phase (Fig. 1D).

It is probable that the acetate formed during the initial period of batch growth accumulated and then was reduced to ethyl alcohol because of the greater reducing capabilities of sporulating cells. When uniformly labeled ^{14}C-acetate was used to follow the fate of acetate and to trace the origin of ethyl alcohol, the results (Fig. 2) indicated that the labeled acetate was converted

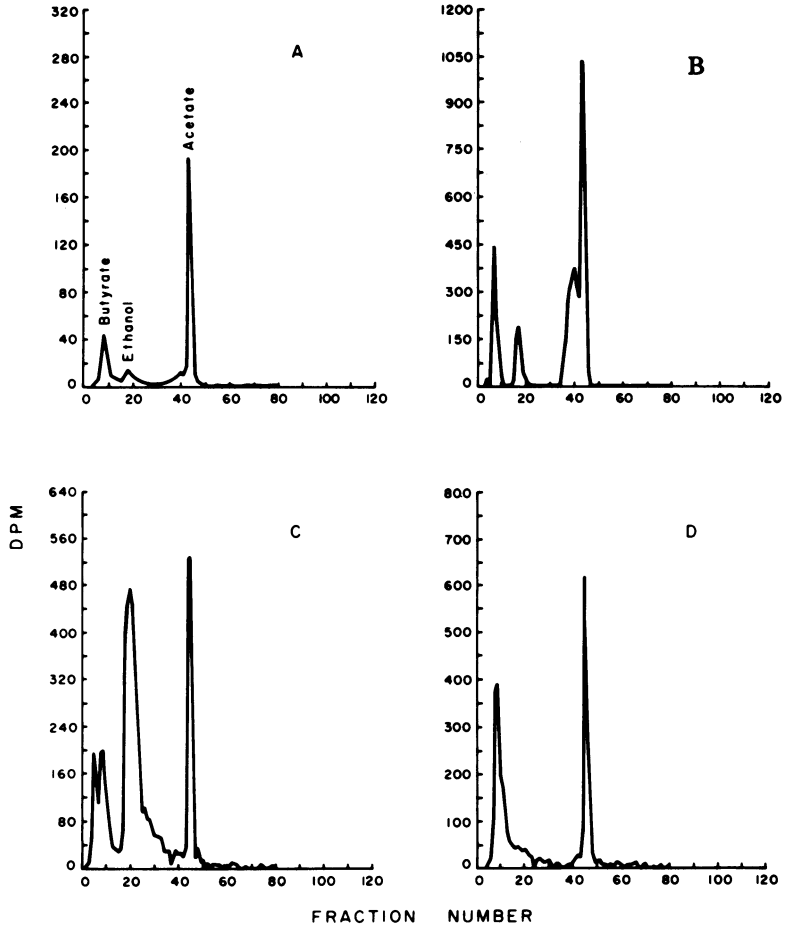

FIG. 1. *Chromatographic profiles from silica gel columns demonstrating differences in glucose metabolism in sporulating and nonsporulating cultures. Conditions were those of restricted growth achieved by the slow feeding of glucose containing 15 μc of uniformly labeled ^{14}C-glucose per 100 ml. Samples for isotopic analysis were removed at the times indicated for each profile. (A) After 2 hr of restricted growth; cells elongating but no sporangia apparent. (B) After 8 hr of restricted growth; cells elongated and numerous sporangia. (C) After 30 hr of restricted growth; cells elongated, maximal number of sporangia, many containing refractile spores. (D) Profile obtained from nonsporulating cells in glucose batch culture (unrestricted growth). The ^{14}C-glucose was added to the glucose solution in a reservoir at the time the feeding was started.*

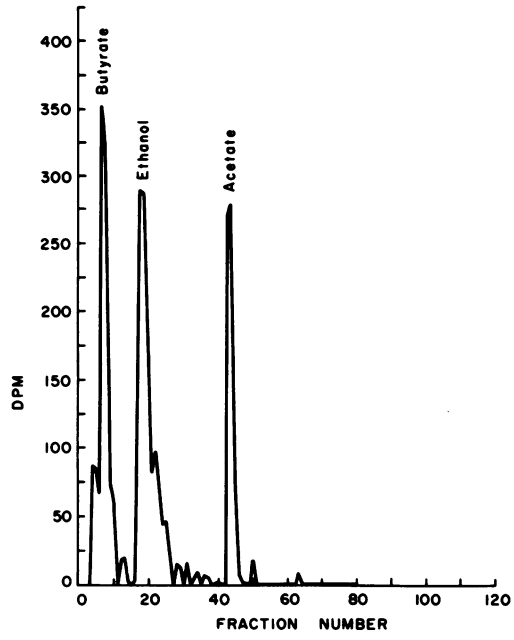

FIG. 2. *Fermentation end products of a sporulating culture separated by silica gel column chromatography. Conditions were those of restricted growth achieved by the slow feeding of glucose to which was added 15 μc of uniformly labeled ^{14}C-acetate. Samples for isotopic analysis were removed after 30 hr of restricted growth.*

to ethyl alcohol and butyrate during sporulation. The spores of this organism are high in lipid (12). This would require an elevated level of an NADPH generating system.

The pattern of end products was essentially the same during sporulation induced in starch medium and during sporulation induced by the mechanical limitation of glucose (Fig. 3). Other less readily utilized substrates, such as galactose, starch, and many glucosides, also showed similar results (8). In all cases, the end products were characterized by the accumulation of ethyl alcohol in addition to butyrate and acetate. The specific growth rate was also substantially reduced. The data suggest the dependence of end products on the catabolic rate rather than on the chemical nature of the substrate.

Incorporation of glucose. To examine further the correlation between metabolic change and sporulation, the quantitative incorporation of uniformly labeled ^{14}C-glucose into cell materials was determined. The labeled substrate was added directly at the time of inoculation. To determine the uptake of ^{14}C by both cell types, samples were withdrawn after six to seven generations of growth and were centrifuged; pellets of cells were washed and extracted. The vegetative culture (glucose) was incubated for approximately 8 hr, and its growth rate constant, k, was calculated to be 0.58 hr^{-1}. The sporulating culture (starch), incubated for 40 hr, had a k value of 0.15 hr^{-1}. However, both cell types were present in this sporulating culture. The cell population consisted of 65% sporangia and 35% vegetative cells.

The results with 8-hr vegetative cells and 40-hr sporulating cells indicated that, although ^{14}C was incorporated into both cell types, the vegetative cells assimilated glucose at only 18% of the rate of sporulating cells (Table 1). The differences in the pattern of incorporation, as evidenced by the changes in the percentage in the four cell fractions, suggest that in sporulating cells a greater amount is used for synthetic reactions.

Incorporation of acetate. A comparison made of the incorporation of ^{14}C into cellular fractions when the label was supplied either by uniformly labeled ^{14}C-acetate or ^{14}C-glucose is presented in Fig. 4. When the ^{14}C was supplied by acetate, a high percentage was located in the lipid fraction. These results emphasize the importance of acetate incorporation into the lipid fraction and fur-

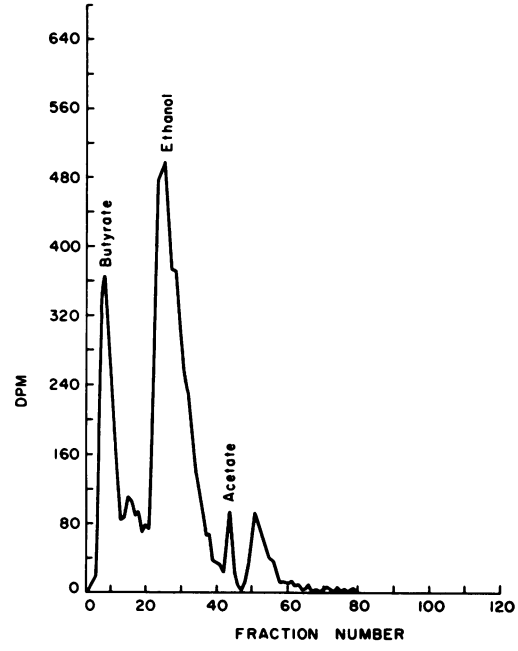

FIG. 3. *Fermentation end products of a sporulating batch culture spearated by silica gel column chromatography. Cells were induced to sporulation by using soluble starch as the source of carbon and energy. Isotopic labeling was achieved by the addition of 15 μc of uniformly labeled ^{14}C-glucose per 100 ml of media. The sample analyzed was removed from the culture after a 40-hr incubation period.*

TABLE 1. *Incorporation of ^{14}C from glucose-U-^{14}C[a] into sporulating and nonsporulating cells of C. thermosaccharolyticum*

Cell fraction	Percentage of total incorporation[b]	
	Sporulating cells[b] (495,072 dpm)	Vegetative cells[b] (84,288 dpm)
Lipid	34.5	62.7
Cold trichloroacetic acid soluble	9.2	27.2
Hot trichloroacetic acid soluble	23.5	6.1
Residual protein	32.8	4.0

[a] Addition of 15 μc of uniformly labeled ^{14}C-glucose per 100 ml of medium.

[b] Total incorporation of unfractioned cells (disintegrations per minute) is given in parentheses. The sporulating cells were from a 40-hr batch culture containing 0.290% starch. The vegetative cells were from a 10-hr batch culture containing 0.290% glucose.

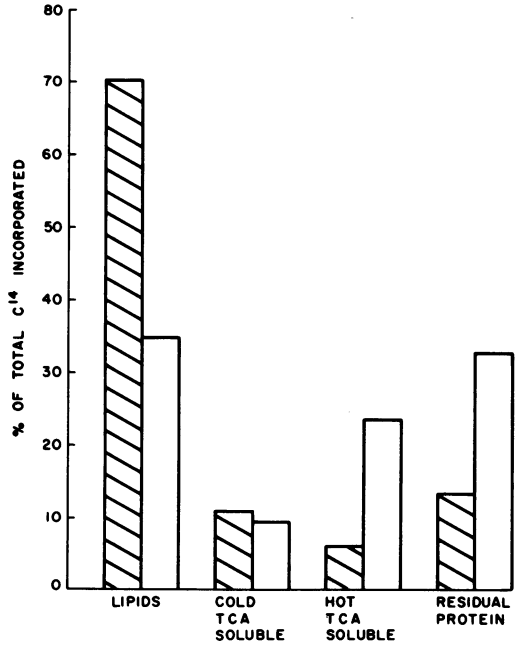

FIG. 4. *Differences in the incorporation of ^{14}C tracer in sporulating cells when the ^{14}C is supplied by uniformly labeled ^{14}C-acetate (slanted) or glucose (open). The cells were induced to sporulation in batch culture by using soluble starch as the carbon and energy source; 15 μc of the indicated ^{14}C compound was added to the soluble starch medium at the time of inoculation. The cells were harvested after 40 hr of incubation and were analyzed.*

ther demonstrate that sporulating cells can utilize exogenous acetate or glucose for the synthesis of cell material.

The next step, therefore, was to determine the role of acetate assimilation at each stage of sporulation. For this purpose, the extent of incorporation of uniformly labeled ^{14}C-acetate was followed during sporogenesis in the continuous-feeding system previously described. The cells were harvested and analyzed 2, 8, and 30 hr after the feeding had started (Fig. 5). During the earlier stages of culture development, only small amounts of label were found in the lipid fraction. When the sporangia were numerous and sporulation was definitely in progress, the amount of label found dramatically increased. Only 2.5% of the label was incorporated into the lipid fraction of vegetative cells, whereas 59.5% was incorporated

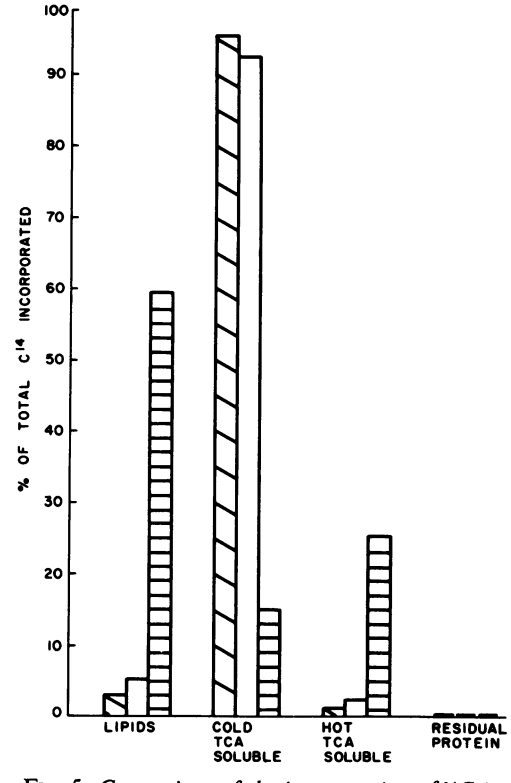

FIG. 5. *Comparison of the incorporation of ^{14}C into cellular fractions when sporulation was induced by the slow feeding of glucose (restricted growth). Uniformly labeled ^{14}C-acetate (15 μc per 100 ml of glucose solution) was used as the isotopic label. The cells were harvested and analyzed after 2 hr (slanted) of incubation, cells elongating but no sporangia; after 8 hr (open), cells elongated with numerous sporangia; and after 30 hr (parallel lines), cells elongated with a maximal number of sporangia, many containing refractile spores.*

into the cellular material consisting of sporangia and mature spores. In the 2- and 8-hr samples, a high percentage of the label was found in the cold trichloroacetic acid-soluble fraction, suggesting that the label was located primarily in the various intermediary metabolic compounds. It is interesting to note that, under the conditions of this experiment, the residual protein fraction contained essentially no label. Under these restricted conditions, there was little or no cell division. The morphological changes were elongation and spore formation.

The differences, then, between the vegetative cell and sporulating cell with regard to acetate metabolism include: (i) an increase in the overall rate of acetate incorporation during sporulation, (ii) an increase in the reduction of acetate to ethyl alcohol by sporulating cells, and (iii) a redistribution of incorporated acetate among the various cell fractions during maturation of sporangia.

Enzyme pattern of vegetative culture versus sporulating culture. An initial comparison of the enzymatic activity in sporulating and nonsporulating cells demonstrated a distinct difference in the activity of alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase (Table 2). In vegetative cells, enzymatic activity was low or nondetectable, whereas in sporulating cells there was definite enzymatic activity. The last two enzymes are involved in the hexose-monophosphate pathway and suggest that a shift had taken place producing a system more favorable to the synthesis of lipid material. The hexose-monophosphate pathway would result in a higher level of NADPH, which is necessary for lipid synthesis. Preliminary cytological studies, with the use of a Sudan Black B stain, also demonstrated the presence of distinct intracellular lipid in the sporulating cells.

Effect of increasing glucose concentration on enzyme pattern during vegetative growth. Inasmuch as the nature of the intracellular catabolite is uncertain, it was felt that a variation in the initial concentration of glucose could best simulate the sequence of glucose utilization and catabolite repression throughout the entire growth period in a batch-culture system. Therefore, the activities of alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were determined for cells grown in the basal medium containing glucose ranging in concentration from 7.2×10^{-4} to 2.2×10^{-2} M (Table 3). It is apparent that, as the glucose concentration of the medium was increased, there was a corresponding decrease in the activity of these three enzymes. A significant decrease was observed with 1.4×10^{-3} M glucose. As the glucose concentration was further increased above 1.1×10^{-2} M, the increase in repression became less pronounced. Although the precise time of glucose exhaustion was not determined, no residual glucose could be detected at the end of the 12-hr incubation period. These results therefore suggest that, as the glucose was utilized from the culture medium, its repressing

TABLE 3. *Effect of increasing glucose concentration on enzyme specific activities in Clostridium thermosaccharolyticum*

Initial concn of glucose source in medium (M)	Specific activity[a]		
	Ethyl alcohol dehydrogenase	Glucose-6-phosphate dehydrogenase	6-Phospho-gluconate dehydrogenase
2.2×10^{-2}	6.91×10^{4}	0.80×10^{4}	0.00
1.1×10^{-2}	16.31×10^{4}	2.06×10^{4}	2.04×10^{4}
5.6×10^{-3}	16.08×10^{4}	2.46×10^{4}	2.46×10^{4}
2.8×10^{-3}	36.03×10	2.72×10^{4}	4.29×10^{4}
1.4×10^{-3}	44.00×10^{4}	6.60×10^{4}	4.40×10^{4}
7.2×10^{-4}	112.89×10^{4}	9.41×10^{4}	14.11×10^{4}

[a] Specific enzyme activities are expressed as units of enzymes formed per unit of OD. Cells were grown in glucose medium and harvested after 12 hr.

TABLE 2. *Alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase activity of cell-free extracts prepared from vegetative and sporulating cells of C. thermosaccharolyticum*

Enzyme	Specific activity[a]	
	Sporulating cells[b]	Vegetative cells[c]
Alcohol dehydrogenase	129.29×10^{4}	1.72×10^{4}
Glucose-6-phosphate dehydrogenase	4.69×10^{4}	0.08×10^{4}
6-Phosphogluconate dehydrogenase	18.78×10^{4}	0.00

[a] Specific enzyme activities are expressed as units of enzyme per unit of OD.
[b] Cells were grown in starch medium and harvested after 40 hr.
[c] Cells were grown in glucose medium and harvested after 12 hr.

effect on the alcohol dehydrogenase activity was relieved. The activities of glucose dehydrogenase and gluconate dehydrogenase, enzymes representative for the hexose-monophosphate shunt, were high during sporulation in starch medium (Table 2) and were substantially repressed during vegetative growth in glucose medium. Extremely low concentrations of glucose seem to derepress their formation.

Although detailed morphological studies were not made in these experiments, all samples were examined microscopically. Bacilli from batch cultures containing the higher glucose concentrations tended to be shorter and less granular than the samples from the cultures having the reduced glucose levels. Likewise, under these more crude conditions, tests for ethyl alcohol were essentially negative. As this organism has the ability to assimilate glucose rapidly, it is probable that the culture medium lacked a suitable exogenous energy source for sporulation to occur (7). However, the data demonstrate that the activity of these enzymes is markedly increased under cultural conditions which stimulate sporulation.

DISCUSSION

The evidence presented here has confirmed that acetate is an important metabolite in both sporulating and nonsporulating cultures. It has also demonstrated that formation of ethyl alcohol increases as sporulation proceeds. Maximal amounts were found in cultures where advanced sporulation was evident.

Lee and Ordal (10) found that, when glucose was fermented by resting cells of *C. thermosaccharolyticum*, a low level of *n*-butyric acid and a large amount of ethyl alcohol were produced. This is in contrast to previous findings by Sjolander (17), who observed the production of relatively large amounts of *n*-butyric acid but no ethyl alcohol in a growing culture of *C. thermosaccharolyticum* (in a malt sprout medium). Campbell and Ordal (3) used a growing culture of this organism and observed that a 5 mM level of fluoroacetic acid (FAA) resulted in a complete inhibition of sporulation and largely stopped the production of ethyl alcohol. Other effects of FAA were an increase in the amount of lactic acid and a decrease in the amount of butyric acid during the growth of FAA-inhibited sporulating cultures. Although the site of inhibitory action of FAA was not indicated, it may be that an inhibitor which prevents either the formation or utilization of a precursor for ethyl alcohol also inhibits sporulation.

Furthermore, growth of this organism in a pyruvate medium (10) resulted in an apparent solitary production of acetate, indicating the phosphoroclastic utilization of pyruvate. Possibly butyrate is formed less readily from pyruvate because of the need for extra hydrogen, which pyruvate does not supply.

In the present work, the accumulation of ethyl alcohol in sporulating cultures would seem not to be indicative of an alternative route of ethyl alcohol formation from the carbon source, but to be a reflection of the fact that an elevated NADPH generating system is subsequently available for the reduction reactions leading to ethyl alcohol.

An extensive investigation of glycolytic enzymes (10) suggested that pyruvate-grown cells are able to utilize glucose chiefly via the hexose-monophosphate pathway. Such an alternative pathway must, therefore, be of significance in sporogenesis, because it would afford a means for generating NADPH necessary for synthesis of fatty acids and for the concomitant reduction of acetate to ethyl alcohol during sporogenesis. This suggests that fatty acid synthesis and ethyl alcohol formation during sporogenesis are linked to an oxidative pathway of glucose metabolism.

Evidence against the operation of the tricarboxylic acid cycle has been reported (3). Reduction of the label acetate to ethyl alcohol during sporulation (Fig. 2) suggests that ethyl alcohol and acetic acid arise from a common precursor, as in the case of *Escherichia coli* (4). If, during sporulation, formation of adenosine triphosphate and reducing conditions is favored, then the result should be an increase in the NADPH generating system of the culture. This should facilitate the formation of ethyl alcohol as well as the synthesis of spore material.

The results presented in this report can be summarized as indicating that the organism converts acetate, formed as end product of the phosphoroclastic cleavage, by two pathways. The first pathway is the condensation of two acetyl coenzyme A to butyrate via the crotonyl pathway, as was previously suggested by Pheil and Ordal (12). The butyrate thus formed can then be utilized in the building up of spore material such as lipid. The second pathway would be the reduction of acetyl phosphate to ethyl alcohol when the NADPH generating system is depressed during sporulation. M. F. Campbell (Ph.D. Thesis, Univ. of Illinois, Urbana, 1968) reported the formation of acetate, ethyl alcohol, and butyrate in a sporulating culture of *C. thermosaccharolyticum*. However, under his experimental conditions he was unable to detect the formation of large amounts of ethyl alcohol when L-arabinose was used as the substrate. Campbell's results also indicated an increased CO_2 production by sporulating cells from carbon 1 of glucose.

This would indicate the need for an active hexose-monophosphate pathway. However, we made no attempt to assess the relative contribution of the oxidative pathway to the general flow of carbon.

We have demonstrated that glucose can support sporulation only under conditions in which growth is restricted. When glucose or a similar readily metabolized carbohydrate is present in excess, the vegetative enzymes are dominant and the sporulation enzymes are not derepressed. Our studies so far also suggest that rapid growth exerts a catabolic repression of the NADPH generating system. This repression is sufficient to prevent sporulation.

The repression of the dehydrogenase formation and the alteration in the end products of carbohydrate metabolism, therefore, seem to provide for a cellular economy which permits the organism to undergo cellular differentiation only under certain physiological conditions. Further work on the transportation mechanism for carbohydrate uptake, with the use of uniformly labeled ^{14}C-α-methyl-glucoside as a tracer, may provide more direct evidence that sporulation is a response to the restricted supply of the carbon source. The evidence presented, however, is sufficient to provide a working model for future studies.

ACKNOWLEDGMENTS

This investigation was supported by the U.S. Department of Agriculture through Agricultural Research Service, Western Utilization Research and Development Division, Albany, Calif., under grant 12-14-100-7651 (74). One of us (E. J. H.) is grateful for a University of Missouri Faculty Research Grant held during a part of this investigation.

The outstanding technical assistance of Helo Zink is gratefully acknowledged.

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