INFECTION AND IMMUNITY, Oct. 1996, p. 4319–4323 Vol. 64, No. 10
0019-9567/96/$04.00+0

Genetic and Physiological Analysis of the Lethal Effect of L-(+)-Lactate Dehydrogenase Deficiency in *Streptococcus mutans*: Complementation by Alcohol Dehydrogenase from *Zymomonas mobilis*

JEFFREY D. HILLMAN,[1]* ANPING CHEN,[1] AND JACKY L. SNOEP[2]

Department of Oral Biology, University of Florida College of Dentistry, Gainesville, Florida 32610,[1] and Department of Microbial Physiology, Vrije Universiteit, NL-1081 HV Amsterdam, The Netherlands

Received 18 December 1995/Returned for modification 7 February 1996/Accepted 15 July 1996

CH4ts is a previously isolated recombinant mutant of *Streptococcus mutans* NG8 which produces a thermolabile L-(+)-lactate dehydrogenase (LDH) activity. It does not grow at 42°C under a variety of cultivation conditions. In this study, we show that a batch culture of CH4ts shifted from 30 to 42°C underwent rapid cessation of growth and accelerated cell death. The mutant grew at 42°C in continuous culture under glucose-limiting conditions. Under these conditions, lactate production was replaced by production of ethanol and, to a smaller extent, acetoin. The cloned *Zymomonas mobilis* gene for alcohol dehydrogenase II, placed under the control of the *S. mutans spaP* regulatory signals, complemented LDH deficiency. The alcohol dehydrogenase-complemented mutant grew as well or better than NG8 on a variety of carbon sources at 42°C and produced significant amounts of ethanol in place of lactic acid. These results are in accord with other approaches indicating that *S. mutans* has other enzymatic activities, including pyruvate formate-lyase and pyruvate dehydrogenase, for pyruvate metabolism. However, at high glucose concentrations, the levels of activity of these enzymes are apparently insufficient to compensate for the absence of LDH.

The cariogenic potential of *Streptococcus mutans* is known to depend on its production of lactic acid (15). Consequently, L-(+)-lactate dehydrogenase (LDH) deficiency has been proposed as one aspect of a strategy to construct an effector strain of *S. mutans* for the replacement therapy of dental caries.

We recently reported (13) an unsuccessful attempt to make *S. mutans* JH1005, which has a demonstrated ability to colonize the human oral cavity (14), LDH deficient by introduction of a cloned, insertionally inactivated *ldh* gene. The results from this study suggested that LDH deficiency was lethal to *S. mutans*. Subsequently, we mutagenized the cloned, wild-type *ldh* gene with hydroxylamine in vitro and isolated an *Escherichia coli* transformant which expressed a thermolabile LDH activity (6). The *ldh*(Ts) gene from this clone was introduced into the naturally transformable *S. mutans* strain NG8 on a suicide vector to create a heterodiploid expressing both wild-type and thermolabile LDH activities. Self-recombinants which had only one *ldh* gene were isolated. One of these clones, CH4ts, possessed only the thermolabile LDH activity. This isolate grew like its parent at 30°C but did not grow at 42°C under a variety of cultivation conditions. We interpreted these results as providing strong evidence that LDH deficiency is lethal in *S. mutans*.

Early studies of *S. mutans* characterized it as a homolactic fermentor (9, 21). LDH was shown to be produced constitutively, but its activity was found to be entirely dependent on the presence of fructose 1,6-diphosphate (3). Under growth conditions where intracellular fructose 1,6-diphosphate concentrations are low, such as with limiting glucose, fermentation end products other than lactate have been observed. Anaerobically, the activity of pyruvate formate-lyase accounts for the observed production of formate, acetate, and ethanol (1). In an aerobic environment, pyruvate formate-lyase is inactivated by oxygen, and instead, pyruvate dehydrogenase activity can account for the observed production of acetate (5) and acetoin (12).

As was shown to be the case for the closely related *Streptococcus rattus* (12), it was initially presumed that these alternate pathways for pyruvate dissimilation would enable an LDH-deficient mutant of *S. mutans* to thrive under both aerobic and anaerobic cultivation conditions. The finding that LDH deficiency was lethal in *S. mutans* suggests that these pathways are less active than they are in *S. rattus*, resulting in growth inhibition from NAD-NADH imbalance and/or the accumulation of glycolytic intermediates.

In the present study, we demonstrate that LDH lethality in *S. mutans* can be overcome by limiting the supply of glucose. We also show that a supplemental alcohol dehydrogenase (ADH) activity, when expressed in *S. mutans*, can complement LDH deficiency. This was accomplished by fusing the *Zymomonas mobilis* ADH gene (*adh*) to the transcription regulation signals of the *S. mutans spaP* gene. When this genetic construct was introduced into the chromosome of CH4ts on a suicide vector, the resulting clone grew as well as or better than wild-type *S. mutans* under a variety of cultivation conditions.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *S. mutans* strains CH4ts (6) and NG8 (16) have been described previously. *E. coli* DH5α was used for cloning. Plasmid pLOI286 (7) containing the cloned *Z. mobilis* ADH II (*adh*) gene was provided by L. O. Ingram. pCR3-8, a pCR2-based plasmid containing a portion of the cloned *S. mutans spaP* gene including its promoter and ribosome binding site (8), was provided by A. S. Bleiweis.

Batch and continuous cultures of CH4ts were grown in the defined medium of Carlsson (4) supplemented with 0.5% tryptone and 1% glucose. Cells for assay of ADH activity were grown in Todd-Hewitt broth supplemented with 1% glu-

* Corresponding author. Mailing address: University of Florida College of Dentistry, Department of Oral Biology, Box 100-424, Gainesville, FL 32610. Phone: (352) 846-0792. Fax: (352) 392-3070. Electronic mail address: jhillman@dental.ufl.edu.

cose. Ampicillin (50 μg/ml), tetracycline (10 μg/ml), and erythromycin (5 μg/ml) were used where appropriate.

Assays of cultures. Samples of batch and continuous cultures were removed at indicated times, and their pH and A_{550} values were determined. Cells were removed by centrifugation at 4°C, and the resulting cell-free liquors were assayed enzymatically (Sigma Chemical Co., St. Louis, Mo.) for glucose, ethanol, and lactic acid and by gas-liquid chromatography (12) for acetoin. The data presented in the figures are the averages of duplicate experiments.

Genetic methods. Plasmid pADH was constructed to contain the *Z. mobilis adh* open reading frame fused to the regulatory sequences of the *S. mutans spaP* gene. It was constructed as shown in Fig. 1. Plasmid pCR3-8 was digested with *Sac*I and *Dra*III and then treated with mung bean nuclease to remove the entire *spaP* gene except for its promoter, ribosome binding site, and 52 bases of the 5′ end of the open reading frame; a 4.2-kbp fragment was recovered by agarose gel electrophoresis. PCR was used to amplify the entire open reading frame of the *Z. mobilis adh* gene except for its translation start codon (ATG) plus one additional (G) base. The plasmid pLOI286 served as template, and forward (5′ CTTCTTCAACTTTTTATATTCCTTTCG) and reverse (5′ CGGAGGCATTG TTTG) primers were synthesized by the University of Florida Biotechnology Core Facility. The amplified fragment was prepared with *Taq* DNA polymerase as described in the manufacturer's (Amersham, Arlington Heights, Ill.) directions. It was recovered by agarose gel electrophoresis, treated with mung bean nuclease, and ligated into the pCR3-8 fragment to form the genetic fusion with *spaP*. Transformants of DH5α were selected on LB medium containing ampicillin. Restriction enzyme digestions were used to confirm the size (5.4 kbp) and proper orientation of the insert in the resulting plasmid, pADH. For cloning into *S. mutans*, pADH was converted to pADH-tet by inserting the 3.5-kbp *Hin*cII fragment of pVA981 containing a tetracycline resistance gene derived from *S. mutans* (22) into the *Sca*I site located in the *bla* gene of pADH. Transformants of DH5α selected on LB medium with tetracycline were shown to be susceptible to ampicillin. The methods of Perry and Kuramitsu (19) were used to transform pADH-tet into *S. mutans*.

Assay of ADH activity. Cells from 200-ml overnight cultures of *E. coli* and *S. mutans* strains were recovered by centrifugation (5,000 × *g* for 15 min) at 4°C and washed twice in 100 mM KPO_4 buffer (pH 8.5). The cell pellets were resuspended in 1 ml of buffer and broken by passage through a French press at 14,000 lb/in^2. Cell debris was removed by centrifugation (14,000 × *g* for 30 min) at 4°C. The cell extracts were kept on ice and assayed for the NAD-dependent oxidation of ethanol by the methods of Neale et al. (18).

RESULTS

Physiology studies. Overnight batch cultures of CH4ts [*ldh*(Ts)] and its parent, NG8, were grown at 30°C. Washed cells were diluted 1:1,000 in fresh medium with and without glucose and incubated at 42°C. At time points indicated in Fig. 2, viable cell counts were determined by spreading appropriately diluted samples on brain heart infusion plates which were incubated at 30°C. The results show that in the absence of glucose, CH4ts and NG8 behaved identically; i.e., growth ceased after 3 h and was followed by slow cell death (half-life, 23.5 h). However, unlike its parent, CH4ts stopped growing shortly after being placed in the presence of glucose and underwent accelerated cell death (half-life, 12.1 h) relative to that of cells incubated in the absence of glucose.

The previous experiment demonstrated that LDH-deficient *S. mutans* cells incubated in the presence of glucose do not simply stop growing but are actually prone to cell death. The following study was performed to determine if regulating the supply of glucose could overcome this effect. CH4ts was grown to constant cell biomass at 30°C in the reaction vessel of a chemostat. Medium inflow providing a dilution rate of 0.1 h^{-1} was maintained for 72 h before the temperature of the culture was shifted to 42°C. Within 3 h following the temperature shift, the cell density began to decline at a rate equivalent to the theoretical washout rate (Fig. 3), indicating a cessation of cell

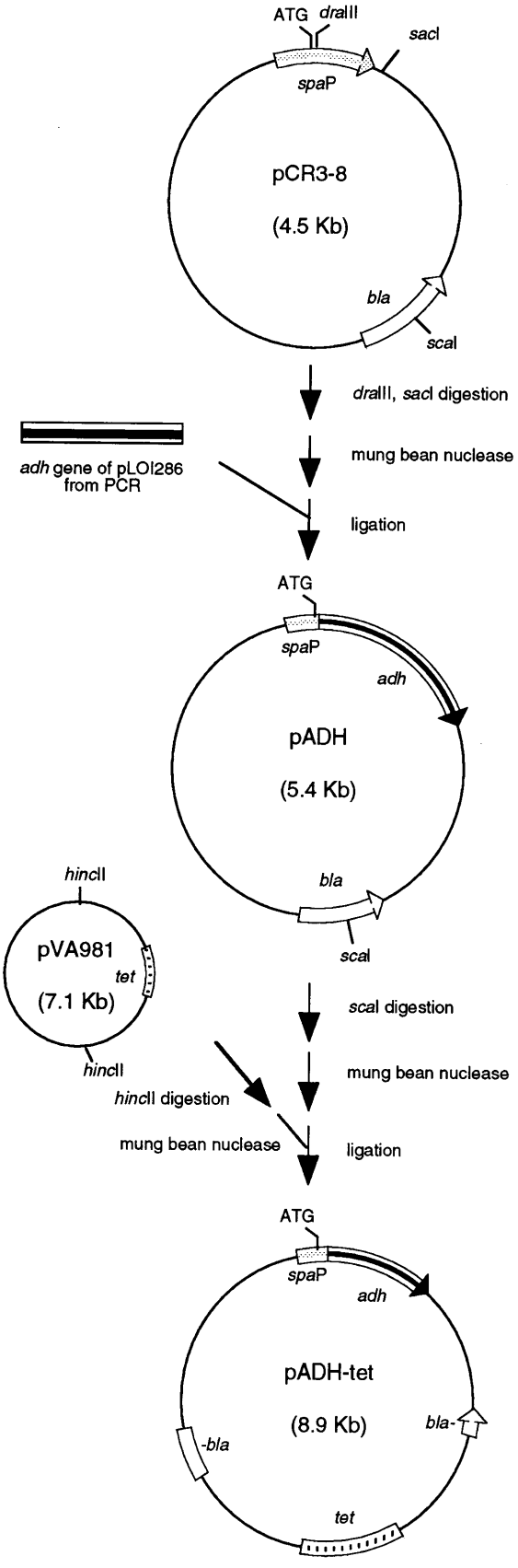

FIG. 1. Schematic diagram of the procedure used for construction of pADH-tet. In the first step, the *Z. mobilis adh* gene, obtained by PCR, was fused in frame to the 5′ end of the *S. mutans spaP* open reading frame. In the second step, the ampicillin resistance (*bla*) gene on the pCR3-8 backbone was inactivated by insertion of a fragment from pVA981 containing a tetracycline resistance (*tet*) gene.

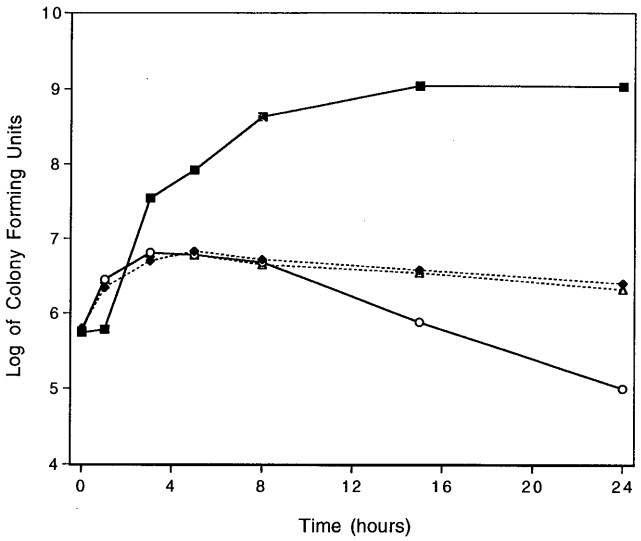

FIG. 2. Effect of glucose on the growth and survival of CH4ts and control. At 0 time, washed cells of CH4ts and NG8 were placed into medium with or without 1% glucose. Samples at the indicated times were analyzed for viable cells. Symbols: ■, NG8 with glucose; ◆, NG8 without glucose; ○, CH4ts with glucose; △, CH4ts without glucose.

growth. This correlated with an increase in the glucose concentration observed in the reaction vessel.

When the continuous culture experiment was repeated with a dilution rate of 0.006 h^{-1}, stable growth of CH4ts was achieved when the temperature was shifted to 42°C (Fig. 4). Immediately following the temperature shift, lactic acid concentrations declined in agreement with the predicted washout rate. Although no attempt was made in this experiment to determine an accurate carbon balance, it was noted that cessation of lactate production was accompanied by initiation of ethanol and acetoin production. Other likely fermentation end products, such as formate and acetate, were not measured. The production of neutral ethanol and acetoin in place of lactic acid resulted in an increase in pH of the continuous culture. Glucose concentrations remained undetectable throughout the experiment.

Genetic studies. *E. coli* DH5α transformed with plasmid pADH containing the *Z. mobilis adh* open reading frame fused to the *S. mutans spaP* regulatory sequences expressed the cloned ADH activity (specific activity, 6.95 μmol/min/mg of protein). This result was in accord with previously published data indicating that the *spaP* promoter and ribosome binding site are active in *E. coli* (16). The level of activity was approximately 17% of that found in DH5α transformed with pLOI286 (specific activity, 41.8 μmol/min/mg of protein), which has the original cloned *adh* gene and regulatory sequences from *Z. mobilis*. Interruption of the ampicillin resistance gene on the pCR2 backbone by insertion of the tetracycline resistance gene from pVA981 to create pADH-tet did not affect the level of ADH activity.

One microgram of pADH-tet was used to transform CH4ts to growth on medium containing tetracycline. From several hundred clones which arose, one randomly selected isolate called CH4ts::adh was found to contain substantially more ADH activity (specific activity, 60.43 μmol/min/mg of protein) than its wild-type grandparent NG8 (specific activity, <1.0 μmol/min/mg of protein) when grown aerobically standing at 30°C in glucose-supplemented Todd-Hewitt broth.

CH4ts::adh grew in batch culture at 42°C. Its rates of growth and cell yields were found to be comparable to those of NG8 when glucose, lactose, or sucrose served as the carbon source (Table 1). CH4ts::adh had substantially shorter doubling times and higher yields than NG8 when galactose, sorbitol, or mannitol served as the carbon source. No lactic acid was detected in culture liquors of CH4ts::adh grown on any of the carbon sources, and ethanol was present in much greater amounts than was found in culture liquors of NG8. The final pH

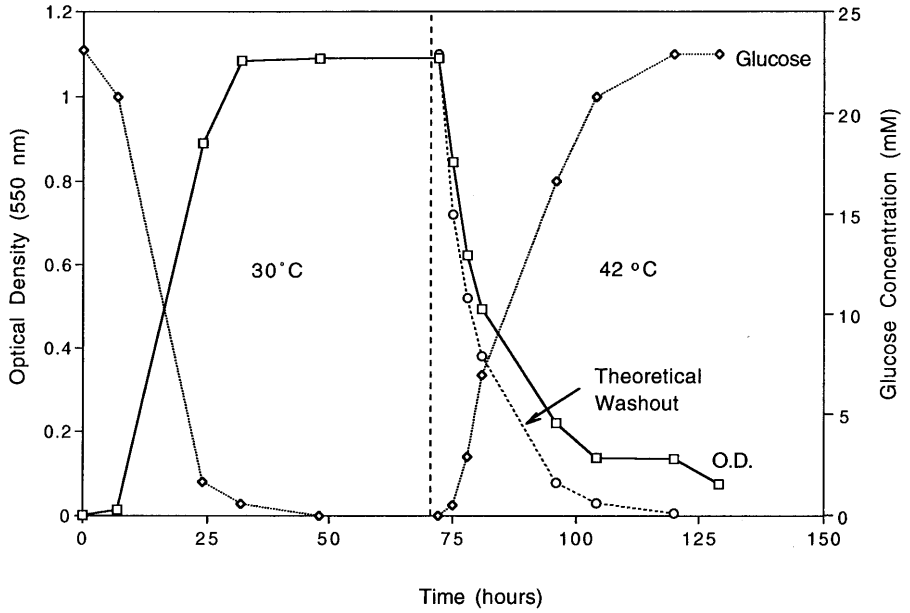

FIG. 3. Continuous culture of CH4ts with dilution rate equal to 0.1 h^{-1}. At the indicated times, samples were analyzed for cell density and glucose concentration. At 72 h, the temperature of the reaction vessel was shifted from 30 to 42°C. Symbols: ◇, glucose concentration; □, optical density at 550 nm; ○, theoretical washout rate.

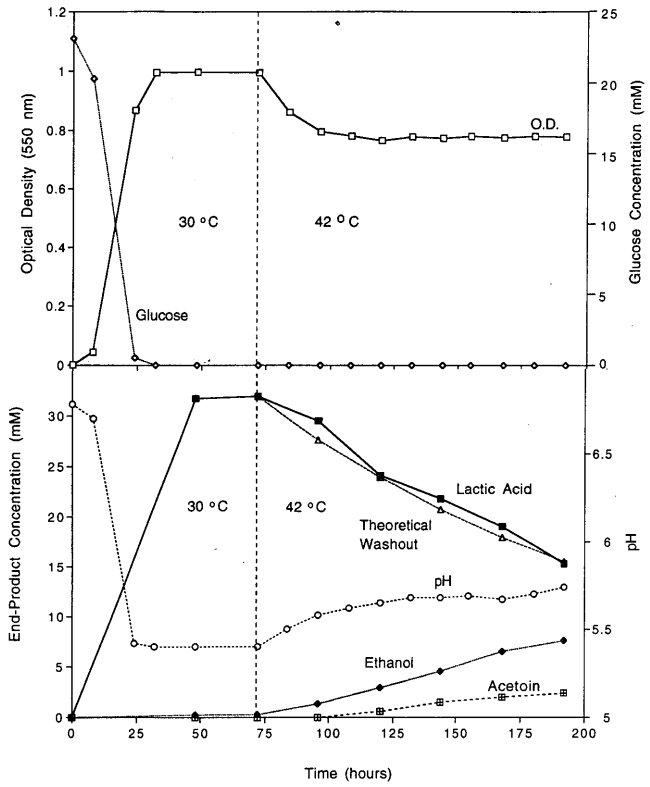

FIG. 4. Continuous culture of CH4ts with dilution rate equal to 0.006 h^{-1}. At the indicated times, samples were analyzed for cell density, pH, and glucose, lactic acid, ethanol, and acetoin concentrations. At 72 h, the temperature of the reaction vessel was shifted from 30 to 42°C. Symbols: ◇, glucose concentration; □, optical density at 550 nm; ○, pH; ◆, ethanol concentration; ⊞, acetoin concentration; ■, lactic acid concentration; △, theoretical washout rate of lactic acid.

achieved by CH4ts::adh in glucose-containing medium was 4.7; this was significantly higher than the value of 4.0 achieved by NG8.

DISCUSSION

Abhyankar et al. (2) isolated an LDH-deficient mutant of *S. mutans* by chemical mutagenesis of a fresh isolate which showed relatively high ethanol and acetate production when grown anaerobically in the presence of limiting glucose. This strain likely possessed elevated levels of one or more enzymes involved in alternative pathways for pyruvate dissimilation. In the absence of such a preexisting metabolic alteration, other strains of *S. mutans* were not mutable to LDH deficiency (11a). To confirm the metabolic basis for these results, we constructed a strain of *S. mutans*, CH4ts, possessing a thermolabile LDH activity. We found that this strain was unable to grow at the nonpermissive temperature aerobically or anaerobically on a variety of carbon sources (6). We hypothesized that this was due either to the accumulation of toxic metabolic intermediates or to an imbalance in NAD-NADH metabolism.

When subcultured into medium without glucose at 42°C, both NG8 and CH4ts ceased growing after 3 h, presumably as a result of exhaustion of endogenous stores of metabolizable carbon, and then showed a slow decline in viable cell numbers (Fig. 2). In the presence of glucose, CH4ts also stopped growing after 3 h but then showed an accelerated rate of cell death. This result shows that some aspect of glucose metabolism is toxic in LDH-deficient *S. mutans* rather than being merely growth inhibitory. It was found that intracellular pyruvate concentrations transiently increased more than 30-fold in CH4ts immediately prior to the onset of accelerated cell death (data not shown). However, this finding should not be taken as evidence that pyruvate accumulation was the direct cause of cell death, since the levels of other metabolites such as acetaldehyde and NADH (see below) likely changed also.

Stable growth of CH4ts at 42°C was achieved in continuous culture under glucose-limiting conditions. Under these conditions, CH4ts behaved like chemically derived LDH-deficient mutants of *S. rattus* (12) with regard to increased ethanol and acetoin production in place of lactic acid production. These results are in accord with fermentation and enzymology studies indicating that *S. mutans* has regulated, alternative pathways for pyruvate metabolism. As described above, under appropriate conditions of cultivation, wild-type *S. mutans* uses pyruvate formate-lyase or pyruvate dehydrogenase to produce substantial amounts of formate, acetate, ethanol, and acetoin (1, 5, 12). These alternative pathways are evidently sufficient to compensate for the absence of LDH when appropriate limitation of glucose is provided but are apparently insufficient in the presence of high glucose concentrations. For example, pyruvate formate-lyase is significantly inhibited by glyceraldehyde 3-phosphate or dihydroxyacetone phosphate, the levels of which are relatively high in *S. mutans* growing in the presence of excess glucose (20).

In an attempt to overcome the toxic effects caused by LDH deficiency, we introduced a supplementary ethanol dehydrogenase activity into CH4ts. Previous attempts to express the *Z. mobilis adh* gene in several gram-positive bacteria were unsuccessful (14a). Therefore, the coding region for the *Z. mobilis adh* gene was fused to the *S. mutans spaP* gene. As expected from previous studies (16) in which it was shown that the *spaP* regulatory signals are recognized in *E. coli*, this construct pro-

TABLE 1. Growth properties of wild-type and ADH-complemented LDH-deficient *S. mutans* strains[a]

Carbon source	Doubling time (h)		Yield (OD_{550})[b]		Lactate concn (mM)		Ethanol concn (mM)	
	NG8	CH4ts::adh	NG8	CH4ts::adh	NG8	CH4ts::adh	NG8	CH4ts::adh
Glucose	2.1	2.0	0.94	1.08	20.1	0	1.4	21.2
Galactose	4.8	2.4	0.68	0.99	19.3	0	1.3	18.9
Lactose	1.9	2.2	0.95	1.03	19.9	0	0.1	19.9
Mannitol	4.5	2.0	0.36	0.94	5.9	0	4.5	26.2
Sorbitol	8.0	3.0	0.35	0.97	3.8	0	3.6	26.1
Sucrose	2.6	2.4	0.78	0.74	20.4	0	0	19.7

[a] Cultures were grown aerobically standing at 42°C in defined medium with 0.5% tryptone and carbon sources at 1% (wt/vol) for 48 h. Results are the average of two independent experiments. Other fermentation end products besides lactate and ethanol were not determined.

[b] OD, optical density.

duced ADH activity when transformed into strain DH5α. Campbell-type recombination was used to install the *spaP*::*adh* fusion into the chromosome of CH4ts. The site of insertion was not identified but may reside at a region of homology provided by the *spaP* gene.

Crude cell extracts of a CH4ts::adh transformant possessed substantially higher levels of ADH activity than did extracts of the control strain. CH4ts::adh grew at the nonpermissive temperature at high glucose concentrations, indicating that the cloned ADH activity circumvented the metabolic blockade caused by LDH deficiency. These results indicate that the concentration of the native ADH was not present in excess in the metabolism of pyruvate in LDH-deficient mutants of *S. mutans*. If, as hypothesized here, ADH activity controls the metabolic flux in LDH-deficient mutants, NADH and the substrate for ADH, acetaldehyde, should be elevated. The results presented here do not allow us to determine whether increased pyruvate, acetaldehyde, or NADH is responsible for the toxic effect of glucose. Additional studies to answer this question are being performed.

CH4ts::adh grew at a rate similar to that of NG8 when glucose, lactose, or sucrose served as the sole carbon source, and growth yields were also comparable. Higuchi (11) showed that the growth on mannitol was severely inhibited by oxygen in some *S. mutans* strains. This finding was correlated with a relative lack of NADH oxidase activity, suggesting that accumulation of NADH was responsible for growth inhibition. NG8 grew poorly on both mannitol and sorbitol under aerobic conditions, but CH4ts::adh grew well. This result indicates that the cloned ADH activity provided sufficient NADH oxidation activity to serve as a suitable sink for excess electrons generated during polyol metabolism. NG8 also grew poorly on galactose. It is not clear why CH4ts::adh should grow better than NG8 on this carbon source, which is metabolized via the tagatose 6-phosphate pathway in *S. mutans* (10), particularly since their growth levels on lactose were comparable.

The good growth and the decreased acid production of CH4ts::adh indicate that augmentation of ADH activity can provide a useful approach for constructing an LDH-deficient effector strain derived from JH1005 for use in replacement therapy of dental caries. ADH augmentation can clearly be achieved by using the cloned *Z. mobilis* structural gene fused to an appropriate regulatory sequence. Alternatively, the ADH gene from *S. rattus* may also be useful in circumventing the lethal metabolic blockade caused by LDH deficiency.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant DEO4529 from the National Institute of Dental Research.

REFERENCES

1. **Abbe, K., S. Takahashi, and T. Yamada.** 1982. Involvement of oxygen-sensitive pyruvate formate-lyase in mixed-acid fermentation by *Streptococcus mutans* under strictly anaerobic conditions. J. Bacteriol. **152:**175–182.
2. **Abhyankar, S., H. J. Sandham, and K. H. Chan.** 1985. Serotype c *Streptococcus mutans* mutable to lactate dehydrogenase deficiency. J. Dent. Res. **64:**1267–1271.
3. **Brown, A. T., and C. L. Wittenberger.** 1972. Fructose-1,6-diphosphate-dependent lactate dehydrogenase from a cariogenic streptococcus: purification and regulatory properties. J. Bacteriol. **110:**604–615.
4. **Carlsson, J.** 1970. Chemically defined medium for growth of *Streptococcus sanguis*. Caries Res. **4:**297–304.
5. **Carlsson, J., U. Kujala, and M.-B. K. Edlund.** 1985. Pyruvate dehydrogenase activity in *Streptococcus mutans*. Infect. Immun. **49:**674–678.
6. **Chen, A., J. D. Hillman, and M. Duncan.** 1994. L-(+)-lactate dehydrogenase deficiency is lethal in *Streptococcus mutans*. J. Bacteriol. **176:**1542–1545.
7. **Conway, T., G. W. Sewell, Y. A. Osman, and L. O. Ingram.** 1987. Cloning and sequencing of the alcohol dehydrogenase II gene from *Zymomonas mobilis*. J. Bacteriol. **169:**2591–2597.
8. **Crowley, P. J., and A. S. Bleiweis.** 1995. Analysis of gene regulation of a major cell surface adhesin (SpaP) from *Streptococcus mutans*. J. Dent. Res. **74:**200. (Abstract.)
9. **Drucker, D. B., and T. H. Melville.** 1968. Fermentation end-products of cariogenic and non-cariogenic streptococci. Arch. Oral Biol. **13:**563–570.
10. **Hamilton, I. R., and H. Lebtag.** 1979. Lactose metabolism by *Streptococcus mutans*: evidence for induction of the tagatose 6-phosphate pathway. J. Bacteriol. **140:**1102–1104.
11. **Higuchi, M.** 1984. The effect of oxygen on the growth and mannitol fermentation of *Streptococcus mutans*. J. Gen. Microbiol. **130:**1819–1826.

11a. **Hillman, J. D.** Unpublished data.

12. **Hillman, J. D., S. W. Andrews, and A. L. Dzuback.** 1987. Acetoin production by wild-type strains and a lactate dehydrogenase-deficient mutant of *Streptococcus mutans*. Infect. Immun. **55:**1399–1402.
13. **Hillman, J. D., A. Chen, M. Duncan, and S.-W. Lee.** 1994. Evidence that L-(+)-lactate dehydrogenase deficiency is lethal in *Streptococcus mutans*. Infect. Immun. **62:**60–64.
14. **Hillman, J. D., A. L. Dzuback, and S. W. Andrews.** 1987. Colonization of the human oral cavity by a *Streptococcus mutans* mutant producing increased bacteriocin. J. Dent. Res. **66:**1092–1094.

14a. **Ingram, L. O.** Personal communication.

15. **Johnson, C. P., S. M. Gross, and J. D. Hillman.** 1980. Cariogenic potential *in vitro* in man and *in vivo* in the rat of lactate dehydrogenase mutants of *Streptococcus mutans*. Arch. Oral Biol. **25:**707–713.
16. **Lee, S. F., A. Progulske-Fox, and A. S. Bleiweis.** 1988. Molecular cloning and expression of a *Streptococcus mutans* major surface protein antigen, P1 (I/II), in *Escherichia coli*. Infect. Immun. **56:**2114–2119.
17. **Lee, S. F., A. Progulske-Fox, G. W. Erdos, D. A. Piacentini, G. Y. Ayakawa, P. J. Crowley, and A. S. Bleiweis.** 1989. Construction and characterization of isogenic mutants of *Streptococcus mutans* deficient in major surface protein antigen P1 (I/II). Infect. Immun. **57:**3306–3313.
18. **Neal, A. D., R. K. Scopes, J. M. Kelly, and R. E. H. Wettenhall.** 1986. The two alcohol dehydrogenases of *Zymomonas mobilis*: purification by differential dye ligand chromatography, molecular characterization and physiological role. Eur. J. Biochem. **154:**119–124.
19. **Perry, D., and H. K. Kuramitsu.** 1981. Genetic transformation of *Streptococcus mutans*. Infect. Immun. **32:**1295–1297.
20. **Takahashi, S., K. Abbe, and T. Yamada.** 1982. Purification of pyruvate formate-lyase from *Streptococcus mutans* and its regulatory properties. J. Bacteriol. **149:**1034–1040.
21. **Tanzer, J. M., M. I. Krichevsky, and P. H. Keyes.** 1969. The metabolic fate of glucose catabolized by a washed stationary-phase caries-conducive streptococcus. Caries Res. **3:**167–177.
22. **Tobian, J. A., M. L. Cline, and F. L. Macrina.** 1984. Characterization and expression of a cloned tetracycline resistance determinant from the chromosome of *Streptococcus mutans*. J. Bacteriol. **160:**556–563.

Editor: J. R. McGhee