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Steady-State Measurements of Lactic Acid Production in a Wild-Type and a Putative D-Lactic Acid Dehydrogenase-Negative Mutant of *Zymomonas mobilis*

Influence of Glycolytic Flux

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Abstract

This work represents a continuation of our investigation into environmental conditions that promote lactic acid synthesis by *Zymomonas mobilis*. The characteristic near theoretical yield of ethanol from glucose by *Z. mobilis* can be compromised by the synthesis of D- and L-lactic acid. The production of lactic acid is exacerbated by the following conditions: pH 6.0, yeast extract, and reduced growth rate. At a specific growth rate of 0.048/h, the average yield of DL-lactate from glucose in a yeast extract-based medium at pH 6.0 was 0.15 g/g. This represents a reduction in ethanol yield of about 10% relative to the yield at a growth rate of 0.15/h. Very little lactic acid was produced at pH 5.0 or using a defined salts medium (without yeast extract) Under permissive and comparable culture conditions, a tetracycline-resistant, D-*ldh* negative mutant produced about 50% less lactic acid than its parent strain Zm ATCC 39676. D-lactic acid was detected in the cell-free spent fermentation medium of the mutant, but this could be owing to the presence of a racemase enzyme. Under the steady-state growth conditions provided by the chemostat, the specific rate of glucose consumption was altered at a constant growth rate of 0.075/h. Shifting from glucose-limited to nitrogen-limited growth, or increasing the temperature, caused an increase in the specific rate of glucose catabolism. There was good correlation between an increase in glycolytic flux and a decrease in lactic acid yield from glucose. This study points to a mechanistic link between the glycolytic flux and the control of end-product glucose metabolism. Implications of reduced glycolytic flux in pentose-fermenting recombinant *Z. mobilis* strains, relative to increased byproduct synthesis, is discussed.

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Index Entries: *Zymomonas mobilis*; lactic acid; end-product selectivity; D-lactate dehydrogenase; glycolytic flux; steady state.

Introduction

The bacterium *Zymomonas mobilis* has been studied extensively as a biocatalyst for fuel ethanol production because of its superior yield and productivity characteristics *(1–4)*. Technoeconomic analyses of projected commercial-scale biomass-to-ethanol processes indicate that major cost reductions can be achieved through technologic advances in both process design (configuration) and biocatalyst performance *(5,6)*. More advanced processes employ continuous flow bioreactors for improved productivity *(7)*. There are many process patents for ethanol production that incorporate *Zymomonas (8–10)*. More advanced microbial biocatalysts can ferment the C5 sugars of hemicellulose, which can comprise as much as 30% of lignocellulosic biomass.

Recently, our research *(11,12)* has focused on assessing the C6/C5 cofermentation characteristics of genomically integrated pentose-fermenting recombinants of *Z. mobilis* that have been developed at the National Renewable Energy Laboratory (NREL)*(13)*. A feature of these metabolically engineered strains is an increased synthesis of alternative fermentation end products *(11,12)*. Although metabolic flux analyses of wild-type and recombinant Zm, using noninvasive in vivo ^{13}C and ^{31}P nuclear magnetic resonance techniques, have been helpful in describing metabolic bottlenecks, they have not yet provided definitive answers regarding metabolic regulation in terms of end-product selectivity *(14,15)*. These studies on metabolic flux in *Z. mobilis* make no mention of lactic acid dehydrogenase in the context of the regulation of pyruvate metabolism.

In their comprehensive review on the biology of *Zymomonas*, Swings and DeLey *(16)* noted that, on the subject of reduced ethanol yield owing to lactic acid production, the literature was conflicted; however, they did not speculate as to possible reasons for the large differences in the fermentation balances reported by different investigators. For example, molar yields of lactic acid from glucose ranged from highs of 0.15 *(17)* and 0.2 *(18)* to lows of 0.02 *(19)* and 0.053 *(20)*. On a mass basis, these yields range from 0.01 to 0.1 g/g. Where Beläich and Senez *(18)* observed a high production of lactate, the pH of the nutrient-rich medium was buffered at 6.8–7.0.

In a review entitled "Byproducts from *Zymomonas mobilis*," Johns et al. *(21)* stated that "*Z. mobilis* is a microorganism that is not only an extremely efficient producer of alcohol, but also is capable of producing other metabolites under the correct conditions." This statement strongly suggests that, apart from any perturbations that may be introduced as a consequence of metabolic engineering, there is a role for environmental conditions in determining the end-product selectivity. With this in mind, we have been examining the correlation between growth rate and the control of pyruvate metabolism in glucose fermentations with wild-type *Z. mobilis (22)*. In a glucose-limited chemostat at pH 6.0, the sugar-to-ethanol conversion effi-

ciency was observed to decrease from 95% at a specific growth rate of 0.2/h to only 82% at 0.042/h. The decrease in ethanol yield was caused by the pH-dependent shift in pyruvate metabolism from ethanol to lactic acid as a fermentation end product *(22)*. At the lowest growth rate, the yields for ethanol and lactic acid were 0.42 g/g and 0.16 g/g, respectively *(22)*. At growth rates <0.1/h, the chemical composition of the medium has also been implicated as a potential contributing factor in determining the level of lactate produced *(22)*. In the context of considering growth rate as a factor influencing ethanol yield, it is important to remember that most batch fermentations are run under optimal growth conditions in which the growth rate is maximal. Likewise, to achieve maximal productivity, most continuous fermentations are operated near maximum dilution rate (i.e., relatively high growth rate). Furthermore, since the optimal pH for growing *Z. mobilis* is in the range of 5.0–5.5, most literature reports of Zm fermentations were done in this pH range. Hence, these conditions, in both batch and continuous mode, are consistent with typical reports of near theoretical maximum ethanol yields in wild-type *Z. mobilis* glucose-based fermentations *(1–4)*. On the other hand, the growth rate of recombinant Zm is markedly reduced with xylose as the sole fermentable sugar *(23–25)*. In pH-stat batch fermentations with single sugars, the specific rate of sugar consumption was fourfold lower with xylose compared to glucose *(25)*. Consequently, to achieve near complete sugar utilization in C6/C5 continuous fermentations with rec Zm, the maximum dilution rate is significantly lower than for glucose fermentations *(26–28)*. Another factor known to contribute to increased lactate formation by wild-type *Z. mobilis* is elevated pH *(18,22)*. Biomass hydrolysates contain acetic acid, which is known to be inhibitory to microbial growth and fermentation. Therefore, our studies with C6/C5 cofermentations with NREL's recombinant Zm strains have used a higher pH control set point (5.75–6.0) to reduce acetic acid inhibition *(11,12)*.

While working on methods to achieve strategic chromosomal integration of exogenous pentose metabolism genes in *Z. mobilis* ATCC 39676, a mutant was created at NREL in which the gene for tetracycline resistance was inserted into the region of the chromosome located between the genes for phosphoglycerate mutase *(pgm)* and alcohol dehydrogenase I (*adhA*) *(29)*. This DNA has been assigned as the gene for a D-isomer-specific 2-hydroxyacid dehydrogenase (*ddh*) *(30)*. The designation of this gene as *ddh* was made on the basis of the correlation in both identity (31.5%) and similarity (55.4%) between its transcription product (331 amino acid polypeptide with an NAD-binding domain) and the D-isomer lactic acid dehydrogenase from *Lactobacillus plantarum (30)*. Herein we refer to this gene as D-*ldh* since it seems reasonable to assume that it would be responsible for the synthesis of D-lactic acid.

In the present study, we explored the role of the putative D-specific lactic acid dehydrogenase in contributing to the production of lactic acid under previously established *(22)* permissive conditions. For this purpose, we used NREL's tetracycline- resistant, D-*ldh*-negative mutant of *Z. mobilis*

Table 1
Zymomonas Media Formulations

Ingredient	Medium designation and formulation[a]		
	ZM[b]	ZM-AC	DS[c]
Yeast extract (g)	variable	variable	0
NH_4Cl (g)	0.8	0	1.6
KH_2PO_4 (g)	3.48	3.48	3.48
$MgSO_4 \cdot 7H_2O$ (g)	0.5	0.5	0.5
$FeSO_4 \cdot 7H_2O$ (g)	0.01	0.01	0.01
Citric acid (g)	0.21	0.21	0.21
Ca pantothenate (mg)	—	—	1.0
Biotin (mg)	—	—	1.0
Distilled water (L)	1.0	1.0	1.0

[a]AC, ammonium chloride; DS, defined salts
[b]Reproduced from ref. *37*.
[c]Reproduced from ref. *22*.

ATCC 39676 *(29)*. We also examined the relationship between glycolytic flux and lactic acid synthesis under steady-state growth conditions. The specific rate of glucose metabolism was increased independent of the growth rate by imposing a condition of nitrogen limitation on the chemostat cultures at a fixed dilution rate of 0.075/h. Increasing the temperature was another means of altering glycolytic flux independent of the growth rate. The performance of the D-*ldh*(–) mutant was compared with that of the parent strain, *Z. mobilis* ATCC 39676.

Materials and Methods

Organisms

Wild-type *Z. mobilis* ATCC 39676 was obtained from the American Type Culture Collection (Rockland, MD), *Z. mobilis* (39676) Tc^R D-*ldh* negative mutant *(29)* was obtained from Dr. Min Zhang (NREL, Golden, CO), under the terms of a materials transfer agreement and a thermotolerant mutant of Zm ATCC 31821 designated as *Z. mobilis* C107 *(31)* was a gift from Dr. Jacques C. Baratti (Université de Province, Marseille, France).

Fermentation Media, Equipment and Operating Conditions

Table 1 gives composition of the different media used. Bacto yeast extract was obtained from Difco (Detroit, MI). Other chemicals were laboratory-grade purity. Glass distilled water was used to prepare all media. Tetracycline 10 µg/mL was added to all D-*ldh*-negative mutant experiments.

Continuous fermentations were conducted with 750-mL MultiGen bioreactors (Model F1000; New Brunswick Scientific, Edison, NJ) except

that the glass vessel had an overflow outlet. The working volume of the chemostats was about 350 mL. Steady state was assumed only after a minimum of 3 vol had exchanged and when assays of successive samples produced similar results.

Analytical Procedures

Growth was measured turbidometrically at 600 nm (1-cm light path) (Unicam spectrophotometer, model SP1800). In all cases, the blank cuvet contained distilled water. Dry cell mass (DCM) was determined by microfiltration of an aliquot of culture followed by washing and drying of the filter to constant weight under an infrared heat lamp. Fermentation media and cell-free spent media were compositionally analyzed by high-performance liquid chromatography (HPLC) with a refractive index detector and computer-interfaced controller/integrator (Hewlett Packard HP 1100 series; Agilent, Palo Alto, CA). Separations were performed at 65°C using an HPX-87H column (300 × 7.8 mm) (Bio-Rad, Richmond, CA) as described previously *(26)*. HPLC measurements were for total lactic acid. L-Lactic acid was estimated using an L (+) lactate assay kit (826-A; Sigma, St. Louis, MO). The enzyme and standards were replaced with D-lactic dehyrogenase and D-lactic acid for assays of D-lactate (Sigma). Ethanol yield ($Y_{p/s}$) and total lactic acid yield ($Y_{LA/s}$) were calculated as the concentration of end product divided by the concentration of glucose consumed. For the purpose of carbon balancing (percentage of C recovery), the carbon content of the cell mass (48.7%) was considered to be constant. Carbon dioxide was not measured but was assumed to be produced at a molar equivalent to ethanol *(32)*.

Results and Discussion

Batch Fermentations

In stirred-tank batch fermentations with the pH controlled at 6.0, neither the wild-type culture *Z. mobilis* 39676 nor the tetracycline-resistant, D-*ldh*-negative mutant produced significant amounts of lactic acid. For both cultures, the ethanol yield was close to theoretical maximum (Table 2). For the wild-type strain, the observed low level of lactic acid production in batch fermentation was predictable from our previous study on this same subject *(22)*. *Z. mobilis* characteristically exhibits a high selectivity in terms of ethanol as fermentation end product. In the batch mode of growth, for any given specified physicochemical environment, a culture expresses its maximum specific growth rate and maximum glycolytic flux (i.e., specific rate of glucose metabolism). The relatively low level of lactic acid observed in our batch fermentations at pH 6.0 is consistent with the suggestion that lactic acid synthesis is inhibited at faster rates of growth *(22)*.

In this aspect of our work, the lactic acid was measured by HPLC analysis and no attempt was made to assess the amount of D- or L-isomer.

Table 2
Summary of Batch Fermentation Parameters
for Wild-Type Zm 39676 and D-*ldh*-Negative Mutant[a]

	Wild-type ATCC 39676	D-*ldh* mutant (Tc^R)
Final cell mass (g DCM/L)	2.04	1.56
$Y_{x/s}$ (g/g)	0.033	0.026
Ethanol (g/L)	30.61	29.67
$Y_{p/s}$ (g/g)	0.50	0.50
Lactic acid (g/L)	0.43	0.13
$Y_{LA/s}$	0.007	0.002
Carbon recovery (%)	103.0	101.0

[a] Medium = ZM (3 g/L of yeast extract and 60 g/L of glucose); temperature = 30°C; pH = 6.0.

However, note that in describing the creation of the D-*ldh*-negative mutant, Zhang et al. *(29)* reported that a batch culture of the parent (Zm 39676) produced almost exclusively D-lactic acid (about 1 g/L), whereas the D-*ldh* negative mutant produced very little D- or L-lactic acid (0.1 g/L).

Continuous Fermentations

The present work extends our previous chemostat studies by comparing the lactic acid yield of the D-*ldh* negative mutant to its parent wild-type strain Zm ATCC 39676. Steady-state levels of lactic acid were determined as a function of dilution rate (*D*) under a condition of glucose-limited growth with the temperature constant at 30°C and the pH controlled at either 6.0 or 5.0. Under this condition, the rate at which the medium is fed to the chemostat (i.e., the dilution rate) determines the specific growth rate. Figure 1 presents the behavior of the wild-type strain. The pH-dependent pattern of decreasing lactic acid yield with increasing growth rate confirms our previously reported observations with this same culture *(22)*. We have observed similar results with several other *Z. mobilis* cultures: ATCC 29191 *(22)* and strains CP4 (NRRL B-14023), ATCC 31821, ATCC 10988, and Zm C107 (unpublished results). Figure 2 shows that, under these growth conditions, the D-*ldh* mutant produces about half as much lactic acid. In experiments with the mutant strain, the presence of tetracycline in the medium limits the likelihood of opportunistic infection by lactic acid–producing organisms. In all our experiments, several measures were incorporated into the protocol to ensure culture purity. These measures included employing single-colony isolates for generation of inocula, periodic microscopic inspection, and plating on media permissive to potential contaminant organisms but not permissive for *Z. mobilis* (e.g., nutrient agar without glucose).

Unfortunately, the results of specific assays for D- and L-lactate were equivocal; however, we concluded from these assays that both the mutant and its parent produced mixtures of D- and L-lactic acid. These mixtures

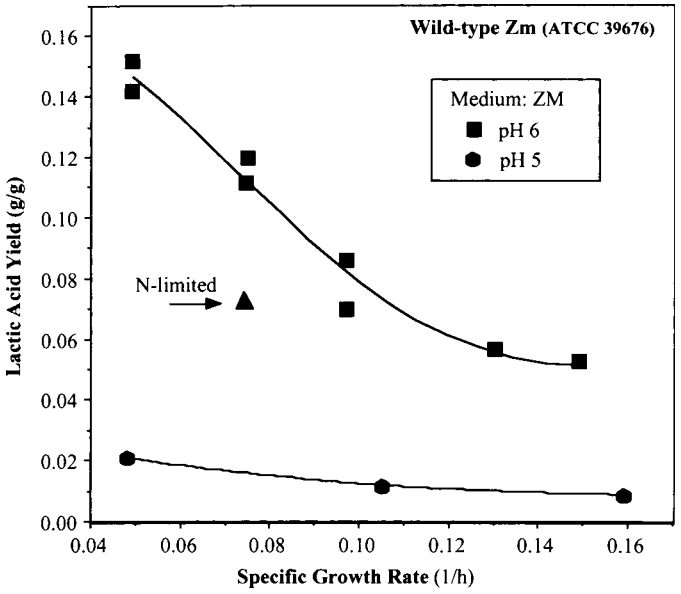

Fig. 1. Lactic acid yield as function of specific growth rate for *Z. mobilis* ATCC 39676. Steady-state measurements of lactic acid were made with glucose-limited chemostat cultures. The ZM medium contained 6% (w/v) glucose, the temperature was maintained at 30°C, and the pH was controlled at either 5.0 or 6.0. The arrow indicates a separate experiment in which the culture was N limited ($D = 0.075/h$) using a ZM-AC medium containing 2 g/L yeast extract. Details of the composition of the media are given in Table 1.

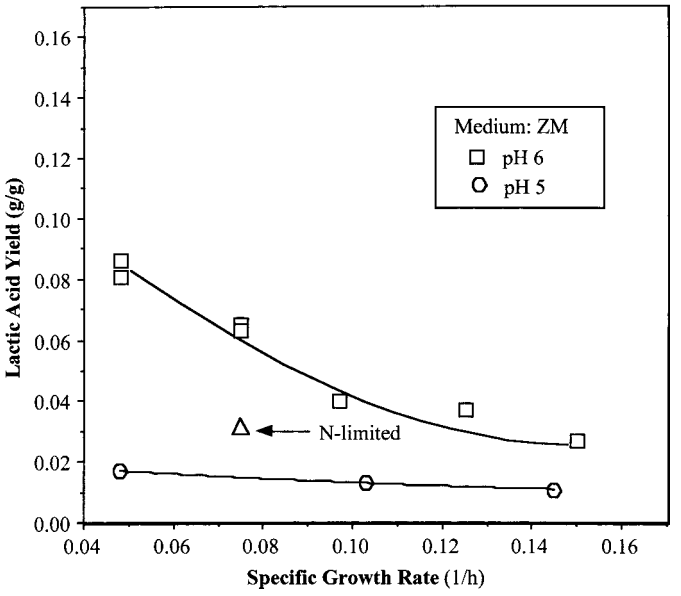

Fig. 2. Lactic acid yield as function of specific growth rate for D-*ldh*-negative mutant Zm 39676. Experimental conditions were the same as described for Fig. 1 except that the media contained 10 mg/L of tetracycline.

may well be the consequence of an uninhibited racemase activity that could be expected to create a DL-racemic mixture with the ratio of D- to L-isomer depending on the equilibrium characteristics of an enzyme. In subsequent determinations of lactic acid, we abandoned the isomer-specific assays and relied solely on the HPLC analytical data for total lactic acid concentrations. Therefore, we were only able to corroborate the observation that the mutant produces less lactic acid than the parent *(29)*. Since the mutant did not produce exclusively L-lactic acid, our results cannot offer direct support for the suggestion that the reduction in the amount of lactate synthesized by the mutant is a consequence of the absence of any synthesis of D-lactic acid *(29)*.

The Role of Glycolytic Flux in Lactic Acid Synthesis

Analyses of metabolic flux in *Zymomonas* have not revealed any correlation to changes in ethanol selectivity (i.e., increased production of byproduct) *(14,15)*. For fructose metabolism, in which lactic acid production is increased *(33,34)*, a possible role for fructose-6-phosphate has been suggested *(14)*. Fructose bisphosphate is known to play a regulatory role in lactic acid dehydrogenase activity in certain bacteria *(35)*, and although it is not an intermediate of the glucose dissimilation pathway in *Zymomonas* *(16)*, fructose-6-phosphate is produced during xylose metabolism as a product of the transketolase activity in recombinant *Z. mobilis* .

Since the glycolytic flux (determined as the specific rate of glucose consumption, q_s) is proportional to the specific growth rate (μ) ($q_s=\mu/Y_{x/s} + m_e$ (in which $Y_{x/s}$ is the growth yield and m_e is the maintenance energy coefficient) *(36)*, the data in Figs. 1 and 2 were replotted to show the relationship between the steady-state lactic acid yield and the glycolytic flux (Fig. 3).

Effect of Nitrogen-Limited Growth on Production of Lactic Acid

We were interested in seeing the effect of altering the glycolytic flux independent of the growth rate. Our first approach was to cause a decrease in the growth yield ($Y_{x/s}$) by creating a condition of nitrogen-limited growth and assessing the effect of the lactic acid yield at constant dilution rate (growth rate). Using a medium in which yeast extract is the sole source of assimilable nitrogen (medium ZM-AC; see Table 1), the steady-state cell mass concentration in the chemostat at $D = 0.075/h$ was directly proportional to the concentration of yeast extract in the medium (Fig. 4). The growth yield for yeast extract (i.e., the slope in line in the plot of cell mass vs yeast extract) is 0.45 g of dry cell mass/g of yeast extract (Fig. 4) *(37)*. To create the condition of N limitation, the ZM-AC medium was supplemented with 2 g/L yeast extract. The steady-state cell mass concentration for both cultures was about 0.9 g of DCM/L (Fig. 4). For comparison purposes, the steady-state growth and fermentation parameters for the glucose-limited and nitrogen-limited cultures are summarized in Table 3. The lactic acid yield of the N-limited cultures is indicated by the triangles in Figs. 1, 2 ,

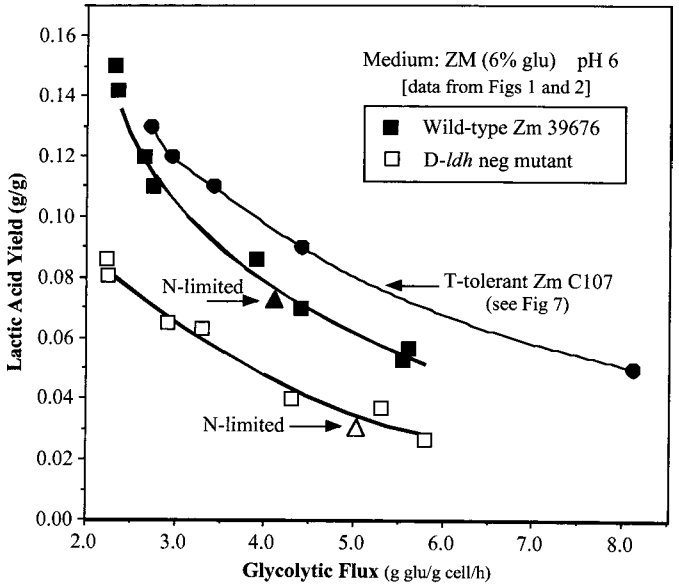

Fig. 3. Relationship between lactic acid yield and specific rate of glucose metabolism (glycolytic flux) for wild-type Zm 39676 and the D-*ldh* mutant. Data are taken from the experiments shown in Figs. 1 and 2. Also shown are the results of a separate experiment with thermotolerant Zm strain C107 in which the temperature was increased incrementally from 30 to 42°C (see Fig. 7).

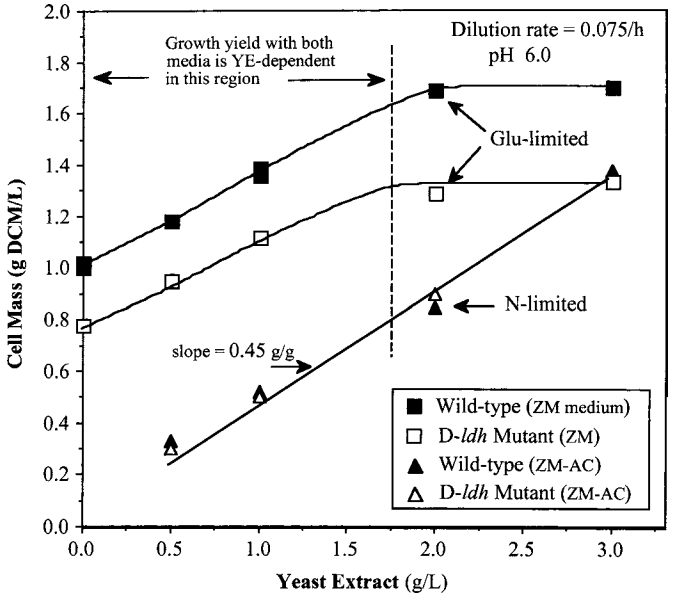

Fig. 4. Steady-state cell mass concentration for wild-type Zm 39676 and D-*ldh*-negative mutant as function of yeast extract in medium. The ZM medium contained 0.8 g/L of ammonium chloride (AC), but the ZM-AC medium did not (see Table 1). The dilution rate was maintained at 0.075/h, the temperature was 30°C, and the pH was 6.0.

Table 3
Summary of Growth and Fermentation Parameters for Glucose-Limited and N-Limited Chemostat Cultures of Zm 39676 and D-*ldh* Negative Mutant[a]

	Zm 39676	D-*ldh* mutant
C-limited growth		
Glucose used (g/L)	60.0	60.0
$Y_{p/s}$ (g/g)	0.45	0.48
Total lactic acid	6.6	2.9
Lactic Acid yield (g/g)	0.11	0.063
Cell mass (g DCM/L)	1.68	1.32
Growth yield ($Y_{x/s}$) g/g	0.028	0.022
q_s (g glu/[g cell·h])	2.68	3.41
N-limited growth		
Glucose used (g/L)	50.0	59.0
$Y_{p/s}$ (g/g)	0.46	0.48
Total lactic acid	3.7	2.2
Lactic acid yield (g/g)	0.073	0.033
Cell mass (g DCM/L)	0.85	0.90
Growth yield ($Y_{x/s}$) (g/g)	0.017	0.015
q_s (g glu/[g cell·h])	4.41	4.92

[a] Conditions: pH = 6.0; temperature = 30°C; D = 0.075/h. Media: for C-limitation, ZM medium (2 g/L of yeast extract + 60 g/L of glucose); for N limitation, ZM-AC medium (2 g/L of yeast extract + 60 g/L of glucose). q_s = specific rate of glucose metabolism (glycolytic flux).

and 3. At D = 0.075/h, changing from carbon (energy)-limited growth to N-limited growth caused about a 30–40% decrease in the lactic acid yield (Table 3, Figs. 1 and 2). However, since N limitation resulted in a significant increase in the specific rate of glucose consumption, both the N-limited cultures conformed very well to the curve describing the relationship between the lactic acid yield and the glycolytic flux (see triangles in Fig. 3). This observation offers support for our hypothesis of a link between the production of lactic acid and the glycolytic flux.

Effect of Yeast Extract on Lactic Acid Production

Figure 5 shows the relationship between the lactic acid yield and the amount of yeast extract in the nitrogen-sufficient ZM medium in a chemostat operating at a constant dilution rate of 0.075/h and pH 6.0. Both the wild-type and mutant cultures show a linear reduction in lactic acid production over the range of yeast extract from 0 to 1.75 g/L, with the mutant producing about half as much lactic acid as the parent culture. Theoretically, the amount of ammonium chloride in the ZM medium is sufficient growth nitrogen to yield a cell mass concentration of about 1.5 g of DCM/L *(37)*. Figure 4 reveals that both the wild-type and mutant cultures were sensitive to the level of yeast extract in the nitrogen-sufficient ZM medium at concentrations of yeast extract over the range of 0–2 g/L.

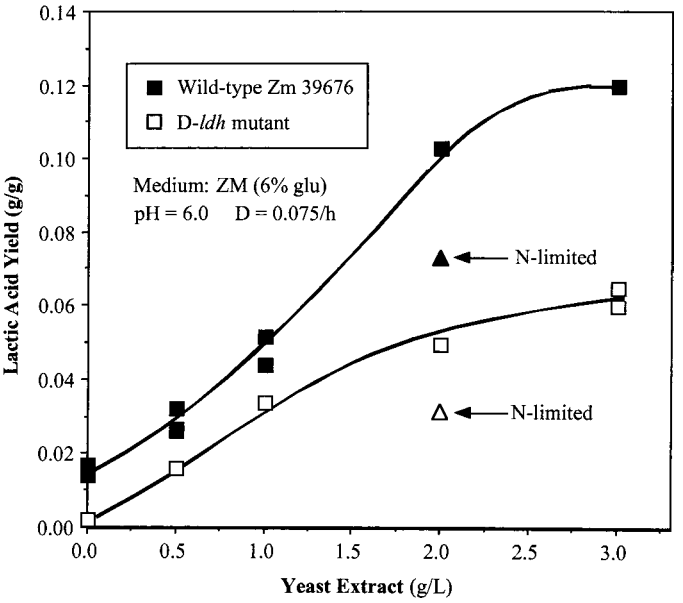

Fig. 5. Lactic acid yield as function of yeast extract extract in medium. Data are from experiments shown in Fig. 4 using nitrogen-sufficient ZM medium (with 0.8 g/L of ammonium chloride). The arrows indicate N-limited cultures using ZM-AC medium with 2 g/L of yeast extract.

Over this range of yeast extract in the ZM medium, the cultures were not N limited, but rather "yeast extract limited" (Fig. 4). Hence, it seemed appropriate to assess the effect of yeast extract on lactic acid production relative to glycolytic flux, independent of growth rate, using the ZM medium containing varying amounts of yeast extract over the range of 0–2 g/L (Fig. 6). Interestingly, the trajectories of the parent and mutant in Fig. 6 have regions where they are almost superimposable. This observation also points to a possible connection between lactic acid synthesis and the rate of specific glucose consumption. These observations also confirm our previous suggestion regarding the involvement of some unidentified component of yeast extract (also tryptone; results not shown) in promoting the synthesis of lactic acid *(22)*.

Effect of Temperature on Lactic Acid Production

Temperature is known to affect the growth rate. To examine the effect of increasing temperature on lactic acid production independent of the effect of temperature on growth, we used a chemostat culture of a thermotolerant mutant of Zm ATCC 31821 (also known as strain ZM4). This mutant was designated as Zm strain C107 *(31)*. The chemostat was operated under a condition known to be conducive to lactate production: glucose limitation at a fixed dilution rate (i.e. growth rate) of 0.07/h and pH 6.0. The temperature was increased incrementally over the range of 30 to 42°C. Figure 7 shows a plot of the steady-state levels of lactic acid and the

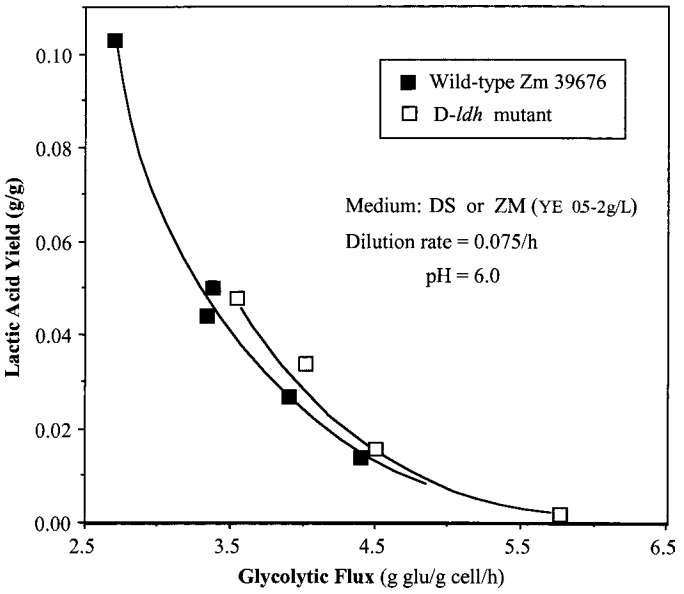

Fig. 6. Effect of yeast extract extract on relationship between lactic acid yield and glycolytic flux for wild-type Zm 39676 and D-*ldh* mutant. The media were DS (no yeast extract) or ZM containing 0.5–2.0 g/L of yeast extract. The dilution rate was maintained at 0.075/h, the temperature was 30°C, and the pH was 6.0.

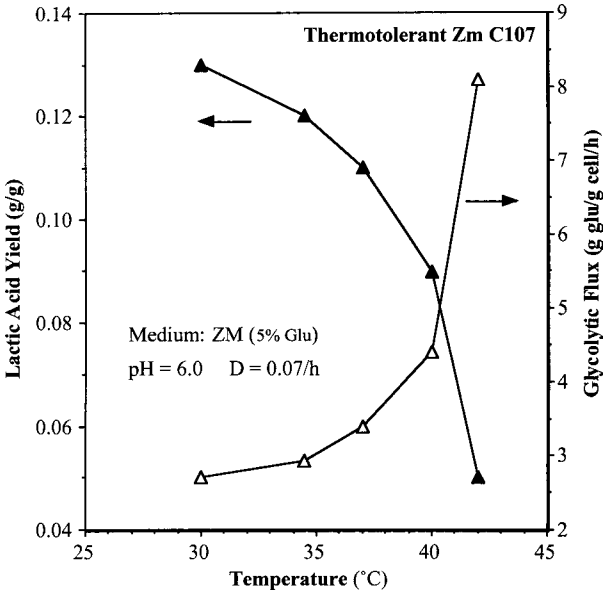

Fig 7. Lactic acid yield and glycolytic flux as function of temperature for thermotolerant Zm strain C107. The ZM medium contained 5% (w/v) glucose. The dilution rate was maintained at 0.07/h, the pH was 6.0, and the temperature was increased incrementally from 30 to 42°C.

glycolytic flux as a function of temperature. For comparison purposes, the glycolytic flux data for this experiment are plotted in Fig. 3 (solid circle). The similarity between the curves in Fig. 3 for the wild-type Zm 39676 and the thermotolerant mutant Zm C107 provides additional evidence to support the concept of a link between lactic acid synthesis and the specific rate of glucose metabolism.

Conclusion

Under permissive and comparable conditions, the D-*ldh*-negative mutant produced about 50% of the amount of lactic acid of its parent culture perhaps because of the involvement of a racemase activity; the stereospecific assays yielded equivocal results. Consequently, it was not possible to quantitate the relative amounts of the D- and L-specific isomers of lactic acid produced by both cultures. However, the mutant did not appear to produce exclusively L-lactic acid. When lactic acid synthesis was maximal, the ethanol yield from glucose was reduced by about 15% with the parent, but only 8% with the D-*ldh*-negative mutant. We also found that increasing the pH from 5.0 to 6.0 could cause a shift in glucose metabolism from solventogenesis (ethanol) to acidogenesis (lactic acid). In addition, an unidentified component of yeast extract appeared to stimulate the synthesis of lactic acid from glucose, and increasing the temperature appeared to reduce lactic acid synthesis. Finally, at pH 6.0, lactic acid synthesis was associated with a decreased glycolytic flux (specific rate of glucose metabolism).

Acknowledgments

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