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Fermentation of D-Xylose and L-Arabinose to Ethanol by *Erwinia chrysanthemi*

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***Erwinia* spp. are gram-negative facultative anaerobes within the family *Enterobacteriacae* which possess several desirable traits for the conversion of pentose sugars to ethanol, such as the ability to ferment a broad range of carbohydrates and the ease with which they can be genetically modified. Twenty-eight strains of *Erwinia carotovora* and *E. chrysanthemi* were screened for the ability to ferment D-xylose to ethanol. *E. chrysanthemi* B374 was chosen for further study on the basis of its superior (4%) ethanol tolerance. We have characterized the fermentation of D-xylose and L-arabinose by the wild type and mutants which bear plasmids containing the pyruvate decarboxylase gene from *Zymomonas mobilis*. Expression of the gene markedly increased the yields of ethanol (from 0.7 up to 1.45 mol/mol of xylose) and decreased the yields of formate, acetate, and lactate. However, the cells with pyruvate decarboxylase grew only one-fourth as fast as the wild type and tolerated only 2% ethanol. Alcohol tolerance was stimulated by the addition of yeast extract to the growth medium. Xylose catabolism was characterized by a high saturation constant K_s (4.5 mM).**

The genus *Erwinia* consists of several species of plant-pathogenic bacteria within the family *Enterobacteriacae* (6). *Bergey's Manual of Systematic Bacteriology* (17) recognizes 15 species within the genus, 3 of which are as follows: *Erwinia amylovora*, which causes fireblight and wilt disease, utilizes a restricted range of carbon compounds, and requires organic nitrogen for growth; *Erwinia herbicola*, which is isolated from plants and from the enteric tracts of humans; and *Erwinia carotovora*, which is a soft-rot pathogen and is the most biochemically active species. There has been much interest in *Erwinia* spp. as plant pathogens, for the production of L-asparaginase, and for the control of ice formation on plants (25). However, there have been few studies of the products of sugar fermentation by these bacteria (16, 26).

Erwinia spp. have several desirable traits for the conversion of pentose sugars to ethanol as an economic fuel source (22). *E. carotovora* strains grow anaerobically and ferment the pentose sugars xylose and arabinose (7). Some strains have been reported to hydrolyze naturally occurring polymers of xylose (23). Furthermore, vectors have been developed for genetic manipulations of *Erwinia* spp. (30, 32).

The goal of this study was to evaluate the fermentation of pentose sugars to ethanol by *Erwinia* spp. Twenty-eight *E. carotovora* and *Erwinia chrysanthemi* strains were screened for ethanol tolerance and rate of anaerobic growth on D-xylose. Principally on the basis of its superior ethanol tolerance, *E. chrysanthemi* B374 was chosen for more detailed study of fermentation end products, sugar catabolism, and nutrient requirements and as the recipient strain for a genetic implant to increase its ethanol production.

E. chrysanthemi and *E. carotovora* dissimilate glucose by the Embden-Meyerhof pathway to yield coliform-type mixed acid fermentation products: butanediol, lactate, acetate, formate, and a small amount (less than 1 mol/mol of glucose) of ethanol (5, 16). The ethanol yield is much higher (up to 2 mol/mol of glucose) in a yeast-type fermentation, in which the decarboxylation of pyruvate to acetaldehyde is catalyzed by pyruvate decarboxylase (EC 4.1.1.1), represented by the dashed line in Fig. 1. Acetaldehyde is subsequently reduced to ethanol by alcohol dehydrogenase (EC 1.1.1.1), with concurrent oxidation of NADH. Pyruvate decarboxylase is not present in *E. chrysanthemi* but is found in yeasts and a few bacteria, including *E. amylovora* and *Zymomonas mobilis* (4, 9, 10). The dissimilation of pentose sugars by the soft-rot *Erwinia* species is presumed to be similar to that shown in Fig. 1 for glucose; 3 mol of pentose yield 5 mol of pyruvate, since enteric bacteria utilize transketolase and transaldolase enzymes rather than the phosphoketolase pathway typical of some fungi and lactic acid bacteria (5).

MATERIALS AND METHODS

Cultures. *E. chrysanthemi* B374 was kindly supplied by A. Toussaint. Monthly subcultures of the *Erwinia* strains used in the initial screening were made on 2% Difco agar slants with 1% Difco nutrient broth and stored under refrigeration (*E. carotovora* subsp. *carotovora*) or at 20°C (*E. chrysanthemi*).

Escherichia coli S17-1 (pZM15) was kindly supplied by B. Bräu (3). Plasmid pZM15 comprises the *pdc* gene from *Z. mobilis* and its promoter and the chloramphenicol resistance gene. *E. coli* S17-1 is a histidine auxotroph. Plasmid pZM15 was conjugated into *E. chrysanthemi* by filter mating by G. Schatz and S. V. Beer, Department of Plant Pathology, Cornell University. The conjugated *E. chrysanthemi* cells were cultivated as the wild type, with the addition of 20 mg of chloramphenicol per liter unless otherwise indicated.

Cultivations. All cultivations and incubations were at 30°C in medium consisting of the following (grams per liter of deionized water): KH_2PO_4, 12.5 (reduced to 4.0 after initial screening experiments); $(NH_4)_2SO_4$, 1.0; K_2HPO_4, 4.0; $MgSO_4 \cdot 7H_2O$, 0.2; $FeSO_4 \cdot xH_2O$, 0.002; D-(+)-glucose, L-(+)-arabinose (Sigma Chemical Co.), or D-(+)-xylose (Sigma), 5.0 or as indicated. The growth medium was adjusted to the desired pH by the addition of NaOH pellets or 2 N H_2SO_4. All chemicals were reagent grade. Difco yeast extract (0.5 g/liter or as indicated), absolute ethanol, and other supplements were added as needed. The *E. chrysanthemi* B374 transconjugants were cultivated as above, with the addition of 20 mg of chloramphenicol per liter.

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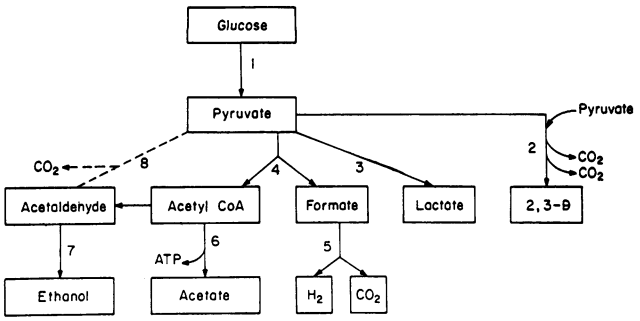

FIG. 1. Fermentation pathways of *Erwinia* spp. 1, Embden-Meyerhof enzymes and intermediates; 2, intermediates of butanediol pathway; 3, lactate dehydrogenase; 4 pyruvate formate-lyase; 5, formate hydrogenlyase; 6, phosphotransacetylase and acetate kinase; 7, alcohol dehydrogenase; 8, pyruvate decarboxylase (not present in wild-type *Erwinia* strains).

Preliminary growth and alcohol tolerance experiments were done in stationary anaerobic broth tubes. Tubes were made anaerobic before sterilization by being heated in a boiling-water bath to remove dissolved oxygen and then being closed under a stream of oxygen-free nitrogen (Matheson Scientific, Inc.). Liquid inocula (2 to 5%) from 48-h cultures, the carbon source, and any additional supplements were added by syringe. The sugars were separately sterilized by being autoclaved. Ethanol, vitamins, and chloramphenicol were filter sterilized. Growth was monitored by periodically measuring the optical density directly in the tubes; samples were withdrawn by syringe for measurement of sugar consumption or products.

Batch fermentations were done in a 2-liter water-jacketed vessel containing 1.5 to 1.7 liters of magnetically stirred medium. After 45 min of steam sterilization the fermentor was gassed with nitrogen for 30 min to attain anaerobic conditions. The pH was controlled to within ±0.1 units by the automatic addition of 2 N NaOH. During continuous fermentations the liquid level was maintained at 1.5 liter by pumping out any liquid that exceeded this level. Masterflex pumps (Cole-Parmer Instrument Co.) were used for feed and base addition and effluent removal. Silicone tubing (Cole-Parmer) was used on all lines. The liquid flow rate was measured by collecting effluent in a graduated vessel for 1-h periods. Base addition accounted for less than 5% of the total liquid flow. Steady-state conditions were assumed to exist after a constant optical density was maintained for at least five residence times.

Analytical methods. Broth samples were analyzed after centrifugation to remove the cells. Ethanol, acetate, and 2,3-butanediol were determined by gas chromatography (no. 3700, with a flame ionization detector; Varian). The 0.25 in. (0.64-cm), 4-mm-inside-diameter glass column was packed with 2 m of 10% SP1000 on 100/200 mesh Chromosorb W-AW and 1% H_3PO_4 (Supelco, Inc.) recommended for the determination of volatile acid and neutral products (28). The flow rate of the carrier gas, He, was 35 ml/min, and the temperatures of the injection port, column, and detector were 180, 152, and 180°C, respectively. Glucose, xylose, and arabinose were determined with dinitrosalicylic acid reagent (18). Formate was determined colorimetrically by reaction with citric acid (24). Lactic acid was measured colorimetrically by reaction with *p*-phenylphenol (19). In some cases, total gas evolution was measured by displacement of acidified water in an inverted tube. The effluent gas was also absorbed into 0.2 M $Ba(OH)_2$ and the precipitated $BaCO_3$ was weighed to determine carbon dioxide evolution. Growth was monitored by measuring the optical density at 600 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. Dry weights were measured by centrifuging 35-ml samples of the culture, washing with distilled water, centrifuging, and then weighing the sediment after drying it overnight at 105°C. Viable cell counts were determined by triplicate platings at several dilutions. Retention of the pyruvate decarboxylase-encoding plasmid in conjugated cells was measured by making viable cell counts on agar with and without 20 mg of chloramphenicol per liter.

RESULTS

Growth and ethanol tolerance. All but three of the strains tested grew on a xylose-plus-salts medium; doubling times ranged from 8 to 18 h for *E. carotovora* and from 9 to 24 h for *E. chrysanthemi*. No correlation was found between growth rate and original host or phenotypic properties of the strains (R. S. Dickey, private communication). The doubling time of selected strains was measured with glucose or arabinose as the carbon source. In all cases, growth was most rapid with glucose (anaerobic doubling time, 1.5 to 3 h), intermediate with arabinose (4 to 6 h), and slowest with xylose.

As a further screening, the ability of several *Erwinia* strains to grow in a xylose-mineral salts medium with ethanol added was measured. Although some strains of *E. carotovora* grew better in the absence of ethanol, *E. chrysanthemi* B374 exhibited the best growth in 3% (wt/vol) added ethanol and even showed some growth up to 5% (wt/vol) ethanol. The other *E. chrysanthemi* strains grew less rapidly at all ethanol concentrations. It is not evident why *E. chrysanthemi* B374 exhibits a higher ethanol tolerance than the other strains tested. One noteworthy observation, however, is that *E. chrysanthemi* B374 was the only strain to form visible (diameter, 1 to 3 mm) clumps of cells when grown in a minimal medium. The clumps were easily dispersed by shaking the culture tubes. No clumps were formed when the cells were grown in a rich medium.

The enhancement of ethanol tolerance and xylose consumption by the addition of medium supplements for *E. chrysanthemi* B374 is shown in Fig. 2. Little xylose was consumed after 4 days in a xylose-mineral salts medium with 2% added ethanol; consumption of xylose increased markedly with the addition of casein hydrolysate in less than 3% ethanol. The enhancement in ethanol tolerance attained by adding yeast extract to the medium suggests that vitamin or mineral requirements are increased by the presence of a high ethanol concentration. The doubling time in xylose-yeast extract increased from 10 h (no ethanol) to 24 h (4% ethanol). By contrast, there was little difference in growth rate between cultures with 0 and 3% added ethanol when glucose was the carbon source (data not shown).

In a rich medium with no added ethanol, the cells were highly motile rods. A filamentous growth became predominant when the ethanol concentration exceeded 2%; the filaments were 20- to 50-fold longer than single cells. As the ethanol concentration increased, the number of colonies formed per unit dry weight increased. Similar filament formation has been observed with *E. coli* in 2 to 3% ethanol (8) and *Z. mobilis* at 7 to 9% ethanol (11). Attempts to improve the ethanol tolerance of *E. chrysanthemi* by supplementing the medium with 0.3 M sodium chloride to prevent cell lysis (12) or oleic acid for the membrane (13) showed no significant effect.

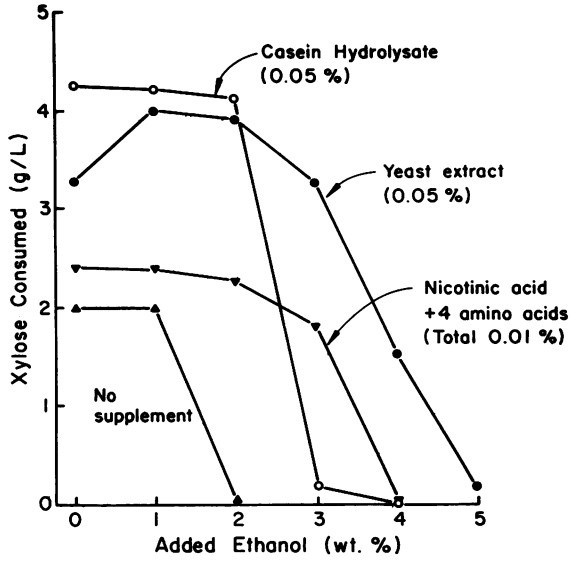

FIG. 2. Consumption of xylose in 88 h by *E. chrysanthemi* B374. Vitamin and amino acid supplements to the basal medium aid xylose consumption.

Fermentation products. The products of the batch fermentation of arabinose and xylose by *E. chrysanthemi* B374 at constant pH 6 and 7 are shown in Fig. 3. Cell mass was estimated, from initial experiments, as 6 to 8% of the carbon source consumed, and CO_2 was estimated from the phosphoroclastic cleavage of pyruvate (5), which gives the molar balance

$$[CO_2] + [\text{formate}] = [\text{acetate}] + [\text{ethanol}] \quad (1)$$

where brackets denote concentration. The estimate of CO_2 includes a small mass of hydrogen. Note that no 2,3-butanediol was detected. By contrast, the fermentation of glucose of *E. carotovora* DSM 30169 yielded 0.18 mol of butanediol per mol of glucose (data not shown), and a value of 0.15 mol/mol of glucose has been reported in the literature (16). All of the runs showed a coliform-type mixed-acid fermentation, with ethanol, lactate, formate, and acetate as the major products. Ethanol represented 18 to 22% of the sugar fermented. Formate production was higher at pH 7 than pH 6, while lactate production from arabinose was higher at pH 6; both patterns are typical for coliform and heterolactic fermentations (31). Very little lactate was produced from xylose, possibly because the slow utilization of xylose resulted in a low intracellular level of fructose 1,6-diphosphate, a positive effector for lactate dehydrogenase (EC 1.1.1.27) (27).

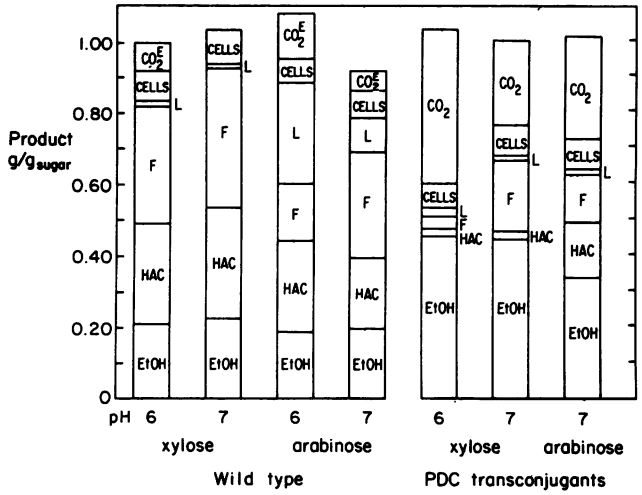

FIG. 3. *E. chrysanthemi* end products of the fermentation of xylose and arabinose, by weight fraction, at constant pH values. Abbreviations: EtOH, ethanol; HAC, acetate; F, formate; L, lactate; CELLS, cell dry weight (estimated as 6 and 8% of the sugar fermented at pH 6 and 7, respectively); CO_2, carbon dioxide (superscript E indicates estimate from equation 1). The *pdc* transconjugants produced much more ethanol than did the wild type, with concurrent decreases in acetate, formate, and lactate.

TABLE 1. Continuous fermentations of arabinose[a]

Dilution rate (h^{-1})	Lactate yield (g/g of arabinose)	Cell yield (g/g of arabinose)
0.11	0.12	0.080
0.07	0.10	0.075
0.03	0.01	0.059
Batch	0.10	0.08

[a] pH 7; 5 g of arabinose per liter plus minimal medium.

Table 1 shows the production of lactate and cell yield from arabinose in continuous fermentations. In a chemostat, the growth rate u equals the dilution rate D, where D is defined as fresh feed rate (in milliliters per hour)/fermentor volume (in milliliters) (21). By using low dilution rates, we obtained low growth rates and decreased the amount of lactic acid produced, possibly owing to a decreased internal pool of fructose 1,6-diphosphate (27). The use of low dilution rates had no significant effect on fermentation products from glucose or xylose (date not shown). The low cell yield observed at the lowest dilution no doubt reflects a high maintenance energy (2).

The maximum ethanol concentration produced by *E. chrysanthemi* was of interest. Since growth is inhibited by high concentrations of pentose sugars, 20 g of xylose per liter was fed after an initial 20 g/liter was consumed. The products of fermentation at pH 7.0 (data not shown) did not differ significantly from those for 5 g of xylose per liter (Fig. 3). Xylose was consumed at a maximum rate of 7.2 g/liter per day, and the final ethanol concentration was 7.4 g/liter. The fermentation was terminated after 120 h, when extensive cell lysis was observed. From the results in Fig. 2, it seems that 7.4 g of ethanol accumulation per liter should not be toxic. To determine which, if any, product was toxic, fresh culture was inoculated into broth tubes supplemented with the sodium salts of formate, acetate, and lactate. Formate inhibited growth at 7 g/liter and caused lysis at 11 g/liter; the other acids had little effect on growth at the concentrations encountered (data not shown).

Sugar dissimilation. The anaerobic growth rate of *E. chrysanthemi* B374 as a function of glucose, xylose, and arabinose concentration is shown in Fig. 4. Data were obtained by two methods, (i) steady-state measurements of sugar concentration and dilution rate in a continuous fermentor at pH 7, and (ii) initial growth rates in broth tubes. The maximum growth rate, 0.28/h, was independent of glucose concentration in the range observed. When arabinose was the substrate, the maximum growth rate, 0.12/h, occurred at a sugar level of 1 g/liter. The growth rate on xylose increased up to a xylose concentration of 8 g/liter.

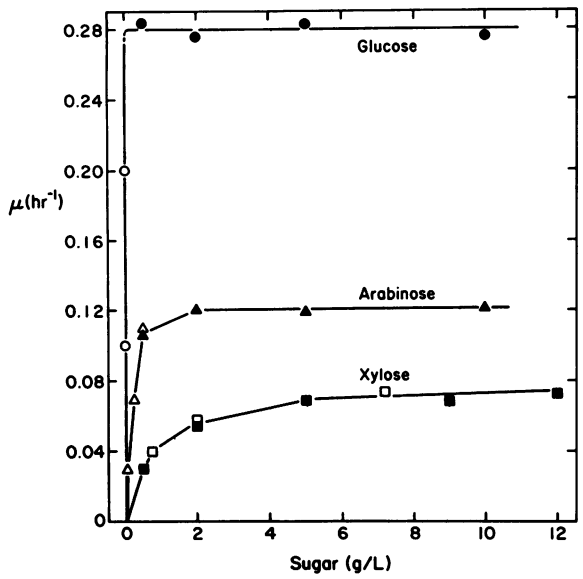

FIG. 4. Growth rate as a function of sugar concentration for *E. chrysanthemi*. Points indicated by solid symbols were based on initial growth rates in broth tubes; open symbols represent steady-state conditions in a continuous fermentation. The growth rate increased gradually with the xylose concentration up to 8 g/liter, a reflection of the high (670 mg/liter) K_s value.

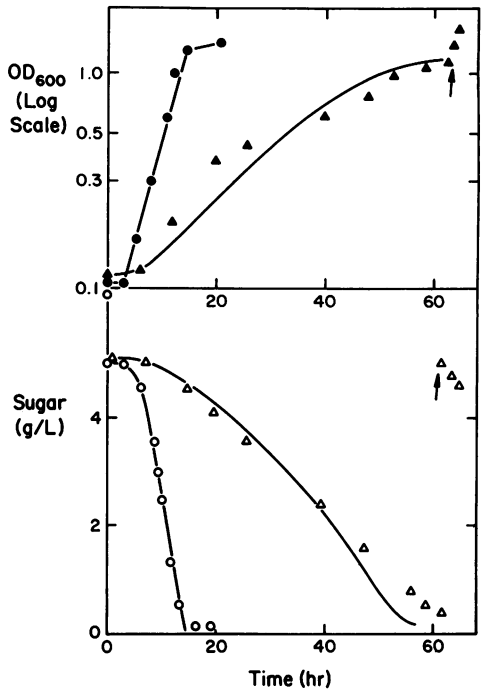

FIG. 5. Growth and sugar consumption with glucose and xylose as substrates. Lines represent equations 2 and 3 with, for glucose (●, ○) μ_M = 0.28/h, S_0 = 5 g/liter, K_s = 8 mg/liter, Y = 0.08 g of cells per g of substrate, lag = 4 h, X_o = 0.04 g/liter, and optical density at 600 nm (OD_{600}) = (3.0 liters/g)X. For xylose (▲, △), the same parameters were used, except that μ_M = 0.08/h, K_s = 670 mg/liter, and lag = 6 h. At the time indicated by the arrow, the xylose concentration was restored to 5 g/liter. Initial growth and consumption rates are recovered, an indication that xylose was the limiting nutrient.

The data from Fig. 4 were recast into a Lineweaver-Burk plot ($1/\mu$ versus $1/S$) to determine the Monod parameters μ_m (maximum growth rate) and K_s (Monod saturation constant) (2).

The K_s value (saturation parameter) for glucose was below the sensitivity of the assay (0.025 g/liter); it may be close to 8 mg/liter, the anaerobic value for *E. coli* (1). The K_s for arabinose was 0.05 g/liter, and that for xylose was 0.67 g/liter, which is comparable to values reported for *Clostridium acetobutylicum* (0.75 g/liter) (20) and *Klebsiella oxytoca* (1.1 g/liter) (14).

A consequence of the high K_s for xylose is that the growth rate in a batch culture starts to diminish well before exhaustion of the sugar. Such a situation can be described by mass balances for substrate and biomass, assuming constant cell yield and Monod kinetics (21). The expression relating substrate concentration with time is

$$t = \frac{K_s}{\mu_M(S_0 + X_0/y)} \ln\left\{\left(\frac{S_0}{S}\right)\left[1 + \frac{Y}{X_0}(S_0 - S)\right]\right\} + \frac{1}{\mu_M} \ln\left[1 + \frac{Y}{X_0}(S_0 - S)\right] \quad (2)$$

where t is time (hours), K_s is the Monod saturation constant (grams per liter), μ_M is the maximum growth rate (reciprocal hours), S_0 is the initial sugar concentration (grams per liter), S is the sugar concentration (grams per liter), X_0 is the initial biomass (grams per liter), X is the biomass (grams per liter), and Y is the biomass yield from sugar (gram of cells per gram of substrate). An analogous expression is obtained for cell mass as a function of time (21):

$$t = \frac{K_s}{\mu_M(S_0 + X_0/y)} \ln\left\{\left[\frac{S_0}{S_0 - (X - X_0)/Y}\right]\left(\frac{X}{X_0}\right)\right\} + \frac{1}{\mu_M} \ln\left(\frac{X}{X_0}\right) \quad (3)$$

The entire first term on the right side of equation 3 is negligible if S_0 is much larger than K_s; thus the familiar exponential growth is obtained. When S_0 is not significantly larger than K_s, deviations from exponential growth occur early in the batch cycle. The predictions of equations 2 and 3 for growth and sugar disappearance are shown in Fig. 5 along with experimentally measured values of the growth parameters. Because the predictions of equations 2 and 3 fit the data reasonably well, the fermentation of both xylose and glucose by *E. chrysanthemi* can be described by Monod kinetics, with sugar limiting. Furthermore, when 5 g of xylose per liter was added to a culture that had reduced an initial 5 g of xylose per liter to less than 0.2 g/liter, the initial growth rate was reestablished (Fig. 5). This is direct evidence of xylose limitation, rather than end product inhibition or limitation by other nutrients.

***E. chrysanthemi* B374 transconjugants.** The fermentation

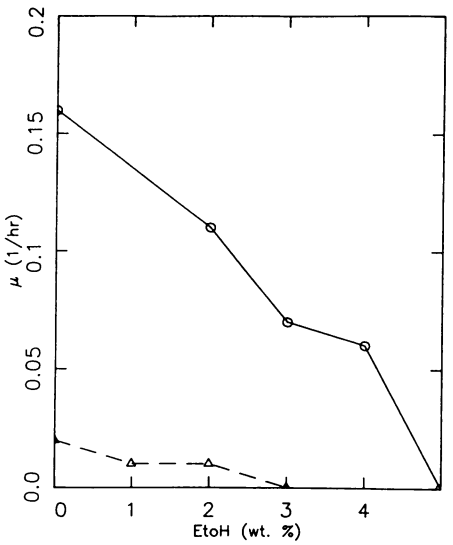

FIG. 6. Growth of conjugated *E. chrysanthemi* (△), compared with wild type (○), with glucose substrate. Growth rate and ethanol tolerance sharply diminished in the conjugated cells.

products of the *E. chrysanthemi* transconjugants are shown in Fig. 3 with xylose and arabinose substrates. Much more ethanol and CO_2 and a much lower level of acids were produced than by the wild type; the ethanol yields of 1.45 mol/mol of xylose, 1.1 mol/mol of arabinose, and up to 1.46 mol/mol of glucose (not shown) are clear evidence for the expression of the *Zymomonas* pyruvate decarboxylase.

The transconjugants grew poorly on both pentose sugars, with an anaerobic doubling time of 45 h with 5 g of substrate per liter. The growth rate was nearly doubled when up to 27 g of sugar per liter was used, indicating a limitation to substrate uptake. Only 0.9 g of xylose and 1.3 g of arabinose per liter were consumed in batch fermentations. Growth with glucose as the carbon source was more rapid and more complete than with the pentose sugars (data not shown); the doubling time was 8 h.

The transconjugant exhibited less tolerance for ethanol and salts than the wild type did. Little growth occurred at an ethanol concentration of more than 2% (Fig. 6). The addition of 1 to 2% ethanol caused cells to become somewhat shorter; the wild type became filamentous in the presence of ethanol. Sodium salts, including sodium chloride, prevented growth at concentrations greater than 12 g/liter. The wild type grew in media containing up to 45 g of added NaCl per liter (data not shown). Such osmosensitivity of the transconjugant was not observed in later work with *Klebsiella* spp. (29).

The addition of 20 mg of chloramphenicol per liter ensured retention of the plasmid for at least three anaerobic batch growth cycles. The plasmid was lost by more than 90% of the cells within one anaerobic batch growth cycle when chloramphenicol was omitted from the medium. During aerobic growth, however, the plasmid was maintained for at least three batch growth cycles without addition of chloramphenicol.

DISCUSSION

Our data showed a significant strain-to-strain variation in the anaerobic growth rate of *E. chrysanthemi* and *E. carotovora* on pentose sugars. Some strains did not ferment xylose, whereas others grew with doubling time of 8 to 24 h on a xylose-salts medium. The growth rates did not correlate with serotypes, plant host, or other known characteristics of *Erwinia* spp. There were also variations in fermentation products (mixed acids with or without 2,3-butanediol) and ethanol tolerance (2 to 4%).

E. chrysanthemi B374 has several desirable traits for the production of ethanol from pentose sugars. Of all the strains tested, it tolerated the highest ethanol concentration (4%). *E. chrysanthemi* B374 produced no 2,3-butanediol and grew as fast as most of the *Erwinia* strains. However, in relation to the requirements for an economically feasible conversion of pentoses to ethanol (15), *E. chrysanthemi* grows and catabolizes sugars much too slowly and produces too little ethanol.

The reason for the slow growth and xylose uptake of *E. chrysanthemi* is not clear; it is also not evident why growth is faster with arabinose as the substrate than with xylose. There may be low, nonspecific permease activity for xylose, as suggested by the high K_s value. Key enzymes in the pathway for xylose catabolism, such as xylose isomerase (EC 5.3.1.5) and xylulokinase (EC 2.7.1.17) (5), may be rate limiting. In particular, xylose isomerase also functions as glucose isomerase, and so there may be a regulatory conflict between hexose and pentose metabolism (R. P. Mortlock, private communication).

The *Zymomonas* pyruvate decarboxylase gene (*pdc*) was expressed quite well in *E. chrysanthemi*; the ethanol yield increased from 0.72 to 1.45 mol/mol of xylose. In addition, production of the toxic product, formate, decreased. However, growth rate, ethanol tolerance, and salt tolerance were sharply diminished in the conjugated cells. Bräu and Sahm (3) found that the *Z. mobilis pdc* gene was similarly expressed in *E. coli*: the ethanol yield was 1.66 mol/mol of glucose in the conjugated cells versus 0.44 mol/mol of glucose in the wild type. Apparently the *E. coli* transconjugants tolerate only 0.23% ethanol. The reduced production of acetic acid may decrease the growth rate, because an additional molecule of ATP is generated for each molecule of acetate formed (Fig. 1). The decrease in acetate and formate production implies a reduced carbon flow through pyruvate formate-lyase. Consequently insufficient acetyl coenzyme A may be available for membrane fatty acid synthesis, a deficiency which may contribute to lower ethanol tolerance.

E. amylovora, which grows poorly anaerobically and does not ferment xylose (6), is reportedly an alternative source of pyruvate decarboxylase (9, 10). *E. amylovora* ferments glucose to ethanol in high yield, 1.55 mol/mol of glucose (26). In preliminary experiments, however, we have observed a coliform-type mixed-acid fermentation in *E. amylovora*, with little alcohol formation. As described in the companion paper (29), *pdc* transconjugants of *Klebsiella planticola* ferment xylose almost as rapidly as the wild type with little loss in ethanol tolerance.

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